

Effects of Estradiol on Neural Reward System and Depression in the Perimenopause (PEERS)

NCT number	NCT02255175
Document Date	4/12/2016

**AN OBSERVATIONAL, CASE-CONTROL, SINGLE-CENTER
STUDY TO DETERMINE THE PERIMENOPAUSAL EFFECTS
OF ESTRADIOL ON REWARD RESPONSIVENESS (PEERS)**

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

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

Protocol Number: 13-3572

IND Number: N/A

Verion 0.1: 9/9/14
Version 0.2: 10/17/2014
Version 1.0: 9/14/2015
Version 2.0: 4/12/2016

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List of Abbreviations

AE	Adverse Event
BOLD	Blood-oxygen-level-dependent
BRIC	Biomedical Research Imaging Center
CHD	Coronary heart disease
CRF	Case report form
DSMB	Data and Safety Monitoring Board
DVT	Deep vein thrombosis
EC	Ethics Committee
EPDS	Edinburgh Postnatal Depression Scale
EPT	Combined estrogen and progestin treatment (EPT)
FDA	Food and Drug Administration
fMRI	Functional magnetic resonance imaging
FSL	Brain imaging analysis software
GnRH	Gonadotropin-releasing hormone
HIPAA	Health Insurance Portability and Accountability Act
HRSD	Hamilton Rating Scale for Depression
IDAS	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
MID	Monetary Incentive Delay (fMRI task)
MDD	Major Depressive Disorder
PHI	Protected health information
PPD	Postpartum depression
ROI	Region of interest
SAE	Serious Adverse Event
SCID	Structured Clinical Interview for DSM-IV-TR Axis-I Disorders
STRAW	Stages of Reproductive Aging Workshop
UNC-CH	University of North Carolina at Chapel Hill
WHI	Women's Health Initiative

Study Summary

Title	Perimenopausal Effects of Estradiol on Reward Responsiveness
Short Title	PEERS
Protocol Number	IRB# 13-3572
Phase	N/A
Methodology	Experimental, case-control study
Study Duration	12-24 months
Study Center(s)	Single-center
Objectives	<p>There are three practical objectives:</p> <p>1. To measure the frontostriatal response to reward in perimenopausal MDD and test the effects of estradiol on neural activation in perimenopausal women. We will use fMRI at baseline and following estradiol treatment in women with and without MDD to probe frontostriatal reward circuitry. Implementation of this protocol will provide hands-on training in fMRI research, the experimental manipulation of sex steroids in humans, and the interpretation of fMRI results based on a pharmacologic manipulation.</p> <p>2. To quantify motivated behavior at baseline and following estradiol administration in perimenopausal women with and without MDD. Motivated behavior will be operationally defined as the response latency to reward versus non-reward during the fMRI reward task. Pursuit of this aim will provide training in behavioral markers of psychopathology in the context of fMRI.</p> <p>3. To measure the psychological correlates of the frontostriatal response to reward in women with perimenopausal MDD at baseline and following estradiol administration. Depressive symptoms will be assessed at baseline and following estradiol administration. Training associated with this aim will include assessing associations between neural activity and psychological symptoms.</p>
Number of Subjects	50
Diagnosis and Main Inclusion Criteria	<p><u>Group 1: Women with Perimenopausal Depression</u> 1) ≥ 2 skipped cycles and an interval of amenorrhea ≥ 60 days, consistent with the late menopause transition (stage -1), and who demonstrate an FSH level higher than two SDs above the mean for follicular phase premenopausal women based on UNC Hospitals laboratory levels ($x= 7$ IU/mL); 2) 44-55 years old; 3) current diagnosis of MDD with an onset associated with menstrual cycle irregularity, and no history of psychiatric illness during the 2 years before the onset of the current depressive episode as determined by the Structure Clinical Interview for DSM-IV-TR for Axis I Disorders (SCID)</p> <p><u>Group 2: Healthy Controls</u> 1) Controls will meet all inclusion criteria specified above except they must not have any past or present psychiatric disorder assessed by the SCID</p>
Study Product, Dose, Route, Regimen	<p>Study Product: Estradiol Active ingredient: micronized estradiol Chemical name: estra-1,3,5,(10)-triene-3, 17β-diol</p> <p>Progesterone Active ingredient: micronized progesterone Chemical name: pregn-4-ene-3, 20-dione</p>

