# *Effects of a Tissue Selective Estrogen Complex on Depression and The Neural Reward System in the Perimenopause (Duavee)*

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# *EFFECTS OF A TISSUE SELECTIVE ESTROGEN COMPLEX ON DEPRESSION AND THE NEURAL REWARD SYSTEM IN THE PERIMENOPAUSE (DUAVEE)*

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**Study Product:**

**Conjugated estrogens/bazedoxifene (Duavee)**
Active ingredients: conjugated estrogens, bazedoxifene
Chemical name: sodium estrone sulfate and sodium equilin sulfate, and sodium sulfate conjugates, 17α-dihydroequilin, 17α-estradiol, and 17β-dihydroequilin; 1*H*-Indol-5-ol, 1-[[4-[2-(hexahydro-1*H*-azepin-1-yl) ethoxy]phenyl]methyl]-2-(4-hydroxyphenyl)-3-methyl-, monoacetate

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## List of Abbreviations

| | |
|---|---|
| AE | Adverse Event |
| BOLD | Blood-oxygen-level-dependent |
| BRIC | Biomedical Research Imaging Center |
| CHD | Coronary heart disease |
| COVID-19 | Coronavirus Infection |
| 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 |
| HIPAA | Health Insurance Portability and Accountability Act |
| HRSD | Hamilton Rating Scale for Depression |
| IDAS | Inventory of Depression and Anxiety Symptoms |
| IDS | Investigational Drug Service |
| 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 |
| TSEC | Tissue Selective Estrogen Complex |
| UNC-CH | University of North Carolina at Chapel Hill |
| WHI | Women’s Health Initiative |

# Study Summary

<table>
<tr><td>Title</td><td>Effects of a Tissue Selective Estrogen Complex on Depression and the Neural Reward System in the Perimenopause</td></tr>
<tr><td>Short Title</td><td>Duavee</td></tr>
<tr><td>Protocol Number</td><td>IRB# 18-2129</td></tr>
<tr><td>Phase</td><td>N/A</td></tr>
<tr><td>Methodology</td><td>Experimental study</td></tr>
<tr><td>Study Duration</td><td>12-24 months</td></tr>
<tr><td>Study Center(s)</td><td>Single-center</td></tr>
<tr><td>Objectives</td><td>There are two practical objectives:<br>1. To determine the extent to which TSEC reduces anhedonia and other depressive symptoms in PM-MDD. Anhedonia and other depressive symptoms will be assessed at baseline and following 3 weeks of TSEC administration.<br>2. To quantify the effect of TSEC on the neural reward system in PM-MDD. We will use fMRI at baseline and following TSEC treatment in PM-MDD to probe frontostriatal reward circuitry. We also will compare the effects of TSEC and ERT in PM-MDD.</td></tr>
<tr><td>Number of Subjects</td><td>20</td></tr>
<tr><td>Diagnosis and Main Inclusion Criteria</td><td><u>Women with Perimenopausal Depression</u><br>1)The anchor for the staging system is the last menstrual period (LMP) we will enroll women who have ≥ 2 skipped cycles and an interval of amenorrhea ≥ 60 days, consistent with the late menopause transition (stage -1). Women who meet these criteria will not require an FSH level. Women who have taken oral contraceptives continuously for relief of perimenopausal symptoms and women who have had hysterectomies (with ovaries remaining intact) will be exempt from our LMP criteria, and their perimenopausal status will be determined by FSH level instead (FSH ≥ 14).<br>2) 44-55 years old;<br>3) current diagnosis of MDD with an onset associated with menstrual cycle irregularity. Past mania, psychosis, suicide attempts, and alcohol or drug dependence, as determined by the SCID are exclusionary. All other current comorbid psychiatric illnesses, as determined by the SCID, will be reviewed with our primary investigator (PI) to determine eligibility on a case-by-case basis.</td></tr>
<tr><td>Study Product, Dose, Route, Regimen</td><td>Study Product<br><br>Conjugated estrogens/bazedoxifene (Duavee)<br>Active ingredients: conjugated estrogens, bazedoxifene acetate<br>Chemical name: sodium estrone sulfate and sodium equilin sulfate, and sodium sulfate conjugates, 17α-dihydroequilin, 17α-estradiol, and 17β-dihydroequilin; 1H-Indol-5-ol, 1-[[4-[2-(hexahydro-1H-azepin-1-yl) ethoxy]phenyl]methyl]-2-(4-hydroxyphenyl)-3-methyl-, monoacetate<br><br>
<table>
<tr><th>Drug</th><th>Dosage</th><th>Formulation</th></tr>
<tr><td>Conjugated estrogens/bazedoxifene</td><td>0.45 milligrams/20 milligrams/day</td><td>Oral tablet</td></tr>
</table>
Route:</td></tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><th>Drug</th><th>Route of Administration</th></tr>
<tr><td>Conjugated estrogens/bazedoxifene</td><td>Oral</td></tr>
</table>
<br>Regimen:<br>Once determined eligible and physically well, participants with perimenopausal depression will receive a tissue selective estrogen complex (TSEC) (conjugated estrogens/bazedoxifene, 0.45mg/20mg) for 3 weeks. Prior to beginning medication, women will have a basic lab panel and serum pregnancy test, pelvic exam, breast exam, and PAP smear. Participants who can provide record of a pelvic exam and breast exam within the past year and normal pap results within the past three years will be permitted to decline the GYN exam. Women will be seen in the clinic each week during TSEC administration to assess blood levels of estradiol and mood symptoms.
</td></tr>
<tr><td>Duration of administration</td><td>3 weeks</td></tr>
<tr><td>Reference population</td><td>Perimenopausal women, non-depressed</td></tr>
<tr><td>Statistical Methodology:</td><td>We will test our H1 hypothesis using a paired t-test of MASQ Anhedonia scores at visit 1 (pre-treatment) and visit 6 (post-treatment). We will test our H2 hypothesis using a paired t-test to compare activity in 3 regions of interest (ROIs)—caudate, putamen, and nucleus accumbens—at the pretreatment and posttreatment scans.</td></tr>
</table>

