Characterizing the Neural Substrates of Irritability in Women: an Experimental Neuroendocrine Model

NCT number NCT04051320
Document Date 10/06/2020

Characterizing the neural substrates of irritability in women: an experimental neuroendocrine model

Regulatory Sponsor:

David R. Rubinow, MD
UNC Department of Psychiatry
10514 Neurosciences Hospital, 101 Manning Drive
Campus Box 7160
Chapel Hill, NC
27599-7160
919-966-4738

Funding Sponsor:

National Institute of Mental Health
6001 Executive Boulevard, Room 6200, MSC 9663
Bethesda, MD 20892-9663

Study Product:

Estradiol
Active ingredient: micronized estradiol
Chemical name: estra-1,3,5,(10)-triene-3, 17β-diol

Lupron Depot
Active ingredient: leuprolide acetate for depot suspension
Chemical name: 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide

Progesterone
Active ingredient: micronized progesterone
Chemical name: pregn-4-ene-3, 20-dione

IRB Protocol Number: *19-0401*

IND Number: *N/A*

Study Personnel:

Principal Investigator: Crystal Edler Schiller, PhD
Perinatal Psychiatrist: David Russel Rubinow, MD
OBGYN: Lauren Schiff, MD
Neuroimaging Expert: Gabriel Dichter, PhD
Biostatistician: Kai Xia, PhD
fMRI Acquisition and Analysis Specialist: Josh Bizzell, MS
Data Manager: Russel Dean, MS
Study Coordinator: Laura Lundegard, BA

Initial version: 1/25/19
Amended: 10/6/20

Table of Contents

List of Abbreviations

AE	Adverse Event
BAS	Behavioral approach system
BIS	Behavioral Inhibition system
BOLD	Blood-Oxygen-Level-Dependent
BRIC	Biomedical Research Imaging Center
CHD	Coronary Heart Disease
CRF	Case Report Form
COVID-19	Coronavirus Infection
DSMB	Data and Safety Monitoring Board
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DVT	Deep Vein Thrombosis
EC	Ethics Committee
EPDS	Edinburgh Postnatal Depression Scale
EPT	Combined Estrogen and Progestin Treatment (EPT)
ERT	Estrogen Replacement Therapy

FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FNR	Frustrative Non-Reward
FSH	Follicle Stimulating Hormone
FSL	Brain Imaging Analysis Software
GnRH	Gonadotropin-Releasing Hormone
gPPI	Generalized Psychophysiological Interaction
HIPAA	Health Insurance Portability and Accountability Act
HCG	Human Chorionic Gonadotropin
HS+	Hormone Sensitive: Defined as a 30% or greater change in any of the mood scales of the IDAS-II, which include dysphoria, suicidal ideation, ill temper (i.e., irritability), and wellbeing (i.e., reverse-keyed), from baseline to hormone addback or withdrawal.
HS-	Non-Hormone Sensitive: Defined as less than a 30% change in any of the mood scales of the IDAS-II, which include dysphoria, suicidal ideation, ill temper (i.e., irritability), and wellbeing (i.e., reverse-keyed), from baseline to hormone addback or withdrawal.
IDAS-II	Inventory of Depression and Anxiety Symptoms
IDS	Investigational Drug Service
IM	Intramuscular

IRB	Institutional Review Board
IVF	In Vitro Fertilization
LH	Luteinizing Hormone
MASQ-AD	Mood and Anxiety Symptom Questionnaire – Anhedonic Depression Subscale
MATLAB	An Interactive Environment for Numerical Computation, Visualization, and Programming
NIH	National Institutes of Health
PAG	Amygdala-Hypothalamic-Periaqueductal Gray
PFC	Prefrontal Cortex
PHI	Protected Health Information
PMDD	Premenstrual Dysphoric Disorder
PMS	Premenstrual Syndrome
PND	Perinatal Depression
PPD	Postpartum Depression
ROI	Region of Interest

SAE	Serious Adverse Event
SCID	Structured Clinical Interview for DSM-V-TR Axis-I Disorders
SNAP	Schedule for Nonadaptive and Adaptive Personality
STRAW	Stages of Reproductive Aging Workshop
UNC-CH	University of North Carolina at Chapel Hill
WHI	Women’s Health Initiative

Study Summary

Title	Characterizing the neural substrates of irritability in women: an experimental neuroendocrine model
Short Title	WIN Study
Protocol Number	IRB# 19-0401
Phase	II
Methodology	Double-blind, placebo controlled, longitudinal cohort-comparison study
Study Duration	36-48 months
Study Center(s)	Single-center
Objectives	The objective of the current project is to examine whether hormone sensitive women (HS+; n=15) show differences in the behavioral activation system relative to non-hormone sensitive women (HS-; n=15) under baseline and hormone challenge conditions using functional magnetic resonance imaging (fMRI) and behavioral tests.
Number of Subjects	30
Diagnosis and Main Inclusion Criteria	<u>Group 1: Women with a history of postpartum depression</u> 1) A history of a DSM-IV major depressive episode that began during the third trimester or within 6 weeks of childbirth (as determined by a SCID interview) and remitted at least one year prior to enrollment in the study; 2) has been well for a minimum of one year; 3) a regular menstrual cycle for at least three months; 4) age 21-45; 5) not pregnant, not lactating and in good medical health; 6) medication free (including birth control pills); 7) no history of puerperal suicide attempts or psychotic episodes requiring hospitalization. <u>Group 2: Healthy Controls</u> 1) Controls will meet all inclusion criteria specified above except they must not have any past or present Axis I diagnosis or evidence of menstrually related mood disorders.
Study Product, Dose, Route, Regimen	**Study Product:** **Estradiol** Active ingredient: micronized estradiol Chemical name: estra-1,3,5,(10)-triene-3, 17β-diol

Lupron Depot
Active ingredient: leuprolide acetate for depot suspension
Chemical name: 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide

Progesterone
Active ingredient: micronized progesterone
Chemical name: pregn-4-ene-3, 20-dione

Dose:

Drug	**Dosage**	**Formulation**
Estradiol	2 mg bid	Oral capsule
Lupron Depot	3.75 mg/month	Intramuscular injection
Progesterone	200 mg bid	Oral capsule

Route:

Drug	**Route of Administration**
Estradiol	Oral
Lupron Depot	Intramuscular injection
Progesterone	Oral

Regimen:

Induced Hypogonadism. After the baseline period, participants will receive their first injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron) 3.75 mg/month via intramuscular injection, which is administered to produce a stable hypogonadal condition (after the initial “flair”). The first Lupron

	injection will be administered on day six of the participants' first menstrual cycle after the baseline period and at monthly intervals thereafter until follow-up. During the month of GnRH agonist administration, all participants will receive placebo progesterone and placebo estradiol tablets and will be told that at some point the placebo pills will be switched to active medication. Addback. After the fourth week of Lupron-alone treatment (i.e., hypogonadism), high plasma levels of estradiol and progesterone will be attained via micronized progesterone and estradiol tablets for two weeks (with continued Lupron administration). Estradiol will be administered at a dose of 2 mg bid (i.e., a total of 4 mg per day). Progesterone will be administered at a dose of 200 mg bid (i.e., a total of 400 mg per day). The blood levels that we expect to achieve and sustain in each woman will be approximately 500 pg/ml of estradiol and 30-40 ng/ml of progesterone. Withdrawal. After two weeks of hormone replacement, active hormone tablets will be replaced with placebo tablets to induce a precipitous drop in plasma estradiol and progesterone levels. Lupron will maintain hypogonadal levels for the two-week withdrawal phase. The second fMRI session will take place at the beginning of the withdrawal period.
Duration of administration	8 weeks
Reference therapy	Placebo
Statistical Methodology	This study has one between-subjects factor (group: HS+ versus HS-) crossed with one within-subjects factor (time: baseline versus hormone addback) across each outcome (behavioral index or percent signal change in a particular region of interest). Thus, for a task/outcome combination, there is a 2-by-2 repeated measures design.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Irritability is a dimensional construct—defined as a predisposition to exhibit anger (Vidal-Ribas et al., 2016) and characterized by dysregulated approach-avoidance motivation (Leibenluft, 2017) —which cuts across neuropsychiatric disorders, including bipolar (American Psychiatric Association, 2013) , depressive (Fava et al., 2010; Perlis et al., 2005), anxiety (Leibenluft et al., 2006; Price & Stolk-Cooke, 2015), trauma-related (Price & Stolk-Cooke, 2015; Van Voorhees et al., 2018), psychotic (Bilgi et al., 2017; Penn et al., 1996), neurodevelopmental (McGuire et al., 2016), neurocognitive (Alper et al., 2002; Lopez et al., 2003), and personality disorders (Scott et al., 2015). Despite the near ubiquity of irritability across disorders, the neural dysfunction underlying the vulnerability to, onset of, and exacerbation of irritability is understudied and poorly understood (Leibenluft, 2017). Although not included in the DSM criteria, irritability is increasingly being recognized as a prominent, defining symptom of perinatal depression (PND) (Williamson et al., 2015). The perinatal period (i.e., pregnancy and first month following childbirth) is characterized by predictable, marked changes in the female reproductive hormones estradiol and progesterone, which powerfully regulate approach-avoidance motivation (Schiller, Johnson, Abate, Schmidt, & Rubinow, 2016). Given that approach-avoidance dysregulation is implicated in irritability (Leibenluft, 2017), PND offers a unique opportunity to identify physiological regulators of irritability in women. However, studying women with PND is challenging for two reasons. First, the existence of multiple putative etiologic pathways (i.e., vulnerability to the effects of hormonal change versus the psychosocial stress of childbirth) results in a heterogeneous phenotype (Postpartum Depression: Action Towards Causes and Treatment (PACT) Consortium, 2015). Second, there are obvious logistical problems entailed in studying a mother who is depressed and requires treatment. A solution to these problems is provided by experimentally manipulating reproductive hormones in non-pregnant, euthymic women to create a scaled down version of the changes that occur during and after pregnancy (Bloch et al., 2000).

We have used this manipulation to identify a subgroup of “hormone sensitive” women in whom perinatal-like changes in reproductive hormones precipitated acute but transient affective symptoms, the most prominent and immediate of which was irritability (measured by the Inventory of Depression and Anxiety Symptoms—IDAS-II) (see Section 1.4 below). However, that study focused on anhedonia and dysphoria, and given that the primacy of irritability was unexpected (i.e., irritability is neither a diagnostic criterion of PND (American Psychiatric Association, 2013) nor included in the gold-standard self-report instrument of PND severity (J. L. Cox, Holden, & Sagovsky, 1987), yet recent research suggests it is, in fact, the cardinal symptom of PND (Williamson et al., 2015), the fMRI tasks were not selected to investigate irritability. Irritability has been conceptualized as an aberrant “approach” response both to potential threat, mediated by cortico-limbic circuit dysfunction, and to frustration, mediated by striatal circuit dysfunction (Leibenluft, 2017). The threat hypothesis of irritability posits that

dysregulation in the amygdala-hypothalamic-periaqueductal gray (PAG) threat response circuitry results in approach behavior in response to threat rather than freezing or flight (Blair, 2016; Salum et al., 2017).

Evidence supporting the threat hypothesis of irritability includes: 1) irritability is associated with anxiety in cross-sectional (Stringaris et al., 2009), longitudinal (Savage et al., 2015; Stringaris et al., 2009), and genetic studies (Savage et al., 2015), 2) irritability is characterized by aberrant connectivity within the PAG threat response circuitry when viewing threatening faces (Stoddard et al., 2017), and 3) irritability is associated with dysfunctional threat processing, including attention bias toward threatening faces (Hommer et al., 2014; Salum et al., 2017). A related but distinct process is dysfunctional reward processing, specifically pertaining to aberrant responses to frustrative nonreward (FNR). FNR is defined as reactions induced by the withdrawal or prevention of reward that one has been conditioned to expect, and the frustration hypothesis of irritability predicts increased approach behavior (i.e., aggression), relative to peers, in response to FNR (Brotman et al., 2017). Evidence supporting the frustration hypothesis of irritability includes: 1) compared with healthy controls, irritable children report greater frustration in response to FNR (Deveney et al., 2013), 2) irritable children respond in an aggressive manner toward others, including in response to ambiguous behavior (Vitaro et al., 2006), and 3) irritable children show dysregulation of the amygdala and striatum in response to FNR (Deveney et al., 2013). Thus, both threat and reward processing are implicated in irritability, and the concepts (i.e., Gray's Fear = frustration model (Gray, 1987)) and neural systems implicated are interrelated (McNaughton & Corr, 2004) and consistent with a dysregulated behavioral approach-avoidance system (Leibenluft, 2017). However, critical gaps remain: this work been done almost exclusively with children and adolescents (Leibenluft, 2017), and the extent to which it generalizes to irritability in adults is unclear; few studies have tested both the threat and frustration hypotheses concurrently, which has precluded an integrated neural systems model; and to our knowledge, none of the existing studies has attempted to distinguish state from trait irritability in terms of clinical presentation, course, or underlying neurobiology in adult women. The proposed research will address all three of these gaps in the literature.

The objective of the current study is to determine whether hormone sensitive women (HS+; n=15) show differences in the behavioral activation system relative to non-hormone sensitive women (HS-; n=15) under baseline and hormone challenge conditions using functional magnetic resonance imaging (fMRI) and behavioral tests. In essence, with a hormonal manipulation strategy, we will induce a physiologic condition that enables us to both provoke the effect of interest (i.e., irritability) and then, with behavioral challenges, identify the systems substrate using neuroimaging. Subjects serve as their own controls across time, carrying with them varying levels of trait vulnerability to irritability and then expressing an irritable mood state, also with varying degrees of severity. This approach will therefore allow us to disentangle the neural substrates of the vulnerability to, onset of, and exacerbation of irritability while simultaneously testing both the threat and frustration hypotheses of irritability, which will change the field in two important ways: First, this novel manipulation will allow us to create a reversible condition simulating both the prominent symptom of irritability and the hormonal state that precipitates it. By studying women with proved hormone sensitivity, we can be assured that the hormone-related irritability phenotype is selected and exploited for investigation of the underlying systems neurobiology of irritability. Second, the outcome of this project is expected to be a greater understanding of irritability

in PND, which remains vastly under-recognized. Despite the prominence of irritability as a core feature of PND, the gold-standard measure (J. L. Cox et al., 1987) for screening in clinical contexts (E. Q. Cox et al., 2017) and for quantifying severity in research contexts (Spinelli et al., 2016) doesn't assess irritability. Thus, this research has the potential not only to provide critical information about the neural systems underlying irritability, one of the most pervasive neuropsychiatric symptoms, but also to provide important information about irritability in the context of PND and other reproductive endocrine-related mood disorders.

1.2 Investigational Agent

Estradiol

Description

Estradiol Tablets USP for oral administration contains 0.5, 1 or 2 mg of micronized estradiol per tablet. Estradiol (17β-estradiol) is a white, crystalline solid, chemically described as estra-1,3,5,(10)-triene-3, 17β-diol. The structural formula is:

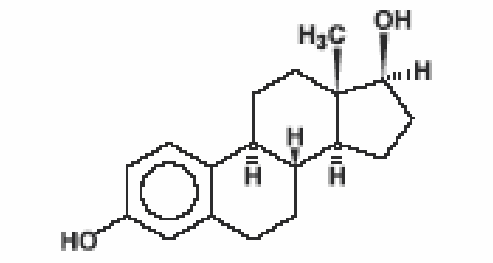


Figure 1. Structural formula for estra-1,3,5,(10)-triene-3, 17β-diol.

Inactive Ingredients: Colloidal silicon dioxide, corn starch, dibasic calcium phosphate, lactose monohydrate, magnesium stearate, and sodium starch glycolate. In addition, the 1 mg also contains FD&C blue no. 1 aluminum lake and D&C red no. 27 aluminum lake. The 2 mg also contains FD&C blue no. 1 aluminum lake and FD&C yellow no. 5 (tartrazine) aluminum lake.

Pharmacology

Endogenous estrogens are largely responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. Although circulating estrogens exist in a dynamic equilibrium of metabolic interconversions, estradiol is the principal intracellular human estrogen and is substantially more potent than its metabolites, estrone and estriol at the receptor level.

The primary source of estrogen in normally cycling adult women is the ovarian follicle, which secretes 70 to 500 mcg of estradiol daily, depending on the phase of the menstrual cycle. After menopause, most endogenous estrogen is produced by conversion of androstenedione, secreted by the adrenal

cortex, to estrone by peripheral tissues. Thus, estrone and the sulfate conjugated form, estrone sulfate, are the most abundant circulating estrogens in postmenopausal women.

Estrogens act through binding to nuclear receptors in estrogen-responsive tissues. To date, two estrogen receptors have been identified. These vary in proportion from tissue to tissue.
Circulating estrogens modulate the pituitary secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), through a negative feedback mechanism. Estrogens act to reduce the elevated levels of these hormones seen in postmenopausal women.

LUPRON DEPOT

Description
Leuprolide acetate is a synthetic nonapeptide analog of naturally occurring gonadotropin-releasing hormone (GnRH or LH-RH). The analog possesses greater potency than the natural hormone. The chemical name is 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt) with the following structural formula:

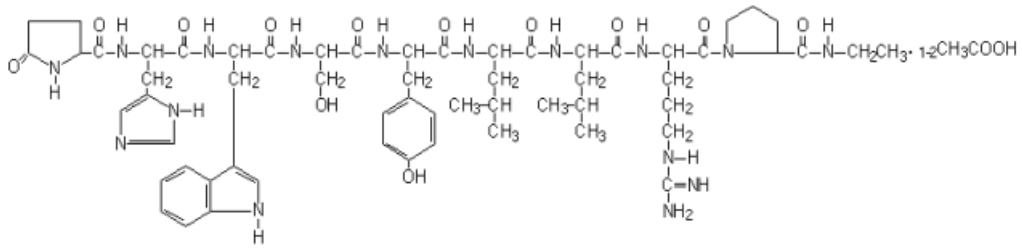


Figure 2. Structural formula for leuprolide acetate (5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-prolinamide acetate).

LUPRON DEPOT is available in a prefilled dual-chamber syringe containing sterile lyophilized microspheres which, when mixed with diluent, become a suspension intended as a monthly intramuscular injection.

The front chamber of LUPRON DEPOT 3.75 mg prefilled dual-chamber syringe contains leuprolide acetate (3.75 mg), purified gelatin (0.65 mg), DL-lactic and glycolic acids copolymer (33.1 mg), and D-mannitol (6.6 mg). The second chamber of diluent contains carboxymethylcellulose sodium (5 mg), D-mannitol (50 mg), polysorbate 80 (1 mg), water for injection, USP, and glacial acetic acid, USP to control pH. During the manufacture of LUPRON DEPOT 3.75 mg, acetic acid is lost, leaving the peptide.

Pharmacology
Leuprolide acetate is a long-acting GnRH analog. A single monthly injection of LUPRON DEPOT 3.75 mg results in an initial stimulation followed by a prolonged suppression of pituitary gonadotropins.

Repeated dosing at monthly intervals results in decreased secretion of gonadal steroids; consequently, tissues and functions that depend on gonadal steroids for their maintenance become quiescent. This effect is reversible on discontinuation of drug therapy.
Leuprolide acetate is not active when given orally. Intramuscular injection of the depot formulation provides plasma concentrations of leuprolide over a period of one month.

PROGESTERONE

Description
PROMETRIUM (progesterone, USP) Capsules contain micronized progesterone for oral administration. Progesterone has a molecular weight of 314.47 and a molecular formula of C21H30O2. Progesterone (pregn-4-ene-3, 20-dione) is a white or creamy white, odorless, crystalline powder practically insoluble in water, soluble in alcohol, acetone and dioxane and sparingly soluble in vegetable oils, stable in air, melting between 126° and 131°C. The structural formula is:

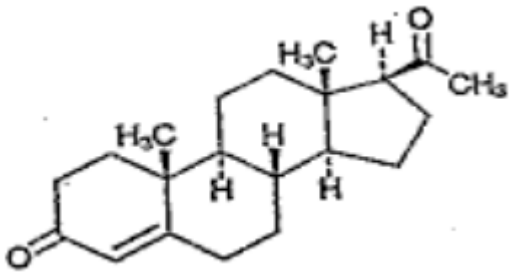


Figure 3. Structural formula for progesterone, USP (pregn-4-ene-3, 20-dione)

Progesterone is synthesized from a starting material from a plant source and is chemically identical to progesterone of human ovarian origin. PROMETRIUM Capsules are available in multiple strengths to afford dosage flexibility for optimum management. PROMETRIUM Capsules contain 100 mg or 200 mg micronized progesterone.
The inactive ingredients for PROMETRIUM Capsules 100 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C Yellow No. 10, and FD&C Red No. 40.
The inactive ingredients for PROMETRIUM Capsules 200 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C Yellow No. 10, and FD&C Yellow No. 6.

Pharmacology
PROMETRIUM Capsules are an oral dosage form of micronized progesterone which is chemically identical to progesterone of ovarian origin. The oral bioavailability of progesterone is increased through micronization.

Summary of Previous Human Experience

The proposed monthly 3.75-mg injection of leuprolide acetate (Lupron Depot) is FDA-approved for use in premenopausal women to treat endometriosis and uterine fibroids. Lupron has also been widely researched in healthy premenopausal women (Flory et al., 2002; Grigorova et al., 2006; Matthews et al., 1998; Sánchez et al., 2002) and to treat a variety of conditions, including ovarian epithelial tumor cells (Thompson et al., 1991), insulin resistance (Elkind-Hirsch, Valdes, & Malinak, 1993), endometrial stromal sarcoma (Scribner Jr. & Walker, 1998), and infertility (Padilla et al., 1991).

Several studies have investigated the use of Lupron and hormone addback to treat the symptoms of premenstrual syndrome and premenstrual dysphoric disorder (Lee et al., 2012; Mitwally et al., 2002; Schmidt et al., 1998; Wyatt et al., 2004), which has resulted in combined Lupron and estrogen/progestin supplementation as a recommended long-term treatment for premenstrual syndrome (Kaur et al., 2004; Mezrow et al., 1994).

Healthy women undergoing in vitro fertilization (IVF) routinely receive luteal supplementation of 600 mg progesterone daily along with 6 mg oral micronized estradiol (Lukaszuk et al., 2005) in combination with GnRH agonists (e.g., Lupron).

GnRH agonist treatment combined with hormone addback (i.e., high-dose estradiol and progesterone supplementation) has been previously studied in large randomized controlled trials for the purpose of IVF (Bourgain et al., 1990; Damario et al., 1999; Fatemi et al., 2006; Friedler et al., 1999; Humaidan et al., 2005; Lukaszuk et al., 2005). Of particular relevance to the current study, researchers induced a hypogonadal state using 3.75 mg Decapeptyl, followed by high-dose (800 mg) oral progesterone treatment in 32 women without an adverse event (Friedler et al., 1999). Several studies have examined the use of combined Lupron treatment (1 mg daily or 3.75 mg monthly), oral micronized estradiol (max doses ranged from 4 mg to 9 mg daily), and either oral (900 mg daily), vaginal (90-600 mg daily), or IM (50-200 mg daily) progesterone administration in over 1800 healthy women (Bourgain et al., 1990; Bustillo et al., 1995; Damario et al., 1999; Engmann et al., 2008; Friedler et al., 1999; Legro et al., 1993; F. Licciardi et al., 2001; F. L. Licciardi et al., 1999; Mirkin et al., 2003; Nagy et al., 2009; Noyes et al., 2001; Paulson et al., 1997; Peress & Philips, 1998; Sauer et al., 1992; Tao & Tamis, 1997; Tonguc et al., 2011). Of note, Damario et al. (Damario et al., 1999) reported the use of combined Lupron treatment, 9 mg oral micronized estradiol, and 100 mg i.m. progesterone in 238 healthy women presenting for IVF.

Dr. Rubinow's team at the NIH has examined the combined administration of Lupron and estradiol/progesterone in healthy premenopausal women in several studies without a significant adverse event (Berman et al., 1997; Bloch et al., 2005; Daly et al., 2001; Roca et al., 2003; Schmidt et al., 2009) Moreover, the same drug protocol proposed in the current study has been employed previously by Dr. Rubinow's research team at the NIH (Bloch et al., 2005) and is currently being used in ongoing projects at the NIH under the direction of Dr. Peter Schmidt and Dr. Pedro Martinez.

Thus, a large number of healthy women have previously received combined Lupron with high-dose oral estradiol and progesterone supplementation either as part of a research study or routine IVF treatment without significant side effects or adverse events.

Status of Drug in Other Countries

To our knowledge, the proposed drugs have not been withdrawn from investigation or marketing in any other country.

1.3 Preclinical Data

N/A

1.4 Clinical Data to Date

Hormone Challenge Protocol. Dr. Rubinow's team at the NIH previously employed the proposed protocol to examine reproductive-related affective dysfunction in 16 women(Bloch et al., 2000). Women with a history of postpartum depression showed significant increases in depressive symptoms during both hormone addback and withdrawal compared with baseline, whereas the control group did not (Bloch et al., 2000). To our knowledge, this was the first direct evidence to support the involvement of ovarian hormones in the development of PND in a subgroup of susceptible women. We replicated this finding in a larger group of women (N=30; 15 with a history of PND and 15 controls without such a history) and included both fMRI and more comprehensive depression and anxiety assessments, which revealed that the mood changes in HS+ are primarily driven by increased irritability.

Recruitment and Retention. Our successful completion of the previous study (N=30) involving the proposed hormone challenge and neuroimaging demonstrates both the feasibility of the proposed protocol and our ability to recruit and retain participants. Participants were recruited primarily via social media (57%) and other online recruitment tools (30%). Of those who began the hormone protocol, only 3 women (9%) dropped out: one who experienced a traumatic life event and consequent psychiatric symptoms unrelated to the study, and two because of logistical problems involved with attending biweekly study visits at UNC Hospital. Consequent to these dropouts, we modified the protocol to conduct all biweekly study visits in participants' homes, which subsequently improved retention. We have estimated an equivalent attrition rate in the current study despite the truncated protocol and the provision of home visits for all but the OBGYN and fMRI sessions, which will likely reduce attrition. Of note, none of the women in this or any of Dr. Rubinow's previous studies employing hormone challenge protocols experienced serious adverse events.

Use of the Hormone Challenge Protocol to Provoke Irritability and Dysregulate Striatum in HS+. Women with a history of PND reported increased irritability, anxiety, dysphoria, and anhedonia during the hormone challenge. Eleven out of 15 (73%) women with a history of PND were HS+ (i.e., showed a 30% increase in IDAS-II affective symptoms during early addback, late addback, or withdrawal compared with baseline). Irritability was the primary symptom for the majority (72%) of HS+, and because irritability distinguished HS+ and HS- as early as 2 weeks into hormone addback ($t=2.97$, $p<.004$; Figure 4), we propose to truncate hormone addback in the current study to 2 weeks (down from 8 weeks in the previous study) and conduct the second fMRI at that time. During the monetary incentive delay (MID) task, there was a significant group x time interaction in the bilateral putamen ($F=5.9$,

p<.022), such that HS- showed greater activation than HS+ during hormone withdrawal (*t*(26)=2.8, *p*<.01) but not at baseline (*t*(26)=-0.6, *p*=.54) (see Figure 5).

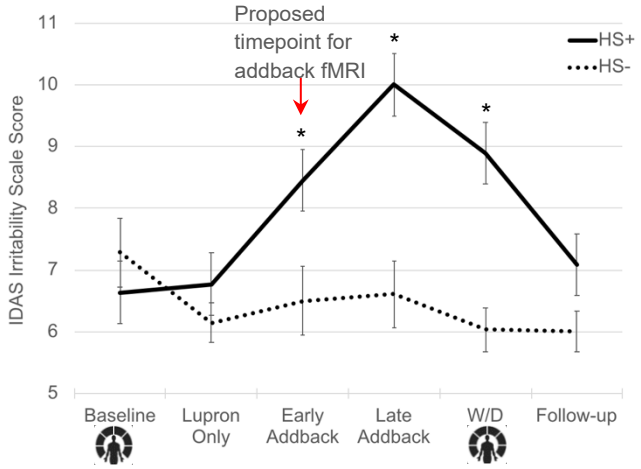


Figure 4. Irritability self-ratings during each study phase. HS+ (*n*=11) showed significantly increased irritability at early addback, compared with baseline and compared with HS- (*n*=14). The early addback assessment occurred 2 after two weeks of hormone addback (plus Lupron). Symbols: fMRI session.

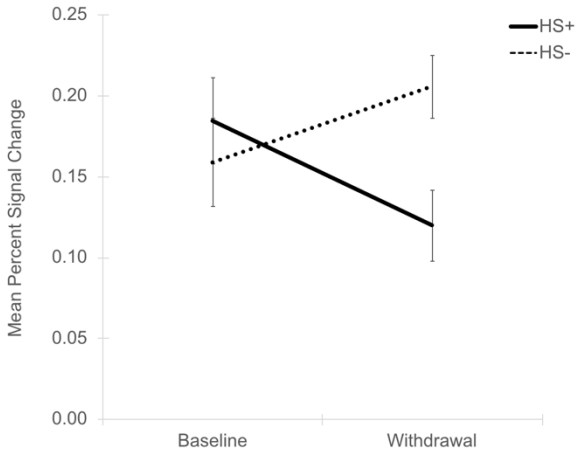


Figure 5. Percent signal change in bilateral putamen activity during monetary reward at baseline and during hormone withdrawal. Consistent with the literature, as HS+ (*n*=11) became depressed, they showed reduced striatal activation to reward, compared with HS- (*n*=14; non-depressed).