	<p>Dose:</p> <table border="1" data-bbox="501 254 1416 348"> <thead> <tr> <th>Drug</th> <th>Dosage</th> <th>Formulation</th> </tr> </thead> <tbody> <tr> <td>Estradiol</td> <td>100 micrograms/day</td> <td>Transdermal</td> </tr> <tr> <td>Progesterone</td> <td>200 miligrams/day</td> <td>Oral capsule</td> </tr> </tbody> </table> <p>Route:</p> <table border="1" data-bbox="501 411 1416 506"> <thead> <tr> <th>Drug</th> <th>Route of Administration</th> </tr> </thead> <tbody> <tr> <td>Estradiol</td> <td>Transdermal</td> </tr> <tr> <td>Progesterone</td> <td>Oral</td> </tr> </tbody> </table> <p>Regimen: Once determined eligible and physically well, participants will receive transdermal estradiol (100 µg/day) for 3 weeks. Prior to estradiol administration women will receive a pelvic and breast exam and Papanicolaou test. Participants who can provide record of a pelvic exam within the past year and normal pap results within the past 3 years will be permitted to decline the GYN exam. Women will also have a basic lab panel and serum pregnancy test performed, and FSH and LH will be assayed. Women will be seen in the clinic each week during estradiol administration to assess blood levels of estradiol and mood symptoms. Women will receive an additional week of combined estradiol and micronized progesterone (200 mg/day) at the end of the study to precipitate menstruation.</p>	Drug	Dosage	Formulation	Estradiol	100 micrograms/day	Transdermal	Progesterone	200 miligrams/day	Oral capsule	Drug	Route of Administration	Estradiol	Transdermal	Progesterone	Oral
Drug	Dosage	Formulation														
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Progesterone	Oral															
Duration of administration	3 weeks															
Reference population	Perimenopausal women, non-depressed															
Statistical Methodology	<p>This study has one between-subjects factor (group: MDD versus control) crossed with one within-subjects factor (time: baseline versus estradiol treatment) with the outcome (percent signal changed) assessed in response to the MID task in the structurally defined frontostriatal ROIs, including the nucleus accumbens, caudate, thalamus, frontopolar cortex, and dorsolateral prefrontal cortex. Thus, for a task/ROI combination, there is a 2-by-2 repeated measures design. We will analyze this design using an ROI analysis and mixed models with an unstructured covariance structure. To test whether perimenopausal women with MDD show reduced frontostriatal activity and motivated behavior in response to reward at baseline, we will examine group differences in BOLD signal and response latency at baseline. To measure the psychological correlates of the frontostriatal response to reward, we will examine correlations between the change in frontostriatal ROI activation and change in depressive symptoms in the MDD group between baseline and post-treatment; also within the MDD group, we will examine the correlation between frontostriatal ROI activity at baseline and the change in depressive symptoms between baseline and post-treatment. Finally, to examine whether estradiol treatment has greater effects on women with MDD than those without, we will assess whether the groups differ with respect to the change from baseline to post-treatment in percent activation in frontostriatal ROIs and response latency.</p>															

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

Despite decades of clinical research, affective disorders continue to affect 20.9 million Americans each year and remain the leading cause of disability worldwide¹. Unraveling the pathophysiology of affective disorders has been uniquely challenging because depressive syndromes are heterogeneous and have diverse etiologies². Attempts to identify genetic and neural biomarkers that would improve the prediction of susceptibility, course of illness, and treatment response have yielded inconsistent results. One way to solve the problem of etiological diversity and diagnostic heterogeneity is to identify a homogeneous depression subtype with an identified etiology. Reproductive affective disorders (i.e., premenstrual dysphoric disorder, postpartum depression, and perimenopausal depression) represent more homogeneous subtypes, and a clear role for reproductive steroids has been established in these conditions³. Thus, we propose to study the pathophysiology of affective dysfunction by examining neural function in women with a specific depression subtype, perimenopausal depression.

Women with perimenopausal depression represent the ideal population in which to study the neural biomarkers of reproductive affective dysfunction because the presumptive etiology—ovarian hormone withdrawal—is objectively measurable and amenable to manipulation. Evidence supporting the role of ovarian hormone withdrawal in the etiology of perimenopausal depression includes the following: perimenopausal women show a temporal association between ovarian hormone withdrawal and the onset of mood symptoms in the late perimenopause⁴; treatment with estrogen reduces mood symptoms⁵; and blinded estradiol withdrawal re-precipitates depression in women with a history of perimenopausal depression (manuscript in preparation). Thus, perimenopausal depression represents a relatively pure phenotype with respect to the presence of hormone sensitive depression. Focusing on a homogeneous etiology will increase the likelihood of identifying meaningful neurobiological markers⁶.

One of the most powerful tools for understanding the neural mediators of complex behaviors is brain imaging⁷. Although the mechanisms by which reproductive steroids modulate human behavior is relatively unknown, the application of both modern imaging techniques and knowledge of the neurocircuitry underlying fundamental human and animal behaviors should permit us to define more precisely the means by which changes in reproductive steroids elicit depression in some, but not other, individuals.

A fundamental behavior that is dysregulated in depression is reward responsiveness⁸. Reduced responsiveness to rewards contributes to the clinical phenomenon of anhedonia (i.e., loss of interest or pleasure in rewarding activities)⁵, a cardinal feature of depression⁹. In individuals with depression, anhedonia is associated with frontostriatal hypoactivity during reward processing¹⁰. Frontostriatal reward circuit dysregulation may be particularly central to perimenopausal depression. Estradiol modulates reward responsiveness in rodents^{11, 12} and a recent human study demonstrated an association between estradiol levels and frontostriatal reactivity to reward during the menstrual cycle in healthy women¹³. Thus, the frontostriatal reward system is regulated by estradiol and implicated in major depression. However, the effects of estradiol withdrawal and administration on frontostriatal reward circuitry have never been examined directly, and the extent to which frontostriatal dysregulation mediates the effects of estradiol withdrawal on depressive symptoms remains unknown.

Thus, we will examine the effects of estradiol on the neural reward system in perimenopausal women. The proposed experimental study will allow us to answer the following questions: 1) Does the reward system differ in women with and without perimenopausal depression? 2) Does the reward system respond to estradiol differently in those with and without perimenopausal depression? 3) Does reward system activity at baseline predict the antidepressant effects of estradiol in women with perimenopausal depression? Our results will provide critical information about the neuroendocrine pathophysiology of perimenopausal depression and may subsequently contribute to the development of novel pharmacologic interventions.