# 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 worldwide[1]. Unraveling the pathophysiology of affective disorders has been uniquely challenging because depressive syndromes are heterogeneous and have diverse etiologies[2]. 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 conditions[3]. 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 perimenopause[4]; treatment with estrogen reduces mood symptoms[5]; 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 markers[6].

One of the most powerful tools for understanding the neural mediators of complex behaviors is brain imaging[7]. 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 responsiveness[8]. 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 depression[9]. In individuals with depression, anhedonia is associated with frontostriatal hypoactivity during reward processing[10]. 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 women[13]. 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.

While estrogen replacement therapy acts as an antidepressant, many women elect not to take estrogen and many physicians discourage its use because of the risk of long-term negative health effects, including breast and uterine cancer. Estrogens can be paired with selective estrogen receptor modulators (SERMs), synthetic non-steroidal agents that have estrogen agonist and antagonist effects in different tissues, to block the negative effects of estrogen on non-target tissues, including the reproductive organs and cardiovascular system, while allowing estrogen to exert effects on target tissues like the brain [14]. The first such tissue selective estrogen complex (TSEC) approved by the FDA is Duavee, which combines conjugated estrogens and the SERM bazedoxifene [14]. Bazedoxifene has antiestrogenic effects in the endometrium and breast, protecting against uterine and breast cancer, and estrogenic effects in bone, making it a potent preventive agent and treatment for osteoporosis. Duavee has been shown to alleviate hot flashes and vaginal atrophy and prevent osteoarthritis. As a result, Duavee may be an ideal agent to reduce depressive symptoms without the attendant risks of estrogen replacement therapy (ERT) or

adjunctive progesterone treatment. However, the effects of Duavee on depression and the neural reward circuit have never been tested, and one can't infer that estrogen will have the same antidepressant effects in the presence of bazedoxifene (which partially blocks the effects of estrogen in certain tissues). The purpose of our additional treatment arm is to 1) test the antidepressant effects of TSEC, and 2) determine whether TSEC restores neural reward sensitivity, which is blunted in the absence of estrogen.

Thus, we will examine the effects 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 respond to TSEC in those with perimenopausal depression? 2) Does reward system activity at baseline predict the antidepressant effects of TSEC 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. It will also allow us to measure frontostriatal responses to TSEC treatment in perimenopausal depression, which has not 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 examine the neural pathophysiology of reproductive mood disorders and conduct experimental hormone investigations of the neural circuits contributing to affective illness in women.

The use of ERT to treat perimenopausal major depressive disorder (PM-MDD) is a relatively nascent area of investigation but one of great importance given the large and increasing number of women who enter the perimenopause each year [15, 16] and are potentially at risk for depression [17, 18]. Untreated mood disorders in perimenopausal women contribute significantly to cardiovascular deaths [19]. Perimenopausal women with depressive symptoms seek treatment from gynecologists, psychiatrists, and general practitioners, yet there is neither consensus nor practical guidelines for preventing and treating perimenopausal depression. Moreover, as mentioned above, many women and physicians do not pursue ERT because of the potential adverse consequences and the need for adjunctive treatment with progesterone to protect the endometrium, which can have negative mood consequences in women who are hormone sensitive. Further, traditional antidepressants are not a viable therapeutic option for PM-MDD consequent to limited efficacy [20, 21] and fears regarding side effects and stigma [22, 23]. Because TSEC addresses the main potential problems of ERT, this research will provide important information for 1) developing new medications for PM-MDD, and 2) understanding the neural mechanisms that contribute to depression during periods of reproductive hormone change.