1.5 Dose Rationale and Risks/Benefits

Dose Rationale

The rationale for the dose of each drug administered in the proposed study is based on the study referenced immediate above. As shown in Figure 4, we found a statistically significant difference in irritability between HS+ and HS- after only 2 weeks of hormone addback, whereas we had administered hormones for a total of 8 weeks in the previous study. Moreover, the first two weeks of hormone addback involved doses (2 mg E2 bid and 200 mg P4 bid), which were half that given in the last 6 weeks of hormone addback. Based on these data, our proposed hormone addback protocol will include 2 mg E2 bid and 200 mg P4 bid for a period of 2 weeks. This shorted addback period will further allow us to reduce the number of Lupron injections from 4 in the previous protocol to 2 in the current protocol. Thus the drug protocol, including the route of administration, dosage, dosage regimen, and dosage period, mirrors a truncated version of our previous study which included a 7-month-long hormone manipulation protocol that included higher dose hormones and a previous study conducted at the NIH, which both successfully modeled the hormonal events of pregnancy and parturition and precipitated the onset of mood symptoms in susceptible women (Bloch et al., 2000, 2005).

Risks/Benefits

We do not expect any adverse side effects associated with the hormonal manipulations outlined in this protocol for the following reasons: First, we will be administering the physiologically relevant steroid hormones (estradiol and progesterone) and not the substituted steroids (such as ethinyl estradiol or norethindrone) present in many oral contraceptives and which have been reported to have a potentially more serious profile of side effects. Second, the doses of Lupron, estradiol and progesterone, and the duration for which they will be administered in this protocol, will result in plasma hormone levels comparable to those commonly used for in vitro fertilization (IVF) protocols lasting 1-3 months. Therefore, based on current IVF procedures we do not anticipate any adverse incidents arising from the proposed doses of estradiol and progesterone (Chetkowski et al., 1986; Rice et al., 1993; Saleh et al., 1995). Third, no adverse reactions or events were encountered in past studies conducted with this protocol in Dr. Rubinow's lab at the NIH. Finally, comparable extended, uninterrupted gonadal steroid treatment (such as oral contraceptives) for 6 to 12 weeks has been shown to be well-tolerated (Sulak et al., 1997).

Lupron: The most frequent adverse effect of Lupron is hot flushes (flashes) reportedly occurring in 4-89% of patients receiving the drug. Lupron-induced hot flushes have ranged in severity from occasional mild flushing to frequent sweating. Episodes of flushing appear to decrease with continued therapy in most patients receiving Lupron; however, in at least one study, the incidence of hot flushes did not appear to decrease with continued therapy. In a recently completed study of 400 women of reproductive age with either uterine fibroids or endometriosis who each received 3.75 mgs depot Lupron every month for a period of six months, the most common side effects reported to occur were as follows: 1) hot flashes of mild to moderate intensity (89%), 2) headache (22%), 3) nervousness or irritability (11%), and 4) insomnia (10%). Local irritation at the injection site was complained of in less than 10% of the patients in this sample, and there was a mean decrease in bone density, as measured by bone densitometry, of 3.4 to 4.0%, which totally reversed after the medication had been discontinued for six months. Approximately ten patients of the original sample of 400 found the side effects to be severe enough to discontinue therapy. In the majority of women regular menstrual cycle function returned within two months following the last injection of depot Lupron (Tapp Pharmaceuticals, personal communication). Blurred vision, myalgias, lethargy, memory disorder, and numbness have been reported in less than 3% of patients receiving the drug. Thrombophlebitis, pulmonary embolus, and congestive heart failure have occurred rarely in patients receiving Lupron, but a causal relationship to the drug has not been established. Adverse GI effects occurring in 2% or more of patients receiving Lupron include nausea and/or vomiting, constipation, and anorexia. Diarrhea and a sour or unusual taste in the mouth have been reported less frequently. Other adverse effects of Lupron occurring in less than 3% of patients include decreased hematocrit and hemoglobin concentration, fatigue, fever, facial swelling, rash, hives, hair loss, and itching. From our experiences with a longer protocol using the same medications in 15 women with a history of PPD and 15 controls, Lupron is well tolerated (no dropouts) with the most common side effect being hot flushes. Limited information is available on the acute toxicity of Lupron. Following subcutaneous administration of Lupron in rats at dosages 250-500 times the usual human dosage, dyspnea, decreased activity, and local irritation at the injection site were observed; however, there is no evidence to date that overdosage in humans produces similar adverse effects. Lupron dosages up to 20 mg daily for up to two years have not produced unusual adverse effects in humans. There has been one report of an anaphylactic reaction in a patient following administration of a GnRH agonist. Recent longitudinal follow-up studies of girls and boys

receiving GnRH agonists as a treatment for precocious puberty report the development of normal reproductive function, skeletal growth, and fertility (Feuillan et al., 1999, 2000).

Estradiol: Nausea is the most common side effect of estrogen administration. At conventional replacement doses, higher than those employed in this protocol, this complaint seldom interferes with eating, and no weight loss has been reported. Breast engorgement, endometrial hyperplasia and bleeding are also common side effects of estrogen administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of estrogen; however, at the dosage and for the duration of estrogen administration in this protocol this risk is small.

The relationship between estrogen, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation (Gambrell, 1986). Numerous retrospective case control studies published since 1975 have indicated that post-menopausal exposure to unopposed estrogens for more than one year results in a two to 12 fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen replacement therapy appears to decrease the risk of endometrial hyperplasia and endometrial cancer to equal or below that of women receiving no hormonal treatment. Recent studies suggest that the optimal regimen to prevent hyperplasia during long term ERT and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progesterone treatment each month when estrogens are administered (Nieman & Loriaux, 1992). There is an increase in thromboembolism in women receiving non-contraceptive estrogen therapy (Cushman et al., 2004; Daly et al., 2001; F. Grodstein et al., 1996; Jick et al., 1996). Additionally, some but not all studies report an increase in risk of stroke (Stampfer et al., 1991; Wassertheil-Smoller et al., 2003) in older women taking estrogen therapy. However, these complications are unlikely at the dose and duration of estrogen replacement employed in this protocol, and in the younger age group of women who participate in this study. One study (Chetkowski et al., 1986)reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use (Mandel et al., 1983; Melis et al., 1984; Nieman & Loriaux, 1992). Blood pressure, on average, appears to be unaffected by estrogen therapy, although both increases and decreases have been reported. In observational studies, post-menopausal estrogen therapy has been observed to lower the relative risk of cardiovascular disease in some but not all studies (Barrett-Connor & Bush, 1991; Stampfer et al., 1991). In contrast, recent randomized controlled trials in older postmenopausal women (e.g., Women's Health Initiative [WHI]) report an increased risk of cardiovascular disease (Manson et al., 2003). Emerging data suggest that these disparities in findings may be related to the timing of initiation of estrogen therapy in relation to the proximity of menopause. Subgroup analyses of the combined estrogen and progestin (EPT) arm of the WHI demonstrated a significant interaction between coronary heart disease (CHD) risk and time since initiation of EPT, with an increased risk in the early years following initiation and a decreased risk in later years. Additionally, the increased risk of CHD was observed in older but not younger perimenopausal women (Francine Grodstein et al., 2006; Hsia et al., 2006; Lobo, 2004; Manson et al., 2003; Prentice et al., 2005, 2006). High doses of oral estrogens have been reported to elevate hepatocellular enzyme levels and, less commonly, cause cholestatic jaundice. The risk for gallstones and hepatocellular adenomas has been reported to be increased in association with oral contraceptive

use, and although uncommon these complications may also occur with the use of replacement doses of estrogen (Cirillo et al., 2005; Petitti et al., 1988). Estrogen therapy also may increase the risk of urinary incontinence in older postmenopausal women (F. Grodstein et al., 2004; Hendrix et al., 2005; Steinauer et al., 2005). Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use(Beral, 1997; Chen et al., 2006; Chlebowski et al., 2003; Colditz et al., 1995; Gann & Morrow, 2003; Grady & Ernster, 1991; Li et al., 2003; Rossouw et al., 2002; Stefanick et al., 2006; Sturmer & Manson, 2004; Wingo et al., 1987), similar to the risk expected if the onset of menopause was delayed for a comparable length of time. Due to the publicity surrounding the cancellation of the treatment arm of the Women's Health Initiative study (Rossouw et al., 2002) that involved the administration of combined conjugated estrogens and medroxyprogesterone acetate (Prempro), we have included the following statement in the consent documents:

Adverse Events Related to Combined Hormone Replacement and the Results of the Women's Health Initiative (WHI): The WHI study demonstrated that continuous administration of one form of estrogen (conjugated estrogens) in combination with one form of progesterone (medroxyprogesterone acetate) is associated with an increased risk of dementia, heart attacks, stroke, blood clots, and breast cancer. Estradiol, the form of estrogen that we use in this study, is administered as a sole agent (with the exception of one week's combination with progesterone) and, consequently, we do not expect that it will pose the increased risks observed with the chronic combination of the conjugated estrogens and medroxyprogesterone administered in the WHI study. Indeed, while the estrogen alone arm of the WHI trial was shown to be associated with an increased risk of stroke, no increased risk of either heart disease or breast cancer was observed (Anderson et al., 2004; Rossouw et al., 2002). Estrogens may precipitate migraine headaches, and depression has also been reported to occur with the use of estrogens. In general, considering the dose and duration of treatment that we propose to use in this protocol, the risk of developing such side effects is negligible.

Progesterone: Progesterone and the synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty (Henzel, 1986). Side effects reported in women taking progestins may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression, easy fatigue and sedation, lack of initiative, and chloasma. Since progestins are often used in women with antecedent menstrual irregularity, it is not clear whether the breakthrough bleeding represents an effect of the medication or refractoriness to treatment. In the large majority of patients, menstruation occurs predictably following withdrawal of progestins and is usually more regular than in spontaneous cycles. In a recent study, an average dose of 1750 mg of oral micronized progesterone was given to 59 women with PMS for a period of three months and was well tolerated by this sample. The side effects reported on progesterone were lightheadedness, fatigue, forgetfulness, and headaches. These were very mild and caused no dropouts.

For the sake of completeness, we will also describe the side effects reported when estradiol and progesterone are combined in the form of oral contraceptives. Side effects observed in patients receiving combined oral contraceptives include nausea, breast soreness, vaginal discharge, fluid retention, hypertension, and clotting abnormalities that have been associated with the estradiol component of the oral contraceptive. Thromboembolic disorders including thrombophlebitis, pulmonary

embolism, and cerebral and coronary thrombosis appear to occur with greater frequency in women taking oral contraceptives. While the increased incidence of these disorders has been associated with the estradiol component of the oral contraceptives, it is now believed that the progestogen component may, to a lesser extent, contribute to the increased risk. There are relatively few reports associating oral contraceptives with the development of carcinomas (vaginal, uterine, hepatic, and mammary) despite the vast use of these agents, although this may reflect the latent period needed for cellular transformation. Finally, several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies.

Lupron, Estradiol, and Progesterone Combined Administration: As discussed in Section 1.2, the proposed monthly 3.75-mg injection of leuprolide acetate (Lupron Depot) is FDA-approved for use in premenopausal women to treat endometriosis and uterine fibroids. Lupron has also been widely researched in healthy premenopausal women (Flory et al., 2002; Grigorova et al., 2006; Matthews et al., 1998; Sánchez et al., 2002) and to treat a variety of conditions, including ovarian epithelial tumor cells (Thompson et al., 1991), insulin resistance (Elkind-Hirsch et al., 1993), endometrial stromal sarcoma (Scribner Jr. & Walker, 1998), and infertility (Padilla et al., 1991).Several studies have investigated the use of Lupron and hormone addback to treat the symptoms of premenstrual syndrome and premenstrual dysphoric disorder (Lee et al., 2012; Mitwally et al., 2002; Wyatt et al., 2004), which has resulted in combined Lupron and estrogen/progestin supplementation as a recommended long-term treatment for premenstrual syndrome (Kaur et al., 2004; Mezrow et al., 1994).Healthy women undergoing in vitro fertilization (IVF) routinely receive luteal supplementation of 600 mg progesterone daily along with 6 mg oral micronized estradiol (Lukaszuk et al., 2005) in combination with GnRH agonists (e.g., Lupron).

GnRH agonist treatment combined with hormone addback (i.e., high-dose estradiol and progesterone supplementation) has been previously studied in large randomized controlled trials for the purpose of IVF (Damario et al., 1999; Fatemi et al., 2006; Friedler et al., 1999; Humaidan et al., 2005; Lukaszuk et al., 2005). Of particular relevance to the current study, researchers induced a hypogonadal state using 3.75 mg Decapeptyl, followed by high-dose (800 mg) oral progesterone treatment in 32 women without an adverse event (Friedler et al., 1999). Several studies have examined the use of combined Lupron treatment (1 mg daily or 3.75 mg monthly), oral micronized estradiol (max doses ranged from 4 mg to 9 mg daily), and either oral (900 mg daily), vaginal (90-600 mg daily), or i.m. (50-200 mg daily) progesterone administration in over 1800 healthy women (Bourgain et al., 1990; Bustillo et al., 1995; Daly et al., 2001; Damario et al., 1999; Engmann et al., 2008; Friedler et al., 1999; Legro et al., 1993; F. Licciardi et al., 2001; F. L. Licciardi et al., 1999; Mirkin et al., 2003; Nagy et al., 2009; Noyes et al., 2001; Paulson et al., 1997; Peress & Philips, 1998; Roca et al., 2003; Sauer et al., 1992; Tao & Tamis, 1997; Tonguc et al., 2011). Of note, Damario et al. (1999) reported the use of combined Lupron treatment, 9 mg oral micronized estradiol, and 100 mg i.m. progesterone in 238 healthy women presenting for IVF.

Dr. Rubinow's team at the NIH has examined the combined administration of Lupron and estradiol/progesterone in healthy premenopausal women in several studies without a significant adverse event (Berman et al., 1997; Bloch et al., 2005; Daly et al., 2001; Roca et al., 2003; Schmidt et al., 2009). Moreover, the same drug protocol proposed in the current study has been employed

previously by our research team and Dr. Rubinow's research team at the NIH (Bloch et al., 2000, 2005) and is currently being used in ongoing projects at the NIH under the direction of Dr. Peter Schmidt and Dr. Pedro Martinez. Thus, a large number of healthy women have previously received combined Lupron with high-dose oral estradiol and progesterone supplementation either as part of a research study or routine IVF treatment without significant side effects or adverse events.

The prior protocol most relevant to the current proposal is the trial we completed most recently, which used higher doses of hormones (at least twice the doses of both E2 and P4 proposed here) and for a longer duration (8 weeks versus 2 weeks) and was classified as IND exempt by the FDA. To clarify differences between the protocol described in our IND application and that proposed here, we have summarized the medication regimens in the table below.

Table 2. Medication Regimens in previous IND Exempt Protocol and the Proposed Protocol

	Past IND Exempt Protocol				***Current Protocol***		
Phase	*1: Hypogonadism*	*2: Low Dose Addback*	*3: High Dose Addback*	*4: High Dose Withdrawal*	*1: Hypogonadism*	*2: Low Dose Addback*	*3: Low Dose Withdrawal*
Duration	*4 weeks*	*2 weeks*	*6 weeks*	*4 weeks*	*4 weeks*	*2 weeks*	*2 weeks*
Lupron	*3.75 mg IM dose #1*	*3.75 mg IM dose #2*	*3.75 mg IM dose #3*	*3.75 mg IM dose #4*	*3.75 mg IM dose #1*	*3.75 mg IM dose #2*	*N/A*
Estrace	*Placebo*	*2 mg bid*	*5 mg bid*	*Placebo*	*Placebo*	*2 mg bid*	*Placebo*
Prometrium	*Placebo*	*200 mg bid*	*400 mg bid*	*Placebo*	*Placebo*	*200 mg bid*	*Placebo*

Below (Table 3) is a list of the adverse events that occurred in the previous, IND exempt protocol conducted at UNC using the same hormone protocol as that proposed in the current study. We have listed the adverse events that occurred during phases 1 and 2 (highlighted in yellow), which involved administration of medications, doses, and durations that are identical to those proposed in the current study, separately from those that occurred in phases 3 and 4 (highlighted in blue), which involved Estrace and Prometrium administration and withdrawal from doses that were at least twice those proposed in the current study and durations that were three times as long.

Table 3. Adverse Events in the Previous IND Exempt Protocol

	1: Hypogonadism	***2: Low Dose Addback***	***3: High Dose Addback***	***4: High Dose Withdrawal***	**5: Follow-up**	**TOTAL**
Cognitive:						
Drowsiness	1	1	4	1		7
Sedation		3	4			7
Dizziness	1	5	3			9
Lightheadedness		1	4			5
Memory impairment			1			1

Psychological:						
Depression		2	2			4
Anxiety		1				1
Irritability		2			1	3
Mood Swings		1	1			2
Trouble concentrating	1					1
Night Terrors		1				1
Gastrointestinal:						
Nausea		1	1			2
Diarrhea	1					1
Upset Stomach				1		1
Constipation	1				1	2
Heart Burn	1	1				2
Cardiac:						
Transient Heart Palpitations*	1					1
Chest Pain*		1	1			2
Arrhythmia*	1					1
Bradycardia*	1					1
Menstrual:						
Spotting	1		3			4
Prolonged menstrual bleeding			1	1		2
Heavy menstrual bleeding			1			1
Breast Tenderness	2	2				4
Vaginal itching			1			1
Cramps					1	1
Physical/Somatic:						
Weight Gain					1	1
Hot Flashes	1		1			2
Headache	3	1		2	1	7
Hair Loss				1		1
Cracked Nipple		1	1			2
Rash on legs	1					1
Tingling		1				1
Dry Mouth		1				1
Frequent Urination		1				1
Hip pain	1					1

Note: Bolded individual symptoms were rated as severe. Headaches were rated as severe two times and all other bolded symptoms were rated as severe only once. All others symptoms were rated minimal, mild, or moderate.

**Symptoms were determined by an independent physician to be a result of participant's excessive physical training for Iron Man combined with poor self-care (i.e., working 3rd shift, sleeping 4 hours/night, and poor diet) rather than the study. All symptoms resolved when participant reduced training schedule and increased time available for sleep.*

All of the symptoms summarized in Table 3 were either transient and remitted without intervention or were addressed either by consolidating the dose to nighttime or by decreasing the dose of estradiol by 2 mg or progesterone by 200 mg, depending on the symptom. There were no serious adverse events in this study.

COVID-19 Risks

Participants may experience the potential risk of COVID-19 transmission during required in-person study visits such as at the Biomedical Research Imaging Center (BRIC) and when in close contact with study team members during Lupron injections or blood draws when study staff will need to maintain less than 6ft distance. The study team will be following UNC guidelines and BRIC guidelines to take every possible precaution to protect study participants and their staff. Including virtual video or telephone visits when possible, shortening duration of in-person contact, requiring masks at all times for study staff and participants, maintaining 6ft distance when possible, temperature checks on arrival to BRIC, COVID-19 pre-screening 24 hours in advance of any in-person contact and the day of, frequent cleaning with 90% alcohol or 1:10 bleach solution, cleaning equipment between each participant, handwashing required every hour and before or after contact with any participants. While the study team will be taking every pre-caution possible to participants and the study staff there may still be a risk of COVID-19 transmission due to the possibility of the COVID-19 virus lingering in enclosed spaces from asymptomatic carriers. However this risk should not be any greater than being in an essential public setting such as a grocery store. If a participant or a member of the study team has had direct or even secondary contact with any suspected or confirmed cases of COVID, the study team will reschedule any upcoming visits until the case is confirmed negative or to quarantine for at least 14 days and monitor symptoms. If a participant or study team member experiences any symptoms of COVID-19 including but not limited to: cough, fever, chest pain, trouble breathing, loss of smell or taste, nausea, abdominal pain, vomiting, etc. participants will be asked to notify the study team immediately, contact their Primary Care Provider and their local COVID-19 hotline to get tested for COVID-19.

2 Study Objectives

The objective of the current project is to extend this line of work by examining whether hormone sensitive women (HS+; n=15) show differences in the behavioral activation system relative to non-hormone sensitive women (HS-; n=15) under baseline and hormone challenge conditions using functional magnetic resonance imaging (fMRI) and behavioral tests. Our central hypothesis is that reproductive hormone changes are associated with dysregulated threat and reward processing and consequent irritability in HS+. The rationale for the proposed study is that employing a scaled down model of puerperal hormonal events permits the identification of a group of individuals homogeneous for hormone sensitivity (HS+), hence creating the best opportunity for disentangling the specific changes in brain function due to reproductive hormones (i.e., HS-) from those accompanying reproductive hormone-precipitated affective dysfunction (i.e., HS+). Because women will act as their own controls across time, this study design also allows for a powerful evaluation of state and trait variables that may contribute to irritability, including both threat processing and reward processing. Identifying both state and trait markers of irritability, and disentangling them from the effects of reproductive hormones on brain and behavior, will allow for the identification of neural substrates of irritability susceptibility that can be investigated across neuropsychiatric disorders. We plan to accomplish the objectives of this application by pursuing the following specific aims:

Aim 1: To determine the extent to which irritability is characterized by dysfunctional threat processing during reproductive hormone challenge relative to baseline in HS+ and HS-.

Hypothesis 1: We hypothesize that HS+ is characterized by behavioral activation in the context of potential threat. Given this hypothesis, we expect that:

> Hypothesis 1a: Compared with HS-, HS+ will show a greater degree of threat attention bias as measured by the visual dot probe task during hormone addback relative to baseline.
>
> Hypothesis 1b: Compared with HS-, HS+ will show reduced amygdala-medial PFC connectivity in response to threatening stimuli during hormone addback relative to baseline.
>
> Hypothesis 1c (secondary): HS+ will show a significant association between self-reported irritability and 1) threat attention bias, and 2) amygdala-ventrolateral PFC connectivity during hormone addback.

Aim 2: To determine the extent to which irritability is characterized by dysfunctional reward processing during reproductive hormone challenge relative to baseline in HS+ and HS-.

Hypothesis 2: We hypothesize that HS+ is characterized by increased frustration in the context of non-reward (i.e., when reward is expected). Given this hypothesis, we expect that:

Hypothesis 2a: Compared with HS-, HS+ will show greater reactive aggression assessed with the Point Subtraction Paradigm during hormone addback relative to baseline.

Hypothesis 2b: Compared with HS-, HS+ will show reduced activation of the amygdala and ventral striatum in response to FNR during hormone addback relative to baseline.

Hypothesis 2c (secondary): HS+ will show a significant association between self-reported irritability and 1) reactive aggression, and 2) striatal activation during hormone addback.

3 Study Design

3.1 General Design

This is a double-blind, placebo controlled, longitudinal cohort-comparison study, single-center study using Lupron depot, estradiol, and progesterone to examine whether hormone sensitive women (HS+; n=15) show differences in the behavioral activation system relative to non-hormone sensitive women (HS-; n=15) under baseline and hormone challenge conditions using functional magnetic resonance imaging (fMRI) and behavioral tests. This study will include 8 study visits, and the hormone protocol will last 8 weeks. Out of those 8 visits, 2 of them will require subjects to come to UNC's campus for their MRI visits at BRIC, while the rest of the appointments will be done virtually through HIPAA compliant Zoom, Webex, or phone when zoom is not available. Out of these 6 virtual visits, 2 of them will require brief in-person contact for phlebotomy or an IM injection of Lupron, these very brief points of contact will be performed outside the subject's home when possible (i.e. backyard, back porch, or screened porch). In the context of COVID-19, all participants and staff will complete the appropriate screening as recommended by UNC and BRIC policy prior to any in-person contact. During in person visits participants and the study team will be required to wear masks at all times and maintain 6 feet distance when possible to minimize the possibility of COVID-19 transmission. Participants will undergo a scaled down hormonal model of pregnancy and parturition (using ovarian suppression with the GnRH agonist Lupron and hormone addback with E2 and progesterone P4) in order to examine how hormones influence neural function and irritability in experimentally confirmed HS+ and HS-. FMRI sessions will occur at baseline and following two weeks of hormone addback. The study timeline is depicted in **Figure 6**, and the specific procedures that will take place are outlined in **Table 1** and detailed below in Section 6.

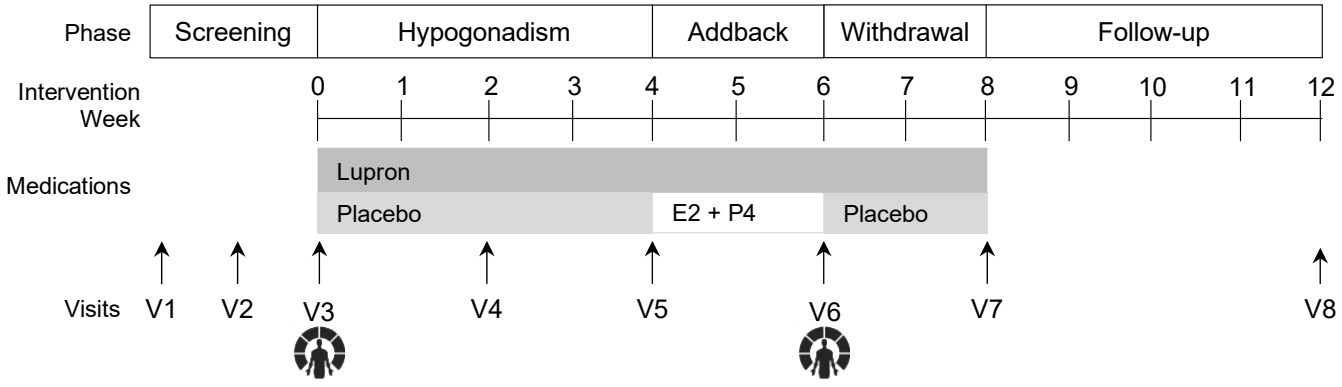


Figure 6. Protocol Timeline. Symbols: fMRI session. The timeline shown displays the 12 week study, time points of each medication to be administered along with the phase of the study which it occurs.

3.2 Outcome Variables

The primary endpoints for this aim are 1) threat attention bias assessed during the visual dot-probe paradigm, 2) amygdala-medial PFC connectivity in response to threatening faces on the implicit emotion face processing task in HS+ (compared with HS-) during hormone challenge relative to baseline. The secondary endpoints for this aim are the 1) correlation between IDAS-II irritability and treat attention bias, and 2) correlation between IDAS-II irritability and amygdala-medial PFC connectivity in HS+.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Participants will enroll healthy, euthymic 21-45 year old women with a history of postpartum depression (*n*=23) and women without such a history (*n*=16). Only participants capable of giving informed consent will be enrolled. Participants will be compensated upon completion of the study.

Inclusion Criteria.
Group 1: Women with a history of postpartum depression
1) A history of a DSM-V major depression episode that occurred within 6 weeks of childbirth (as determined by a SCID interview) and remitted at least one year prior to enrollment in the study;
2) Has been well for a minimum of one year;
3) A regular menstrual cycle for at least three months;
4) Age 21-45;
6) Medication free (including birth control pills);

Group 2: Healthy Controls
1) Controls will meet all inclusion criteria specified above.

A structured clinical interview for DSM-V (SCID) will be administered to all women prior to study entry. Any woman with a <u>current</u> axis I psychiatric diagnosis will be excluded from participating in this protocol.

4.2 Exclusion Criteria

Patients will not be permitted to enter this protocol if they have important clinical or laboratory abnormalities including any of the following:

- current axis I psychiatric diagnosis (based on a structured clinical interview for DSM-V (SCID));
- endometriosis;
- undiagnosed enlargement of the ovaries;
- liver disease;
- breast cancer;
- a history of blood clots in the legs or lungs;

- undiagnosed vaginal bleeding;
- porphyria;
- diabetes mellitus;
- malignant melanoma;
- gallbladder or pancreatic disease;
- heart or kidney disease;
- cerebrovascular disease (stroke);
- cigarette smoking;
- a history of suicide attempts or psychotic episodes requiring hospitalization;
- recurrent migraine with aura;
- pregnancy-related medical conditions such as hyperemesis gravidarum, pretoxemia and toxemia, deep vein thrombosis (DVT) and bleeding diathesis;
- Any woman with a first degree relative (immediate family) with premenopausal breast cancer or breast cancer presenting in both breasts or any woman who has multiple family members (greater than three relatives) with postmenopausal breast cancer will also be excluded from participating in this protocol;
- Any woman meeting the Stages of Reproductive Aging Workshop Criteria (STRAW) for perimenopause will be excluded from participation. Specifically, we will exclude any woman with an elevated plasma FSH level (> 14 IU/L) and with menstrual cycle variability of > 7 days different from their normal cycle length.
- Pregnant women will be excluded from participation (patients will be warned not to become pregnant during the study and will be advised to employ barrier contraceptive methods), and women who become pregnant (although unlikely because of the hormone manipulation) will be withdrawn. The use of leuprolide acetate is not recommended during pregnancy. Prior to treatment, a complete physical, including a serum β-HCG test for pregnancy. Participants will be seen at the outpatient clinic on a regular biweekly basis. All participants will be required to use non-hormonal forms of birth control (e.g., barrier methods) to avoid pregnancy during this study. Participants will also undergo urine toxicology and pregnancy tests on the day of each of the two fMRI scans. If a woman becomes pregnant during the study, she will not complete the fMRI scan, and the hormone protocol will be discontinued.