The proposed study design will allow us to examine the role of frontostriatal hypoactivity in the pathophysiology of perimenopausal depression (by assessing baseline differences between perimenopausal depression and controls). It will also allow us to measure frontostriatal responses to

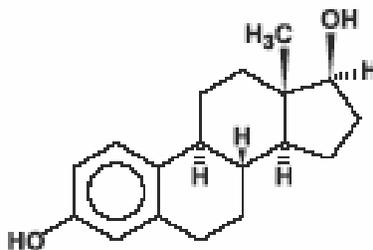
estradiol treatment in both perimenopausal depression and control women, neither of which have been previously characterized. This information is critical for understanding the pathophysiology of perimenopausal depression and the mechanisms by which estradiol withdrawal and supplementation have differential effects on mood across women (i.e., why some but not all women become depressed in the perimenopause). These aims are of central relevance to my training goals to gain the skills necessary to examine the neural pathophysiology of reproductive mood disorders and conduct experimental hormone investigations of the neural circuits contributing to affective illness in women. We have elected not to include a placebo control condition, as many studies in the field of affective neuroscience forgo placebo controls when examining the effects of drugs on neural circuitry^{14, 15}. If, however, the expense associated with fMRI and sample size were not concerns, the inclusion of a placebo condition would allow us to control for the potential variance associated with repeated fMRI and to examine the potential placebo effects of medication taking on frontostriatal circuitry. Thus, the proposed study will provide pilot data for a larger, randomized, placebo-controlled study with sufficient power to compare treatment and placebo responders and non-responders, which will allow us to further elucidate the precise relationships between changes in frontostriatal activation, estradiol, and changes in mood. Nevertheless, results of the proposed study will provide critical information about the involvement of frontostriatal circuitry in the pathophysiology of perimenopausal depression and the antidepressant effects of estradiol treatment.

1.2 Investigational Agent

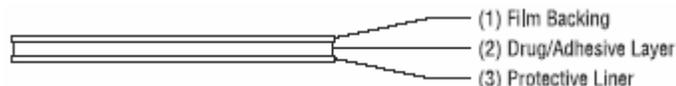
ESTRADIOL

Description

Climara®, estradiol transdermal system, is designed to release estradiol continuously upon application to intact skin. Six (6.5, 9.375, 12.5, 15, 18.75 and 25 cm²) systems are available to provide nominal *in vivo* delivery of 0.025, 0.0375, 0.05, 0.06, 0.075 or 0.1 mg respectively of estradiol per day. The period of use is 7 days. Each system has a contact surface area of either 6.5, 9.375, 12.5, 15, 18.75 or 25 cm², and contains 2, 2.85, 3.8, 4.55, 5.7 or 7.6 mg of estradiol USP respectively. The composition of the systems per unit area is identical. Estradiol USP is a white, crystalline powder, chemically described as *estra-1,3,5(10)-triene-3, 17β-diol*. It has an empirical formula of C₁₈H₂₄O₂ and molecular weight of 272.39. The structural formula is:



The Climara system comprises three layers. Proceeding from the visible surface toward the surface attached to the skin, these layers are (1) a translucent polyethylene film, and (2) an acrylate adhesive matrix containing estradiol USP. A protective liner (3) of siliconized or fluoropolymer-coated polyester film is attached to the adhesive surface and must be removed before the system can be used.



The active component of the system is estradiol. The remaining components of the system (acrylate copolymer adhesive, fatty acid esters, and polyethylene backing) are pharmacologically inactive.

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.

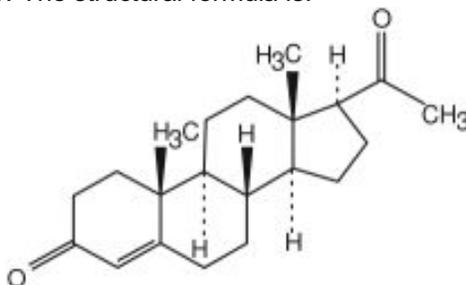
Estrogens act through binding to nuclear receptors in estrogen-responsive tissues. To date, two estrogen receptors have been identified. These vary in proportion from tissue to tissue.

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.

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:



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

We will enroll perimenopausal women to undergo estradiol supplementation using an existing recruitment infrastructure. Drs. Girdler and Rubinow are currently conducting a 12-month RCT (MHR01087619) designed to identify predictors of mood and cardiovascular benefit of transdermal estradiol in perimenopausal women. Over the 23 months of recruitment, with ads that specify no current depression, 2,029 women were screened, 248 (12%) of whom were not eligible based on current MDD symptoms. Our data underscore the prevalence of both depressive symptoms for women in this stage of life and the likelihood of recruiting women with MDD. These results also show an attrition rate of less than 20% post-randomization in the context of a 12-month clinical trial. Although attrition may be increased in women with MDD, the proposed study is substantially shorter and should be associated with reduced attrition.

Support for the hypotheses comes from my own research and that of my mentors:

1. **Estradiol withdrawal precipitates anhedonia in rats, and decreasing estradiol levels are associated with increasing negative mood symptoms in women**¹¹. These results support my ability to conduct successful research in the area of reproductive mood disorders. Our current hypotheses are consistent with this prior research and suggest that the results may have implications for reproductive mood disorders.
2. **Estradiol withdrawal is associated with the onset of perimenopausal MDD, and estradiol treatment reduces perimenopausal MDD symptoms compared with placebo**. In a 5-year longitudinal study of 29 women, a 14-fold increase in MDD was observed in the 24 months surrounding menopause⁴. In a larger cross sectional study (n=116), depressive episodes were significantly more likely during the late perimenopause compared with the pre-, early-, and post-menopause¹⁶. Although estradiol levels are highly variable in regularly cycling women and during the pre- and early perimenopause, the average level of circulating estradiol declines dramatically during the transition from early to late perimenopause²⁰, which is accompanied by an increased incidence in MDD^{4, 16}. Depressive symptoms significantly decreased following 3 weeks of estradiol treatment in 34 women with perimenopausal MDD compared with baseline and women taking placebo⁵ (Figure 1c). Moreover, despite comparable ovarian hormone levels in women with perimenopausal MDD compared with controls^{4, 17}, women with a history of perimenopausal MDD (but not those lacking that history) rapidly experience depression recurrence when blindly withdrawn from estradiol. Thus, estradiol withdrawal appears to trigger perimenopausal MDD in susceptible women, and three weeks of treatment with estradiol is sufficient to treat perimenopausal MDD.
3. **Active MDD symptoms and MDD risk are associated with frontostriatal hypoactivity**. Individuals at high risk for MDD show reduced frontostriatal activation during the proposed monetary incentive delay task, and the degree of activation is associated with the severity of self-reported rumination among those at high risk for MDD¹⁸.
4. **Estradiol levels are associated with increased reward-related neural function during the menstrual cycle in non-depressed women**. Estradiol levels were positively correlated with activation of the dorsolateral and frontopolar cortices¹⁹, which is consistent with our hypothesis that estradiol supplementation will increase activation in frontal regions.
5. **The proposed monetary incentive delay (MID) task induces frontostriatal activation during monetary reward in control women**. At baseline, control women enrolled in our R21 study of the effects of a scaled down hormonal model of pregnancy and parturition on mood and brain function showed increased activity in frontostriatal regions (specifically the nucleus accumbens, caudate, thalamus, dorsolateral prefrontal cortex, and frontopolar cortex) during monetary reward. These areas were selectively responsive to reward and not activated during non-reward, indicating that the task reliably activates the regions of interest associated with reward in previous studies.

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

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.

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 investigation²¹. 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 administered²². There is an increase in thromboembolism in women receiving non-contraceptive estrogen therapy²³⁻²⁶. Additionally, some but not all studies report an increase in risk of stroke^{27,28} 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 study²⁹ reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use^{22,30,31}. 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^{27, 32}. In contrast, recent randomized controlled trials in older postmenopausal women (e.g., Women's Health Initiative [WHI]) report an increased risk of cardiovascular disease³³. 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 women³⁴⁻³⁹. High doses of oral estrogens have been reported to elevate hepatocellular enzyme levels and, less commonly, cause cholestatic jaundice. The risk for gall stones 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^{40,41}. Estrogen therapy also may increase the risk of urinary incontinence in older postmenopausal women⁴²⁻⁴⁴. Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use⁴⁵⁻⁵⁵, 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 study⁵⁵ 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^{55,56}. 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 puberty⁵⁷. 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.

1.5 Dose Rationale and Risk/Benefits

As previously stated Drs. Girdler and Rubinow are currently conducting a 12-month RCT (MHR01087619) designed to identify predictors of mood and cardiovascular benefit of transdermal estradiol in perimenopausal women. This study will enroll perimenopausal women to undergo estradiol supplementation using their existing infrastructure. Many studies have identified perimenopausal women as being at high risk for mood disorders, especially during the late menopause transition, but only a few studies have systematically assessed this biological vulnerability. The benefits of this study are three-fold: 1) increased knowledge of the neuroendocrine mechanisms underlying both the triggering of and susceptibility to depression in women, 2) improved identification of perimenopausal women for whom hormone replacement therapy is likely to have beneficial neuroregulatory effects, and 3) increased information regarding novel targets for pharmacologic intervention. While there are scientific benefits to this study, the benefit to patients will be minimal. Estradiol administration may reduce depressive symptoms in some of the participants, and this information may be helpful to women as they plan their future health care in relation to menopause symptom management.

2 Study Objectives

The long-term research objectives are to 1) advance our understanding of the effects of ovarian hormones on the female brain and how these effects contribute to the triggering of and susceptibility to mood disorders; and 2) to identify neural and endocrine markers of depression risk, thereby improving our ability to prevent affective illness in women.

The central hypothesis is that the neural reward system is hypoactive in perimenopausal MDD, and the antidepressant effects of a three-week transdermal estradiol intervention will be mediated by increased activity in the neural reward system, assessed using fMRI. We will test our hypothesis by executing the following aims:

Aim 1: To measure the frontostriatal response to reward in perimenopausal MDD and test the effects of estradiol on neural activation in perimenopausal women. We will use fMRI at baseline and following estradiol treatment in women with and without MDD to probe frontostriatal reward circuitry. Implementation of this protocol will provide hands-on training in fMRI research, the experimental manipulation of sex steroids in humans, and the interpretation of fMRI results based on a pharmacologic manipulation.

H1a: We expect women with perimenopausal MDD to show reduced frontostriatal reactivity to reward at baseline compared with perimenopausal women without MDD. Specific regions of interest include the nucleus accumbens, caudate, and putamen.

H1b: We also expect that estradiol administration will be associated with greater increases in frontostriatal responsivity to reward in those with MDD compared with those without MDD.

Aim 2: To quantify motivated behavior at baseline and following estradiol administration in perimenopausal women with and without MDD. Motivated behavior will be operationally defined as the response latency to reward versus non-reward during the fMRI reward task. Pursuit of this aim will provide training in behavioral markers of psychopathology in the context of fMRI.

H2a: Women with perimenopausal MDD will show greater response latencies during reward trials but not non-reward trials compared with those without MDD.