We will be able to determine whether Duavee can be used to treat PM-MDD and the brain circuits involved in depression and its treatment. This research will also provide information essential for 1) identifying which patients will benefit most from hormone therapies and 2) developing new medications for PM-MDD.

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, TSEC, 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 TSEC treatment.

## *1.2 Investigational Agent.*

**CONJUGATED ESTROGENS/BAZDOXIFENE (DUAVEE)**

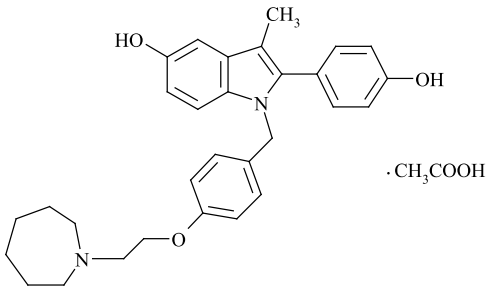
Description

DUAVEE (conjugated estrogens/bazedoxifene) tablets contain conjugated estrogens with bazedoxifene, an estrogen agonist/antagonist. Conjugated estrogens are purified from pregnant mares' urine and

consist of the sodium salts of water-soluble estrogen sulfates blended to represent the average composition of material derived from pregnant mares' urine. Conjugated estrogens are a mixture of sodium estrone sulfate and sodium equilin sulfate, and also contain as concomitant components, sodium sulfate conjugates, 17α-dihydroequilin, 17α-estradiol, and 17β-dihydroequilin.

Bazedoxifene is supplied as the acetate salt (bazedoxifene acetate) and has the chemical name 1*H*-Indol-5-ol, 1-[[4-[2-(hexahydro-1*H*-azepin-1-yl) ethoxy]phenyl]methyl]-2-(4 hydroxyphenyl)-3-methyl-, monoacetate. The empirical formula is C30H34N2O3 • C2H4O2, and the molecular weight is 530.65.

Bazedoxifene acetate is a white to tan powder. The aqueous solubility of bazedoxifene is pH- dependent. Solubility is higher at lower pH. The solubility of bazedoxifene acetate in unbuffered sterile water was measured to be 923 microgramsA/mL at pH 5.4. The following represents the chemical structure of bazedoxifene acetate:




DUAVEE is available for oral administration as tablets containing 0.45 mg of conjugated estrogens with 20 mg of bazedoxifene (equivalent to 22.6 mg of bazedoxifene acetate). Each tablet of DUAVEE contains the following inactive ingredients: calcium phosphate tribasic, hydroxypropyl cellulose, microcrystalline cellulose, powdered cellulose, hypromellose, lactose monohydrate, magnesium stearate, polyethylene glycol, sucrose, ascorbic acid, sucrose palmitic acid ester, hydroxyethylcellulose, titanium dioxide, red iron oxide, yellow iron oxide, black iron oxide, povidone, polydextrose, maltitol, poloxamer 188, propylene glycol, and isopropyl alcohol.

Pharmacology

DUAVEE pairs conjugated estrogens with bazedoxifene. Conjugated estrogens and bazedoxifene function by binding to and activating estrogen receptors (ER) α and β, which vary in proportion from tissue to tissue. Conjugated estrogens are composed of multiple estrogens and are agonists of ER- α and β. Bazedoxifene is an estrogen agonist/antagonist that acts as an agonist in some estrogen-sensitive tissues and an antagonist in others (e.g., uterus). The pairing of conjugated estrogens with bazedoxifene produces a composite effect that is specific to each target tissue. The bazedoxifene component reduces the risk of endometrial hyperplasia that can occur with the conjugated estrogens component.

Pharmacodynamic studies have not been conducted with DUAVEE.

**Summary of Previous Human Experience**

We will enroll perimenopausal women to undergo TSEC supplementation using an existing recruitment infrastructure. We currently are assessing the neural reward system in PM-MDD using functional magnetic resonance imaging (fMRI) at baseline and following ERT in our “Effects of Neural Reward System and Depression in the Perimenopause” study (#13-3572). Over the 45 months of

recruitment with ads that specify no current depression, 10,721 women were screened, 244 (2%) of whom were not eligible based on current MDD symptoms.