4.3 Subject Recruitment and Screening

Methods of recruiting for this study include:

We plan to use a similar recruitment strategy to that of our previous study (N=30) involving a 7-month-long hormone challenge and two neuroimaging session. The core of our recruitment plan is to focus on the following resources:

• Targeted social media advertising (e.g., Facebook, Instagram)
• Monthly university-wide recruitment emails
• Online classifieds (e.g., Craigslist)

• Online research registries (ResearchMatch.org, Join the Conquest)
• Research registry specific to Dr. Schiller's and Dr. Rubinow's previous study involving hormone challenge and two neuroimaging sessions
• Flyers in the community

We also will partner with the North Carolina Translational and Clinical Sciences Institute (NC TraCS) Data Access and Informatics Core to identify a cohort of UNC Hospital patients for recruitment. UNC uses the EPIC electronic medical record system, which enables communication to potential research participants via email and through the MyChart patient interface. This makes communication with a large number of potential research participants cost effective and highly efficient because it will enable potential participants to access our online screening tool simply by clicking a link in their email or MyChart electronic message. Additionally, we will capitalize on the TraCS Research Recruitment Service's expertise in enrolling members of communities historically underrepresented in research, including local American Indian tribes.

Eligibility screening will include:

- An initial phone or online screening that includes questions about past medical and mental health history to assess potential participants eligibility based on the criteria listed in sections 4.1 and 4.2.
- Participants will undergo a Clinical and Health Screening process to determine whether they are healthy enough to participate in this study. This screening will include past medical and mental health history and physical exam. During this evaluation, they will be asked questions about past and present psychiatric symptoms. They will also be asked to complete questionnaires about psychiatric symptoms. They may choose not to answer any or all of the questions for any reason.
- Participants will complete a safety questionnaire to determine whether they have any foreign iron or steel metal objects in their bodies, such as a pacemaker, shrapnel, metal plate, or metal debris. If they have any such objects in their bodies, they cannot participate in the MRI session.
- All participants will receive a pregnancy test and urine drug screening. No pregnant women will be entered into the study, because the study drugs (Lupron, estrogen, and progesterone) may be associated with birth defects.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Participants with significant clinical or laboratory abnormalities will be discontinued from the study prior to GnRH agonist administration.

As the population being studied in this protocol may be at high risk to develop subsequent depressions and as gonadal steroids have been implicated in the etiology of postpartum depression, some depressive reactions, including suicidal ideation, may occur during this protocol. However, based on

experience with Lupron and estradiol/progesterone addback in women with menstrually-related mood disorders, we would expect mood symptoms to be of mild to moderate severity.

Adverse mood symptoms, including suicidal ideation, will be monitored on a bi-weekly basis by administering the Inventory of Depression and Anxiety Symptoms (IDAS-II). The IDAS-II will be scored during the study visit (before the patient leaves the hospital). IDAS-II Suicidality Scale scores of > 8 are observed at any period in the protocol or any subject with suicidal ideation, or anyone with concerns about their ability to continue in the study, will be considered to have severe mood symptoms and be discontinued from the protocol.

In the event of the occurrence of severe mood symptoms, either the protocol will be terminated, or if symptoms occur during the withdrawal phase, hormone replacement will be re-instituted. Should this step prove to be unsuccessful, conventional medication will be prescribed as needed. Further, although we do not anticipate severe adverse reactions, participants may be hospitalized in a UNC Neurosciences Hospital Inpatient Psychiatry Clinic if symptoms are otherwise unmanageable.

Any patient experiencing clinically significant side effects such as nausea, hypertension, vomiting or extreme fluid retention from the medication will have the dose titrated to achieve relief of the symptoms. If adequate relief cannot be achieved in this manner, drug treatment will be discontinued. Similarly, if menopausal-like symptoms occurring secondary to GnRH agonist treatment are intolerable, drug treatment will be discontinued. Additionally, study medication will be terminated in subjects that experience any grade 3 or higher adverse events that limit their activities of daily living, that cannot be otherwise addressed by modifying a medication dose.

In the event of three grade 3 or higher adverse events (i.e. a life threatening adverse event that may require hospitalization) possibly or definitely associated with the study intervention, the study will be suspended and the study's Data and Safety Monitoring Board will be notified.

The determination of when and how to withdrawal subjects will be overseen by the study's Data and Safety Monitoring Board and will be reported to the UNC Biomedical IRB.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Participants who elect to discontinue the hormone protocol or are discontinued for safety reasons will be asked to continue to complete self-report ratings through the end of the proposed study period. However, fMRI exams will not be done if participants discontinue the hormone protocol.

5 Study Drug

5.1 Description

Drug	Dosage	Formulation
Estradiol	2 mg bid (total of 4 mg/day)	Oral capsule
Lupron Depot	3.75 mg/month	Intramuscular injection
Progesterone	200 mg bid (total of 400 mg/day)	Oral capsule

5.2 Treatment Regimen

Regimen

Induced Hypogonadism (weeks 0-4). After the baseline period, participants will receive their first injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron) 3.75 mg/month via intramuscular injection, which is administered to produce a stable hypogonadal condition (after the initial "flair"). The first Lupron injection will be administered on day six of the participants' first menstrual cycle after the baseline period and at monthly intervals thereafter until follow-up. During the first month of GnRH agonist administration, all participants will receive placebo progesterone and placebo estradiol tablets and will be told that at some point the placebo pills will be switched to active medication.

Addback (weeks 4-6). After the month of Lupron-alone treatment (i.e., hypogonadism), high plasma levels of estradiol and progesterone will be attained via micronized progesterone and estradiol tablets for two weeks (with continued Lupron administration). Estradiol will be administered at a dose of 4 mg/day. Progesterone will be administered at 400 mg/day. The blood levels that we expect to achieve and sustain in each woman will be approximately 500 pg/ml of estradiol and 30-40 ng/ml of progesterone.

Withdrawal (weeks 6-8). After two weeks of hormone replacement, active hormone tablets will be replaced with placebo tablets to induce a precipitous drop in plasma estradiol and progesterone levels. Lupron will maintain hypogonadal levels for the two-week withdrawal phase. The second fMRI session will take place at the start of the withdrawal period.

5.3 Method for Assigning Subjects to Treatment Groups

This is a longitudinal cohort-comparison study, and all subjects will receive the same drug protocol.

5.4 Preparation and Administration of Study Drug

All study drugs will be stored, prepared and dispensed from the UNC Investigational Drug Service (IDS).

Contact:
Sue Pope, Manager (or her replacement)
Investigational Drug Service
Department of Pharmacy
UNC Hospitals
CB 7600, Room 3001
101 Manning Drive
Chapel Hill, NC 27514
Office: 919-966-1766
Fax: 919-966-6359

5.5 Subject Compliance Monitoring

We will monitor participants' compliance with the drug regimen by assaying blood levels of estradiol and progesterone collected at monthly study visits. The drug protocol will be reviewed at each study session, and participants who are significantly non-compliant with the study treatment regimen will be discontinued from the study.

5.6 Prior and Concomitant Therapy

Women are required to be medication free to enroll in this study; however, prior medication usage will not preclude participation in the study.

5.7 Packaging

The UNC Investigational Drug Service will receive the active drug from their Pharmacy storeroom and will provide the capsules for blinding. Lupron will be administered IM by a study nurse or physician's assistant at monthly study visits. Pill bottles containing estradiol, progesterone, and placebo that are provided to participants at bi-weekly study visits will contain a two-week supply of capsules.

5.8 Blinding of Study Drug (if applicable)

Placebo and active medication will be in like-colored capsules with identical labeling.

5.9 Receiving, Storage, Dispensing and Return

The UNC Investigational Drug Service will receive the study drugs from the UNC Pharmacy Storeroom and will dispense the drug to the PI to deliver to participants. Any unused drug will be returned to the UNC Investigational Drug Service by the PI.

5.9.1 Receipt of Drug Supplies

Upon receipt of the study treatment supplies, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. The designated study staff will count and verify 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 will notify the UNC Pharmacy Storeroom of any damaged or unusable study treatments that were supplied to the Investigational Drug Service.

5.9.2 Storage

Lupron, Estradiol, and Progesterone will be stored at 20° to 25° C in a temperature-controlled facility.

5.9.3 Dispensing of Study Drug

Drugs will be dispensed in tight, light-resistant containers and defined in the USP, with a child-resistant closure. Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug accountability form, and signed and dated by the study team.

5.9.4 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.

6 Study Procedures

Overview. This is a double-blind, placebo controlled, longitudinal cohort-comparison study, single-center study using Lupron depot, estradiol, and progesterone to examine whether hormone sensitive women (HS+; n=15) show differences in the behavioral activation system relative to non-hormone sensitive women (HS-; n=15) under baseline and hormone challenge conditions using functional magnetic resonance imaging (fMRI) and behavioral tests. This study will include 8 study visits, and the hormone protocol will last 8 weeks. Out of those 8 visits, 2 of them will require subjects to come to UNC's campus for their MRI visits at BRIC, while the rest of the appointments will be done virtually through HIPAA compliant Zoom, Webex, or phone when zoom is not available. Out of these 6 virtual visits, 2 of them will require brief in-person contact for phlebotomy or an IM injection of Lupron these very brief points of contact will be performed outside the subject's home when possible (i.e. backyard, back porch, or screened porch). In the context of COVID-19, all participants and staff will complete the appropriate screening as recommended by UNC and BRIC policy prior to any in-person contact. During in person visits participants and the study team will be required to wear masks at all times and maintain 6 feet distance when possible to minimize the possibility of COVID-19 transmission. Participants will undergo a scaled down hormonal model of pregnancy and parturition (using ovarian suppression and hormone addback) in order to examine how hormones influence neural function and irritability in experimentally confirmed HS+ and HS-. FMRI sessions will occur at baseline and following two weeks of hormone addback. The study timeline is depicted in **Figure 6** in Section 3, and the specific procedures that will take place are outlined in **Table 1** and detailed below.

Table 1. Study Procedures and Timeline

Study Visit	1	2	3	4	5	6	7	8
Estimated maximum duration of in-person visit (hours)		1	2		0.15	2		
Informed Consent	x							
Psychological Interview (SCID)	x							
Physical Exam		x						
fMRI Session			x			x		
Pulse Oximetry			x			x		
Lupron injection			x		x			
Venipuncture		x	x			x		
Self-Report Questionnaires								
Daily Rating Form	x	x	x	x	x	x	x	x
IDAS-II	x		x	x	x	x	x	x
BITe	x		x	x	x	x	x	x

Misophonia questionnaire	x		x	x	x	x	x	x
SNAP	x							
Demographic Questionnaire	x							
Trauma History Questionnaire	x							
Handedness Questionnaire	x							
Hormone History Questionnaire	x							
BIS/BAS	x		x			x		
COVID Stress Scale (CSS)			x			x		
Behavioral Tests								
Visual Dot Probe			x			x		
Point Subtraction Aggression			x			x		

Note: H/G = Hypogonadism; W/D = Withdrawal; F/U = Follow-up

Participants. Healthy, euthymic, unmedicated (including oral contraceptives), 21-45-year-old women with regular menstrual cycles and either with or without a history of PND will be recruited from an existing research registry of euthymic women with and without a history of PND screened for Dr. Schiller's and Rubinow's last NIH-sponsored study and with the social media advertising pipeline used for the last study. All women will have one or more biological children and will be at least one year past their most recent childbirth. In order to maximize the likelihood of identifying HS+, those with a history of PND must have a history of at least one episode of DSM-V major depression accompanied by irritability, with an onset within six weeks postpartum (Forty et al., 2006), and no history of DSM-V major depression with an onset outside of the postpartum period. In order to maximize the likelihood of identifying HS-, the control group will comprise women without a history of any past or present psychiatric disorder, including PMDD, and without first-degree relatives with a history of PND. Additional inclusion and exclusion criteria required for participant safety are detailed in the human subjects section. Hormone sensitivity will be prospectively assessed using the hormone protocol described below.

We have defined HS+ as a 30% or greater change in any of the IDAS-II mood scales, which include dysphoria, suicidal ideation, ill temper (i.e., irritability), and wellbeing (i.e., reverse-keyed) during hormone addback or withdrawal, compared with the individual's own baseline using the equation below:

$$\% \text{ symptom change} = (\text{Addback score} - \text{Baseline score})/\text{Baseline score}$$

Percent symptom change will be calculated for each of the IDAS-II scales listed above at both the addback visit (V6) and withdrawal visit (V7). If a participant has a percent symptom change of 30% or

greater on any of the IDAS-II scales listed, she will be classified as HS+. HS- is defined as less than 30% change in any of the IDAS-II mood scales.

We will only include those with confirmed HS+ and HS- in our planned analyses. Those with a history of PND confirmed to be HS- will be excluded from planned analyses. However, if we have enough subjects with a history of PND who are HS-, then they may be included as a separate group in exploratory analyses. Alternatively, if those with a history of PND who are HS- are not significantly different from those without a history of PND who are HS- in terms of our key outcome variables, then we will pool the groups to increase power.

Hormone Administration. The hormone administration protocol shown in **Figure 6** and described below will be administered in a double-blind manner and replicates the methods of our recent study and Bloch et al (2000), modified as follows: the hormone addback period is truncated and the hormone doses have been reduced based on our finding that HS+ can be distinguished from HS- after two weeks of low-dose addback (see Preliminary Data). To clarify double-blinding, all statisticians in the study will be blinded to the treatment intervention given to participants. As all participants will be completing digital behavioral tasks and self-report questionnaires throughout the study, which will be analyzed upon study completion.

Baseline. Prior to medication administration, participants will complete a physical exam and comprehensive psychological assessment, including interviews and self-report questionnaires. The first fMRI session will be completed during a period of low reproductive hormone levels (days 4-8, the mid-follicular phase), which will be confirmed using a plasma estradiol assay. Conducting the baseline fMRI session during the midfollicular phase promotes consistency in hormone profiles across participants and provides the optimal contrast for affective symptoms during the period of reproductive hormone addback.

Induced Hypogonadism. After the baseline period, participants will receive their first injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron) 3.75 mg/month via i.m. injection, which is administered to produce a stable hypogonadal condition (after the initial “flair”). The first Lupron injection will be administered at the end of Study Visit 3 on day six of the participants’ first menstrual cycle after the screening period and again one month later. During the first month of GnRH agonist administration, all participants will receive placebo capsules (identical in appearance and number to the hormone capsules they will receive later) and will be told that at some point the placebo pills will be switched to active medication.

Addback. After one month of Lupron-alone treatment (i.e., hypogonadism), high plasma levels of estradiol and progesterone will be attained via micronized progesterone and estradiol tablets for two weeks (with continued Lupron administration). Participants will take 2 mg of estradiol twice daily (for a total of 4 mg/day) and 200 mg of progesterone twice daily (for a total of 400 mg/day). The second fMRI session will take place at the conclusion of the 2 weeks of hormone addback. Withdrawal. After 2 weeks of hormone addback, active hormone tablets will be replaced with placebo tablets to induce a precipitous drop in plasma estradiol and progesterone levels. Lupron will maintain hypogonadal levels for the 2-week withdrawal phase.

Follow-up. Participants will be followed for four more weeks while unmedicated. Based on our past experience, estradiol, progesterone, and mood symptoms are expected to return to baseline levels

in all participants by follow-up week 4. The withdrawal and follow-up phases, along with the addback phase, are used for the purpose of ensuring correct classification of subjects as HS+ and HS-.

Clinical Assessments

The Structured Clinical Interview for DSM-V-TR Axis-I Disorders (SCID) (First et al., 2015), and the Schedule for Nonadaptive and Adaptive Personality (SNAP) (Clark, 1993) will be administered during screening to assess past and present psychiatric illness and personality pathology. Each morning, starting at Study Visit 1, participants will complete the Daily Rating Form (Endicott et al., 2006) online, a 20-item questionnaire that assesses physical and mood symptoms that accompany ovarian hormone changes. Ratings are transmitted to the study team in real-time, which **will be used to monitor rapid mood changes throughout the study and ensuring participant safety.**

The following measures will be administered at according to table 1:

1. The Inventory of Depression and Anxiety Symptoms (IDAS-II) (Watson et al., 2007) is a 64-item self-report questionnaire that comprehensively assesses anxiety and depression symptoms, including ill temper (i.e., irritability), dysphoria, panic, social anxiety, appetite change, lassitude, well-being, suicidality, traumatic intrusions, and insomnia. The IDAS-II has excellent psychometric properties and has been validated in PND (Watson et al., 2007). The dysphoria, ill temper, wellbeing (inverse), panic, and suicidality scales will be used to determine hormone sensitivity status. Replicating our previous study, HS+ will be defined as a 30% increase in mood symptoms during hormone addback. The ill temper scale will be used as the measure of irritability to examine associations with behavioral and fMRI data (Hypotheses 1c and 2c).

2. The Behavioral Inhibition/Behavioral Activation (BIS/BAS) Scales (Carver & White, 1994) is comprised of four subscales—BIS, Reward Responsiveness, Drive, and Fun Seeking—and 20 items total that assess behavioral inhibition (anxiety) and behavioral activation (impulsivity). We will also include the 5-item Frustrative Nonreward Responsiveness Subscale (Wright et al., 2009) to quantify lowered approach motivation following FNR (Hypothesis 2a). The BIS/BAS scales are associated with neural markers of psychopathology (Beaver et al., 2006; Simon et al., 2010). Exploratory analyses will be conducted to assess associations between BIS/BAS, IDAS-II irritability, behavioral, and fMRI data.

   In addition, to assess potential covariates that may impact the associations above, we have included a demographic questionnaire(including self-reported history of pain and poor sleep following past pregnancies, **in response to the SRC's comments**), the Edinburgh Handedness Scale(because left handedness may impact the validity of fMRI data), a hormone history questionnaire that we created for previous studies to explore whether menstrual, reproductive, or family history may be associated with HS+, and the Trauma History Questionnaire(given that PND is associated with increased prevalence of trauma history. While variables have been shown to predict PND, it is unclear the extent to which they are related to HS+ specifically, and as such, we will not exclude women for experiencing a history of trauma, pain, or sleep dysregulation (i.e., the experience of these symptoms following a past childbirth likely does not preclude a woman from also being hormone sensitive, which will be experimentally confirmed using the hormone protocol). However, if these variables are significantly associated with either

the independent or dependent variable for any of the planned analyses, they will be entered as a covariate in the statistical model. In addition we will also be using the Brief Irritability Test (BITe) which is a brief 5-item self-report questionnaire and the Misophonia Questionnaire which is 19-tem self-report questionnaire both will help to assess irritability. To account for the additional level of stress posed by the COVID-19 pandemic, a COVID Stress Scale (CSS) will also be included during in-person MRI scans. The CSS is a 36-item measure to better understand COVID related distress during these in-person visits (Taylor et al., 2020).

Depressive symptoms resulting from this hormone regimen, although significant, are relatively brief, lasting approximately 7-10 days (Price & Stolk-Cooke, 2015). Despite the use of exclusion criteria designed to reduce risk to participants, depressive symptoms are monitored closely. Any subject who develops severe depressive symptoms (e.g., suicidal ideation) will be discontinued from the study and offered treatment. If inpatient hospitalization becomes necessary as a result of the study, that participant or their insurance will be responsible for covering the costs.

Functional MRI. The following tasks will be included in each of the two, one hour-long fMRI sessions:

The implicit face emotion processing task(Stoddard et al., 2017) will test whether HS+ is characterized by reduced amygdala-medial PFC connectivity in response to threat (Hypothesis 1b),, that robustly activates key limbic regions, including the amygdala. During the task, participants are asked to identify the gender of angry, happy, and fearful faces at 50%, 100%, and 150% emotion intensity presented in random order for 2000 milliseconds followed by jittered fixation. Trials will appear in 3 blocks, generating 30 trials of each emotion at each intensity and 90 neutral face emotion trials. Adolescents with irritability and anxiety show reduced amygdala-PFC connectivity compared with those without anxiety during this task (Stoddard et al., 2017).

The Affective Posner Task (Tseng et al., 2017) will test whether HS+ is characterized by reduced activation of the amygdala and ventral striatum in response to frustration (Hypothesis 2b), a well validated (Deveney et al., 2013; Rich et al., 2005, 2007, 2010) adaptation of the Posner spatial cueing task that includes both reward and FNR and activates key limbic and striatal regions, including the amygdala, caudate, putamen, and nucleus accumbens (Deveney et al., 2013). This event-related task is divided into 3 runs: during Run 1 (practice run conducted outside of the scanner), participants receive accurate feedback about their performance on the task and do not win or lose money; during Run 2, participants receive accurate feedback about their performance on the task and win or lose 50 cents per trial, based on their accuracy and reaction time; and during Run 3 (FNR), participants are told they must respond accurately to win money, but participants are given feedback that they responded too slowly on 60% of accurate trials, regardless of their performance (Deveney et al., 2013). During the FNR run, participants with mood dysregulation/irritability have demonstrated decreased amygdala and striatal activation relative to euthymic controls (Deveney et al., 2013). This task also has good convergence with self-reported irritability and frustration and excellent test retest reliability (Tseng et al., 2017), making it ideal for a repeated-measures study design. For the purpose of exploratory analysis, we will also include a resting state fMRI scan.

FMRI Data Acquisition and Image Processing.

Scanning is performed using a Siemens Prisma scanner at the UNC BRIC, which includes built in pulse oximetry capability. During each scanning session, participants will wear a pulse oximeter on their finger to record heart rate for the duration of time in the scanner. Evidence supports that monitoring autonomic nervous system activity during fMRI can provide insight into stress adaptability (Butle & Wartolowska, 2017). Whole-brain functional images will be acquired using a single-shot, gradient recalled echoplanar pulse sequence sensitive to blood-oxygen-level-dependent (BOLD) contrast. Each of 2 runs will consist of the acquisition of 195 successive brain volumes. FMRI image preprocessing, processing, and analysis will be conducted using FSL and custom MATLAB scripts.

FMRI analyses will include a block-design analysis for the implicit face emotion processing task and event-related BOLD response analysis for the Affective Posner Task within a priori selected regions of interest, including corticolimbic regions (amygdala, medial PFC) for the implicit face task and amygdala and striatum (caudate, putamen, nucleus accumbens) for the Affective Posner. Image analyses will control for any group differences in reaction time or accuracy.

Behavioral Assessments. The following tasks will be conducted outside the scanner at the end of visits 3 & 6: To test whether HS+ show a greater degree of threat attention bias (Hypothesis 1a), participants will complete a visual dot-probe paradigm (Salum et al., 2017.), which measures attention bias toward angry faces. Each trial will start with a fixation cross, followed by a pair of faces (one on each side of the screen; in anger trials, one face is angry and the other is neutral), followed by an asterisk (half of the time presented on the same side as the angry face and half of the time presented on the side of the neutral face). Trials will be of three types (angry-neutral, happy-neutral, and neutral-neutral) and presented in random order. Threat attention bias will be calculated for the angry-neutral trials by subtracting the mean reaction time on trials where the asterisk appears on the same side as the angry face from the mean reaction time on trials where the asterisk appears on the same side as the neutral face. Using this task, previous studies have demonstrated that both irritable youths (Hommer et al., 2014; Salum et al., 2017) and anxious adults (Bradley et al., 1999; Mogg & Bradley, 1999, 2005) have greater threat attention bias. To test whether HS+ show greater relational aggression (approach behavior) in response to frustration (Hypothesis 2a), participants will complete the brief Point Subtraction Aggression Paradigm (Golomb et al., 2007), which measures relational aggression and has been validated in adult women (Dougherty et al., 1999). In the task, participants press a button to accrue money or press another button to subtract money from a (fictional) partner at no direct gain to themselves. Frustration is induced by periodic subtractions of their own money, which is attributed to the partner. Relational aggression is operationalized as number of point subtractions the participant makes.

Physical Exam

Participants will receive a physical exam prior to drug administration to rule out exclusionary health problems. Results of physical exam will be added to participants' UNC Hospital medical record. Additionally, participants will be required to provide documentation of a normal gynecological exam within the last three years, which is consistent with guidance from the American Academy of Family Medicine (Qaseem et al., 2014).

Venipuncture

Participants will undergo venipuncture at 3 study visits. Initial blood tests will include a CBC, Hcg serum, BUN, creatinine, electrolytes (Sodium, Potassium, Chloride, and Calcium) and a hepatic panel to rule out exclusionary health problems. Lab results will be added to participants' medical records. Subsequent venipuncture will be for the purpose of assaying estradiol, allopregnanolone and progesterone levels to ensure participant compliance at visits 3 and 6 and to ensure medication adherence and explore associations between hormone levels and mood symptoms. During the context of COVID-19, venipuncture will occur only at in-person visits, 3 and 6 to reduce unnecessary close contact. The risk of checking medication adherence, outweigh the benefits to the study, given that we will be in close contact with participants. To promote additional medication adherence in absence of repeated venipuncture, we will implement behavioral health techniques to encourage adherence, such as having participants set an alarm or reminder on their phone to take their medication. Additionally we will create a medication adherence plan for them to incorporate taking the medication into their routine. This would include items such as, encouraging them to put their medication next to their toothbrush to remember to take it morning and evening. Additionally we will be in regular contact with the participants via phone and email to check-in and see how they are doing, or if they have missed any medication doses.

Drug Administration

Participants will receive estradiol, progesterone, and placebo at study visits 3-7. Participants will receive Lupron injections at visits 3 and 5.

7 Statistical Plan

Kai Xia, PhD, is the biostatistician who will oversee all aspects of the statistical analyses. Dr. Schiller, in collaboration with Dr. Dichter and Josh Bizzell, will conduct the fMRI analyses.

7.1 Sample Size Determination

We approximated power and sample size using a simpler model comparing the two groups on change scores in behavioral effects (threat attention bias, reactive aggression) and brain activation (amygdala and striatal response to non-reward) from baseline to addback. With 15 subjects per group, we have 80% power to detect effect sizes of 1.10, which are large and consistent with our previous study (see Preliminary Data). We posit that if treatment with supra physiologic levels of reproductive hormones does not produce large enough differences in neural circuits between HS+ and HS- to be detected in a relatively small sample of women, then this line of research, which involves significant participant burden, is not worth pursuing. Moreover, given the experimental nature of the proposed research, our effect size estimates are more similar to those seen in experimental animal research than in traditional human clinical trials. Nonetheless, our own and other previous clinical research supports our hypothesized large effect. Studies comparing participants with major depression and controls have yielded effect sizes (Cohen's d) ranging from 1.15 to 2.05 in dorsomedial PFC responsivity to negative emotional stimuli (Grimm et al., 2008; Hooley et al., 2005; Moses-Kolko et al., 2010) and from 1.0 to 1.56 in ventral striatum reactivity to reward (Epstein et al., 2006; Moses-Kolko et al., 2011; Moria J Smoski et al., 2009). Studies examining the association between amygdala activation and depressive symptom severity have yielded effect sizes (Cohen's d) ranging from 1.62 to 2.76 (Hamilton & Gotlib, 2008; Moses-Kolko et al., 2010).

Our target number of subjects to include in the fMRI analyses is N=30 (15 confirmed HS+ and 15 confirmed HS-). Because we have not yet identified a mechanism by which we can predict hormone sensitive status at baseline with 100% accuracy, we will need to over-recruit women with a history of PND to account for those who end up not being HS+. In our previous study 11 of the 15 women (73%) with a history of PND were HS+. For the current study, we therefore plan to over-enroll women with a history of PND in order to reach our target of 15 women with confirmed HS+. If 73% of women with a history of PND and no history of non-puerperal major depressive disorder are HS+, then we will need to enroll 21 women to find 15 with confirmed HS+. Based on the 9% dropout rate of our prior trial, we estimated a 9% dropout rate, and as such, we have budgeted for an additional 3 subjects to account for dropouts. In the prior study 2/3 of the women who withdrew from the protocol had a history of PND, so we anticipate 2 women with a history of PND and 1 control (without a history of PND) will withdraw from the proposed study. These numbers are summarized in the following table:

Recruitment Group	Target Enrollment	Confirmed HS+/HS- (included in fMRI/behavioral analysis)	Not HS+/HS- (excluded from fMRI/behavioral analysis)	Withdrawn
History of PND	23	15 HS+	6 not HS+	2
Control (no history of PND)	16	15 HS-	0 not HS-	1
TOTAL	39	30	6	3

Thus, we plan to enroll a total of 39 subjects (n=16 without a history of PND, n=23 with a history of PND).

7.2 Statistical Methods

We have defined HS+ as a 30% or greater change in any of the IDAS-II mood scales, which include dysphoria, suicidality, ill temper (i.e., irritability), and wellbeing (i.e., reverse-keyed) during hormone addback or withdrawal, compared with the individual's own baseline using the equation below:

% symptom change = (Addback score-Baseline score)/Baseline score

Percent symptom change will be calculated for each of the IDAS-II scales listed above at both the addback visit (V6) and withdrawal visit (V7). If a participant has a percent symptoms change of 30% or greater on any of the IDAS-II scales listed, she will be classified as HS+. HS- is defined as less than 30% change in any of the IDAS-II mood scales.

We will only include those with confirmed HS+ and HS- in our planned analyses. Those with a history of PND confirmed to be HS- will be excluded from planned analyses. However, if we have enough subjects with a history of PND who are HS-, then they may be included as a separate group in exploratory analyses. Alternatively, if those with a history of PND who are HS- are not significantly different from those without a history of PND who are HS- in terms of our key outcome variables, then we will pool the groups to increase power.