H2b: We also expect that response latency to reward will be attenuated with estradiol administration in women with MDD.

Aim 3: To measure dysphoria at baseline and following estradiol administration. Dysphoria will be assessed at baseline and following estradiol administration. Training associated with this aim will include assessing associations between neural activity and psychological symptoms.

H3a: Women with perimenopausal MDD will report a significant reduction in depressive symptoms following estradiol administration, and the degree of symptom improvement will be associated with the change in frontostriatal responsivity to reward.

3 Study Design

3.1 General Design

Overview. This is an observational, case-control, single-center study using estradiol and progesterone to examine the role of frontostriatal hypoactivity in the pathophysiology of perimenopausal MDD (by assessing baseline differences between perimenopausal MDD and controls.) It will also allow us to measure frontostriatal responses to estradiol treatment in both perimenopausal MDD and control women. This study will include 6 study visits that will take place over the course of approximately 6 weeks. Participants will receive transdermal estradiol for 3 weeks and FMRI sessions will occur at baseline and at the end of the third week of estradiol administration. The 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
Consent	x					
OBGYN Exam		x				
fMRI			x			x
PRT			x			x
Venipuncture		x	x	x	x	x
SCID	x					
SNAP	x					

HAM-D	x		x	x	x	x
IDAS	x		x	x	x	x
PANAS	x		x	x	x	x
MASQ	x		x	x	x	x
GCS	x		x	x	x	x
PSQI			x			x
Estradiol Rx provided			x	x	x	x
Progesterone Rx provided						x
Payment	\$100	\$30	\$150	\$35	\$35	\$150

Participants. Participants will include healthy, unmedicated perimenopausal women ages 44-55 either with ($n=20$) or without ($n=20$) current major depression.

Hormone Administration.

In the OBGYN visit, women will have a basic lab panel and serum pregnancy test performed, and FSH and LH will be assayed. Prior to estradiol administration women will receive a pelvic exam, breast exam and Papanicolaou test. Participants who can provide record of a pelvic exam within the past year and a normal pap results within the past 3 years will be permitted to decline the GYN exam and. Women will be seen in the clinic each week during estradiol administration to assess blood levels of estradiol and mood symptoms. Women will receive an additional week of combined estradiol and micronized progesterone (200 mg/day) at the end of the study to precipitate menstruation. Blood will be batched until the end of data collection, at which point estradiol will be assayed.

Clinical Assessments

The Structured Clinical Interview for DSM-IV-TR Axis-I Disorders (SCID)⁵⁸ and the Schedule for The SCID1 will be administered at baseline to determine study eligibility. The following standard measures will be administered at baseline, both fMRI sessions, and weekly clinic visits:

- The Positive and Negative Affect Schedule⁵⁹ is a 20-item questionnaire that measures both positive affect (PA) and negative affect (NA) and is sensitive to subtle changes in affect over time.
- The Hamilton Rating Scale for Depression (HRSD)⁶⁰ is a 21-item researcher-rated standardized scale that assesses depressive symptoms and is widely used as a measure of symptom change over time.
- The Inventory of Depression and Anxiety Symptoms (IDAS)⁶¹ is a 64-item self-report questionnaire that comprehensively assesses anxiety and depression symptoms on 10 subscales. The IDAS has excellent psychometric properties and has been validated for assessing reproductive mood disorders.
- The Mood and Anxiety Symptom Questionnaire - Anhedonic Depression Subscale (MASQ-AD)⁶² is a 22-item scale that assesses anhedonia (i.e., loss of interest and low positive affect). MASQ-AD scores have been associated with blunted striatal response to reward in previous studies of patients with depression⁶³.
- The Greene Climacteric Scale (GCS)⁶⁴ is a 21-item scale that is the gold-standard measure of four domains of climacteric symptoms: vasomotor, somatic, anxiety, and depression.
- The Pittsburgh Sleep Quality Index (PSQI)⁶⁵ is a 9-item questionnaire that measures the quality and sleep patterns in older adults. *This measure will only be administered at the two fMRI sessions.

While the SCID and HAM-D are researcher administered, the other measures will be collected through self-report questionnaires using *Qualtrics* or paper and pencil questionnaires.

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 paid for by the study. If inpatient hospitalization becomes

necessary as a result of the study, UNC Health Care will cover the cost. The following language has been added to the study consent form to reflect this policy:

“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 cover the costs of such outpatient exams and treatment at UNC Health Care.

“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 reimburse you for reasonable and necessary 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 you 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.”

FMRI Tasks

The following task will be included in each of the two fMRI sessions:

The Monetary Incentive Delay (MID) Task⁶⁶ engages the reward circuitry during monetary incentive anticipation and outcomes. Each of two MID runs consists of 90 6-second trials during which subjects are presented with one of nine cue shapes, a fixation crosshair (for a variable duration), the target, and performance (win/loss/neutral) feedback. The cue indicates whether it is an incentive (gain, loss) or non-incentive trial. During incentive trials, participants can either gain or lose money by pressing a button during target presentation. Task difficulty is based on participant reaction times. This task elicits reliable frontostriatal dysregulation in people with remitted MDD⁶⁷. Resting state scans will also be conducted at the beginning and end of each session. The Hariri Emotional Faces Task is an emotional face-matching task that engages neuronal regions implicated in emotional processing, including the amygdala and dorsomedial PFC. This block design paradigm consists of 4 blocks of a perceptual face-processing task interleaved with 5 blocks of a sensorimotor control task. Participants are presented with a target stimulus and asked to select one of 2 images, presented on the low half of the screen, which match the target. Each face-processing block consists of fear and anger sub-blocks. During the sensorimotor control blocks, subjects view a trio of simple geometric shapes. Notably, this task elicited differences in amygdala activation between women with PPD and euthymic postpartum women in a previous study of PPD.