Support for the hypotheses comes from our lab's own research and that of others, to include:

1. **Estradiol withdrawal precipitates anhedonia in rats, and decreasing estradiol levels are associated with increasing negative mood symptoms in women**[11]. These results support our 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 menopause[4]. 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-menopause[16]. 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 perimenopause[20], 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 placebo[5] (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 MDD[18].
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 cortices[19], 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: We will be administering estrogen paired with selective estrogen receptor modulators (SERMs) to a subset of women with perimenopausal women. SERMs are synthetic non-steroidal agents that have estrogen agonist and antagonist effects in different tissues, to block the negative effects of estrogen on non-target tissues, including the reproductive organs and cardiovascular system, while allowing estrogen to exert effects on target tissues like the brain, and thus decreasing risk for side effects.

**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. TSECs, the form of estrogen that we use in this study, is administered as a sole agent 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.

**Conjugated Estrogens/Bazdoxifene (Duavee):** The first such tissue selective estrogen complex (TSEC) approved by the FDA is Duavee, which combines conjugated estrogens and the SERM bazedoxifene [1]. Bazedoxifene has antiestrogenic effects in the endometrium and breast, protecting against uterine and breast cancer, and estrogenic effects in bone, making it a potent preventive agent and treatment for osteoporosis. Duavee has been shown to alleviate hot flashes and vaginal atrophy and prevent osteoarthritis. The safety of conjugated estrogens/bazedoxifene was evaluated in four Phase 3 clinical trials (sponsored by Wyeth) ranging from 12 weeks to 24 months in duration and enrolling 6,210 postmenopausal women age 40 to 75 years (mean age 55 years). A total of 1,224 patients were treated with DUAVEE and 1,069 patients received placebo. The incidence of all-cause mortality was 0.0% in the DUAVEE group and 0.2% in the placebo group. The incidence of serious adverse reactions was 3.5% in the DUAVEE group and 4.8% in the placebo group, and 7.5% of the DUAVEE group and 10.0% of the placebo group withdrew [1]. The most common adverse reactions leading to discontinuation were hot flashes, upper abdominal pain, and nausea. Other less common adverse reactions included diarrhea, dyspepsia, muscle spasms, neck pain, dizziness and oropharyngeal pain [1]. The incidence of endometrial hyperplasia was less than 1% in both clinical studies examining the effect of Duavee on the endometrium [1, 2, 3, 4]. The SMART study examined safety and efficacy of Duavee and found that women reported low incidences of breast pain and high incidences of cumulative amenorrhea, in addition to relief of vasomotor symptoms [5].

## *1.5 Dose Rationale and Risk/Benefits*

This study will enroll perimenopausal women to undergo TSEC 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. TSEC 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.

### *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 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 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. Our central hypothesis is that the neural reward system is hypoactive in PM-MDD, and the antidepressant effects of a three-week TSEC intervention will be associated with increased activity in the neural reward system, assessed using functional magnetic resonance imaging (fMRI).

**Aim 1: Determine the extent to which TSEC reduces anhedonia and other depressive symptoms in PM-MDD.** Anhedonia and other depressive symptoms will be assessed at baseline and following 3 weeks of TSEC administration.

**Aim 2 (Exploratory): Quantify the effect of TSEC on the neural reward system in PM-MDD.** We will use fMRI at baseline and following TSEC treatment in PM-MDD to probe frontostriatal reward circuitry. We also will compare the effects of TSEC and ERT in PM-MDD.

Null hypothesis ($H_0$): TSEC has no salutary effect on depression.

Alternative hypothesis ($H_1$): TSEC reduces depression as evidenced by 1) a statistically significant decrease in average MASQ Anhedonia scale score following 3 weeks of treatment, and 2) a clinically significant increase of 1 SD in MASQ Anhedonia scale score.

We will test this hypothesis using a paired t-test of MASQ Anhedonia scores at visit 1 (pre-treatment) and visit 6 (post-treatment).

**Aim 2 (Exploratory): Quantify the effect of TSEC on the neural reward system in PM-MDD.** We will use fMRI at baseline and following TSEC treatment in PM-MDD to probe frontostriatal reward circuitry. We also will compare the effects of TSEC and ERT in PM-MDD.

Null hypothesis ($H_0$): TSEC has no meaningful effect on activity of the neural reward circuit in response to rewards.

Alternative hypothesis ($H_1$): TSEC reduces increases neural responsiveness to reward in the frontostriatal reward circuitry as evidenced by increased activation of the caudate, putamen, and nucleus accumbens in PM-MDD in response to reward following TSEC administration (compared with baseline).