The IDAS-II is scored using a 5-point Likert scale, ranging from 1 "Not at all" to 5 "Extremely." The scales we will use to determine hormone sensitivity and their ranges are listed in the table below:

IDAS-II Scale	Minimum Score	Maximum Score
Dysphoria	10	50
Suicidality	6	30
Ill Temper	5	25
Wellbeing	8	40

Missingness. We will use multiple imputation to correct for data determined to be missing at random, given that it is a reliable method for obtaining valid inferences in both behavioral and fMRI research (Rubin, 1987; Vaden et al., 2012). Data for dropouts will be compared with data for those who complete

the study to determine the extent to which excluding their data will introduce selection bias, and selection bias will be identified as a weakness in any resulting publications or presentations.

Sensitivity Analysis. In addition to the statistical tests described below, we will use sensitivity analyses to evaluate the robustness of the study's main results to reasonable perturbations of the statistical methods and assumptions used and to help ensure reproducibility of the main results. Sensitivity analysis will address the inclusion/exclusion of questionable data values (i.e., outliers), the use of alternative methods of coping with missing values and dropouts, and modeling assumptions.

Hypothesis testing. We have detailed both the null and alternative hypotheses we will test below. For all hypothesis tests, we will use the Bonferroni method to correct for multiple comparisons. All hypothesis tests that are observed to be not statistically significant will be reported as being inconclusive. In accordance with the ICMJE and Consort Statement, all statistical estimates of population parameters will be tabulated along with confidence intervals. The interpretation of confidence intervals will be an integral part of the data analysis in concert with the statistical hypothesis tests.

Power calculations overview. We have based our power calculations on previous studies of similar effects (i.e, not pilot studies) with *N* greater than or equal to 30. Given that prior literature suggests large effect sizes for Aims 1 and 2, we believe that point-estimates will yield relatively accurate power calculations.

Aim 1: To determine the extent to which irritability is characterized by dysfunctional threat processing during reproductive hormone challenge relative to baseline in HS+ and HS-.

Null hypothesis 1: HS+ is not characterized by behavioral activation in the context of potential threat during periods of hormone change in the target population.

Alternative hypothesis 1: HS+ is characterized by behavioral activation in the context of potential threat during periods of hormone change in the target population. Given this hypothesis, we will test the following sub-hypotheses:

> Null hypothesis 1a: HS+ is not characterized by threat attention bias during hormone addback relative to baseline in the target population.
>
> Alternative hypothesis 1a: HS+ is characterized by threat attention during hormone addback relative to baseline in the target population.
>
> > Independent variables: Group (HS+ versus HS-) and Phase (Visit 3-baseline versus Visit 6-hormone addback)
> >
> > Dependent variable: Threat attention bias will be measured using the visual dot probe task at study visits 3 and 6. Threat attention bias will be calculated for the angry-neutral trials by subtracting the mean reaction time (measured in milliseconds) on trials where the asterisk

appears on the same side as the angry face from the mean reaction time on trials where the asterisk appears on the same side as the neutral face.

Analysis: 2x2 Repeated Measured ANOVA will be used to analyze the effects of Group (HS- vs. HS+) and Phase (baseline vs. hormone addback) on threat attention bias.

Power: We used G*Power 3.1 for Mac to calculate power. We anticipate an effect size f=0.3 based on a previous study of threat attention bias in those with and without mood dysregulation (Hommer et al., 2014). If we set α=.05, we will have 87% power to reject the null hypothesis with a total sample size of N=30.

Null hypothesis 1b: HS+ is not characterized by reduced amygdala-medial PFC connectivity in response to threatening stimuli during hormone addback relative to baseline in the target population.

Alternative hypothesis 1b: HS+ is characterized by reduced amygdala-medial PFC connectivity in response to threatening stimuli during hormone addback relative to baseline in the target population.

Independent variables: Group (HS+ versus HS-) and Phase (Visit 3-baseline versus Visit 6-hormone addback)

Dependent variable: Amygdala-medial PFC connectivity will be assessed during the implicit face emotion processing fMRI task at study visits 3 and 6. Functional connectivity expresses the statistical dependency among activations in different brain regions and results in undirected (symmetrical) connectivity matrices and will be calculated according to standard procedures (Walsh et al., 2017). We will use a generalized psychophysiological interaction (gPPI) approach for detecting context-dependent functional connectivity (Cisler et al., 2014; McLaren et al., 2012). Replicating the methods of Walsh et al. (2017), we will use an ROI approach to target the amygdala, which will be defined using the Harvard-Oxford subcortical atlas. Voxel-wise models will be used to evaluate connectivity with the amygdala seed. For each participant, mean fMRI time courses will be extracted from the amygdala seed using the “fslmeants” program in FSL, then multiplied by each psychological regressor of interest (i.e., task condition) to form the PPI interaction terms. The gPPI model will include physiological and psychological regressors, as well as their interaction terms to describe the unique effect of these interactions above and beyond the man effects of seed time courses and task conditions. Our primary contrast of interest will evaluate the difference between connectivity during presentation of threatening stimuli versus non-threatening stimuli. This allows for the characterization of connectivity patterns specific to threat processing.

Analysis: Group and phase differences will be evaluated with respect to seed-based connectivity specific to threat processing using a 2x2 mixed effects analysis with Bayesian estimation techniques with FMRIB Local Analysis of Mixed Effects (Smith et al., 2004).

Clusters will be thresholded at $Z>2.58$ or $p<.005$ corrected with a minimum contiguous cluster size of 8 (Walsh et al., 2017).

Power: We used G*Power 3.1 for Mac to calculate power. While few studies have examined changes in amygdala-PFC between those with and without depression following treatment, the few extant studies yielded large effects (d=1.3) (Straub et al., 2017). If we anticipate an effect size f=.5 and set α=.05, we will have greater than 99% power to reject the null hypothesis with a total sample size of N=30.

Null hypothesis 1c (secondary): In HS+, the degree of irritability, threat attention bias, and amygdala-medial PFC connectivity during hormone addback are unrelated in the target population.

Alternative hypothesis 1c (secondary): In HS+, irritability is associated with 1) threat attention bias, and 2) amygdala-medial PFC connectivity during hormone addback in the target population.

"Independent" variable: Irritability will be defined as scores on the IDAS-II ill temper scale at study visit 6.

"Dependent" variables: Threat attention bias at study visit 6 will be calculated in the same way as for hypothesis 1a. Amygdala-medial PFC connectivity at study visit 6 will be calculated in the same way as for hypothesis 1b.

Analysis: Pearson's r will be calculated for correlations between irritability and 1) threat attention bias and 2) amygdala medial-PFC connectivity during hormone addback (study visit 6) in HS+.

Power: We have not powered to the study to assess secondary hypotheses. We used G*Power 3.1 for Mac to calculate power. Prior research suggests the association between irritability and threat attention bias has a medium effect size (Spearman's ρ=.3) (Hommer et al., 2014). If we anticipate an effect size ρ=.3 and set α=.05 (two-tailed), we will have less than 20% power to reject the null hypothesis with a HS+ sample size of n=15. There is not enough information to predict the strength of the association between irritability and amygdala medial-PFC connectivity.

Aim 2: To determine the extent to which irritability is characterized by dysfunctional reward processing during reproductive hormone challenge relative to baseline in HS+ and HS-.

Null hypothesis 2: HS+ is not characterized by increased frustration in the context of non-reward (i.e., when reward is expected) during hormone addback relative to baseline in the target population.

Alternative hypothesis 2: HS+ is characterized by increased frustration in the context of non-reward during hormone addback relative to baseline in the target population. Given this hypothesis, we will test the following sub-hypotheses:

Null hypothesis 2a: HS+ is not characterized by reactive aggression during hormone addback relative to baseline in the target population.

Alternative hypothesis 2a: HS+ is characterized by reactive aggression during hormone addback relative to baseline in the target population.

Independent variables: Group (HS+ versus HS-) and Phase (Visit 3-baseline versus Visit 6-hormone addback)

Dependent variable: Reactive aggression will be defined as the number of point subtractions the participant makes during the Point Subtraction Aggression Paradigm.

Analysis: 2x2 Repeated Measured ANOVA will be used to analyze the effects of Group (HS- vs. HS+) and Phase (baseline vs. hormone addback) on threat attention bias.

Power: We used G*Power 3.1 for Mac to calculate power. We anticipate a large effect size based on a previous study examining reactive aggression using the Point Subtraction Aggression Paradigm in men who were treated with testosterone versus placebo (*d*=.95) . If we estimate the effect size f=.45 and set α=.05, we will have greater than 99% power to reject the null hypothesis with a total sample size of N=30.

Null hypothesis 2b: HS+ is not characterized by reduced activation of the amygdala and ventral striatum in response to FNR during hormone addback relative to baseline in the target population.

Alternative hypothesis 2b: HS+ is characterized by reduced activation of the amygdala and ventral striatum in response to FNR during hormone addback relative to baseline in the target population.

Independent variables: Group (HS+ versus HS-) and Phase (Visit 3-baseline versus Visit 6-hormone addback)

Dependent variable: Activation in amygdala and ventral striatum (caudate, putamen, nucleus accumbens) ROIs during the Affective Posner Task. Event types include three categories: trial type (money, no money), validity (valid, invalid), and feedback (positive, negative, error). Combination of these three factors will be modeled using individual linear regression with a fixed-shape, gamma-variate response function, convolved with a boxcar function of the stimulus duration. FNR will be defined as the contrast between positive valid money trials and negative valid money trials (i.e., the difference between expecting money and getting it and expecting money and not getting it).

Analysis: The model will include each of the two event-type regressors (to model FNR), the six motion parameters, and baseline drift for each of the runs. Beta coefficients and t-statistics will be calculated for each voxel and regressor. Group level analyses will be conducted on the amygdala and striatum (caudate, putamen, and nucleus accumbens), as defined by the Harvard-Oxford subcortical atlas. Mean signal intensity will be extracted from each ROI for each of the event types using FSL. Signal intensity values will be submitted to a Group x Trial type x Feedback x Phase x Region ANCOVA in SPSS.
Power: We used G*Power 3.1 for Mac to calculate power. We anticipate a large effect size based on previous studies examining changes in amygdala activation with various treatments (*d*=1.58 to 1.96) (Hauner et al., 2012; Riem et al., 2011). If we estimate the effect size f=.5 and set α=.05, we will have greater than 99% power to reject the null hypothesis with a total sample size of N=30.

Null hypothesis 2c (secondary): The degree of irritability, reactive aggression, and subcortical (amygdala, caudate, putamen, and nucleus accumbens) activation in HS+ during hormone addback are unrelated in the target population.

Alternative hypothesis 2c (secondary): In HS+, self-reported irritability is associated with 1) reactive aggression, and 2) subcortical (amygdala, caudate, putamen, and nucleus accumbens) activation during hormone addback in the target population.

"Independent" variable: Irritability will be defined as scores on the IDAS-II ill temper scale at study visit 6.

"Dependent" variables: Reactive aggression at study visit 6 will be calculated in the same way as for hypothesis 2a. Subcortical activation at study visit 6 will be calculated in the same way as for hypothesis 2b.

Analysis: Pearson's r will be calculated for correlations between irritability and 1) reactive aggression and 2) subcortical activation during hormone addback (study visit 6) in HS+.

Power: We have not powered to the study to assess secondary hypotheses. We used G*Power 3.1 for Mac to calculate power. Prior research suggests the association between irritability and reactive aggression has a large effect size (Pearson's *r*=.6) in a civilian population (Geniole et al., 2017). If we anticipate an effect size *r*=.3 and set α=.05 (two-tailed), we will have 70% power to reject the null hypothesis with a HS+ sample size of n=15. There is not enough information to predict the strength of the association between irritability and subcortical activation in response to FNR.

7.3 Subject Population(s) for Analysis

The rationale for the proposed study is that employing a scaled down model of puerperal hormonal events permits the identification of a group of individuals homogeneous for hormone sensitivity (HS+),

hence creating the best opportunity for disentangling the specific changes in brain function due to reproductive hormones (i.e., HS-) from those accompanying reproductive hormone-precipitated affective dysfunction (i.e., HS+). Thus, we will only include those with confirmed HS+ and HS- in our planned analyses as described in section 7.2.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

· Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)

· Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,

· Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

· results in study withdrawal
· is associated with a serious adverse event
· is associated with clinical signs or symptoms
· leads to additional treatment or to further diagnostic tests
· is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

· fatal
· life-threatening
· requires or prolongs hospital stay
· results in persistent or significant disability or incapacity
· a congenital anomaly or birth defect
· an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events will be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Pre-existing Condition
A pre-existing condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor will also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

· Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
· Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
· Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

COVID-19
Any participant that develops symptoms of COVID-19 including by not limited to: fever, cough, shortness of breath, nausea, abdominal pain, headache, loss of taste or smell, or has known contact with a COVID-19 positive carrier will be asked to contact their PCP, their local COVID-19 hotline and the study team immediately. All study procedures will be put on hold until the participant has received a negative test result or has quarantined for 14 days while monitoring their symptoms. If a participant is confirmed positive for COVID-19, the appropriate case report forms will be completed in accordance with CDC guidelines and UNC Policy.

8.2 Recording of Adverse Events

At each contact or virtual visit with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, though will be grouped under one diagnosis.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:
· related to study participation,
· unexpected, and
· serious or involve risks to subjects or others
(see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, will be reported to the study sponsor and DSMB by telephone within 24 hours of the event. To report such events, an FDA Form 3500A will be completed by the investigator, signed by the sponsor, and faxed to the DSMB within 24 hours. The investigator will keep a copy of this FDA Form 3500A on file at the study site.

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed FDA Form 3500A, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor and the FDA.

8.3.2 Investigator reporting

For reportable deaths, the initial submission to the UNC IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
- An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.

- Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
- A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.3 Investigator reporting: *Notifying UNC if affiliate site*

N/A

8.3.4 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

· ***Within 7 calendar days***

Any study event that is:

– associated with the use of the study drug
– unexpected,
– fatal or life-threatening, and

· ***Within 15 calendar days***

Any study event that is:

– associated with the use of the study drug,
– unexpected, and
– serious, but not fatal or life-threatening

-or-

– a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

– suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. All adverse events will be reported to the FDA.

8.3.5 Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

8.4 Unblinding Procedures

In the event of a SAE or discontinuation from the study, subjects will be unblinded.

8.5 Stopping Rules

The Data and Safety Monitoring plan contains rules for discontinuing individual participant and rules for stopping the entire study. In the event of three grade 3 or higher adverse events (i.e. a life threatening adverse event that may require hospitalization) possibly or definitely associated with the study intervention, the study will be suspended and the study's Data and Safety Monitoring Board will be notified.

8.6 Medical Monitoring

The principal investigator will oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Independent Data and Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) will act in an advisory capacity to the University of North Carolina at Chapel Hill (UNC-CH) Institutional Review Board (IRB) to monitor patient safety and evaluate the efficacy of the intervention.

DSMB RESPONSIBILITIES

The initial responsibility of the DSMB will be to approve the initiation of this clinical trial. After this approval and at periodic intervals, following the enrollment and protocol start of 10 subjects or at 6 months intervals during the course of the trail, the DSMB responsibilities are to:

- review plans for data safety and monitoring;
- evaluate the progress of the trial, including safety events, accrual and retention, and other factors that can affect study outcome;
- make recommendations to the PI concerning continuation, termination, or other modifications of the trial based on the reported beneficial or adverse effects of the treatment under study;
- if appropriate, review interim analysis of efficacy in accordance with stopping rules which are clearly defined in advance of data analysis and have the approval of the DSMB;
- assist the UNC-CH IRB by commenting on any problems with enrollment, and sample size.

MEMBERSHIP

The DSMB will consist of at least three members, and the presence of all three members will be required to constitute a quorum. The members, recommended by NCTraCS, will include an obstetrician-gynecologist, a biostatistician, and a psychiatrist (not affiliated with the UNC Department of Psychiatry) and will be subject to approval by the UNC-CH IRB. Membership will consist of persons completely independent of the investigators who have no financial, scientific, or other conflict of interest with the trial. Collaborators or associates of Dr. Schiller are not eligible to serve on the DSMB. Written documentation attesting to absence of conflict of interest is required. The DSMB includes experts in or representatives of the fields of:

• a psychiatrist with relevant clinical experience,

• an obstetrician-gynecologist with relevant clinical experience;

• clinical trial methodology, and

• biostatistics.

The DSMB chairperson will be responsible for overseeing the meetings, developing the agenda in consultation with Dr. Schiller and the UNC-CH IRB, as required. The chair is the contact person for the DSMB

A Safety Officer will be identified by Dr. Schiller at the first meeting. This person will be the contact person for severe adverse event reporting. The Safety Officer will notify the DSMB and UNC-CH IRB of serious adverse events within 24 hours. Procedures for notifying the Chair of the DSMB and UNC-CH IRB will be determined at the first meeting.

BOARD PROCESS

The first meeting will take place face-to-face to discuss the protocol, any modifications of the trial, and to establish guidelines to monitor the study. The DSMB Chairperson and Dr. Schiller will prepare the agenda to address the review of the manual of operating procedures, modification of the study design, initiation of the trial, identification of a safety officer, reporting of adverse events, stopping rules, interim analysis plan, etc.

Meetings of the DSMB will be held two times a year at the call of the Chairperson. Meetings shall be closed to the public because discussions may address confidential patient data. Meetings are attended, when appropriate, by the principal investigator and members of her staff. Meetings may be convened as conference calls as well as in person, although the initial meeting and meetings to discuss interim analysis will be face-to-face. An emergency meeting of the DSMB may be called at any time by the Chairperson or by the UNC-CH IRB should questions of patient safety arise.

MEETING FORMAT

An appropriate format for DSMB meetings consists of an open and closed session. The open sessions may be attended by the principal investigator(s), institution staff and UNC-CH IRB staff, and should always include the study biostatistician. Issues discussed at open sessions will include the conduct and progress of the study, including patient accrual, compliance with protocol, and problems encountered. Patient-specific data and treatment group data may not be presented in the open session.

The closed session will be attended only by voting DSMB members. The DSMB may request others to attend part or all of the closed sessions (e.g., the study statistician, UNC-CH IRB staff). All safety and efficacy data are and must be presented at this session. The discussion at the closed session is completely confidential.

The DSMB will conduct an interim review following the enrollment of 10 subjects, or at 6 month intervals, whichever is soonest.

Should the DSMB decide to issue a termination recommendation, full vote of the DSMB will be required. In the event of a split vote, majority vote will rule, and a minority report should be appended.

REPORTS

1. **Interim Reports:** Interim reports are generally prepared by the study statistician and distributed to the DSMB at least 10 days prior to a scheduled meeting. These interim reports are numbered and provided in sealed envelopes within an express mailing package or by secure email as the DSMB prefers. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be directed by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts:

Part 1 (Open Session Report) provides information on study aspects such as accrual, baseline characteristics, and other general information on study status.

Part 2 (Closed Session Report) may contain data on study outcomes, including safety data, and depending on the study, perhaps efficacy data. The Closed Session Report is considered confidential and should be destroyed at the conclusion of the meeting. Data files to be used for interim analyses should have undergone established editing procedures to the extent possible. Interim analyses of the efficacy data are performed only if they are specified and approved in advance and criteria for possible stopping is clearly defined.

1. **Reports from the DSMB:** A formal report containing recommendations for continuation or modifications of the study prepared by the DSMB Chairperson will be sent to the full DSMB within four weeks of the meeting. Once approved by the DSMB, the formal DSMB recommendation will be forwarded to Dr. Schiller It is Dr. Schiller's responsibility to distribute the formal DSMB recommendation report to tall co-investigators and to assure that copies are submitted to all the IRBs associated with the study.

As previously stated, the formal DSMB report should conclude with a recommendation to continue or to terminate the study. This recommendation should be made by a formal majority vote. A termination recommendation may be made by the DSMB at any time by a majority vote. The DSMB Chairperson is responsible for notifying Dr. Schiller of a decision to terminate the study. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report. The report should not include unblinded data, discussion of the unblinded data, etc.

Mailings to the DSMB: On a scheduled basis (as agreed upon by the DSMB) blinded safety data should be communicated to all DSMB members or to the designated safety officer (to be determined at the first meeting). Any concerns noted should be brought to the attention of the DSMB Chairperson.

Access to Interim Data: Access to the accumulating endpoint data should be limited to as small a group as possible. Limiting the access to interim data to the DSMB members relieves the investigator of the burden of deciding whether it is ethical to continue to enroll patients and helps protect the study from bias in patient entry and/or evaluation.

CONFIDENTIALITY

All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Only study personnel, the PI, the study coordinator, and research assistants will have access to confidential information about study subjects. Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, Dr. Schiller, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

All subject files will be stored in the study coordinator's office in a locked filing cabinet, which will only be accessed by the PI, study coordinator, and their trained research assistants. Lab values, recorded data, subject files, screening and call logs will all be stored digitally on the secure, Schiller lab drive server. Access will only be granted on this electronic drive for study personnel.
Once enrolled in the study, participants will be identified by a specific study ID number assigned to them upon enrollment, to protect their privacy and maintain confidentiality. Any identifying information (i.e. consent forms and HIPAA authorizations) will be stored separately from their individual subject files. Additionally, once enrolled in the study, Qualtrics will be used to collect questionnaires data from study participants will be labeled by their unique study ID number.

9.3 Case Report Forms (as applicable)

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. Reasons for withdrawal (drop-outs) from the study and missing data values will be documented in the database. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9.5 Data Management and Quality

Data will be managed by Laura Lundegard (RA) and Russ Dean (data manager) and overseen by Dr. Schiller with a goal of maximizing data quality.

Ms. Lundegard will monitor data completeness using the case report forms for each subject and document missing data as well as the reason for missingness in the database. Data accuracy will be monitored using descriptive statistics to identify values outside of the possible range as well as outliers.

Interview and Questionnaire Data

Mr. Dean and Ms. Lundegard, in collaboration, will prepare a codebook for the study and design Qualtrics surveys to reduce opportunities for data errors and missingness (e.g., using survey response logic to ask participants to confirm they'd like to skip the questions for any missing questionnaire values).

All research assistants will receive training with the codebook for all items that require hand entry into databases. Ms. Lundegard will examine data on a monthly basis for adherence to the codebook.

Behavioral Data

Ms. Lundegard will visually inspect the behavioral data captured from each of the tasks for completeness immediately following each study visit. If a runtime error occurs during the study visit, the task will be re-started if participant agrees and if there is sufficient time to complete the task. Data accuracy will be monitored using descriptive statistics to identify values outside of the possible range as well as outliers.

fMRI Data

Research assistants will inspect the fMRI QI output to identify possible problems with data quality, including head motion, susceptibility artifact, and scanner problems. Any problems identified after the scan will be reviewed by Dr. Schiller and Mr. Bizzell to determine 1) whether data can be corrected statistically, and 2) whether the problem needs to be addressed prior to future scans (e.g., in the case of a problem with a scan setting).

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Appendix C. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment ___ for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through a R21 grant from the National Institutes of Health.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) will have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All UNC investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

Participants will be compensated the amount specified below upon completion of the study for a total of $1,000.00. Payment will be given through the form of a Visa Gift Card, issued inactive and unloaded through USPS mail to minimize in-person contact during the COVID-19 pandemic. Upon confirmed arrival of the Visa gift card, the card will be loaded with the payment amount and will be able to be activated by the participant. If a subject withdraws or is withdrawn from the study as a result of an adverse event, her compensation will be prorated based on the following schedule:

Initial evaluation and physical exam (1 visit) $60.00
Psychological Interview (1 visit) $60.00
Screening Phase $30.00
FMRI Imaging (2 sessions, combined with clinic visits) $120
Repeated Venipuncture (3) $150
Clinic Visits following screening (6) $180
Daily Mood and Behavioral ratings, medications ($6.50/day) $550

Payment is processed through the UNC Department of Psychiatry.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 References

Alper, K. R., Barry, J. J., & Balabanov, A. J. (2002). Treatment of psychosis, aggression, and irritability in patients with epilepsy. *Epilepsy & Behavior*, *3*(5), 13–18. https://doi.org/10.1016/S1525-5069(02)00500-5

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*. American Psychiatric Pub.

Anderson, G. L., Limacher, M., Assaf, A. R., Bassford, T., Beresford, S. A., Black, H., Bonds, D., Brunner, R., Brzyski, R., Caan, B., Chlebowski, R., Curb, D., Gass, M., Hays, J., Heiss, G., Hendrix, S., Howard, B. V., Hsia, J., Hubbell, A., … Wassertheil-Smoller, S. (2004). Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: The Women's Health Initiative randomized controlled trial. *JAMA : The Journal of the American Medical Association*, *291*(14), 1701–1712. https://doi.org/Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, U.S. Gov't, P.H.S.

Barrett-Connor, E., & Bush, T. L. (1991). Estrogen and coronary heart disease in women. *JAMA : The Journal of the American Medical Association*, *265*(2005736), 1861–1867.

Beaver, J. D., Lawrence, A. D., Ditzhuijzen, J. van, Davis, M. H., Woods, A., & Calder, A. J. (2006). Individual Differences in Reward Drive Predict Neural Responses to Images of Food. *Journal of Neuroscience*, *26*(19), 5160–5166. https://doi.org/10.1523/JNEUROSCI.0350-06.2006

Beral, V. (1997). Breast cancer and hormone replacement therapy: Collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. *Lancet*, *350*(9084), 1047–1059.

Berman, K. F., Schmidt, P. J., Rubinow, D. R., Danaceau, M. A., Van Horn, J. D., Esposito, G., Ostrem, J. L., & Weinberger, D. R. (1997). Modulation of cognition-specific cortical activity by gonadal steroids: A positron-emission tomography study in women. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(16), 8836–8841. https://doi.org/Clinical Trial Controlled Clinical Trial

Bilgi, M. M., Taspinar, S., Aksoy, B., Oguz, K., Coburn, K., & Gonul, A. S. (2017). The relationship between childhood trauma, emotion recognition, and irritability in schizophrenia patients. *Psychiatry Research*, *251*, 90–96. https://doi.org/10.1016/j.psychres.2017.01.091

Blair, R. J. R. (2016). The Neurobiology of Impulsive Aggression. *Journal of Child and Adolescent Psychopharmacology*, *26*(1), 4–9. https://doi.org/10.1089/cap.2015.0088

Bloch, M., Rubinow, D. R., Schmidt, P. J., Lotsikas, A., Chrousos, G. P., & Cizza, G. (2005). Cortisol response to ovine corticotropin-releasing hormone in a model of pregnancy and parturition in euthymic women with and without a history of postpartum depression. *The Journal of Clinical Endocrinology and Metabolism*, *90*(2), 695–699. https://doi.org/10.1210/jc.2004-1388

Bloch, M., Schmidt, P. J., Danaceau, M., Murphy, J., Nieman, L., & Rubinow, D. R. (2000). Effects of gonadal steroids in women with a history of postpartum depression. *American Journal of Psychiatry*, *157*(10831472), 924–930.

Bourgain, C., Devroey, P., Waeshberghe, L. V., Smitz, J., & Steirteghem, A. C. V. (1990). Effects of natural progesterone on the morphology of the endometrium in patients with primary ovarian failure. *Human Reproduction*, *5*(5), 537–543.

Bradley, B. P., Mogg, K., White, J., Groom, C., & Bono, J. D. (1999). Attentional bias for emotional faces in generalized anxiety disorder. *British Journal of Clinical Psychology*, *38*(3), 267–278. https://doi.org/10.1348/014466599162845

Brotman, M. A., Kircanski, K., Stringaris, A., Pine, D. S., & Leibenluft, E. (2017). Irritability in Youths: A Translational Model. *American Journal of Psychiatry*, *174*(6), 520–532. https://doi.org/10.1176/appi.ajp.2016.16070839

Bustillo, M., Krysa, L. W., & Coulam, C. B. (1995). Implantation: Uterine receptivity in an oocyte donation programme. *Human Reproduction*, *10*(2), 442–445.

Butle, D., & Wartolowska, K. (2017). Monitoring cardiac and respiratory physiology during fMRI. *NeuroImage*, *154*, 81–91.

Carver, C. S., & White, T. L. (1994). Behavioral Inhibition, Behavioral Activation, and Affective Responses to Impending Reward and Punishment: The Bis/bas Scales. *Journal of Personality and Social Psychology*, *67*(2), 319–333.

Chen, W. Y., Manson, J. E., Hankinson, S. E., Rosner, B., Holmes, M. D., Willett, W. C., & Colditz, G. A. (2006). Unopposed estrogen therapy and the risk of invasive breast cancer. *Archives of Internal Medicine*, *166*(9), 1027–1032. https://doi.org/Research Support, N.I.H., Extramural

Chetkowski, R. J., Meldrum, D. R., Steingold, K. A., Randle, D., Lu, J. K., Eggena, P., Hershman, J. M., Alkjaersig, N. K., Fletcher, A. P., & Judd, H. L. (1986). Biologic effects of transdermal estradiol. *The New England Journal of Medicine*, *314*(3012339), 1615–1620.