FMRI Data Acquisition and Image Processing

Scanning is performed using a Siemens Magnetom 3T TIM Trio scanner. 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 an event-related BOLD response analysis within *a priori* structurally defined frontostriatal regions of interest (ROIs). Contrasts of interest will include win versus non-win outcomes⁶⁸, although the anticipation of wins will also be explored.

Other Tasks

Probabilistic Reward Task (PRT)⁶⁹ will be conducted outside of the scanner. This task allows us to test the additional hypothesis that perimenopausal depression is characterized by blunted reward learning. During this task, correct identifications of two ambiguous stimuli are differentially rewarded. The task will consist of 300 trials, divided into 3 blocks of 100 trials. Each trial starts with the presentation of a fixation cross for 500 msec followed by a mouthless face. After a delay of 500 msec, either a short mouth (11.5 mm) or a long mouth (13 mm) is presented for 100 msec. Participants will be asked to identify which type of mouth was presented. For each block, the long and short mouths are presented equally often in a pseudorandomized sequence and 40 correct trials are followed by reward feedback immediately after the correct response. An asymmetrical reinforcer ratio will be used in this task, such that for half of the participants, correct identification of the short mouth will be associated with three times more positive feedback (30 of 40) than correct identification of the long mouth (10 of 40). For the other half of the participants, the contingencies will be reversed.

Participants will be paid same-day for the amount earned on the PRT and the MID. Participants will sign a form at that time to confirm they received payment and to confirm the amount they received. The money earned during the PRT and MID will be part of the \$500 compensation.

3.2 Primary Study Endpoints

1. Frontostriatal reactivity to reward during the MID fMRI task
2. Response latency to reward versus non-reward during the MID fMRI task
3. Depressive symptoms

3.3 Secondary Study Endpoints

1. Neural connectivity measured during resting-state fMRI

3.4 Primary Safety Endpoints

N/A

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Participants will include healthy, unmedicated perimenopausal women ages 44-55 either with ($n=20$) or without ($n=20$) current major depression. Thus, only participants capable of giving informed consent will be enrolled. Participants will be compensated upon completion of the study.

Inclusion Criteria.

1. Perimenopause Status: We will employ the Stages of Reproductive Aging Workshop (STRAW) criteria⁷⁰ to confirm perimenopausal status. The stages are primarily based on the characteristics of the menstrual cycle and secondarily on follicle stimulating hormone (FSH) levels. The anchor for the staging system is the final menstrual period (FMP). We will enroll women who have ≥ 2 skipped cycles and an interval of amenorrhea ≥ 60 days, consistent with the late menopause transition (stage -1), and who demonstrate an FSH level higher than two SDs above the mean for follicular phase premenopausal women based on UNC Hospitals laboratory levels ($x= 7$ IU/mL). Because extremes of body weight (BMI < 18 or > 30 kg/m²) or a history of chronic menstrual cycle irregularity can contribute to inaccurate reproductive staging⁷⁰, these will serve as additional exclusion criteria;
2. MDD Group Eligibility Criterion: current diagnosis of MDD with an onset associated with menstrual cycle irregularity, and no history of psychiatric illness during the 2 years before the onset of the current depressive episode as determined by the Structure Clinical Interview for DSM-IV-TR for Axis I Disorders (SCID);

3. Control Group Eligibility Criterion: absence of any past or present psychiatric disorder as assessed by the SCID.

4.2 Exclusion Criteria

. Patients will not be permitted to enter this protocol if they have any of the following:

1. current medication use (i.e., psychotropics, anti-hypertensives, statins, hormonal preparations, or frequent use of anti-inflammatory agents (> 10 times/month)). Women will be allowed to enroll who take medications without known mood effects (e.g. stable thyroid hormone replacement and occasional (< 5 times/month) use of Ambien)*;
2. pregnant, breastfeeding or trying to conceive;
3. FMP more than 12 months prior to enrollment;
4. history of undiagnosed vaginal bleeding;
5. undiagnosed enlargement of the ovaries;
6. polycystic ovary syndrome;
7. history of breast or ovarian cancer;
8. first degree relative with ovarian cancer;
9. first degree relative with premenopausal or bilateral breast cancer;
10. 2+ first degree relatives with breast cancer (regardless of onset);
11. 3+ relatives with postmenopausal breast cancer;
12. abnormal finding in a provider breast exam and/or mammogram;
13. known carrier of BRCA1 or 2 mutation;
14. endometriosis;
15. blood clots in the legs or lungs;
16. porphyria;
17. diabetes mellitus;
18. malignant melanoma;
19. Hodgkin's disease;
20. recurrent migraine headaches that are preceded by aura;
21. gallbladder or pancreatic disease**;
22. heart or kidney disease**;
23. liver disease;
24. cerebrovascular disease (stroke);
25. first degree relative with history of heart attack or stroke;
26. current cigarette smoking;
27. current suicidal ideation or psychosis;
28. past suicide attempts or psychotic episodes requiring hospitalization;
29. chronic depression (i.e., episode(s) lasting 3+ years);
30. recurrent depression (i.e., more than 1 prior episode, not including episodes with postpartum onset)
31. depressive episode(s) within 2 years of enrollment;
32. self-reported claustrophobia
33. peanut allergy

*all reported prescription medications will be reviewed and cleared by a study physician prior to a participant's enrollment;

**participants will be given the opportunity to describe these conditions in the online screening survey. Reported conditions that are acute in nature and/or benign will be reviewed by a study physician and exclusions will be decided case-by-case. All chronic conditions will be exclusionary.