We will test this hypothesis using a paired t-test to compare activity in 3 regions of interest (ROIs)—caudate, putamen, and nucleus accumbens—at the pretreatment and posttreatment scans.

# 3 Study Design

## *3.1 General Design*

*Overview*. This is an observational, single-center study using tissue-selectective estrogen complex (TSEC) to examine the role of frontostriatal hypoactivity in the pathophysiology or perimenopausal MDD. This study will include 6 study visits that will take place over the course of approximately 6 weeks. Out of those 6 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. Five visits will require brief in-person contact for phlebotomy, 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. Perimenopausal MDD women will receive Duavee, a TSEC, for 3 weeks. FMRI sessions will occur at baseline and at the end of the third week of medication administration. The procedures that will take place are outlined in **Table 1** and detailed below.

**Table 1. Study Procedures and Timeline**

| **Study Visits** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Maximum duration of in-person visit (hours) | | 1 | 2 | 0.15 | 0.15 | 2 |
| Consent | X | | | | | |
| OBGYN Exam | | X | | | | |
| fMRI | | | X | | | X |
| PRT | | | X | | | X |
| Venipuncture | | X | X | X | X | X |
| SCID | X | | | | | |
| SNAP | X | | X | X | X | X |
| HAM-D | X | | X | X | X | X |
| IDAS | X | | X | X | X | X |
| GCS | X | | X | X | X | X |
| PSQI | | | X | | | X |
| HHQ | | | | | | X |
| THQ | | | | | | X |
| Duavee Rx Provided | | | X | X | X | |
| Payment | $100 | $30 | $150 | $35 | $35 | $150 |

*Participants*. Participants will include healthy, unmedicated perimenopausal women ages 44-55 with (*n*=20) current major depression.

*Hormone Administration*.
Prior to beginning medication, women will have a basic lab panel and serum pregnancy test, pelvic exam, breast exam, and PAP smear. Participants who can provide record of a pelvic exam and breast exam within the past year and normal pap results within the past three years will be permitted to decline the GYN exam. Women will be seen in the clinic each week during medication administration to assess blood levels of estradiol and mood symptoms.

*Optional Home Visits:* In an effort to increase recruitment and retention, the study team will offer home visits for Study Visits 1, 4, 5, as well as Baseline Labs.

*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 Schedule[59] 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 depression[63].
- 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.
- The Hormone History Questionnaire is a 19-item inventory that assesses women's mental and physical health and was created for the purpose of this study.
- The Trauma History Questionnaire [14] is a 24-item measure that examines participant's experiences with potentially serious or traumatic life events.

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 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."

*FMRI Tasks*

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

The Monetary Incentive Delay (MID) Task[66] 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 of 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 MDD[67]. Resting state scans will also be conducted at the beginning and end of each session.

*FMRI Data Acquisition and Image Processing*

Scanning is performed using a Siemens Magnetom 7T 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 outcomes[68], 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.

## *3.2 Primary Study Endpoints*

1. Frontiostriatal 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 Exploratory 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 with (*n*=20) current major depression. 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) criteria to confirm perimenopausal status. The stages are primarily based on characteristics of the menstrual cycle and secondarily on follicle stimulating hormone (FSH) levels. The anchor for the staging system is the last menstrual period (LMP). We will enroll women who have ≥ 2 skipped cycles with an interval of amenorrhea ≥ 60 days and had a LMP within the last year, consistent with the late menopause transition (stage -1). Women who meet these criteria will not require an FSH level*. Women who have taken oral contraceptives continuously for relief of perimenopausal symptoms and women who have had hysterectomies (with ovaries remaining intact) will be exempt from our LMP criteria, and their perimenopausal status will be determined by FSH level instead (FSH ≥14)*.
2. MDD Eligibility Criterion: current diagnosis of MDD with an onset associated with menstrual cycle irregularity. Past mania, psychosis, suicide attempts, and alcohol or drug dependence, as determined by the Structure Clinical Interview for DSM-IV-TR for Axis I Disorders (SCID) are exclusionary. All other current comorbid psychiatric illnesses, as determined by the SCID, will be reviewed with our primary investigator (PI) to determine eligibility on a case-by-case basis.

## *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:

1. current medication use (i.e., psychotropics, anti-hypertensives, statins,or hormonal preparations). 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. LMP 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 onset 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. current cigarette smoking;
26. current suicidal ideation, mania, psychosis, or alcohol/drug dependence;
27. past suicide attempts, mania, alcohol/drug dependence, or psychotic episodes;
28. chronic depression (i.e., episode(s) lasting 3+ years);
29. depressive episode(s) within 2 years of enrollment;
30. self-reported claustrophobia
31. Allergies to red or yellow dye.