Chlebowski, R. T., Hendrix, S. L., Langer, R. D., Stefanick, M. L., Gass, M., Lane, D., Rodabough, R. J., Gilligan, M. A., Cyr, M. G., Thomson, C. A., Khandekar, J., Petrovitch, H., & McTiernan, A. (2003). Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: The Women's Health Initiative Randomized Trial. *JAMA : The Journal of the American Medical Association*, *289*(24), 3243–3253. https://doi.org/Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

Cirillo, D. J., Wallace, R. B., Rodabough, R. J., Greenland, P., LaCroix, A. Z., Limacher, M. C., & Larson, J. C. (2005). Effect of estrogen therapy on gallbladder disease. *JAMA : The Journal of the American Medical Association*, *293*(3), 330–339. https://doi.org/Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't

Cisler, J. M., Bush, K., & Steele, J. S. (2014). A comparison of statistical methods for detecting context-modulated functional connectivity in fMRI. *NeuroImage*, *84*, 1042–1052. https://doi.org/10.1016/j.neuroimage.2013.09.018

Clark, L. A. (1993). *Schedule for Nonadaptive and Adaptive Personality (SNAP).* University of Minnesota Press.

Colditz, G. A., Hankinson, S. E., Hunter, D. J., Willett, W. C., Manson, J. E., Stampfer, M. J., Hennekens, C., Rosner, B., & Speizer, F. E. (1995). The use of estrogens and progestins and the risk of breast cancer in

postmenopausal women. *The New England Journal of Medicine*, *332*(24), 1589–1593. https://doi.org/Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

Cox, E. Q., Raines, C., Kimmel, M., Richardson, E., Stuebe, A., & Meltzer-Brody, S. (2017). Comprehensive Integrated Care Model to Improve Maternal Mental Health. *Journal of Obstetric, Gynecologic & Neonatal Nursing*, *46*(6), 923–930. https://doi.org/10.1016/j.jogn.2017.08.003

Cox, J. L., Holden, J. M., & Sagovsky, R. (1987). Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *British Journal of Psychiatry*, *150*(3651732), 782–786.

Cushman, M., Kuller, L. H., Prentice, R., Rodabough, R. J., Psaty, B. M., Stafford, R. S., Sidney, S., Rosendaal, F. R., & Investigators, for the W. H. I. (2004). Estrogen Plus Progestin and Risk of Venous Thrombosis. *JAMA: The Journal of the American Medical Association*, *292*(13), 1573–1580. https://doi.org/10.1001/jama.292.13.1573

Daly, R. C., Schmidt, P. J., Davis, C. L., Danaceau, M. A., & Rubinow, D. R. (2001). Effects of gonadal steroids on peripheral benzodiazepine receptor density in women with PMS and controls. *Psychoneuroendocrinology*, *26*(6), 539–549. https://doi.org/Clinical Trial Randomized Controlled Trial

Damario, M. A., Hammitt, D. G., Galanits, T. M., Session, D. R., & Dumesic, D. A. (1999). Pronuclear stage cryopreservation after intracytoplasmic sperm injection and conventional IVF: Implications for timing of the freeze. *Fertility and Sterility*, *72*(6), 1049–1054. https://doi.org/10.1016/S0015-0282(99)00444-6

Deveney, C. M., Connolly, M. E., Haring, C. T., Bones, B. L., Reynolds, R. C., Kim, P., Pine, D. S., & Leibenluft, E. (2013). Neural Mechanisms of Frustration in Chronically Irritable Children. *American Journal of Psychiatry*, *170*(10), 1186–1194. https://doi.org/10.1176/appi.ajp.2013.12070917

Dougherty, D. M., Bjork, J. M., Huckabee, H. C. G., Moeller, F. G., & Swann, A. C. (1999). Laboratory measures of aggression and impulsivity in women with borderline personality disorder. *Psychiatry Research*, *85*(3), 315–326. https://doi.org/10.1016/S0165-1781(99)00011-6

Elkind-Hirsch, K. E., Valdes, C. T., & Malinak, L. R. (1993). Insulin resistance improves in hyperandrogenic women treated with Lupron. *Fertility and Sterility*, *60*(4), 634–641.

Endicott, J., Nee, J., & Harrison, W. (2006). Daily Record of Severity of Problems (DRSP): Reliability and validity. *Archives of Women's Mental Health*, *9*(1), 41–49. https://doi.org/10.1007/s00737-005-0103-y

Engmann, L., DiLuigi, A., Schmidt, D., Benadiva, C., Maier, D., & Nulsen, J. (2008). The effect of luteal phase vaginal estradiol supplementation on the success of in vitro fertilization treatment: A prospective randomized study. *Fertility and Sterility*, *89*(3), 554–561. https://doi.org/Randomized Controlled Trial

Epstein, J., Pan, H., Kocsis, J. H., Yang, Y., Butler, T., Chusid, J., Hochberg, H., Murrough, J., Strohmayer, E., Stern, E., & Silbersweig, D. A. (2006). Lack of ventral striatal response to positive stimuli in depressed versus normal subjects. *The American Journal of Psychiatry*, *163*(10), 1784–1790. https://doi.org/Comparative Study Research Support, Non-U.S. Gov't

Fatemi, H. M., Kolibianakis, E. M., Camus, M., Tournaye, H., Donoso, P., Papanikolaou, E., & Devroey, P. (2006). Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: A randomized controlled trial. *Human Reproduction*, *21*(10), 2628–2632. https://doi.org/10.1093/humrep/del117

Fava, M., Hwang, I., Rush, A. J., Sampson, N., Walters, E. E., & Kessler, R. C. (2010). The importance of irritability as a symptom of major depressive disorder: Results from the National Comorbidity Survey Replication. *Molecular Psychiatry*, *15*(8), 856–867. https://doi.org/10.1038/mp.2009.20

Feuillan, P. P., Jones, J. V., Barnes, K. M., Oerter-Klein, K., & Cutler, G. B. (2000). Boys with precocious puberty due to hypothalamic hamartoma: Reproductive axis after discontinuation of gonadotropin-releasing hormone analog therapy. *The Journal of Clinical Endocrinology and Metabolism*, *85*(11), 4036–4038. https://doi.org/Comparative Study

Feuillan, P. P., Jones, J. V., Barnes, K., Oerter-Klein, K., & Cutler, G. B. (1999). Reproductive axis after discontinuation of gonadotropin-releasing hormone analog treatment of girls with precocious puberty: Long term follow-up comparing girls with hypothalamic hamartoma to those with idiopathic precocious puberty. *The Journal of Clinical Endocrinology and Metabolism*, *84*(1), 44–49. https://doi.org/Comparative Study

First, M. B., Williams, J. B. W., Karg, R. S., & Spitzer, R. L. (2015). Structured clinical interview for DSM-5—Research version (SCID-5 for DSM-5, research version; SCID-5-RV). *Arlington, VA: American Psychiatric Association*.

Flory, J. D., Matthews, K. A., Sistilli, C. G., Caggiula, A. R., Berga, S. L., & F. Owens, J. (2002). Short-term suppression of ovarian function and immune measures in healthy women. *Psychoneuroendocrinology*, *27*(6), 749–768. https://doi.org/10.1016/S0306-4530(01)00078-6

Forty, L., Jones, L., Macgregor, S., Caesar, S., Cooper, C., Hough, A., Dean, L., Dave, S., Farmer, A., McGuffin, P., Brewster, S., Craddock, N., & Jones, I. (2006). Familiality of postpartum depression in unipolar disorder: Results of a family study. *The American Journal of Psychiatry*, *163*(9), 1549–1553. https://doi.org/Comparative Study Multicenter Study Research Support, U.S. Gov't, Non-P.H.S.

Friedler, S., Raziel, A., Schachter, M., Strassburger, D., Bukovsky, I., & Ron-El, R. (1999). Luteal support with micronized progesterone following in-vitro fertilization using a down-regulation protocol with gonadotrophin-releasing hormone agonist: A comparative study between vaginal and oral administration. *Human Reproduction*, *14*(8), 1944–1948. https://doi.org/10.1093/humrep/14.8.1944

Gambrell, R. D. (1986). The role of hormones in the etiology and prevention of endometrial cancer. *Clinics in Obstetrics and Gynaecology*, *13*(4), 695–723.

Gann, P. H., & Morrow, M. (2003). Combined Hormone Therapy and Breast Cancer. *JAMA: The Journal of the American Medical Association*, *289*(24), 3304–3306. https://doi.org/10.1001/jama.289.24.3304

Geniole, S. N., MacDonell, E. T., & McCormick, C. M. (2017). The Point Subtraction Aggression Paradigm as a laboratory tool for investigating the neuroendocrinology of aggression and competition. *Hormones and Behavior*, *92*, 103–116. https://doi.org/10.1016/j.yhbeh.2016.04.006

Golomb, B. A., Cortez-Perez, M., Jaworski, B. A., Mednick, S., & Dimsdale, J. (2007). Point Subtraction Aggression Paradigm: Validity of a Brief Schedule of Use. *Violence and Victims; New York*, *22*(1), 95–103.

Grady, D., & Ernster, V. (1991). Invited Commentary: Does Postmenopausal Hormone Therapy Cause Breast Cancer? *American Journal of Epidemiology*, *134*(12), 1396–1400.

Gray, J. A. (1987). *The Psychology of Fear and Stress*. CUP Archive.

Grigorova, M., Sherwin, B. B., & Tulandi, T. (2006). Effects of treatment with leuprolide acetate depot on working memory and executive functions in young premenopausal women. *Psychoneuroendocrinology*, *31*(8), 935–947. https://doi.org/10.1016/j.psyneuen.2006.05.004

Grimm, S., Beck, J., Schuepbach, D., Hell, D., Boesiger, P., Bermpohl, F., Niehaus, L., Boeker, H., & Northoff, G. (2008). Imbalance between left and right dorsolateral prefrontal cortex in major depression is linked to negative emotional judgment: An fMRI study in severe major depressive disorder. *Biological Psychiatry*, *63*(4), 369–376. https://doi.org/Research Support, Non-U.S. Gov't

Grodstein, F., Lifford, K., Resnick, N. M., & Curhan, G. C. (2004). Postmenopausal hormone therapy and risk of developing urinary incontinence. *Obstetrics and Gynecology*, *103*(2), 254–260. https://doi.org/Comparative Study Research Support, U.S. Gov't, P.H.S.

Grodstein, F., Stampfer, M. J., Goldhaber, S. Z., Manson, J. E., Colditz, G. A., Speizer, F. E., Willett, W. C., & Hennekens, C. H. (1996). Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet*, *348*(8855854), 983–987.

Grodstein, Francine, Manson, J. E., & Stampfer, M. J. (2006). Hormone therapy and coronary heart disease: The role of time since menopause and age at hormone initiation. *Journal of Women's Health*, *15*(16417416), 35–44.

Hamilton, J. P., & Gotlib, I. H. (2008). Neural substrates of increased memory sensitivity for negative stimuli in major depression. *Biological Psychiatry*, *63*(12), 1155–1162. https://doi.org/Research Support, N.I.H., Extramural

Hauner, K. K., Mineka, S., Voss, J. L., & Paller, K. A. (2012). Exposure therapy triggers lasting reorganization of neural fear processing. *Proceedings of the National Academy of Sciences*, *109*(23), 9203–9208. https://doi.org/10.1073/pnas.1205242109

Hendrix, S. L., Cochrane, B. B., Nygaard, I. E., Handa, V. L., Barnabei, V. M., Iglesia, C., Aragaki, A., Naughton, M. J., Wallace, R. B., & McNeeley, S. G. (2005). Effects of estrogen with and without

progestin on urinary incontinence. *JAMA : The Journal of the American Medical Association*, *293*(8), 935–948. https://doi.org/Research Support, U.S. Gov't, P.H.S.

Henzel, M. R. (1986). Contraceptive hormones and their clinical use. In *Reproductive endocrinology: Physiology, pathophysiology and clinical management* (pp. 643–682). W.B. Saunders Company.

Hommer, R. E., Meyer, A., Stoddard, J., Connolly, M. E., Mogg, K., Bradley, B. P., Pine, D. S., Leibenluft, E., & Brotman, M. A. (2014). Attention Bias to Threat Faces in Severe Mood Dysregulation. *Depression and Anxiety*, *31*(7), 559–565. https://doi.org/10.1002/da.22145

Hooley, J. M., Gruber, S. A., Scott, L. A., Hiller, J. B., & Yurgelun-Todd, D. A. (2005). Activation in dorsolateral prefrontal cortex in response to maternal criticism and praise in recovered depressed and healthy control participants. *Biological Psychiatry*, *57*(7), 809–12. https://doi.org/Comparative Study Research Support, Non-U.S. Gov't

Hsia, J., Langer, R. D., Manson, J. E., Kuller, L., Johnson, K. C., Hendrix, S. L., Pettinger, M., Heckbert, S. R., Greep, N., Crawford, S., Eaton, C. B., Kostis, J. B., Caralis, P., Prentice, R., & for the Women's Health Initiative Investigators. (2006). Conjugated Equine Estrogens and Coronary Heart Disease: The Women's Health Initiative. *Arch Intern Med*, *166*(3), 357–365. https://doi.org/10.1001/archinte.166.3.357

Humaidan, P., Bredkjær, H. E., Bungum, L., Bungum, M., Grøndahl, M. L., Westergaard, L., & Andersen, C. Y. (2005). GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: A prospective randomized study. *Human Reproduction*, *20*(5), 1213–1220. https://doi.org/10.1093/humrep/deh765

Jick, H., Derby, L. E., Myers, M. W., Vasilakis, C., & Newton, K. M. (1996). Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet*, *348*(9033), 981–983. https://doi.org/Comparative Study Research Support, Non-U.S. Gov't

Kaur, G., Gonsalves, L., & Thacker, H. L. (2004). Premenstrual dysphoric disorder: A review for the treating practitioner. *Cleveland Clinic Journal of Medicine*, *71*(4), 303–305. https://doi.org/10.3949/ccjm.71.4.303

Kilborn, L. C., & Labbé, E. E. (1990). Magnetic resonance imaging scanning procedures: Development of phobic response during scan and at one-month follow-up. *Journal of Behavioral Medicine*, *13*(4), 391–401. https://doi.org/10.1007/BF00844886

Lee, E. E., Nieman, L. K., Martinez, P. E., Harsh, V. L., Rubinow, D. R., & Schmidt, P. J. (2012). ACTH and Cortisol Response to Dex/CRH Testing in Women with and without Premenstrual Dysphoria during GnRH Agonist-Induced Hypogonadism and Ovarian Steroid Replacement. *Journal of Clinical Endocrinology & Metabolism*, *97*(6), 1887–1896. https://doi.org/10.1210/jc.2011-3451

Legro, R. S., Ary, B. A., Paulson, R. J., Stanczyk, F. Z., & Sauer, M. V. (1993). Pregnancy: Premature luteinization as detected by elevated serum progesterone is associated with a higher pregnancy rate in donor oocyte in-vitro fertilization. *Human Reproduction*, *8*(9), 1506–1511.

Leibenluft, E. (2017). Pediatric Irritability: A Systems Neuroscience Approach. *Trends in Cognitive Sciences*, *21*(4), 277–289. https://doi.org/10.1016/j.tics.2017.02.002

Leibenluft, E., Cohen, P., Gorrindo, T., Brook, J. S., & Pine, D. S. (2006). Chronic Versus Episodic Irritability in Youth: ACommunity-Based, Longitudinal Study of Clinical and Diagnostic Associations. *Journal of Child and Adolescent Psychopharmacology*, *16*(4), 456–466. https://doi.org/10.1089/cap.2006.16.456

Li, C. I., Malone, K. E., Porter, P. L., Weiss, N. S., Tang, M.-T. C., Cushing-Haugen, K. L., & Daling, J. R. (2003). Relationship Between Long Durations and Different Regimens of Hormone Therapy and Risk of Breast Cancer. *JAMA: The Journal of the American Medical Association*, *289*(24), 3254–3263. https://doi.org/10.1001/jama.289.24.3254

Licciardi, F., Berkeley, A. S., Krey, L., Grifo, J., & Noyes, N. (2001). A two- versus three-embryo transfer: The oocyte donation model. *Fertility and Sterility*, *75*(3), 510–513. https://doi.org/10.1016/S0015-0282(00)01755-6

Licciardi, F. L., Kwiatkowski, A., Noyes, N. L., Berkeley, A. S., Krey, L. L., & Grifo, J. A. (1999). Oral versus intramuscular progesterone for in vitro fertilization: A prospective randomized study. *Fertility and Sterility*, *71*(4), 614–618. https://doi.org/10.1016/S0015-0282(98)00515-9

Lobo, R. A. (2004). Evaluation of Cardiovascular Event Rates With Hormone Therapy in Healthy, Early Postmenopausal Women: Results From 2 Large Clinical Trials. *Arch Intern Med*, *164*(5), 482–484. https://doi.org/10.1001/archinte.164.5.482

Lopez, O. L., Becker, J. T., Sweet, R. A., Klunk, W., Kaufer, D. I., Saxton, J., Habeych, M., & DeKosky, S. T. (2003). Psychiatric Symptoms Vary With the Severity of Dementia in Probable Alzheimer's Disease. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *15*(3), 346–353. https://doi.org/10.1176/jnp.15.3.346

Lukaszuk, K., Liss, J., Lukaszuk, M., & Maj, B. (2005). Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization–embryo transfer cycles. *Fertility and Sterility*, *83*(5), 1372–1376. https://doi.org/10.1016/j.fertnstert.2004.11.055

Mandel, F. P., Geola, F. L., Meldrum, D. R., Lu, J. H., Eggena, P., Sambhi, M. P., Hershman, J. M., & Judd, H. L. (1983). Biological effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism*, *57*(6304131), 133–139.

Manson, J. E., Hsia, J., Johnson, K. C., Rossouw, J. E., Assaf, A. R., Lasser, N. L., Trevisan, M., Black, H. R., Heckbert, S. R., Detrano, R., Strickland, O. L., Wong, N. D., Crouse, J. R., Stein, E., & Cushman, M. (2003). Estrogen plus progestin and the risk of coronary heart disease. *The New England Journal of Medicine*, *349*(6), 523–534. https://doi.org/Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

Matthews, K. A., Berga, S. L., Owens, J. F., & Flory, J. D. (1998). EFFECTS OF SHORT-TERM SUPPRESSION OF OVARIAN HORMONES ON CARDIOVASCULAR AND NEUROENDOCRINE REACTIVITY TO STRESS IN WOMEN. *Psychoneuroendocrinology*, *23*(4), 307–322. https://doi.org/10.1016/S0306-4530(98)00013-4

McGuire, K., Fung, L. K., Hagopian, L., Vasa, R. A., Mahajan, R., Bernal, P., Silberman, A. E., Wolfe, A., Coury, D. L., Hardan, A. Y., Veenstra-VanderWeele, J., & Whitaker, A. H. (2016). Irritability and Problem Behavior in Autism Spectrum Disorder: A Practice Pathway for Pediatric Primary Care. *Pediatrics*, *137*(Supplement 2), S136–S148. https://doi.org/10.1542/peds.2015-2851L

McLaren, D. G., Ries, M. L., Xu, G., & Johnson, S. C. (2012). A generalized form of context-dependent psychophysiological interactions (gPPI): A comparison to standard approaches. *NeuroImage*, *61*(4), 1277–1286. https://doi.org/10.1016/j.neuroimage.2012.03.068

McNaughton, N., & Corr, P. J. (2004). A two-dimensional neuropsychology of defense: Fear/anxiety and defensive distance. *Neuroscience & Biobehavioral Reviews*, *28*(3), 285–305. https://doi.org/10.1016/j.neubiorev.2004.03.005

Melis, G. B., Fruzzetti, F., Paoletti, A. M., Carmassi, F., & Fioretti, P. (1984). Fibrinopeptide a plasma levels during low-estrogen oral contraceptive treatment. *Contraception*, *30*(6), 575–583. https://doi.org/10.1016/0010-7824(84)90007-6

Mezrow, G., Shoupe, D., Spicer, D., Lobo, R., Leung, B., & Pike, M. (1994). Depot leuprolide acetate with estrogen and progestin add-back for long-term treatment of premenstrual syndrome. *Fertility and Sterility*, *62*(5), 932–937.

Mirkin, S., Gimeno, T. G., Bovea, C., Stadtmauer, L., Gibbons, W. E., & Oehninger, S. (2003). Factors Associated with an Optimal Pregnancy Outcome in an Oocyte Donation Program. *Journal of Assisted Reproduction and Genetics*, *20*(10), 400–408. https://doi.org/10.1023/A:1026236726568

Mitwally, M. F. M., Gotlieb, L. R., & Casper, R. F. (2002). Prevention of bone loss and hypoestrogenic symptoms by estrogen and interrupted progestogen add-back in long-term GnRH-agonist down-regulated patients with endometriosis and premenstrual syndrome. *Menopause July 2002*, *9*(4), 236–241.

Mogg, K., & Bradley, B. P. (1999). Some methodological issues in assessing attentional biases for threatening faces in anxiety: A replication study using a modified version of the probe detection task. *Behaviour Research and Therapy*, *37*(6), 595–604. https://doi.org/10.1016/S0005-7967(98)00158-2

Mogg, K., & Bradley, B. P. (2005). Attentional Bias in Generalized Anxiety Disorder Versus Depressive Disorder. *Cognitive Therapy and Research*, *29*(1), 29–45. https://doi.org/10.1007/s10608-005-1646-y

Moses-Kolko, E. L., Fraser, D., Wisner, K. L., James, J. A., Saul, A. T., Fiez, J. A., & Phillips, M. L. (2011). Rapid habituation of ventral striatal response to reward receipt in postpartum depression. *Biological*

Psychiatry, *70*(4), 395–399. https://doi.org/Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.

Moses-Kolko, E. L., Perlman, S. B., Wisner, K. L., James, J., Saul, A. T., & Phillips, M. L. (2010). Abnormally reduced dorsomedial prefrontal cortical activity and effective connectivity with amygdala in response to negative emotional faces in postpartum depression. *The American Journal of Psychiatry*, *167*(11), 1373–1380. https://doi.org/Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't

Murphy, K. J., & Brunberg, J. A. (1997). Adult claustrophobia, anxiety and sedation in MRI. *Magnetic Resonance Imaging*, *15*(1), 51–54. https://doi.org/10.1016/S0730-725X(96)00351-7

Nagy, Z. P., Chang, C.-C., Shapiro, D. B., Bernal, D. P., Elsner, C. W., Mitchell-Leef, D., Toledo, A. A., & Kort, H. I. (2009). Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertility and Sterility*, *92*(2), 520–526. https://doi.org/10.1016/j.fertnstert.2008.06.005

Nieman, L. K., & Loriaux, D. L. (1992). Estrogens and progestins. In *Textbook of pharmacology* (pp. 695–716). W.B. Saunders Company.

Noyes, N., Hampton, B. S., Berkeley, A., Licciardi, F., Grifo, J., & Krey, L. (2001). Factors useful in predicting the success of oocyte donation: A 3-year retrospective analysis. *Fertility and Sterility*, *76*(1), 92–97. https://doi.org/10.1016/S0015-0282(01)01823-4

Padilla, S. L., Smith, R. D., & Garcia, J. E. (1991). The Lupron screening test: Tailoring the use of leuprolide acetate in ovarian stimulation for in vitro fertilization. *Fertility and Sterility*, *56*(1), 79–83.

Paulson, R. J., Hatch, I. E., Lobo, R. A., & Sauer, M. V. (1997). Cumulative conception and live birth rates after oocyte donation: Implications regarding endometrial receptivity. *Human Reproduction*, *12*(4), 835–839. https://doi.org/10.1093/humrep/12.4.835

Penn, D. L., Spaulding, W., Reed, D., & Sullivan, M. (1996). The relationship of social cognition to ward behavior in chronic schizophrenia. *Schizophrenia Research*, *20*(3), 327–335. https://doi.org/10.1016/0920-9964(96)00010-2

Peress, M. R., & Philips, H. M. (1998). Quintuplet Gestation Following Transfer of Five Cryopreserved Embryos: Conditions Stimulating Thawed-Embryo Implantation. *Journal of Assisted Reproduction and Genetics*, *15*(8), 521–523. https://doi.org/10.1023/A:1022590605998

Perlis, R. H., Fraguas, R., Fava, M., Trivedi, M. H., Luther, J. F., Wisniewski, S. R., & Rush, A. J. (2005). Prevalence and Clinical Correlates of Irritability in Major Depressive Disorder: A Preliminary Report From the Sequenced Treatment Alternatives to Relieve Depression Study. *The Journal of Clinical Psychiatry*, *66*(02), 159–166. https://doi.org/10.4088/JCP.v66n0202

Petitti, D. B., Sidney, S., & Perlman, J. A. (1988). Increased risk of cholecystectomy in users of supplemental estrogen. *Gastroenterology*, *94*(1), 91–95. https://doi.org/Comparative Study Research Support, U.S. Gov't, P.H.S.

Postpartum Depression: Action Towards Causes and Treatment (PACT) Consortium. (2015). Heterogeneity of postpartum depression: A latent class analysis. *The Lancet Psychiatry*, *2*(1), 59–67. https://doi.org/10.1016/S2215-0366(14)00055-8

Prentice, R. L., Langer, R. D., Stefanick, M. L., Howard, B. V., Pettinger, M., Anderson, G. L., Barad, D., Curb, J. D., Kotchen, J., Kuller, L., Limacher, M., & Wactawski-Wende, J. (2006). Combined analysis of Women's Health Initiative observational and clinical trial data on postmenopausal hormone treatment and cardiovascular disease. *American Journal of Epidemiology*, *163*(7), 589–599. https://doi.org/Research Support, N.I.H., Extramural

Prentice, R. L., Langer, R., Stefanick, M. L., Howard, B. V., Pettinger, M., Anderson, G., Barad, D., Curb, J. D., Kotchen, J., Kuller, L., Limacher, M., & Wactawski-Wende, J. (2005). Combined postmenopausal hormone therapy and cardiovascular disease: Toward resolving the discrepancy between observational studies and the Women's Health Initiative clinical trial. *American Journal of Epidemiology*, *162*(5), 404–414. https://doi.org/Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.

Price, M., & Stolk-Cooke, K. van. (2015). Examination of the interrelations between the factors of PTSD, major depression, and generalized anxiety disorder in a heterogeneous trauma-exposed sample using DSM 5 criteria. *Journal of Affective Disorders*, *186*, 149–155. https://doi.org/10.1016/j.jad.2015.06.012

Qaseem, A., Humphrey, L. L., Harris, R., Starkey, M., & Denberg, T. D. (2014). Screening Pelvic Examination in Adult Women: A Clinical Practice Guideline From the American College of Physicians. *Annals of Internal Medicine*, *161*(1), 67. https://doi.org/10.7326/M14-0701

Rice, V. C., Richard-Davis, G., Saleh, A. A., Ginsburg, K. A., Mammen, E. F., Moghissi, K., & Leach, R. (1993). Fibrinolytic parameters in women undergoing ovulation induction. *American Journal of Obstetrics and Gynecology*, *169*(6), 1549–1553.

Rich, B. A., Holroyd, T., Carver, F. W., Onelio, L. M., Mendoza, J. K., Cornwell, B. R., Fox, N. A., Pine, D. S., Coppola, R., & Leibenluft, E. (2010). A preliminary study of the neural mechanisms of frustration in pediatric bipolar disorder using magnetoencephalography. *Depression and Anxiety*, *27*(3), 276–286. https://doi.org/10.1002/da.20649

Rich, B. A., Schmajuk, M., Perez-Edgar, K. E., Fox, N. A., Pine, D. S., & Leibenluft, E. (2007). Different Psychophysiological and Behavioral Responses Elicited by Frustration in Pediatric Bipolar Disorder and Severe Mood Dysregulation. *American Journal of Psychiatry*, *164*(2), 309–317. https://doi.org/10.1176/ajp.2007.164.2.309

Rich, B. A., Schmajuk, M., Perez-Edgar, K. E., Pine, D. S., Fox, N. A., & Leibenluft, E. (2005). The Impact of Reward, Punishment, and Frustration on Attention in Pediatric Bipolar Disorder. *Biological Psychiatry*, *58*(7), 532–539. https://doi.org/10.1016/j.biopsych.2005.01.006

Riem, M. M. E., Bakermans-Kranenburg, M. J., Pieper, S., Tops, M., Boksem, M. A. S., Vermeiren, R. R. J. M., van Ijzendoorn, M. H., & Rombouts, S. A. R. B. (2011). Oxytocin modulates amygdala, insula, and inferior frontal gyrus responses to infant crying: A randomized controlled trial. *Biological Psychiatry*, *70*(3), 291–297. https://doi.org/10.1016/j.biopsych.2011.02.006

Roca, C. A., Schmidt, P. J., Altemus, M., Deuster, P., Danaceau, M. A., Putnam, K., & Rubinow, D. R. (2003). Differential menstrual cycle regulation of hypothalamic-pituitary-adrenal axis in women with premenstrual syndrome and controls. *The Journal of Clinical Endocrinology and Metabolism*, *88*(7), 3057–3063.

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., Jackson, R. D., Beresford, S. A. A., Howard, B. V., Johnson, K. C., Kotchen, J. M., Ockene, J., & Writing Group for the Women's Health Initiative Investigators. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results From the Women's Health Initiative randomized controlled trial. *JAMA: The Journal of the American Medical Association*, *288*(3), 321–333.

Rubin, D. B. (1987). *Multiple Imputation for Nonresponse in Surveys*. John Wiley & Sons.

Saleh, A. A., Ginsburg, K. A., Duchon, T. A., Dorey, L. G., Hirata, J., Alshameeri, R. S., Dombrowski, M. P., & Mammen, E. F. (1995). Hormonal contraception and platelet function. *Thromb Res*, *78*(7631316), 363–367.