4.3 Subject Recruitment and Screening

Methods of recruiting for this study include:

- Letters
- Flyers
- E-mail announcements

- Facebook advertisements
- Craigslist advertisements

Recruitment materials (e.g., advertisements) will also be placed in university buildings and local businesses, and a university wide email will be used to recruit participants. Advertisements, including the letter, flyer, and email, will instruct participants to call our research lab to complete a phone screen or follow a link to our online eligibility survey. The phone screen and online eligibility survey include same questions (see attached).

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. No pregnant women will be entered into the study, because the study drugs (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 estrogen administration.

Adverse mood symptoms will be monitored by administering the the Hamilton Rating Scale for Depression (HRSD) at each study visit. **If suicidal thoughts or severe mood symptoms are observed at any period in the medication phase, then the suicide item of the HRSD will be administered on consecutive days for three days. Anyone who expresses suicidal thoughts with plans or intent, or who expresses concern about her 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. Although we do not anticipate severe adverse reactions, we have arranged for inpatient hospital admission in the Psychiatry Department 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.

The determination of when and how to withdrawal subjects will be overseen by the study's principal investigator, co-investigators, and research coordinators 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	100 micrograms/day	Transdermal
Progesterone	200 miligrams/day	Oral capsule

5.2 Treatment Regimen

Regimen

Once determined eligible and physically well, participants will receive transdermal estradiol (100 µg/day) for 3 weeks. Women will receive an additional week of combined estradiol and micronized progesterone (200 mg/day) at the end of the study to precipitate menstruation.

5.3 Method for Assigning Subjects to Treatment Groups

This is a case-control study design, 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
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 collected at weekly 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 free of medications that have known CNS effects 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. Estradiol patches will be provided to participants at weekly study visits. The patches release estradiol for a week. Progesterone capsules will be provided to participants at the final study visit.

5.8 Blinding of Study Drug (if applicable)

N/A

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 or research coordinator to deliver to participants. Any unused drug will be returned to the UNC Investigational Drug Service by the PI or research coordinator.

5.9.1 Receipt of Drug Supplies

Upon receipt of the 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

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 as 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

The flowchart outlining the procedures at each study visit is attached.

OBGYN Exam

Participants will receive a gynecological exam prior to drug administration to rule out exclusionary health problems. Results of the exam will be added to participants' UNC Hospital medical record. However, participants who can provide documentation of a normal gynecological exam within the last year will not receive the gynecological exam.

Venipuncture

Participants will undergo venipuncture at 5 study visits. Initial blood tests will include a pregnancy test, FSH, LH, BUN, creatinine, 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 levels to ensure participant compliance.

Drug Administration

Participants will receive estradiol at study visits 3-5. Participants will receive Progesterone at visit 6.

Clinical Assessments

The Structured Clinical Interview for DSM-IV-TR Axis-I Disorders (SCID)⁵⁸ and the Schedule for The SCID1 will be administered at baseline to determine study eligibility. The following standard measures will be administered at baseline, both fMRI sessions, and weekly clinic visits:

- The Positive and Negative Affect Schedule⁵⁹ is a 20-item questionnaire that measures both positive affect (PA) and negative affect (NA) and is sensitive to subtle changes in affect over time.
- The Hamilton Rating Scale for Depression (HRSD)⁶⁰ is a 21-item researcher-rated standardized scale that assesses depressive symptoms and is widely used as a measure of symptom change over time.
- The Inventory of Depression and Anxiety Symptoms (IDAS)⁶¹ is a 64-item self-report questionnaire that comprehensively assesses anxiety and depression symptoms on 10 subscales. The IDAS has excellent psychometric properties and has been validated for assessing reproductive mood disorders.
- The Mood and Anxiety Symptom Questionnaire - Anhedonic Depression Subscale (MASQ-AD)⁶² is a 22-item scale that assesses anhedonia (i.e., loss of interest and low positive affect). MASQ-AD scores have been associated with blunted striatal response to reward in previous studies of patients with depression⁶³.
- The Greene Climacteric Scale (GCS)⁶⁴ is a 21-item scale that is the gold-standard measure of four domains of climacteric symptoms: vasomotor, somatic, anxiety, and depression.
- The Pittsburgh Sleep Quality Index (PSQI)⁶⁵ is a 9-item questionnaire that measures the quality and sleep patterns in older adults. *This measure will only be administered at the two fMRI sessions.

While the SCID and HAM-D are researcher administered, the other measures will be collected through self-report questionnaires using *Qualtrics* or paper and pencil questionnaires.

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, including inpatient hospitalization.

FMRI Tasks

The following task will be included in each of the two fMRI sessions:

The Monetary Incentive Delay (MID) Task⁶⁶ engages the reward circuitry during monetary incentive anticipation and outcomes. Each of two MID runs consists of 90 6-second trials during which subjects are presented with one of nine cue shapes, a fixation crosshair (for a variable duration), the target, and performance (win/loss/neutral) feedback. The cue indicates whether it is an incentive (gain, loss) or non-incentive trial. During incentive trials, participants can either gain or lose money by pressing a button during target presentation. Task difficulty is based on participant reaction times. This task elicits reliable frontostriatal dysregulation in people with remitted MDD⁶⁷. Resting state scans will also be conducted at the beginning and end of each session.