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

**women who have recently taken oral contraceptives continuously for relief of perimenopausal symptoms will be exempt from LMP criteria, and instead, the presence of menstrual irregularity prior to the use of oral contraceptives and elevated FSH will be used to determine their perimenopausal status;

***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.

There is no evidence for first degree relative with history of MI/CVA as a contraindication for short-term ERT, and in our first 6 months of recruitment we have come to find this criterion superfluous for our purposes. Per the Endocrine Society Scientific Statement, thrombosis risk in HRT users is substantially increased with oral HRT administration and obesity; neither of which applies to our participants. Further, the American College of Obstetricians and Gynecologists does not list family history of MI/CVA as a contraindication for HRT. In our recruitment to date we have assessed many women with family history of MI/CVA who are healthy and otherwise appropriate for the study, and we do not want to continue to exclude them unnecessarily.

Santen, R. J., Allred, D. C., Ardoin, S. P., Archer, D. F., Boyd, N., Braunstein, G. D., ... Endocrine Society. (2010). Postmenopausal hormone therapy: an Endocrine Society scientific statement. The Journal of Clinical Endocrinology and Metabolism, 95(7 Suppl 1), s1–s66. ,

ACOG Practice Bulletin No. 141: management of menopausal symptoms. (2014). Obstetrics and Gynecology, 123(1), 202–216.

## *4.3 Subject Recruitment and Screening*

**Methods of recruiting for this study include**:

- Letters
- Flyers
- E-mail announcements
- Facebook advertisements
- Instagram advertisements
- Craigslist advertisements
- Newspaper and magazine advertisements

A number of women from our “Effects of Estradiol on Neural Reward System and Depression in the Perimenopause” (PEERS) study were ineligible on the basis of current depression. These women will be sent a letter as they may qualify for our study and have interest in participating. In addition, participants will be self-identified by responding to flyers in the community. A university wide email will also be used to recruit participants.

The letters described above will be used to recruit potential subjects. 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 participant's 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 (Duavee) may be associated with fetal harm.

## *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 Hamilton Rating Scale for Depression (HRSD) at each study visit. If suicidal thoughts or severe mood symptoms a**re observed at any period in the protocol, then the HRSD will be administered on consecutive days for three days. Anyone scoring > 20 on the HRSD or 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.

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, fMRIs will not be done if participants discontinue the hormone protocol.

# 5 Study Drug

## *5.1 Description*

| Drug | Dosage | Formulation |
|---|---|---|
| Conjugated estrogens/bazedoxifene | 0.45 milligrams/20 milligrams/day | Oral tablet |

## *5.2 Treatment Regimen*

**Regimen**

Once determined eligible and physically well, participants with perimenopausal depression will receive TSEC (conjugated estrogens/bazedoxifene, 0.45mg/20mg) for 3 weeks. Prior to beginning medication, women will have a basic lab panel and serum pregnancy test, pelvic exam, breast exam, and PAP smear. Participants who can provide record of a pelvic exam and breast exam within the past year and normal pap results within the past three years will be permitted to decline the GYN exam. Women will be seen in the clinic each week during TSEC administration to assess blood levels of estradiol and mood symptoms.

## *5.3 Method for Assigning Subjects to Treatment Groups*

N/A

## *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 medication 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 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. All the Duavee tablets will be provided to participants at study visit 3, in its original blister packaging. Duavee tablets are to be taken once daily. The remaining tablets will be returned to the study team at the conclusion of each subject's participation in the study, and returned to IDS for appropriate disposal.

## *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) 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

Duavee 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 the manufacturer's blister packaging. Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining at each study visit. 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 it attached.

*OBGYN Exam*

Prior to beginning medication, women will have a basic lab panel and serum pregnancy test, pelvic exam, breast exam, and PAP smear. Participants who can provide record of a pelvic exam and breast exam within the past year and normal pap results within the past three years will be permitted to decline the GYN exam. Results of the exam will be added to the participants' UNC Hospital medical record.

*Venipuncture*

Participants will undergo venipuncture at 5 study visits. Initial blood tests will include BUN, creatinine, ALT, AST, potassium, chloride, calcium, alkaline phosphatase, and a CBCD 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. During the context of COVID-19 venipuncture will be carried out by fully vaccinated study team members in private outdoor settings when possible, such as the subject's backyard.

*Drug Administration*

Participants will receive their entire supply of Duavee at study visit 3.

*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 Schedule[59] 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 depression[63].
- 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.
- The Hormone History Questionnaire is a 19-item inventory that assesses women's mental and physical health and was created for the purpose of this study.
- The Trauma History Questionnaire [14] is a 24-item measure that examines participant's experiences with potentially serious or traumatic life events.