Salum, G. A., Mogg, K., Bradley, B. P., Stringaris, A., Gadelha, A., Pan, P. M., Rohde, L. A., Polanczyk, G. V., Manfro, G. G., Pine, D. S., & Leibenluft, E. (2017). Association between irritability and bias in attention orienting to threat in children and adolescents. *Journal of Child Psychology and Psychiatry*, *58*(5), 595–602. https://doi.org/10.1111/jcpp.12659

Sánchez, L. A., Morán, C., Reyna, R., Ochoa, T., Boots, L. R., & Azziz, R. (2002). Adrenal progestogen and androgen production in 21-hydroxylase-deficient nonclassic adrenal hyperplasia is partially independent of adrenocorticotropic hormone stimulation. *Fertility and Sterility*, *77*(4), 750–753. https://doi.org/10.1016/S0015-0282(01)03236-8

Sauer, M. V., Paulson, R. J., & Lobo, R. A. (1992). Reversing the Natural Decline in Human FertilityAn Extended Clinical Trial of Oocyte Donation to Women of Advanced Reproductive Age. *JAMA: The Journal of the American Medical Association*, *268*(10), 1275–1279. https://doi.org/10.1001/jama.1992.03490100073030

Savage, J., Verhulst, B., Copeland, W., Althoff, R. R., Lichtenstein, P., & Roberson-Nay, R. (2015). A Genetically Informed Study of the Longitudinal Relation Between Irritability and Anxious/Depressed Symptoms. *Journal of the American Academy of Child & Adolescent Psychiatry*, *54*(5), 377–384. https://doi.org/10.1016/j.jaac.2015.02.010

Schiller, C. E., Johnson, S. L., Abate, A. C., Schmidt, P. J., & Rubinow, D. R. (2016). Reproductive Steroid Regulation of Mood and Behavior. In *Comprehensive Physiology*. John Wiley & Sons, Inc. http://onlinelibrary.wiley.com/doi/10.1002/cphy.c150014/abstract

Schmidt, P. J., Nieman, L. K., Danaceau, M. A., Adams, L. F., & Rubinow, D. R. (1998). Differential Behavioral Effects of Gonadal Steroids in Women with and in Those without Premenstrual Syndrome. *New England Journal of Medicine*, *338*(4), 209–216. https://doi.org/10.1056/NEJM199801223380401

Schmidt, P. J., Steinberg, E. M., Negro, P. P., Haq, N., Gibson, C., & Rubinow, D. R. (2009). Pharmacologically Induced Hypogonadism and Sexual Function in Healthy Young Women and Men. *Neuropsychopharmacology*, *34*(3), 565–576. https://doi.org/10.1038/npp.2008.24

Scott, L. N., Stepp, S. D., Hallquist, M. N., Whalen, D. J., Wright, A. G. C., & Pilkonis, P. A. (2015). Daily shame and hostile irritability in adolescent girls with borderline personality disorder symptoms. *Personality Disorders: Theory, Research, and Treatment*, *6*(1), 53–63. https://doi.org/10.1037/per0000107

Scribner Jr., D. R., & Walker, J. L. (1998). Low-Grade Endometrial Stromal Sarcoma Preoperative Treatment with Depo-Lupron and Megace. *Gynecologic Oncology*, *71*(3), 458–460. https://doi.org/10.1006/gyno.1998.5174

Simon, J. J., Walther, S., Fiebach, C. J., Friederich, H.-C., Stippich, C., Weisbrod, M., & Kaiser, S. (2010). Neural reward processing is modulated by approach- and avoidance-related personality traits. *NeuroImage*, *49*(2), 1868–1874. https://doi.org/10.1016/j.neuroimage.2009.09.016

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*, S208–S219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Smoski, M. J., Felder, J., Bizzell, J., Green, S. R., Ernst, M., Lynch, T. R., & Dichter, G. S. (2009). FMRI of alterations in reward selection, anticipation, and feedback in major depressive disorder. *Journal of Affective Disorders*, *118*(1–3), 69–78. https://doi.org/10.1016/j.jad.2009.01.034

Spinelli, M. G., Endicott, J., Goetz, R. R., & Segre, L. S. (2016). Reanalysis of efficacy of interpersonal psychotherapy for antepartum depression versus parenting education program: Initial severity of depression as a predictor of treatment outcome. *The Journal of Clinical Psychiatry*, *77*(4), 535–540. https://doi.org/10.4088/JCP.15m09787

Stampfer, M. J., Colditz, G. A., Willett, W. C., Manson, J. E., Rosner, B., Speizer, F. E., & Hennekens, C. H. (1991). Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. *The New England Journal of Medicine*, *325*(11), 756–762. https://doi.org/Research Support, U.S. Gov't, P.H.S.

Stefanick, M. L., Anderson, G. L., Margolis, K. L., Hendrix, S. L., Rodabough, R. J., Paskett, E. D., Lane, D. S., Hubbell, F. A., Assaf, A. R., Sarto, G. E., Schenken, R. S., Yasmeen, S., Lessin, L., & Chlebowski, R. T. (2006). Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. *JAMA : The Journal of the American Medical Association*, *295*(16609086), 1647–1657.

Steinauer, J. E., Waetjen, L. E., Vittinghoff, E., Subak, L. L., Hulley, S. B., Grady, D., Lin, F., & Brown, J. S. (2005). Postmenopausal hormone therapy: Does it cause incontinence? *Obstetrics and Gynecology*, *106*(5 Pt 1), 940–945. https://doi.org/Randomized Controlled Trial Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't

Stoddard, J., Tseng, W.-L., Kim, P., Chen, G., Yi, J., Donahue, L., Brotman, M. A., Towbin, K. E., Pine, D. S., & Leibenluft, E. (2017). Association of Irritability and Anxiety With the Neural Mechanisms of Implicit Face Emotion Processing in Youths With Psychopathology. *JAMA Psychiatry*, *74*(1), 95–103. https://doi.org/10.1001/jamapsychiatry.2016.3282

Straub, J., Metzger, C. D., Plener, P. L., Koelch, M. G., Groen, G., & Abler, B. (2017). Successful group psychotherapy of depression in adolescents alters fronto-limbic resting-state connectivity. *Journal of Affective Disorders*, *209*, 135–139. https://doi.org/10.1016/j.jad.2016.11.024

Stringaris, A., Cohen, P., Pine, D. S., & Leibenluft, E. (2009). Adult Outcomes of Youth Irritability: A 20-Year Prospective Community-Based Study. *American Journal of Psychiatry*, *166*(9), 1048–1054. https://doi.org/10.1176/appi.ajp.2009.08121849

Sturmer, T., & Manson, J. E. (2004). Estrogens and breast cancer: Does timing really matter? *Journal of Clinical Epidemiology*, *57*(8), 763–765.

Sulak, P. J., Cressman, B. E., Waldrop, E., Holleman, S., & Kuehl, T. J. (1997). Extending the duration of active oral contraceptive pills to manage hormone withdrawal symptoms. *Obstetrics and Gynecology*, *89*(9015017), 179–183.

Tao, J., & Tamis, R. (1997). Application of assisted hatching for 2-Day-old, frozen-thawed embryo transfer in a poor-prognosis population. *Journal of Assisted Reproduction and Genetics*, *14*(2), 128–130. https://doi.org/10.1007/BF02765783

Taylor, S., Landry, C. A., Paluszek, M. M., Fergus, T. A., McKay, D., & Asmundson, G. J. G. (2020). Development and initial validation of the COVID Stress Scales. *Journal of Anxiety Disorders*, *72*, 102232. https://doi.org/10.1016/j.janxdis.2020.102232

Thompson, M. A., Adelson, M. D., & Kaufman, L. M. (1991). Lupron Retards Proliferation of Ovarian Epithelial Tumor Cells Cultured in Serum-Free Medium. *Journal of Clinical Endocrinology & Metabolism*, *72*(5), 1036–1041. https://doi.org/10.1210/jcem-72-5-1036

Tonguc, E., Var, T., Ozyer, S., Citil, A., & Dogan, M. (2011). Estradiol supplementation during the luteal phase of in vitro fertilization cycles: A prospective randomised study. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *154*(2), 172–176. https://doi.org/10.1016/j.ejogrb.2010.10.003

Tseng, W.-L., Moroney, E., Machlin, L., Roberson-Nay, R., Hettema, J. M., Carney, D., Stoddard, J., Towbin, K. A., Pine, D. S., Leibenluft, E., & Brotman, M. A. (2017). Test-retest reliability and validity of a

frustration paradigm and irritability measures. *Journal of Affective Disorders*, *212*, 38–45. https://doi.org/10.1016/j.jad.2017.01.024

Vaden, K. I., Gebregziabher, M., Kuchinsky, S. E., & Eckert, M. A. (2012). Multiple imputation of missing fMRI data in whole brain analysis. *Neuroimage*, *60*(3), 1843–1855. https://doi.org/10.1016/j.neuroimage.2012.01.123

Van Voorhees, E. E., Dennis, P. A., Elbogen, E. B., Fuemmeler, B., Neal, L. C., Calhoun, P. S., & Beckham, J. C. (2018). Characterizing anger-related affect in individuals with posttraumatic stress disorder using ecological momentary assessment. *Psychiatry Research*, *261*, 274–280. https://doi.org/10.1016/j.psychres.2017.12.080

Vidal-Ribas, P., Brotman, M. A., Valdivieso, I., Leibenluft, E., & Stringaris, A. (2016). The Status of Irritability in Psychiatry: A Conceptual and Quantitative Review. *Journal of the American Academy of Child & Adolescent Psychiatry*, *55*(7), 556–570. https://doi.org/10.1016/j.jaac.2016.04.014

Vitaro, F., Barker, E. D., Boivin, M., Brendgen, M., & Tremblay, R. E. (2006). Do Early Difficult Temperament and Harsh Parenting Differentially Predict Reactive and Proactive Aggression? *Journal of Abnormal Child Psychology*, *34*(5), 681–691. https://doi.org/10.1007/s10802-006-9055-6

Walsh, E., Carl, H., Eisenlohr-Moul, T., Minkel, J., Crowther, A., Moore, T., Gibbs, D., Petty, C., Bizzell, J., Smoski, M. J., & Dichter, G. S. (2017). Attenuation of Frontostriatal Connectivity During Reward Processing Predicts Response to Psychotherapy in Major Depressive Disorder. *Neuropsychopharmacology*, *42*(4), 831–843. https://doi.org/10.1038/npp.2016.179

Wassertheil-Smoller, S., Hendrix, S. L., Limacher, M., Heiss, G., Kooperberg, C., Baird, A., Kotchen, T., Curb, J. D., Black, H., Rossouw, J. E., Aragaki, A., Safford, M., Stein, E., Laowattana, S., & Mysiw, W. J. (2003). Effect of estrogen plus progestin on stroke in postmenopausal women: The Women's Health Initiative: A randomized trial. *JAMA : The Journal of the American Medical Association*, *289*(20), 2673–2684. https://doi.org/Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, U.S. Gov't, P.H.S.

Watson, D., O'Hara, M. W., Simms, L. J., Kotov, R., Chmielewski, M., McDade-Montez, E. A., Gamez, W., & Stuart, S. (2007). Development and validation of the Inventory of Depression and Anxiety Symptoms (IDAS). *Psychological Assessment*, *19*(17845118), 253–268.

Williamson, J. A., O'Hara, M. W., Stuart, S., Hart, K. J., & Watson, D. (2015). Assessment of Postpartum Depressive Symptoms: The Importance of Somatic Symptoms and Irritability. *Assessment*, *22*(3), 309–318. https://doi.org/10.1177/1073191114544357

Wingo, P. A., Layde, P. M., Lee, N. C., Rubin, G., & Ory, H. W. (1987). The Risk of Breast Cancer in Postmenopausal Women Who Have Used Estrogen Replacement Therapy. *JAMA: The Journal of the American Medical Association*, *257*(2), 209–215. https://doi.org/10.1001/jama.1987.03390020075030

Wright, K. A., Lam, D. H., & Brown, R. G. (2009). Reduced approach motivation following nonreward: Extension of the BIS/BAS scales. *Personality and Individual Differences*, *47*(7), 753–757. https://doi.org/10.1016/j.paid.2009.06.015

Wyatt, K. M., Dimmock, P. W., Ismail, K. M. K., Jones, P. W., & O'Brien, P. M. S. (2004). The effectiveness of GnRHa with and without 'add-back' therapy in treating premenstrual syndrome: A meta analysis. *BJOG: An International Journal of Obstetrics & Gynaecology*, *111*(6), 585–593. https://doi.org/10.1111/j.1471-0528.2004.00135.x

15 Appendices

Appendix A. Study Consent Form
Appendix B. Study Procedures Flowchart
Appendix C. Data Safety and Monitoring Plan
Appendix D. COVID-19 Information Sheet

Appendix A. Study Consent Form

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: July 21, 2020
IRB Study # 19-0401
Title of Study: Characterizing the neural substrates or irritability in women: an experimental neuroendocrine model
Principal Investigator: Crystal Schiller, Ph.D.
Principal Investigator Department: Psychiatry
Principal Investigator Phone number: 919-966-4810
Principal Investigator Email Address: crystal_schiller@med.unc.edu
Co-Investigators:

Funding Source: National Institutes of Health (NIH)

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to determine the role of hormones (estrogen and progesterone) in postpartum depression. We will investigate the role of these hormones in mood by using a hormonal "challenge." The hormonal challenge involves three phases. During phase one, you will receive Lupron, a medicine that will temporarily turn off your ovaries during the study. Lupron reduces the levels of estrogen and progesterone in your blood. During phase two, you will continue to receive Lupron and you will take pills that contain estrogen and progesterone or placebo. At one point during phase two, the estrogen and progesterone treatment will be stopped, and the levels of both hormones will drop rapidly. As your hormones drop, you may experience symptoms of depression, including sadness and less interest in your daily activities. During phase three, you will stop taking all study medications, and we expect your mood and hormone levels to return to normal. You will receive two scans of your brain during the study. The brain scans will help us understand how changing hormone levels impact your brain function and mood symptoms.

Background:

Previous studies have shown that estrogen and progesterone play a role in postpartum depression. It is remains unclear why changes in estrogen and progesterone cause postpartum depression in some women and not others. You are being asked to participate because you are a woman between the ages of 21 and 45 in good medical health, taking no medication (with the exception of birth control pills), with regular menses. You also have given birth before, and you either had an episode of postpartum depression or no history of depression in the past.

Are there any reasons you should not be in this study?

You will not be permitted to enter this study if you have important clinical or laboratory abnormalities including any history of the following:

- any foreign metal objects or implants in your body as determined by the safety questionnaires
- endometriosis (an illness related to abnormal tissue growth around the uterus)
- enlargement of the ovaries
- liver disease
- breast cancer
- a history of blood clots in the legs or lungs
- undiagnosed vaginal bleeding
- porphyria (a rare genetic blood disorder)
- diabetes mellitus
- malignant melanoma (a type of skin cancer)
- gallbladder or pancreatic disease
- heart or kidney disease
- cerebrovascular disease (stroke)
- cigarette smoking of more than 10 cigarettes per day
- a history of suicide attempts or psychotic episodes

- recurrent migraine headaches with aura
- history of pregnancy-related medical conditions such as excessive vomiting, high blood pressure, deep vein thrombosis, or seizures
- menstrual cycle variability of greater than 7 days different from your normal cycle length or an abnormal level of follicle stimulating hormone
- current psychiatric diagnosis
- body mass index (BMI) greater than 35
- hormonal contraceptives that are implanted (i.e. progestin IUD or implant)

In addition, you will not be permitted to participate if you have a first degree relative (immediate family) with breast cancer that occurred before menopause, or breast cancer presenting in both breasts, or if you have multiple family members (greater than three relatives) with breast cancer.

You may not take part in this study if you are pregnant or receiving any medication. If you are using a hormonal contraceptive, you must enter a washout period (i.e. stop taking the hormonal contraceptives and instead use a barrier contraceptive) and have 3 regular periods before you can begin the study procedures. If you are experiencing medication side effect, the dose will be changed, but you must not change your medication dose without consulting the investigator. If adequate relief cannot be achieved by changing the dose, the drug will be stopped. Dropping out of the study will not interfere with any medical treatment you were receiving at UNC Hospitals.

How many people will take part in this study?

There will be approximately 39 people in this research study.

How long will your participation in this study last?

This study will include 8 visits over the course of 12 weeks. Study visits will range from 30 minutes to 3 hours, and most visits will last approximately 45 minutes. The visits that do not require brain scans may take place virtually through secure video visits or at your home provided that you live within a 60 minute drive of UNC hospital.

What will happen if you take part in the study?

Clinical and Health Screening:

You will undergo a screening process to determine if you are eligible for the study. In order to protect you from adverse medication effects, you will be screened with a complete medical and psychiatric history, physical exam, and laboratory tests. During the initial visit, approximately 2 tablespoons (about 30 mls) of blood will be drawn to test your hormone levels and liver and kidney function. We will also require that you have had a gynecological exam within the last three years. If you are eligible for the study, the results of your medical history, physical exam, and laboratory tests will appear in your UNC Health Care medical record.

During the initial screening, you will be asked questions about your past mental health as well as questions about any symptoms you may be experiencing now. You will also be asked to complete questionnaires about your mood symptoms and trauma history. You may choose not to answer any or all of the questions for any reason.

You will complete a safety questionnaire to determine whether you have any foreign iron or steel metal objects in your body, such as a pacemaker, shrapnel, metal plate, or metal debris. If you have any such objects in your body, you cannot participate in the MRI session. Please ask the experimenter if you are unsure.

You will also receive a urine drug screening prior to each MRI session to make sure that it is safe for you to receive the MRI and that your brain activity is not affected by drug use.

Blood Samples and Screening:

Blood samples will be taken at the start of the study and at times during the course of the protocol. All blood samples will be drawn in the following way: you will be asked to sit down and after ten minutes a small plastic tube (catheter) will be placed in an arm vein, the blood sample will be drawn, and the catheter removed. During each of your in-person MRI visits 30 ml (2 tablespoons) of your blood will be drawn. Estrogen, progesterone, and allopregnanolone levels in your blood will be assessed.

You will receive a pregnancy test before starting the study. You may not participate in this study if you are pregnant because the study drugs (Lupron, estrogen, and progesterone) may be associated with birth defects. We will ask that you use barrier contraceptive methods (diaphragm or condom or both) for the entire time you are in this study. To prevent pregnancy, we strongly recommend that you also continue barrier contraception for at least three months after the last injection of Lupron and until you have two to three regular menstrual cycles after the study has ended.

Hormone Procedures:

Once you finish the screening, we will ask you to have an injection of Lupron into a muscle, once a month for two months. You will attend virtual video appointments every two weeks during the study when possible to minimize the risk of COVID-19 transmission. During the first two months, we will also ask you to take two different kinds of capsules that contain either estrogen and progesterone or placebo. You will not be aware of the real nature (hormones versus placebo) of the capsules you are taking, but you will know that at some point during these three months you will be started on a one- to two-month period of active medication. During this period, you will receive up to 2 mg of estrogen (17-beta estradiol) and 200 mg of progesterone. All medication refills during the study will be dropped off at your home every two weeks by a member of the study team to minimize the risk of COVID-19 transmission. You will not be aware of the exact time the hormone capsules are switched over to placebo capsules. After completing the medication period lasting two months, we will continue your follow-up at the clinic for another month. At the end of the study, we will discuss the study results and their meaning with you.

Brain Imaging:

You will participate in two brain imaging sessions at the UNC Biomedical Research Imaging Center. During the COVID-19 pandemic you will be asked pre-screening questions 24 hours in advance of these visits, and on the day of the visit. You will be asked to maintain 6ft distance from others when possible and wear a surgical mask in all face-to-face visits which will be provided for you by the study team in advance. On arrival to the UNC Biomedical Research Imaging Center you will have a temperature scan prior to entering the building and will be

asked to wait in one of the holding rooms. This will allow for yourself and the member of the study team to walk through the MRI center without possibility of encountering other staff members. In these sessions, magnetic resonance images (MRIs) of your brain will be taken. An MRI is a picture of your brain taken with the use of strong magnetic fields. MRIs do not use x-rays or other radiation, and there are no known risks associated with MRIs. In this study, special MRIs (called functional MRIs or fMRIs) will be taken that provide information about what areas of the brain are made active by particular kinds of stimuli. You will view "stimuli," including pictures of faces and objects such as slot machines and money. The experimenter may ask you to push a button when particular stimuli appear on the screen.

When you understand the task instructions, you will lie down on your back on a platform and your head will be positioned inside a helmet-like circular tube and the platform will be pushed inside the long tube of the MRI machine. The MRI technician will provide padding for your head and knees to make you more comfortable while lying down. If you are uncomfortable or feel pain because of lying down, please tell the technician immediately. You will be able to see outside of the helmet and outside the imaging machine by looking at a mirror. In this way, you will be able to watch the pictures or words displayed on a screen placed near your feet. If sounds are presented, you will hear them through earphones. It is expected that each imaging session will take approximately 2 hours.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. There is little chance you will benefit from being in this research study. However, you may learn about your sensitivity to changes in estrogen and progesterone levels, which may be useful if you plan to have children in the future.

What are the possible risks or discomforts involved from being in this study?

1) Mood changes – If you have never experienced an episode of depression in the past, then your risk of developing mood symptoms during this study are very low, but this risk still exists. If you experience any depression symptoms, including thoughts about death or suicide, we ask that you inform us immediately. We will assess your mood each day during the study and at your bi-weekly clinic visits. The majority of participants complete the daily mood rating every day, and if you miss a day, we will contact you to do a safety assessment over the phone. Once you have finished taking the medication for the study, you will only need complete the mood ratings once a week.

If you experience severe mood symptoms or suicidal thoughts, then you not be allowed to continue in the study. We will provide short-term treatment with medication for depression until your symptoms improve, however these costs will not be covered by the study. If you do not wish not to receive standard treatment with medication for depression, you may have to be transferred to another hospital for treatment. We can only treat you at UNC if you are either in a research study or agree to receive treatment after you have completed the research study. If you receive treatment at another hospital, UNC will not cover the costs.

Given the relatively brief nature and moderate severity of any mood symptoms that you may experience during this study, we do not anticipate that your mood symptoms will adversely impact your children. Nonetheless, we cannot guarantee the absence of effect, nor can we anticipate every risk that may result from your participation in this study.

2) Lupron causes the ovary to stop working for a short period of time (one month). Lupron may cause the same side effects that are seen when the ovaries stop functioning permanently (menopause). Side effects may include hot flushes (flashes), decrease in libido, headaches, nervousness, irritability, muscle aches, trouble sleeping, and a slight decrease in bone density. The decrease in bone density is reversible and should not be related to an increased risk of bone fracture because of the short time you will receive Lupron treatment. Your menstrual periods may be irregular for 2-6 months after the last Lupron injection. Skin irritation may occur at the site of injection. All side effects are reversible after the Lupron treatment is stopped, and many side effects, if they do occur, will be reversed by the estrogen and progesterone given during the study. At the dose of Lupron that we will use, the risk of developing severe side effects is small. Nevertheless, you will be monitored closely to see if side effects develop, and the medication will be stopped if side effects become intolerable.

3) Estrogen capsules (Estrace, Micronized 17-beta-estradiol) – The risk of developing side effects is small because of the dosage and period of time that you will receive estrogen treatment. The most common side effects are nausea, breast tenderness or enlargement, and fluid retention (swelling). There is a significantly increased risk of stroke following estrogen therapy in individuals with migraine with aura, as compared to individuals without aura, and as such, women with a history of recurrent migraine with aura will be excluded. Less common side effects are vomiting, depression, high blood pressure, a spotty darkening of the skin (mostly of the face), or vaginal bleeding. Any side effects that you may experience should stop after the end of the study. In the rare event that you experience spotty skin darkening, it may not disappear completely.

Estrogen use increases the risk of certain medical gallbladder problems, and it may increase the risk of certain types of cancer. However, the risk of cancer is minimal because of the dose and the short period of time estrogen is used in this study. The risk of cancer is also reduced because estrogen is given along with progesterone.

Estrogen and progesterone may also cause an increase in birth defects if they are taken during early pregnancy. Therefore, you should not become pregnant during this study. Pregnancy tests will be given during the screening phase, and you will not be allowed to participate if you are pregnant. The study will cover the cost of the pregnancy tests.

You will be followed closely to see if side effects develop, and the medication will be discontinued if you experience negative or intolerable side effects.

4) Progesterone capsules (Micronized progesterone) - Progesterone is used to treat a variety of gynecological disorders. Side effects are not common and are usually mild. These include breakthrough bleeding (menstruation earlier than expected), edema (swelling), loss or increase of weight, jaundice, rash (with or without itching), breast tenderness, diarrhea, flatulence (gas), vaginal discharge, loss or increase of sexual drive, faintness, uterine cramps, depressed mood, easily fatigued, drowsiness, lack of initiative, and skin color changes. During and shortly after the hormonal replacement you will probably experience some vaginal bleeding.

If you experience symptoms such as severe mood symptoms, nausea, hypertension (high blood pressure), vomiting or extreme fluid retention (bloating or swelling) from the medication, you will have the dose adjusted until you feel relief. If you do not feel relief, then drug treatment will be discontinued. If vaginal bleeding or any other gynecological problem occurs, we will arrange for a visit with a UNC gynecologist, which may include a transvaginal ultrasound examination. If you experience marked discomfort during the insertion of the ultrasound

probe, then the procedure will be discontinued. Otherwise, there are no additional associated risks or discomforts with the ultrasound probe. If the gynecologist finds any abnormality during the ultrasound, further testing may occur. If breakthrough bleeding occurs and is intolerable, you will be withdrawn from hormone replacement (which should precipitate a period).

5) Blood drawing - You may experience some discomfort or temporary pain at the site of the needle entry. There is a small risk of fainting and local infection.

6) MRI sessions - There are no known risks from exposure to magnetic fields and radio waves used in the MRI session. However, it is not assured that harmful effects will not be recognized in the future. A known risk is that strong magnetic fields attract iron or steel metal objects posing a safety risk. Prior to this study you will be given a questionnaire to determine if you have any foreign iron or steel metal objects in your body, such as a pacemaker, shrapnel, metal plate, or metal debris. If you have such objects in your body, you cannot participate in the MRI part of the study.

If you participate in the MRI session, you will be asked to leave any metal objects in lockers provided in the waiting room of the MRI center. You will also be asked to remove any articles of clothing with metal inserts or clasps before entering the magnet room. Please ask the experimenter if you are unsure. It is possible you will feel uncomfortable or confined once inside the imaging machine. This feeling usually passes within a few minutes as the experimenters talk with you and the study begins. However, if this feeling persists, you can tell the investigators over the intercom and you will be removed immediately from the machine. On rare occasions some subjects may experience one or more of the following: momentary dizziness or nausea, a metallic taste, tingling sensations, or muscle twitches. Please tell the investigator over the intercom if any of these sensations occur. Once inside the machine, you will hear loud mechanical clanging sounds. This is part of the normal operation of the machine. You will be given earplugs to reduce the sounds.

6) There may be uncommon or previously unknown risks. If you have any symptoms or unexpected side effects during this protocol, please call the study physicians or nurses right away.

7) COVID-19 Transmission – You may experience the potential risk to COVID-19 transmission during in-person study visits at the MRI center or when in contact with study team members during Lupron injections or blood draws when study staff will need to maintain less than 6ft distance. Our study team and UNC will be taking every precaution possible to protect you and the study team from COVID-19, including virtual video or telephone visits when possible, with limited in-person contact to every degree possible. During the in-person visits we will require masks at all times which will be provided to you by the study team, maintaining 6ft distance when possible, temperature checks on arrival to the UNC Biomedical Research Imaging Center, COVID-19 prescreening for yourself and staff at various timepoints including the day of the study visit, frequent cleaning with 90% alcohol or 1:10 Bleach solution, cleaning between each subject, handwashing requirements every hour for you and all staff, and before and after any contact with any subject. While the study team will be taking every pre-caution possible to protect yourself and the study staff there may still be a risk of COVID-19 transmission due to the possibility of the COVID-19 virus lingering in enclosed spaces from asymptomatic carriers. If you or a member of the study team has had direct or even secondary contact with any suspected or confirmed cases of COVID, the study team will ask to reschedule your visit until the case is confirmed negative or to quarantine for at least 14 days and monitor your symptoms. If you experience any symptoms of COVID-19 including but not limited to: cough,

fever, chest pain, trouble breathing, loss of smell or taste, nausea, abdominal pain, vomiting, etc. you will be asked to notify the study team immediately, contact your Primary Care Provider and your local COVID-19 hotline to get tested for COVID-19. The study team and UNC Research are not responsible for any care or treatment of COVID-19.

Addendum for Patients with a History of Gestational Diabetes, Glucose Intolerance, and Episodic Headaches
1) There is no evidence that estrogen or progesterone directly causes gestational (pregnancy-related) diabetes or diabetes mellitus. Both birth control pills and estrogen replacement can be taken by women who have had pregnancy-related diabetes or who currently have diabetes. However, it is possible that estrogen may influence how your body processes glucose. We will monitor your plasma glucose throughout the study, and if we detect increased blood sugar levels, we will ask you to stop participating in this study.