The Hariri Emotional Faces Task is an emotional face-matching task that engages neuronal regions implicated in emotional processing, including the amygdala and dorsomedial PFC. This block design paradigm consists of 4 blocks of a perceptual face-processing task interleaved with 5 blocks of a sensorimotor control task. Participants are presented with a target stimulus and asked to select one of 2 images, presented on the low half of the screen, which match the target. Each face-processing block consists of fear and anger sub-blocks. During the sensorimotor control blocks, subjects view a trio of simple geometric shapes. Notably, this task elicited differences in amygdala activation between women with PPD and euthymic postpartum women in a previous study of PPD.

Other Tasks

Pizzagalli's Probabilistic Reward Task (PRT)⁶⁹ will be conducted outside of the scanner. This task allows us to test the additional hypothesis that perimenopausal depression is characterized by blunted reward learning. During this task, correct identifications of two ambiguous stimuli are differentially rewarded. The task will consist of 300 trials, divided into 3 blocks of 100 trials. Each trial starts with the presentation of a fixation cross for 500 msec followed by a mouthless face. After a delay of 500 msec, either a short mouth (11.5 mm) or a long mouth (13 mm) is presented for 100 msec. Participants will be asked to identify which type of mouth was presented. For each block, the long and short mouths are presented equally often in a pseudorandomized sequence and 40 correct trials are followed by reward feedback immediately after the correct response. An asymmetrical reinforcer ratio will be used in this task, such that for half of the

participants, correct identification of the short mouth will be associated with three times more positive feedback (30 of 40) than correct identification of the long mouth (10 of 40). For the other half of the participants, the contingencies will be reversed.

7 Statistical Plan

7.1 Sample Size Determination

We approximated power and sample size using a model comparing the two groups on change-scores from baseline to post-treatment. With 20 subjects per group, we have 80% power to detect effect sizes of 0.9, which represents a large effect size. Given our strong preliminary evidence and the experimental nature of the proposed research, we expect to detect statistically significant results. Previous clinical research supports our hypothesized effect size. Studies comparing participants with MDD and controls have yielded effect sizes (Cohen's d) ranging from 1.0 to 1.56 in ventral striatum responsivity to reward 51-53. We will enroll an additional 10 participants to account for attrition, increasing the total N to 50.

7.2 Statistical Methods

This study has one between-subjects factor (group: MDD versus control) crossed with one within-subjects factor (time: baseline versus estradiol treatment) with the outcome (percent signal changed) assessed in response to the MID task in the structurally defined frontostriatal ROIs, including the nucleus accumbens, caudate, thalamus, frontopolar cortex, and dorsolateral prefrontal cortex. Thus, for a task/ROI combination, there is a 2-by-2 repeated measures design. We will analyze this design using an ROI analysis and mixed models with an unstructured covariance structure. To test whether perimenopausal women with MDD show reduced frontostriatal activity (H1) and motivated behavior (H2) in response to reward at baseline, we will examine group differences in BOLD signal and response latency at baseline. To measure the psychological correlates of the frontostriatal response to reward, we will examine change in depressive symptoms between baseline and post-treatment in MDD relative to controls (H3).

7.3 Subject Population(s) for Analysis

Given that we are interested in understanding the effect of estradiol on brain function, only data from protocol-compliant participants will be subjected to the study analysis.

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.

Preexisting Condition

A preexisting 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 any one of the following 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.

8.2 Recording of Adverse Events

At each contact 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
- Study Center
- 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 PI and IRB 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 IRB 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*

Investigators who are not UNC faculty or affiliated with a UNC research site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting

requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.

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

N/A

8.5 Stopping Rules

The protocol only includes rules for discontinuing individual participant, but not rules for stopping the entire study:

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 the Hamilton Rating Scale for Depression (HRSD) at each study visit. **If suicidal thoughts or severe mood symptoms are observed at any period in the medication phase, then the suicide item of the HRSD will be administered on consecutive days for three days. Anyone who expresses suicidal thought with plans or intent, or who expresses concern about her 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. Although we do not anticipate severe adverse reactions, we have arranged for inpatient hospital admission in the Psychiatry Department 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.

The determination of when and how to withdrawal subjects will be overseen by the study's principal investigator, co-investigators, and research coordinators and will be reported to the UNC Biomedical IRB.

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.

The Principal Investigator's responsibilities are to:

- review the research protocol, informed consent documents, and plans for data safety and monitoring;
- 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;
- 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;
- protect the safety of the study participants;
- report on the safety and progress of the trial;
- make recommendations to the UNC-CH IRB, the PI, 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;
- if appropriate, conduct 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;
- ensure the confidentiality of the trial data and the results of monitoring; and,
- assist the UNC-CH IRB by commenting on any problems with study conduct, enrollment, and sample size and/or data collection.

8.6.1 Independent Data and Safety Monitoring Board

N/A

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). 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, 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.

In the event that the research team is unable to contact a subject (either by 3 phone calls, or over 2 days' time), Dr. Schiller will contact the subject's emergency contact, thereby breaching confidentiality. This will be communicated to the subject at the time of consent, and she will be encouraged to inform her emergency contact person of this plan.

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.

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. All missing data must be explained. 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.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Appendix _____. 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 grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

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 \$500.00. 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 Psychological Interview (Visit 1; 2.5 hours) \$100.00
Physical Exam (Visit 2; 1-2 hours) \$30.00
fMRI Imaging combined with clinic visits (Visits 3 and 6) \$150.00/visit -- \$300.00 total
Clinic visits with venipuncture (Visits 4 and 5) \$35.00/visit -- \$70.00

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.

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