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) Task[66] 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 of 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 MDD[67]. 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.

FMRI Data Acquisition and Image Processing. Scanning was initially performed using a Siemens Magnetom 3T TIM Trio scanner. In July 2016, the scanner was upgraded to a Siemens 3T Prisma, and as such, all scanning will be performed on the Prisma system going forward. Scanning parameters on the Prima were set to be identical to the Trio in order to minimize the effects of scanner on the data.

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 outcomes[9], although the anticipation of wins will also be explored.

Pizzagalli's Probabilistic Reward Task (PRT) [12] 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 1 00 trials, in 1 block. 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*

The researchers will have 80% power to detect a large effect size (.7) in 20 women. Our recently completed study of the effects of transdermal estradiol on mood symptoms in the same population showed a reduction in depressive symptoms with the PM-MDD group from pre-treatment to post-treatment ($t=4.3$, $p<0.001$ Cohen's $d=1.15$). We anticipate the TSEC (the active ingredient of which is estrogen) to have similar effects on mood symptoms, and thus, the sample size of 20 is a conservative estimate of the number of subject we will need to detect a statistically significant effect.

## *7.2 Statistical Methods*

Aim 1: Determine the extent to which TSEC reduces anhedonia and other depressive symptoms in PM-MDD. Anhedonia and other depressive symptoms will be assessed at baseline and following 3 weeks of TSEC administration. Null hypothesis (H0): TSEC has no salutary effect on depression. Alternative hypothesis (H1): TSEC reduces depression as evidenced by 1) a statistically significant decrease in average MASQ Anhedonia scale score following 3 weeks of treatment, and 2) a clinically significant increase of 1 SD in MASQ Anhedonia scale score. We will test this hypothesis using a paired t-test of MASQ Anhedonia scores at visit 1 (pre-treatment) and visit 6 (post-treatment).

Aim 2 (Exploratory): Quantify the effect of TSEC on the neural reward system in PM-MDD. We will use fMRI at baseline and following TSEC treatment in PM-MDD to probe frontostriatal reward circuitry. We also will compare the effects of TSEC and ERT in PM-MDD. Null hypothesis (H0): TSEC has no meaningful effect on activity of the neural reward circuit in response to rewards. Alternative hypothesis (H1): TSEC reduces increases neural responsiveness to reward in the frontostriatal reward circuitry as evidenced by increased activation of the caudate, putamen, and nucleus accumbens in PM-MDD in response to reward following TSEC administration (compared with baseline).We will test this hypothesis using a paired t-test to compare activity in 3 regions of interest (ROIs)—caudate, putamen, and nucleus accumbens—at the pretreatment and posttreatment scans.

## *7.3 Subject Population(s) for Analysis*

Given that we are interested in understanding the effect of TSECs 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.

**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.

**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 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 PI and IRB by telephone within 24 hours of the event. To report such events, an unanticipated problems (UAP) form will be completed by the investigator, and faxed to the IRB within 24 hours. The investigator will keep a copy of this UAP form on file at UNC.

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 UAP, 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.

### *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 investigator 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 are responsible for complying with UNC's IRB reporting requirements, though must submit the required reports to the 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

This study is IND exempt, therefore IND safety reports are not required.

**Additional reporting requirements**
N/A

**Reporting Process**
N/A

### 8.3.5 Sponsor reporting: Notifying participating investigators

N/A

## *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:

Adverse mood symptoms will be monitored by administering the Hamilton Rating Scale for Depression (HRSD) at each study visit. If suicidal thoughts or severe mood symptoms a**re observed at any period in the protocol, then the HRSD will be administered on consecutive days for three days. Anyone scoring > 20 on the HRSD or 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.

## *8.6 Medical Monitoring*

The principal investigator will oversee the safety of the study at 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, and the PI, 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;
- 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.

The principal investigator or one of her trained study staff will be present at all visits to assess psychological changes using interviews and scales (i.e. HDRS, SCID, IDAS). If the subject self-reports suicidal ideation, this will be immediately communicated to the principal investigator, who will assess each subject on a case-to-case basis.

### 8.6.1 Independent Data and Safety Monitoring Board

Please see the data and safety monitoring plan in Appendix 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.

### *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 the Attachments. 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 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 Foundation of Hope for Research and Treatment of Mental Illness.

## *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. 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 Psychological Interview (Visit 1; 2.5 hours) $100.00
Physical Exam & Baseline Labs (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.