2) Lupron and estrogen treatment have been reported to aggravate migraine headaches. If you have a history of recurrent migraine headaches you may not participate in this study. Once Lupron is injected into your body its effects last for about four weeks and you may experience an increase in headache frequency while under its influence. If this does happen, we will do our best to manage the pain. If your headaches become severe enough to require repeated medication, we may decide with you to discontinue your participation in this study.

Addendum for Patients with a History of Epilepsy:
There is some evidence that estrogen may increase the potential for seizures in some people. Progesterone has been shown to have the opposite effect. In the present study estrogen levels will not be higher than peak pregnancy levels. In addition, estrogen will be administered along with progesterone. Thus, the likelihood of inducing seizures in someone who has been seizure-free should be very low. However, this possibility exists. If you develop a seizure, your participation in the study will be stopped, and after assuring that you are medically stable, we will return you to the care of your primary physician for any follow up that will be required.

What are the risks to a pregnancy or to a nursing child?
You may not take part in this study if you are nursing or planning to get pregnant. You will take a pregnancy test to make sure that you are not pregnant before starting the study. Pregnant women are cannot participate because the study medications and the MRI sessions may have adverse effects on an unborn child. We request that you use barrier contraceptive methods (diaphragm or condom or both) while you are in this study. We strongly recommend that you continue barrier contraception for at least three months after the last injection of Lupron and until you have two to three regular menstrual periods after the study has ended. If you become pregnant during the study you should notify the researcher right away.

What if we learn about new findings or information during the study?
You will be given any new information gained during the study that might affect your willingness to continue your participation.

How will your privacy be protected?
You will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of our records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of your personal information. In some cases, your information

in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

Your identity will be protected by assigning you a number and omitting your name and other identifying information from scientific reports of the study. The pictures of your brain may be included in a database for future research or scientific reports, and researchers at other institutions may therefore be able to examine your brain pictures. However, your name will not be included in such a database and researchers using these databases cannot learn your identity. If you are enrolled or become enrolled in another research study, we may share your results with researchers from that study, or we may obtain information about you from them. Identifiable data and the file that links your ID with your data will be accessible only to those working on this study, including research assistants and the PI. All computer files are kept on secure computers on a secure network.

Audio recordings of your clinical interviews will be identified by your study ID only and stored on a secure computer accessible only to members of our research team. The recordings will be destroyed at the end of the study. Only your research ID number will be tape recorded along with your interview. You may request to have the audio recording turned off if you are uncomfortable with recording your interview.
Check the line that best matches your choice:
_____ OK to audio record me during the study
_____ Not OK to audio record me during the study

A copy of this consent form will be given to you.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study.

If you become depressed or anxious as a direct result of participating this study, the UNC Department of Psychiatry will provide outpatient medical treatment. If vaginal bleeding or any other gynecological problem occurs, we will arrange for a visit with a UNC gynecologist. The research study will not cover the costs of such outpatient exams and treatment at UNC Health Care, you will need to use your own insurance to cover these costs.

If you become sick or injured as a direct result of participating in this study, and your condition cannot be addressed with outpatient treatment, UNC Health Care will provide all needed inpatient medical treatment. UNC Health Care will not be able to reimburse you for costs of such inpatient medical treatment not covered by your insurance company. No other form of reimbursement for study-related injury or illness is offered by UNC Health Care. You do not give up any legal rights by signing this consent.

If you receive Medicare benefits, UNC Health Care is required by law to report payments made to you for treatment, complications, and injuries that arise from this study. Information that you are taking part in this study, medical treatments received, Medicare claims, and other personal information about your such as your name, social security number, and date of birth, will be provided to the Centers of Medicare and Medicaid Services and its agents and/or contractors for this purpose.

If you seek treatment outside of UNC Health Care, you will be responsible for the costs of your treatment.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will receive a total of $1,000 for your participation in this study. Payment will be given through the form of a Visa Gift Card. Your card will be issued inactive and unloaded through USPS mail. Upon confirmation of receiving Visa gift card, the card will be loaded with the payment amount and you will be able to activate the card. For each of the long study visits, you will receive the following compensation:

Initial visit, psychological interview, and questionnaires (1 visit)	$60.00
MRI Session 1 (1 visit)	$60.00
MRI Session 2 (1 visit)	$60.00

You will receive the remaining $820 upon completion of the study. If you are withdrawn from the study because of an adverse event, you will be compensated for the parts of the study you have completed, as shown below:

Physical/OBGYN exam (1 visit)	$60.00
Screening phase (2 weeks)	$30.00
Clinic visits following initial evaluation and screening (6)	$180.00
Repeated blood draws (3)	$150.00
Daily mood and behavioral ratings, medications ($6.50/day)	$550.00

You will also receive vouchers to park in the Dogwood Deck at UNC Hospitals as instructed by the research team. This should cover the cost of your parking for each of the study visits.

Will it cost you anything to be in this study?
If you enroll in this study, you will have costs, including transportation to the appointments at UNC as well as any incidental expenses, such as child care costs. There will be no other costs to you for participating.

Who is sponsoring this study?
This research is funded by the National Institute of Mental Health (NIMH).

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Option to Participate in Additional Studies:
You may be asked to participate in additional testing sessions, which may or may not be part of the current study. Study participation details will be explained to you at the time we contact you.

If you agree to be contacted to participate in future studies, you may initial the "YES" line. If you wish not to be contact for future studies, you may check the "NO" line.

_____ **YES** : I wish to be contacted about future studies.

_____ **NO** : I do NOT wish to be contacted about future studies.

Participant's Agreement :

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

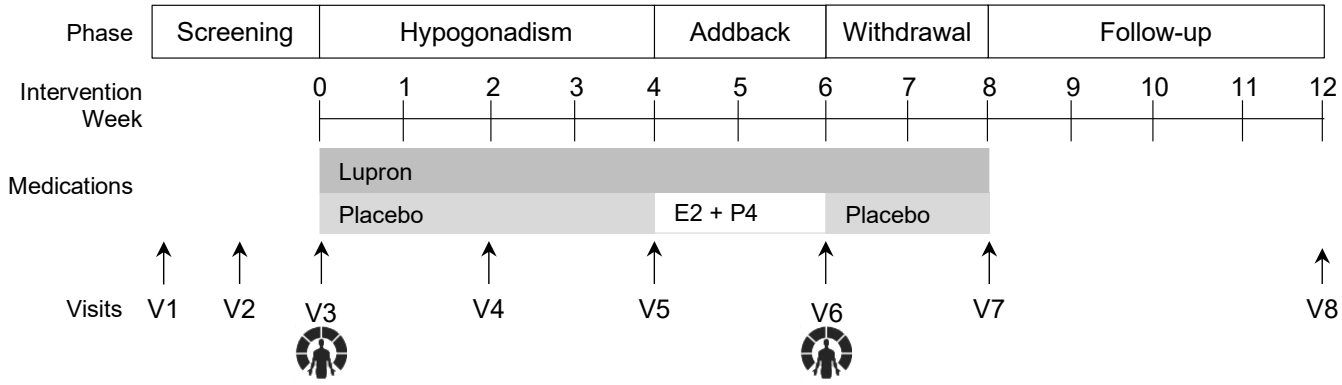
_____ _____
Signature of Research Participant Date

_____
Printed Name of Research Participant

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

Appendix B. Study Procedures Flowchart



Appendix C. Data Safety Monitoring Plan

Data and Safety Monitoring Plan

IRB Study #19-0401
Title of Study: Characterizing the neural substrates or irritability in women: an experimental neuroendocrine model
Principal Investigator: Crystal Schiller, PhD
Principal Investigator Department: Psychiatry
Principal Investigator Phone number: (919) 966-4810
Principal Investigator Email address: crystal_schiller@med.unc.edu

I. Overview

A. Brief description of the purpose of the study and study protocol

The objective of the current project is to by examining whether hormone sensitive women (HS+; n=15) show differences in the behavioral activation system relative to non-hormone sensitive women (HS-; n=15) under baseline and hormone challenge conditions using functional magnetic resonance imaging (fMRI) and behavioral tests. The first aim is to determine the extent to which irritability is characterized by dysfunctional threat processing during reproductive hormone challenge relative to baseline in HS+ and HS-. The primary endpoints for this aim are 1) threat attention bias assessed during the visual dot-probe paradigm, 2) amygdala-medial PFC connectivity in response to threatening faces on the implicit emotion face processing task in HS+ (compared with HS-) during hormone challenge relative to baseline. The secondary endpoints for this aim are the 1) correlation between IDAS-II irritability and treat attention bias, and 2) correlation between IDAS-II irritability and amygdala-medial PFC connectivity in HS+. The second aim is to determine the extent to which irritability is characterized by dysfunctional reward processing during reproductive hormone challenge relative to baseline in HS+ and HS-. The primary endpoints for this aim are 1) reactive aggression during the Point Subtraction Aggression Paradigm, and 2) amygdala and ventral striatum activation in response to frustrative non-reward (FNR) in HS+ (compared with HS-) during hormone challenge relative to baseline. The secondary endpoints for this aim are the 1) correlation between IDAS-II irritability and reactive aggression, and 2) correlation between IDAS-II irritability and amygdala and ventral striatum activation in HS+.

Inclusion Criteria.
Participants will enroll healthy, euthymic 21-45 year old women with a history of postpartum depression (*n*=23) and women without such a history (*n*=16). Thus, only participants capable of giving informed consent will be enrolled. Participants will be compensated upon completion of the study.

Group 1: Women with a history of postpartum depression

1) A history of a DSM-V major depression episode that occurred within 6 weeks of childbirth (as determined by a SCID interview) and remitted at least one year prior to enrollment in the study;
2) has been well for a minimum of one year;
3) a regular menstrual cycle for at least three months;
4) age 21-45;
6) medication free (including birth control pills).

Group 2: Healthy Controls

1) Controls will meet all inclusion criteria specified above.

Exclusion Criteria.

Patients will not be permitted to enter this protocol if they have important clinical or laboratory abnormalities including any of the following:

- current axis I psychiatric diagnosis (based on a structured clinical interview for DSM-V (SCID));
- endometriosis;
- undiagnosed enlargement of the ovaries;
- liver disease;
- breast cancer;
- a history of blood clots in the legs or lungs;
- undiagnosed vaginal bleeding;
- porphyria;
- diabetes mellitus;
- malignant melanoma;
- gallbladder or pancreatic disease;
- heart or kidney disease;
- cerebrovascular disease (stroke);
- cigarette smoking;
- a history of suicide attempts or psychotic episodes requiring hospitalization;
- recurrent migraine with aura;
- pregnancy-related medical conditions such as hyperemesis gravidarum, pretoxemia and toxemia, deep vein thrombosis (DVT) and bleeding diathesis;
- Any woman with a first degree relative (immediate family) with premenopausal breast cancer or breast cancer presenting in both breasts or any woman who has multiple family members (greater than three relatives) with postmenopausal breast cancer will also be excluded from participating in this protocol;
- Any woman meeting the Stages of Reproductive Aging Workshop Criteria (STRAW) for perimenopause will be excluded from participation. Specifically, we will exclude

any woman with an elevated plasma FSH level (> 14 IU/L) and with menstrual cycle variability of > 7 days different from their normal cycle length.

- Pregnant women will be excluded from participation (patients will be warned not to become pregnant during the study and will be advised to employ barrier contraceptive methods), and women who become pregnant (although unlikely because of the hormone manipulation) will be withdrawn. The use of leuprolide acetate is not recommended during pregnancy. Prior to treatment, a complete physical, including a serum β-HCG test for pregnancy. Participants will be seen at the outpatient clinic on a regular biweekly basis. All participants will be required to use non-hormonal forms of birth control (e.g., barrier methods) to avoid pregnancy during this study. Participants will also undergo urine toxicology and pregnancy tests on the day of each of the two fMRI scans. If a woman becomes pregnant during the study, she will not complete the fMRI scan, and the hormone protocol will be discontinued.

B. Adherence statement

The Data and Safety Monitoring Plan (DSMP) outlined below will adhere to the protocol approved by the University of North Carolina at Chapel Hill IRB.

II. Adverse Events

A. Adverse event assessment

This study defines adverse event as any unfavorable and unintended sign, symptom, or disease temporally associated with the subject's participation.

<u>Potential Risks to Human Subjects and Minimization of those Risks</u>

Hormone Protocol

Participants may experience mild to moderate mood symptoms throughout the hormone protocol in the study. Participant's mood symptoms will be monitored each day while on the hormone protocol, if any severe mood symptoms are reported short term treatment may be terminated and medication prescribed for depression as needed until symptoms improve.

Psychological Assessment

Clinical interviews and self-report assessments contain questions regarding sensitive personal information. As a result, participants may become upset or embarrassed when discussing current or past distressing life events. This risk is necessary in order to assess past depressive symptoms and associated psychopathology. However, we have conducted several studies assessing past depressive symptoms in women, and participants are unlikely to become upset. If participants become upset during an assessment, they will be

reminded of their right to discontinue participation, suicidal ideation will be assessed, and appropriate treatment referrals will be provided. Participants in need of medical follow-up or psychological counseling will be referred to the UNC Psychiatry Outpatient Clinic. If a participant reports having suicidal thoughts, intent, plans, and means during the course of the study, she will be taken to the UNC Emergency Department for evaluation.

MRI

The psychological risks associated with the MRI include the discomfort some subjects encounter by the confinement within the bore of the MRI system (i.e., claustrophobia) and the loud noise made by the gradients during imaging. These risks occur for all clinical MRI exams and are not increased by the proposed research. Nonetheless, the steps taken to reduce these risks are described below.

Claustrophobia

The FDA does not provide guidelines concerning claustrophobia. Some subjects may feel uncomfortable or confined once positioned within the MRI system. For some subjects, this confinement results in anxiety. The number of subjects who experience claustrophobic reactions during MRI is uncertain. Approximately 5-10% of patients become claustrophobic during clinical MRI scans (Kilborn & Labbé, 1990). In one study, 14.3% of 949 patients undergoing MRI testing in their hospital required sedation to tolerate the procedure (Murphy & Brunberg, 1997). The numbers reported in these studies are not representative of our experience at BRIC where terminated scans and panic are infrequent. This is not surprising, given that our volunteers choose to participate while patients have less choice. Also, patients may have additional anxieties about their illness or diagnosis that contribute to their discomfort.

We counter the risk of claustrophobia through subject selection and by communication with the subjects. We will exclude from study those subjects who state that they are claustrophobic. We talk to subjects frequently throughout the scan (particularly at its onset). We offer to keep the bore fan running to reduce heat and eliminate any fear of suffocation. We also provide an emergency ‘panic’ button so that he or she knows that help can be immediately summoned.

We explain to the subjects that the sounds they will hear are a normal part of the scanning, and that mild apprehension in enclosed spaces is a normal reaction. Subjects are told that if they feel increasingly anxious during the scan, they can ask to stop the scan. Experimenters listen for telltale signs of growing anxiety or discomfort, such as the subject repeatedly asking how much longer the scan will last. If a subject appears to be more than mildly anxious or declares himself or herself to be anxious, then the experimenter removes the subject from the scanner immediately. These studies are then reported to the IRB at the end of the year as incomplete studies. We have recently constructed a ‘mock scanner’ (or MRI simulator) from parts of a decommissioned GE scanner. The mock scanner has a bed that moves the subject into its bore, and a spare head coil is used for added realism. Recorded scanner noises are played from speakers hidden within the frame. Although there are no actual magnetic fields present, the

experience of being 'scanned' in the mock scanner is very realistic. Whenever possible, we familiarize naïve subjects in the mock scanner so that they know what to expect during the real study. Although we have no objective data yet to report, our expectation is that subjects who experience claustrophobia in the mock scanner will identify themselves to the experimenters at that time, and thus will be excused from further participation in the experimental protocol.

Acoustic Noise

The FDA guidelines limit the peak unweighted sound pressure level to less than 140 dB. The A-weighted root mean square (rms) sound pressure level is limited to 99 dBA with hearing protection in place. The rapid rise and fall of currents within the gradient coils in the presence of the static magnetic field create strong Lorentz forces that cause the gradient coils to move against their mountings. The vibration of the coils and the vibration and flexing of their mountings cause the loud tapping and knocking noises during imaging. The BRIC scanner was tested for sound intensity during image acquisition and the acoustic noise falls within the FDA guidelines. We counter the risks caused by acoustic noise by providing hearing protection to all subjects. We require subjects to use Aearo Classic earplugs that have an EPA Noise Reduction Rate (NRR) of 29 dB (when properly fit), or headphones that are rated for 30 dB of attenuation. We instruct subjects on the proper insertion procedure for the earplug and then inspect the fit to make certain that this procedure has been followed. Subjects are told to contact an experimenter immediately if the scanner is ever uncomfortably or painfully loud.

Confidentiality

A breach of confidentiality could indicate to others a participants' psychiatric history. Risk of breach of confidentiality is minimized by identifying research subjects by a study number on all research documents. Study documents that must contain personal information, including the informed consent document, and the document that links study ID number to personal identifying information are kept in locked filing cabinets in locked rooms. All data will be stored in locked cabinets inside locked offices; electronic data will be stored only on password-protected computers, and data encryption methods will be used during communication between investigators. Only study personnel will have access to these data. All study staff participate in annual human subject training that includes education about responsibilities to the minimize risk of confidentiality breach.

Lupron Side Effects

We do not expect any adverse side effects associated with the hormonal manipulations outlined in this protocol for the following reasons: We have administered Lupron in previous studies in higher doses with minimal side effects, please see table 1 and 2 in MPD outlined in Section 1.5.

Lupron: The most frequent adverse effect of Lupron is hot flushes (flashes) Lupron-induced hot flushes have ranged in severity from occasional mild flushing to frequent

sweating. Episodes of flushing appear to decrease with continued therapy in most patients receiving Lupron. In women of reproductive age with either uterine fibroids or endometriosis who each received 3.75 mgs depot Lupron every month for a period of six months, the most common side effects reported to occur were as follows: 1) hot flashes of mild to moderate intensity, 2) headache, 3) nervousness or irritability, and 4) insomnia. Local irritation at the injection site and a decrease in bone density, which totally reversed after the medication had been discontinued for six months. Blurred vision, myalgias, lethargy, memory disorder, and numbness have been reported in less than 3% of patients receiving the drug. Thrombophlebitis, pulmonary embolus, and congestive heart failure have occurred rarely in patients receiving Lupron, but a causal relationship to the drug has not been established. Adverse GI effects occurring in 2% or more of patients receiving Lupron include nausea and/or vomiting, constipation, and anorexia. Diarrhea and a sour or unusual taste in the mouth have been reported less frequently. Other adverse effects of Lupron occurring in less than 3% of patients include decreased hematocrit and hemoglobin concentration, fatigue, fever, facial swelling, rash, hives, hair loss, and itching. From our experiences with a longer protocol using the same medications in 15 women with a history of PPD and 15 controls, Lupron is well tolerated (no dropouts) with the most common side effect being hot flushes. Limited information is available on the acute toxicity of Lupron. Following subcutaneous administration of Lupron in rats at dosages 250-500 times the usual human dosage, dyspnea, decreased activity, and local irritation at the injection site were observed; however, there is no evidence to date that overdosage in humans produces similar adverse effects. Lupron dosages up to 20 mg daily for up to two years have not produced unusual adverse effects in humans. There has been one report of an anaphylactic reaction in a patient following administration of a GnRH agonist. Recent longitudinal follow-up studies of girls and boys receiving GnRH agonists as a treatment for precocious puberty report the development of normal reproductive function, skeletal growth, and fertility (Feuillan et al., 1999, 2000).

Estradiol: Nausea is the most common side effect of estrogen administration. At conventional replacement doses, higher than those employed in this protocol, this complaint seldom interferes with eating, and no weight loss has been reported. Breast engorgement, endometrial hyperplasia and bleeding are also common side effects of estrogen administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of estrogen; however, at the dosage and for the duration of estrogen administration in this protocol this risk is small.

The relationship between estrogen, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation (Gambrell, 1986). Numerous retrospective case control studies published

since 1975 have indicated that post-menopausal exposure to unopposed estrogens for more than one year results in a two to 12 fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen replacement therapy appears to decrease the risk of endometrial hyperplasia and endometrial cancer to equal or below that of women receiving no hormonal treatment. Recent studies suggest that the optimal regimen to prevent hyperplasia during long term ERT and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progesterone treatment each month when estrogens are administered (Nieman & Loriaux, 1992). There is an increase in thromboembolism in women receiving non-contraceptive estrogen therapy (Cushman et al., 2004; Daly et al., 2001; F. Grodstein et al., 1996; Jick et al., 1996). Additionally, some but not all studies report an increase in risk of stroke (Stampfer et al., 1991; Wassertheil-Smoller et al., 2003) in older women taking estrogen therapy. However, these complications are unlikely at the dose and duration of estrogen replacement employed in this protocol, and in the younger age group of women who participate in this study. One study (Chetkowski et al., 1986)reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use (Mandel et al., 1983; Melis et al., 1984; Nieman & Loriaux, 1992). Blood pressure, on average, appears to be unaffected by estrogen therapy, although both increases and decreases have been reported. In observational studies, post-menopausal estrogen therapy has been observed to lower the relative risk of cardiovascular disease in some but not all studies (Barrett-Connor & Bush, 1991; Stampfer et al., 1991). In contrast, recent randomized controlled trials in older postmenopausal women (e.g., Women's Health Initiative [WHI]) report an increased risk of cardiovascular disease (Manson et al., 2003). Emerging data suggest that these disparities in findings may be related to the timing of initiation of estrogen therapy in relation to the proximity of menopause. Subgroup analyses of the combined estrogen and progestin (EPT) arm of the WHI demonstrated a significant interaction between coronary heart disease (CHD) risk and time since initiation of EPT, with an increased risk in the early years following initiation and a decreased risk in later years. Additionally, the increased risk of CHD was observed in older but not younger perimenopausal women (Francine Grodstein et al., 2006; Hsia et al., 2006; Lobo, 2004; Manson et al., 2003; Prentice et al., 2005, 2006). High doses of oral estrogens have been reported to elevate hepatocellular enzyme levels and, less commonly, cause cholestatic jaundice. The risk for gallstones and hepatocellular adenomas has been reported to be increased in association with oral contraceptive use, and although uncommon these complications may also occur with the use of replacement doses of estrogen (Cirillo et al., 2005; Petitti et al., 1988). Estrogen therapy also may increase the risk of urinary incontinence in older postmenopausal women (F. Grodstein et al., 2004; Hendrix et al.,

2005; Steinauer et al., 2005). Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use(Beral, 1997; Chen et al., 2006; Chlebowski et al., 2003; Colditz et al., 1995; Gann & Morrow, 2003; Grady & Ernster, 1991; Li et al., 2003; Rossouw et al., 2002; Stefanick et al., 2006; Sturmer & Manson, 2004; Wingo et al., 1987), similar to the risk expected if the onset of menopause was delayed for a comparable length of time. Due to the publicity surrounding the cancellation of the treatment arm of the Women's Health Initiative study (Rossouw et al., 2002) that involved the administration of combined conjugated estrogens and medroxyprogesterone acetate (Prempro), we have included the following statement in the consent documents:

Adverse Events Related to Combined Hormone Replacement and the Results of the Women's Health Initiative (WHI): The WHI study demonstrated that continuous administration of one form of estrogen (conjugated estrogens) in combination with one form of progesterone (medroxyprogesterone acetate) is associated with an increased risk of dementia, heart attacks, stroke, blood clots, and breast cancer. Estradiol, the form of estrogen that we use in this study, is administered as a sole agent (with the exception of one week's combination with progesterone) and, consequently, we do not expect that it will pose the increased risks observed with the chronic combination of the conjugated estrogens and medroxyprogesterone administered in the WHI study. Indeed, while the estrogen alone arm of the WHI trial was shown to be associated with an increased risk of stroke, no increased risk of either heart disease or breast cancer was observed (Anderson et al., 2004; Rossouw et al., 2002). Estrogens may precipitate migraine headaches, and depression has also been reported to occur with the use of estrogens. In general, considering the dose and duration of treatment that we propose to use in this protocol, the risk of developing such side effects is negligible.

Progesterone: Progesterone and the synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty (Henzel, 1986). Side effects reported in women taking progestins may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression, easy fatigue and sedation, lack of initiative, and chloasma. Since progestins are often used in women with antecedent menstrual irregularity, it is not clear whether the breakthrough bleeding represents an effect of the medication or refractoriness to treatment. In the large majority of patients, menstruation occurs predictably following withdrawal of progestins and is usually more regular than in spontaneous cycles. In a recent study, an average dose of 1750 mg of oral micronized progesterone was given to 59 women with PMS for a period of three months and was well tolerated by this sample. The side effects reported on

progesterone were lightheadedness, fatigue, forgetfulness, and headaches. These were very mild and caused no dropouts.

For the sake of completeness, we will also describe the side effects reported when estradiol and progesterone are combined in the form of oral contraceptives. Side effects observed in patients receiving combined oral contraceptives include nausea, breast soreness, vaginal discharge, fluid retention, hypertension, and clotting abnormalities that have been associated with the estradiol component of the oral contraceptive. Thromboembolic disorders including thrombophlebitis, pulmonary embolism, and cerebral and coronary thrombosis appear to occur with greater frequency in women taking oral contraceptives. While the increased incidence of these disorders has been associated with the estradiol component of the oral contraceptives, it is now believed that the progestogen component may, to a lesser extent, contribute to the increased risk. There are relatively few reports associating oral contraceptives with the development of carcinomas (vaginal, uterine, hepatic, and mammary) despite the vast use of these agents, although this may reflect the latent period needed for cellular transformation.

Potential Benefits to Human Subjects

This study is designed to benefit society by gaining new knowledge. There is little chance women who participate will directly benefit from being in the study. However, women who participate may learn about your sensitivity to changes in estrogen and progesterone levels, which may be useful if they plan to have children in the future. If a participant is interested at the end of study, she will be welcomed to review any physical or mood related symptoms, she will also have the opportunity to learn more about whether she demonstrated hormone sensitivity throughout the study with the PI.

B. Adverse event reporting

1. This study defines an unanticipated problem as an incident, experience or outcome that is both *unexpected* (in nature, severity, or frequency) and *related or possibly related to the research.*
2. This study defines a serious adverse event as fatal or life threatening, requires or prolongs hospitalization or results in significant or persistent disability or congenital anomaly/birth defect.
3. Adverse events will be reported to the UNC-CH IRB and to the NIH as required. Following UNC-CH Adverse Event guidelines, events that meet the criteria for an unanticipated problems involving risks to subjects or others (UPIRSO) and are also serious adverse events will be reported to the IRB within one (1) week of the investigator becoming aware of the event. Any other events that meet the criteria for a UPIRSO will be reported to the IRB within two (2) weeks of the investigator becoming aware of the problem. In accordance with the terms of the Federal Wide Assurance, the Office for Human Research Protections (OHRP) and the Federal Drug Administration (FDA) are notified in a timely manner of 1) serious or continuing

noncompliance; 2) significant adverse events involving risk to participants or others; or 3) suspension or termination of IRB approval for a study. Unexpected problems involving risk, unless the event is serious and related to the research, are not routinely submitted to the sponsor.

4. Adverse events will be identified through clinical interviews, laboratory results, safety tests, and self-report questionnaires.
5. Every event will be reported to the principal investigator and Dr. David Rubinow by the designated research associates and will be documented. An adverse event report will be generated for each even and will include a description of the event, when and how it was reported, and any official chart records or documentation to corroborate the event.
6. The adverse event report will also include the severity attribution of the adverse event using the Common Toxicity Criteria (CTC) scale: 0 = no adverse event or within normal limits; 1 = mild AE, not requiring treatment; 2 = moderate AE, resolved with treatment; 3 = severe AE, resulted in inability to carry on normal activities and required professional medical attention; 4 = life threatening or disabling AE; 5 = fatal AE.
7. The adverse event report will also include a determination of attribution according to the following scale: 0 = definitely not related; 1 = probably not related; 2 = possibly related; 3 = probably related; 4 = definitely related.

III. Safety Review Plan and Monitoring

Oversight of participant safety includes a review of adverse events as well as study progress, data integrity, and study outcomes.

A. Justification of sample size

We approximated power and sample size using a simpler model comparing the two groups on change scores from baseline to addback. With 15 subjects per group, we have power to detect effect sizes of 1.10, which are large and consistent with our previous study. We posit that if treatment with supra physiologic levels of reproductive hormones does not produce large enough differences in neural circuits between HS+ and HS- to be detected in a relatively small sample of women, then this line of research, which involves significant participant burden, is not worth pursuing. Moreover, given the experimental nature of the proposed research, our effect size estimates are more similar to those seen in experimental animal research than in traditional human clinical trials. Nonetheless, our own and other previous clinical research supports our hypothesized large effect. Studies comparing participants with major depression and controls have yielded effect sizes (Cohen's d) ranging from 1.15 to 2.05 in dorsomedial PFC responsivity to negative emotional stimuli (Grimm et al., 2008; Hooley et al., 2005; Moses-Kolko et al., 2010) and from 1.0 to 1.56 in ventral striatum reactivity to reward (Epstein et al., 2006; Moses-Kolko et al., 2011; Moria J Smoski et al., 2009). Studies examining the association between amygdala activation and depressive symptom severity have yielded effect sizes (Cohen's d) ranging from 1.62 to 2.76 (Hamilton & Gotlib, 2008; Moses-Kolko et al., 2010).

Our target number of subjects to include in the fMRI analyses is N=30 (15 confirmed HS+ and 15 confirmed HS-). Because we have not yet identified a mechanism by which we can predict hormone sensitive status at baseline with 100% accuracy, we will need to over-recruit women with a history of PND to account for those who end up not being HS+. In our previous study 11 of the 15 women (73%) with a history of PND were HS+. For the current study, we therefore plan to over-enroll women with a history of PND in order to reach our target of 15 women with confirmed HS+. If 73% of women with a history of PND and no history of non-puerperal major depressive disorder are HS+, then we will need to enroll 21 women to find 15 with confirmed HS+. We anticipate a dropout rate of 9%, and as such, we have budgeted for an additional 3 subjects to account for dropouts. In the prior study 2/3 of the women who withdrew from the protocol had a history of PND, so we anticipate 2 women with a history of PND and 1 control (without a history of PND) will withdraw from the proposed study. These numbers are summarized in the table below:

Recruitment Group	Target Enrollment	Confirmed HS+/HS- (included in fMRI/behavioral analysis)	Not HS+/HS- (excluded from fMRI/behavioral analysis)	Withdrawn
History of PND	23	15 HS+	6 not HS+	2
Control (no history of PND)	16	15 HS-	0 not HS-	1
TOTAL	39	30	6	3

Thus, we plan to enroll a total of 39 subjects (n=16 without a history of PND, n=23 with a history of PND).

B. Safety and study progress reviews

1. The research coordinator will evaluate the study subjects at appropriate intervals and assess laboratory data and clinical signs for potential adverse events. The research coordinator will assist the PI with gathering information to help the PI determine classification and causality. The research coordinator will also observe and document all adverse events, act on the PI's recommendation, and maintain follow-up until reconciliation.
2. The principal investigator, Dr. Crystal Schiller and Dr. David Rubinow will review the adverse events, immediately after they occur, with follow-up resolution. The principal investigator and Dr. Rubinow will evaluate individual and cumulative participant data when making recommendations regarding the safe continuation of the study. The principal investigator will be notified within 24 hours of an adverse event.
3. Specific responsibilities of the principal investigator include:
   a. Review the research protocol, informed consent documents, and plans for data safety and monitoring;

b. Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial site, and other factors that can affect study outcome;
c. Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
d. Protect the safety of the study participants;
e. Report on the safety and progress of the trial;
f. Make recommendations to the UNC-CH IRB, and, if required to the Food and Drug Administration (FDA) concerning continuation, termination, or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
g. If appropriate, conduct interim analysis of efficacy in accordance with stopping rules which are clearly defined in advance of data analysis;
h. Ensure the confidentiality of the trial data and the results of monitoring; and,
i. Assist the UNC-CH IRB by commenting on any problems with study conduct, enrollment, and sample size and/or data collection.

4. The frequency of the principal investigator's review is detailed below:
   Participant accrual—weekly
   Study adherence and dropouts—Weekly
   Adverse events—As events occur
   Participant confidentiality—As events occur
5. The annual report will include a list and summary of any adverse events; whether the adverse event rates are consistent with pre-study assumptions; a summary of recruitment and retention and reason for dropouts; and whether the study is on track to be completed and accomplish the stated aims.

C. Stopping Rules—Individual Subject

Participants with significant clinical or laboratory abnormalities will be discontinued from the study prior to estrogen administration. Adverse mood symptoms will be monitored by administering the Inventory for Depression and Anxiety Symptoms II (IDAS-II) at each study visit and the DSRPs administered daily. If suicidal thoughts or severe mood symptoms are observed at any period in the protocol, then PI will follow-up with the subject by phone. Anyone expressing concerns about their ability to continue in the study, will be considered to have severe mood symptoms and be discontinued from the protocol. In the event of the occurrence of severe mood symptoms, the protocol will be terminated. Should this step prove to be unsuccessful, conventional medication will be prescribed as needed. Further, although we do not anticipate severe adverse reactions, we have arranged for inpatient hospital admission in the Psychiatry Department if symptoms are otherwise unmanageable.

IV. Informed Consent

Informed consent will be obtained from each subject at entry into the study. Potential participants will complete eligibility screening by telephone/online survey and by laboratory tests conducted at the first study visit. Prior to the eligibility screening, potential participants will give verbal consent via the phone or will read and agree to a consent statement on an online survey before providing any information. No more information will be asked of participants than necessary to obtain eligibility information and to contact those who appear eligible. Dr. Crystal Schiller, or her trained study staff, will obtain written informed consent from those individuals who pass the initial telephone or electronic screening and are interested in participating. During the consenting process, all of the applicable consent forms will be reviewed with each individual, and they will be given as much time as they would like to discuss their participation with their families and decide whether to participate.

V. Data Quality and Management

A. Measures to be taken to review data collection

1. Data will be collected and analyzed as specified in the protocol:

a. Study Design

Once determined eligible and physically well, participants will begin a hormone protocol which will last for 8 weeks. Participants will undergo a scaled down hormonal model of pregnancy and parturition (using ovarian suppression with the GnRH agonist Lupron and hormone addback with E2 and progesterone P4) in order to examine how hormones influence neural function and irritability in experimentally confirmed HS+ and HS-. FMRI sessions will occur at baseline and following two weeks of hormone addback. In the second study visit, women will have a basic lab panel and serum pregnancy test performed. Prior to the hormone protocol women will receive a pelvic exam, breast exam, and Papanicolaou test. Participants who can provide record of a pelvic exam and breast exam within the past year and normal pap results within the past 3 years will be permitted to decline the GYN exam. Women will be seen in the clinic each week during hormone protocol to assess blood levels of E2, P4 and mood symptoms.

Clinical Assessments.
The Structured Clinical Interview for DSM-V-TR Axis-I Disorders (SCID) (First et al., 2015), and the Schedule for Nonadaptive and Adaptive Personality (SNAP) (Clark, 1993) will be administered during screening to assess past and present psychiatric illness and personality pathology. Each morning, starting at Study Visit 1, participants will complete the Daily Rating Form (Endicott et al., 2006) online, a 20-item questionnaire that assesses physical and mood symptoms that accompany ovarian hormone changes. Ratings are transmitted to the study team in real-time, which **will be used to monitor rapid mood changes throughout the study and ensuring participant safety.**

The following standard measures will be administered at each study visit:

The Inventory of Depression and Anxiety Symptoms (IDAS-II) is a 99-item self-report questionnaire that comprehensively assesses anxiety and depression symptoms on 10 subscales. The IDAS-II has excellent psychometric properties and has been validated for assessing reproductive mood disorders.

The Behavioral Inhibition/Behavioral Activation (BIS/BAS) Scales (Carver & White, 1994) is comprised of four subscales—BIS, Reward Responsiveness, Drive, and Fun Seeking—and 20 items total that assess behavioral inhibition (anxiety) and behavioral activation (impulsivity). We will also include the 5-item Frustrative Nonreward Responsiveness Subscale (Wright et al., 2009) to quantify lowered approach motivation following FNR (Hypothesis 2a). The BIS/BAS scales are associated with neural markers of psychopathology (Beaver et al., 2006; Simon et al., 2010). Exploratory analyses will be conducted to assess associations between BIS/BAS, IDAS-II irritability, behavioral, and fMRI data.

Brief Irritability Test (BITe) is a brief 5-item self-report questionnaire that will assess irritability.

Misophonia Questionnaire is 19-tem self-report questionnaire that will assess misophonia and in turn irritability.

fMRI Task and Protocol.

The following tasks will be included in each of the two, one hour-long fMRI sessions:

The implicit face emotion processing task(Stoddard et al., 2017) will test whether HS+ is characterized by reduced amygdala-medial PFC connectivity in response to threat (Hypothesis 1b), that robustly activates key limbic regions, including the amygdala. During the task, participants are asked to identify the gender of angry, happy, and fearful faces at 50%, 100%, and 150% emotion intensity presented in random order for 2000 milliseconds followed by jittered fixation. Trials will appear in 3 blocks, generating 30 trials of each emotion at each intensity and 90 neutral face emotion trials. Adolescents with irritability and anxiety show reduced amygdala-PFC connectivity compared with those without anxiety during this task (Stoddard et al., 2017).

The Affective Posner Task (Tseng et al., 2017) will test whether HS+ is characterized by reduced activation of the amygdala and ventral striatum in

response to frustration (Hypothesis 2b), a well validated (Deveney et al., 2013; Rich et al., 2005, 2007, 2010) adaptation of the Posner spatial cueing task that includes both reward and FNR and activates key limbic and striatal regions, including the amygdala, caudate, putamen, and nucleus accumbens (Deveney et al., 2013). This event-related task is divided into 3 runs: during Run 1 (practice run conducted outside of the scanner), participants receive accurate feedback about their performance on the task and do not win or lose money; during Run 2, participants receive accurate feedback about their performance on the task and win or lose 50 cents per trial, based on their accuracy and reaction time; and during Run 3 (FNR), participants are told they must respond accurately to win money, but participants are given feedback that they responded too slowly on 60% of accurate trials, regardless of their performance (Deveney et al., 2013). During the FNR run, participants with mood dysregulation/irritability have demonstrated decreased amygdala and striatal activation relative to euthymic controls (Deveney et al., 2013). This task also has good convergence with self-reported irritability and frustration and excellent test retest reliability (Tseng et al., 2017), making it ideal for a repeated-measures study design. For the purpose of exploratory analysis, we will also include a resting state fMRI scan.

FMRI Data Acquisition and Image Processing.
Scanning is performed using a Siemens Prisma scanner at the UNC BRIC. High-resolution, T1-weighted anatomical images will be acquired using an MPRAGE sequence. Whole-brain functional images will be acquired using a single-shot, gradient recalled echoplanar pulse sequence sensitive to blood-oxygen-level-dependent (BOLD) contrast. Each of 2 runs will consist of the acquisition of 195 successive brain volumes. FMRI image preprocessing, processing, and analysis will be conducted using FSL and custom MATLAB scripts.

FMRI analyses will include a block-design analysis for the implicit face emotion processing task and event-related BOLD response analysis for the Affective Posner Task within a priori selected regions of interest, including corticolimbic regions (amygdala, medial PFC) for the implicit face task and amygdala and striatum (caudate, putamen, nucleus accumbens) for the Affective Posner. Image analyses will control for any group differences in reaction time or accuracy.

Behavioral Assessments. The following tasks will be conducted outside the scanner at the end of visits 3 & 6: To test whether HS+ show a greater degree of threat attention bias (Hypothesis 1a), participants will complete a visual dot-probe paradigm (Salum et al., 2017.), which measures attention bias toward angry faces. Each trial will start with a fixation cross, followed by a pair of faces (one on each

side of the screen; in anger trials, one face is angry and the other is neutral), followed by an asterisk (half of the time presented on the same side as the angry face and half of the time presented on the side of the neutral face). Trials will be of three types (angry-neutral, happy-neutral, and neutral-neutral) and presented in random order. Threat attention bias will be calculated for the angry-neutral trials by subtracting the mean reaction time on trials where the asterisk appears on the same side as the angry face from the mean reaction time on trials where the asterisk appears on the same side as the neutral face. Using this task, previous studies have demonstrated that both irritable youths (Hommer et al., 2014; Salum et al., 2017) and anxious adults (Bradley et al., 1999; Mogg & Bradley, 1999, 2005) have greater threat attention bias. To test whether HS+ show greater relational aggression (approach behavior) in response to frustration (Hypothesis 2a), participants will complete the brief Point Subtraction Aggression Paradigm (Golomb et al., 2007), which measures relational aggression and has been validated in adult women (Dougherty et al., 1999). In the task, participants press a button to accrue money or press another button to subtract money from a (fictional) partner at no direct gain to themselves. Frustration is induced by periodic subtractions of their own money, which is attributed to the partner. Relational aggression is operationalized as number of point subtractions the participant makes.

The table below outlines the procedures that will occur at each study visit:

Table 1. Study Procedures and Timeline

Study Visit	1	2	3	4	5	6	7	8
Informed Consent	x							
Psychological Interview (SCID)	x							
Physical Exam		x						
fMRI Session			x			x		
Lupron injection			x		x			
Venipuncture		x	x			x		
Self-Report Questionnaires								
Daily Rating Form	x	x	x	x	x	x	x	x
IDAS-II	x		x	x	x	x	x	x
BITe	x		x	x	x	x	x	x

Misophonia questionnaire	x		x	x	x	x	x	x
SNAP	x							
Demographic Questionnaire	x							
Trauma History Questionnaire	x							
Handedness Questionnaire	x							
Hormone History Questionnaire	x							
BIS/BAS	x		x			x		
Behavioral Tests								
Visual Dot Probe			x			x		
Point Subtraction Aggression			x			x		

Note: H/G = Hypogonadism; W/D = Withdrawal; F/U = Follow-up

b. Statistical analysis strategy:
This study has one between-subjects factor (group: HS+ versus HS-) crossed with one within-subjects factor (time: baseline versus hormone addback) with the outcome (percent signal changed) assessed in response to 2 tasks and in each of 2 ROIs. Thus, for a task/ROI combination, there is a 2-by-2 repeated measures design. We will analyze the data using linear mixed-effect models with an unstructured variance-covariance matrix. The principal effect of interest is the interaction, which will assess whether the groups differ with respect to the change from baseline to hormone addback in percent activation. We will also assess the difference between the groups at baseline and at addback. Hypotheses 1c and 2c will be addressed only in HS+, by correlating irritability with behavioral and neural responses to threat (1c) and FNR (2c). All hypothesis tests that are observed to be not statistically significant will be reported as being inconclusive.

2. The principal investigator will evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial site, and other factors that can affect study outcome.

B. Measures taken to insure data integrity and protection of databases

1. All study personnel are responsible for the collection and storage of data. All study staff participate in annual human subject training that includes education about responsibilities to minimize risk that confidentiality may be breached.
2. Participants will be identified by study ID number on all research documents and in electronic data files. All data will be stored in locked cabinets inside locked offices, and electronic data will be stored only on password-protected file servers only accessible from computers in the Psychiatry Department. Only study personnel will have access to these data. Qualtrics will be used to collect self-report questionnaire data for all participants with internet access. Participants will not enter any personally identifying information into the Qualtrics system, and they will be identified by their unique study ID only.
3. All members of the team will have access to the secure file servers on which electronic data are stored. It will be therefore unnecessary to send data files between investigators

VI. Confidentiality

As explained above, research subjects will be identified by their assigned study number on all research documents, electronic files, and specimen. Study documents will be stored in locked cabinets inside locked offices, and electronic data will be stored on secure servers accessible only on password-protected computers.

REPORTABLE EVENTS

The PI will report significant research events to the NIMH in a timely manner in compliances with the NIMH Reportable Events Policy (version date: April 16, 2015). Reportable events include Adverse Events (AEs), Serious Adverse Events (SAEs), Unanticipated Problems Involving Risks to Subjects or Others (UAPs), protocol violations, non-compliance, suspensions or terminations by monitoring entities, and suspensions or terminations by regulatory agencies.

The PI is responsible for the clinical management of the participant and accurate written documentation, investigation, and follow-up of all possible study-related AES. The PI is also responsible for describing and adhering to the procedures for identifying, monitoring, and reporting reportable events according to the timeline below.

Reports will be made in writing to the NIMH Program Official (PO). Reports will indicate that the monitoring entities (i.e., PI, IRB, and DSMB) and appropriate regulatory entities have been notified in accordance with the monitoring plan and federal regulations. Reports will be submitted to the DSMB according to the schedule outlined below, consistent with the NIMH policy.

Reportable Event	**When is Event Reported to the NIMH**	**Reported By**

IRB/ISM/DSMB/OHRP/FDA Suspensions or Terminations	Any suspension or termination of approval must include a statement of the reason(s) for the action and must be reported promptly to the NIMH PO within **3 business days of receipt.**	Regulatory or Monitoring Entity and Investigator
Deaths related to study participation	Deaths must be reported immediately (no later than within **5 business days)** of the principal investigator first learning of the death.	Investigator
Unexpected Serious Adverse Events related to study participation	Reported to the NIMH PO within **10 business days** of the study team becoming aware of the SAE.	Investigator
Unanticipated Problems Involving Risks to Subjects or Others	Reported to the NIMH PO within **10 business days** of the investigator learning of the event.	Investigator
Serious or Continuing Noncompliance	Reported to the NIMH PO within **10 business days** of IRB determination	Institution
Adverse Event	For all AEs and SAEs that are deemed expected and/or unrelated to the study, a summary should be submitted to the NIMH PO with the **annual progress report**.	Investigator
Protocol Violations	With the **annual progress report**.	Investigator

Documentation to be submitted for reportable events will include:
Identifying information for the research protocol
The date on which the event occurred and the date on which the PI became aware of the event
A detailed description of the event, including the impact on participant(s)
A detailed description of the measures taken (including clinical) in response to the event (if any)
Confirmation that the appropriate monitoring entities and regulatory bodies have been notified
A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the event

DSMB RESPONSIBILITIES

The initial responsibility of the DSMB will be to approve the initiation of this clinical trial. After this approval, and at periodic intervals (to be determined) during the course of the trail, the DSMB responsibilities are to:

- review plans for data safety and monitoring;
- evaluate the progress of the trial, including safety events, accrual and retention, and other factors that can affect study outcome;
- make recommendations to the PI concerning continuation, termination, or other modifications of the trial based on the reported beneficial or adverse effects of the treatment under study;
- if appropriate, review interim analysis of efficacy in accordance with stopping rules which are clearly defined in advance of data analysis and have the approval of the DSMB;
- assist the UNC-CH IRB by commenting on any problems with enrollment, and sample size.

MEMBERSHIP

The DSMB will consist of at least three members, and the presence of all three members will be required to constitute a quorum. The members, recommended by NCTraCS, will include an obstetrician-gynecologist, a biostatistician, and a psychiatrist (not affiliated with the UNC Department of Psychiatry) and will be subject to approval by the UNC-CH IRB. Membership will consist of persons completely independent of the investigators who have no financial, scientific, or other conflict of interest with the trial. Collaborators or associates of Dr. Schiller are not eligible to serve on the DSMB. Written documentation attesting to absence of conflict of interest is required. The DSMB includes experts in or representatives of the fields of:

- a psychiatrist with relevant clinical experience,
- an obstetrician-gynecologist with relevant clinical experience;
- clinical trial methodology, and
- biostatistics.

The DSMB chairperson will be responsible for overseeing the meetings, developing the agenda in consultation with the PI and the UNC-CH IRB, as required. The chair is the contact person for the DSMB.

A Safety Officer will be identified by the PI at the first meeting. This person will be the contact person for severe adverse event reporting. The Safety Officer will notify the DSMB and UNC-CH IRB of serious adverse events within 24 hours. Procedures for notifying the Chair of the DSMB and UNC-CH IRB will be determined at the first meeting.

BOARD PROCESS

The first meeting will take place face-to-face to discuss the protocol, any modifications of the trial, and to establish guidelines to monitor the study. The DSMB Chairperson and Dr. Schiller will prepare the agenda to address the review of the manual of operating procedures, modification of study design, initiation of the trial, identification of a safety officer, reporting of adverse events, stopping rules, interim analysis plan, etc.

Meetings of the DSMB will be held two times a year at the call of the Chairperson. Meetings shall be closed to the public because discussions may address confidential patient data. Meetings are attended, when appropriate, by the principal investigator and members of her staff. Meetings may be convened as conference calls as well as in person, although the initial meeting will be face-to-face. An emergency meeting of the DSMB may be called at any time by the Chairperson or by the UNC-CH IRB should questions of patient safety arise.

MEETING FORMAT

An appropriate format for DSMB meetings consists of an open and closed session. The open sessions may be attended by the principal investigator(s), institution staff and UNC-CH IRB staff, and the study biostatistician. Issues discussed at open sessions will include the conduct and progress of the study, including patient accrual, compliance with protocol, and problems encountered. Patient-specific data and treatment group data will not be presented in the open session.

The closed session will be attended only by voting DSMB members. The DSMB may request others to attend part or all of the closed sessions (e.g., the study statistician, UNC-CH IRB staff). All safety and efficacy data will and must be presented at this session. The discussion at the closed session will be completely confidential.

Should the DSMB decide to issue a termination recommendation, full vote of the DSMB will be required. In the event of a split vote, majority vote will rule, and a minority report should be appended.

REPORTS

Interim Reports: Interim reports will be generally prepared by the study statistician and distributed to the DSMB at least 10 days prior to a scheduled meeting. These interim reports will be numbered and provided in sealed envelopes within an express mailing package or by secure email as the DSMB prefers. The contents of the report will be determined by the DSMB. Additions and other modifications to these reports may be directed by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts:

Part 1 (Open Session Report) will provide information on study aspects such as accrual, baseline characteristics, and other general information on study status.

Part 2 (Closed Session Report) may contain data on study outcomes, including safety data, and depending on the study, perhaps efficacy data. The Closed Session Report is considered confidential and should be destroyed at the conclusion of the meeting. Data files to be used for interim analyses will have undergone established editing procedures to the extent possible. Interim analyses of the efficacy data are performed only if they are specified and approved in advance and criteria for possible stopping is clearly defined.

Reports from the DSMB: A formal report containing recommendations for continuation or modifications of the study prepared by the DSMB Chairperson will be sent to the full DSMB within four weeks of the meeting. Once approved by the DSMB, the formal DSMB recommendation will be forwarded to Dr. Schiller. It is Dr. Schiller's responsibility to distribute the formal DSMB recommendation report to tall co-investigators and to assure that copies are submitted to all the IRBs associated with the study.

As previously stated, the formal DSMB report should conclude with a recommendation to continue or to terminate the study. This recommendation should be made by a formal majority vote. A termination recommendation may be made by the DSMB at any time by a majority vote. The DSMB Chairperson is responsible for notifying the PI of a decision to terminate the study. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report. The report should not include unblinded data, discussion of the unblinded data, etc.

Mailings to the DSMB: On a scheduled basis (as agreed upon by the DSMB) blinded safety data should be communicated to all DSMB members or to the designated safety officer (to be determined at the first meeting). Any concerns noted should be brought to the attention of the DSMB Chairperson.

Access to Interim Data: Access to the accumulating endpoint data should be limited to as small a group as possible. Limiting the access to interim data to the DSMB members relieves the investigator of the burden of deciding whether it is ethical to continue to enroll patients and helps protect the study from bias in patient entry and/or evaluation.

CONFIDENTIALITY

All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

Appendix D. COVID-19 Information Sheet

The University of North Carolina at Chapel Hill
Information about participating in a Research Study during COVID-19.

Version Date: 7.15.20
IRB Study # 19-0401
Title of Study: Characterizing the neural substrates of irritability in women: a neuroendocrine model
Principal Investigator: Crystal Edler Schiller, PhD
Study Contact Telephone Number: (919)966-5243
Study Contact Email: laura_lundegard@med.unc.edu

The "you" referenced in this information sheet either refers to the participant, "your child", or the individual you are providing consent on behalf of as a legal authorized representative, as applicable.

The following information should be read as an addition to the original Consent process. Unless specifically stated otherwise in the following paragraphs, all information contained in that original Consent Form is still true and remains in effect. Your participation continues to be voluntary. You may choose not to participate or may withdraw your consent to participate at any time, and for any reason, without jeopardizing your future care at this institution or your relationship with your study doctor.

New or additional information

COVID-19 is a novel (previously unidentified) coronavirus and has been declared a pandemic due to its global spread. The virus causing coronavirus disease 2019 (COVID-19), is not the same as the coronaviruses that commonly circulate among humans and usually cause mild illness, like the common cold. Many countries, states, and local governments have implemented restrictions (e.g., physical distancing requirements, closure of public places) to attempt to limit the spread of the virus. If your community does not permit travel or otherwise limits interaction, please tell the study team. UNC's permission for certain studies to continue is not intended to interfere with a person's ability to follow their community's requirements.

Physical distancing is the primary strategy used to prevent the spread of the virus that causes COVID-19. Physical distancing calls for people to increase the space between one another and to avoid gatherings and crowds. The Centers for Disease Control and Prevention (CDC) says people should maintain a distance of at least six feet from others when possible.

If you choose to continue your participation in research at UNC project research personnel will do their best to follow the below recommendations described in this information sheet that were developed based on guidance from the CDC and in partnership with study teams, departments, and UNC's Infectious Disease specialist. Despite everyone's efforts, there is still the risk that you may already have or may become infected with COVID-19 and may then infect others.

COVID-19 Symptoms

People with COVID-19 have had a wide range of reported symptoms – ranging from mild symptoms to severe illness which may lead to death. Symptoms may appear 2-14 days after exposure to the virus. People with these symptoms may have COVID-19:

- Cough
- Shortness of breath or difficulty breathing
- Fever
- Chills especially repeated shaking chills
- Muscle pain
- Sore throat
- New loss of taste or smell
- Vomiting and diarrhea

This list is from the CDC but may not include all possible symptoms. People experience the virus in many different ways. Please contact your medical provider if you are experiencing symptoms that are concerning to you.

Pandemics can be stressful for everyone. Fear and anxiety about a disease can be overwhelming and cause strong emotions in both adults and children. Please talk with the study team if you are experiencing COVID symptoms, fear or anxiety.

Please seek emergency medical care immediately if experiencing the following symptoms:

- Trouble breathing
- Persistent pain or pressure in the chest
- New confusion
- Inability to wake or stay awake
- Bluish lips or face

*This list is not all possible symptoms. Please call your medical provider for any other symptoms that are severe or concerning to you. Call 911 or call ahead to your local emergency facility: Notify the operator that you are seeking care for someone who has or may have COVID-19.

Children and Multisystem Inflammatory Syndrome (MIS-C)

Multisystem Inflammatory Syndrome (MIS-C), is a condition in children where different body parts can become inflamed, including the heart, lungs, kidneys, brain, skin, eyes, or gastrointestinal organs. We do not yet know what causes MIS-C, however we know that many children with MIS-C had the virus that causes COVID-19, or had been around someone with COVID-19. MIS-C can be serious, even deadly, but most children who have been diagnosed with this condition have gotten better with medical care.

Contact your child's doctor, nurse, or clinic right away if your child is showing symptoms of MIS-C:

- Fever
- Abdominal pain
- Vomiting
- Diarrhea
- Neck pain
- Rash

- Bloodshot eyes
- Feeling extra tired

Be aware that not all children will have all the same symptoms. Seek emergency care right away if your child is showing any of these emergency warning signs of MIS-C or other concerning signs:

- Trouble breathing
- Pain or pressure in the chest that does not go away
- New confusion
- Inability to wake or stay awake
- Bluish lips or face
- Severe abdominal pain

What steps are the study team and UNC taking to prevent the spread of COVID?

To help prevent the spread of COVID study teams across UNC are taking the following steps:

Conducting an individual risk assessment with subjects to determine if the individual is part of a high-risk group as defined by the CDC and discussing with you if the specific research activities that may break physical distancing recommendations is in your best interest. The wellness screen will include questions about whether the you have or had COVID-19, possible exposure to the virus, possible symptoms, and about risk factors.

If the subject would like to participate but is in a high-risk group as defined by the CDC including those over the age of 65, live in a nursing home or long-term care facility, or who have underlying medical conditions, particularly if not well controlled, they should discuss if study activities can be safely delayed with the study team or if participation should continue.

1. Prior to a face-to-face visit, research personnel must confirm your appointment and perform telephone wellness screenings no more than 24 hours prior to the scheduled visit.

    a. You and anyone required to attend the visit with you must be rescreened by front desk staff or research personnel upon arrival before the study visit begins (i.e., to confirm there are no symptoms, no fever, etc.). You and anyone required to attend the visit must be masked upon arrival at the clinic/research site. Anyone who fails rescreening will be immediately isolated in a private room. Clinical study personnel will be contacted and recommendations from **Infection Prevention** regarding referral for testing should be followed. You may be responsible for cost of testing, and treatment of COVID-19 unless this is specifically part of the study you are participating in or otherwise covered. The study specific consent form will cover this if applicable.

2. During face-to-face visits, research personnel and you should maintain a physical distance of 6 feet whenever possible, wear a facemask and eye protection, and perform hand hygiene before and after face-to-face interaction with all participants.

3. Interactions should take place in an outdoor setting, if possible.

4. Research personnel have developed and implemented a regular schedule for frequently cleaning and wiping touched surfaces and objects (e.g., door and cabinet handles, faucets, light switches, keyboards, and other frequently touched objects) with an approved disinfectant or

disinfectant wipes. Research personnel will also follow routine surface decontamination of common equipment like instrumentation and computers. Disinfecting any surfaces that may be thought to be contaminated and use an approved disinfectant such as a 1:10 dilution of bleach or 60% to 90% alcohol solution.

5. Research that involves participants of 10 or fewer individuals in a group, such as a focus group, is allowed. Seating should be arranged to allow 6 feet between group members, and all focus group participants must wear masks.
6. If research involves travel or overnight stays, accommodation and meals should allow for adequate physical distancing (6 feet or more) wherever feasible. Vehicle occupancy should be limited to no more than two people in a standard car, with open windows while travelling, if possible. Vehicle occupants should wear masks.
7. Some locations may have additional safety procedures. If your visit is at such a location, the study team will describe what to expect when they call to confirm your study visit.

If you feel as though the above precautions are not being followed please either talk with the study staff, submit an anonymous request through Ethics Point by calling 1-866-294-8688, or reach out to the UNC OHRE IRB at 919-966-3113.

You should receive a copy of this form when possible. Please discuss with your study team, ask all questions you may have, and confirm whether you would like to continue your participation. The study team may also withdraw you from the study or request to delay study activities if they have concerns about safety.