# 14 References


1. Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R. & Walters, E. E. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* **62,** 617–627 (2005)
2. Krishnan, V. & Nestler, E. J. The molecular neurobiology of depression. *Nature* **455,** 894–902 (2008).

3. Rubinow, D. R. & Schmidt, P. J. Gonadal steroids, brain, and behavior: role of context. *Dialogues Clin. Neurosci.* **4,** 123–137 (2002).
4. Schmidt, P. J., Haq, N. & Rubinow, D. R. A longitudinal evaluation of the relationship between reproductive status and mood in perimenopausal women. *Am. J. Psychiatry* **161,** 2238–2244 (2004).
5. Schmidt, P. J. *et al.* Estrogen replacement in perimenopause-related depression: A preliminary report. *Am. J. Obstet. Gynecol.* **183,** 414–420 (2000).
6. Hasler, G. & Northoff, G. Discovering imaging endophenotypes for major depression. *Mol. Psychiatry* **16,** 604–619 (2011).
7. Peterson, B. S. & Weissman, M. M. A Brain-Based Endophenotype for Major Depressive Disorder. *Annu. Rev. Med.* **62,** 461–474 (2011).
8. Pizzagalli, D. A., Iosifescu, D., Hallett, L. A., Ratner, K. G. & Fava, M. Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. *J. Psychiatr. Res.* **43,** 76–87 (2008).
9. American Psychiatric Association. & American Psychiatric Association. Task Force on DSM-IV. *Diagnostic and statistical manual of mental disorders?: DSM-IV-TR*. (American Psychiatric Association, 2000).
10. Forbes, E. E. *et al.* Altered striatal activation predicting real-world positive affect in adolescent major depressive disorder. *Am. J. Psychiatry* **166,** 64–73 (2009).
11. Schiller, C. The hormone withdrawal hypothesis of postpartum depression: a translational approach. *Theses Diss.* (2011). at <http://ir.uiowa.edu/etd/1261>
12. Bless, E. P., McGinnis, K. A., Mitchell, A. L., Hartwell, A. & Mitchell, J. B. The effects of gonadal steroids on brain stimulation reward in female rats. *Behav. Brain Res.* **82,** 235–244 (1997).
13. Dreher, J. C. *et al.* Menstrual cycle phase modulates reward-related neural function in women. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 2465–70 (2007).
14. Anand, A. *et al.* Antidepressant Effect on Connectivity of the Mood-Regulating Circuit: An fMRI Study. *Neuropsychopharmacology* **30,** 1334–1344 (2005).
15. Fu, M. D. *et al.* Neural Responses to Happy Facial Expressions in Major Depression Following Antidepressant Treatment. *Am. J. Psychiatry* **164,** 599–607 (2007).
16. Steinberg, E. M. *et al.* A cross-sectional evaluation of perimenopausal depression. *J. Clin. Psychiatry* **69,** 973–980 (2008).
17. Schmidt, P. ., Murphy, J. ., Haq, N., Danaceau, M. . & St. Clair, L. S. Basal plasma hormone levels in depressed perimenopausal women. *Psychoneuroendocrinology* **27,** 907–920 (2002).
18. Schiller, C. E., Minkel, J., Smoski, M. J. & Dichter, G. S. Remitted major depression is characterized by reduced prefrontal cortex reactivity to reward loss. *J. Affect. Disord.* **151,** 756–762 (2013).
19. Dreher, J. C., Kohn, P. & Berman, K. F. Neural coding of distinct statistical properties of reward information in humans. *Cereb. Cortex* **16,** 561–73 (2006).
20. Burger, H. The Menopausal Transition—Endocrinology. *J. Sex. Med.* **5,** 2266–2273 (2008).
21. Gambrell, R. D. The role of hormones in the etiology and prevention of endometrial cancer. *Clinics in obstetrics and gynaecology* **13,** 695–723 (1986).
22. Nieman, L. K. & Loriaux, D. L. in *Textbook of pharmacology* 695–716 (W.B. Saunders Company, 1992).
23. Jick, H., Derby, L. E., Myers, M. W., Vasilakis, C. & Newton, K. M. Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet* **348,** 981–3 (1996).
24. Daly, E. *et al.* Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet* **348,** 977–980 (1996).
25. Grodstein, F. *et al.* Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet* **348,** 983–987 (1996).
26. Cushman, M. *et al.* Estrogen Plus Progestin and Risk of Venous Thrombosis. *JAMA: The Journal of the American Medical Association* **292,** 1573–1580 (2004).
27. Stampfer, M. J. *et al.* Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. *The New England journal of medicine* **325,** 756–62 (1991).
28. Wassertheil-Smoller, S. *et al.* 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,** 2673–84 (2003).
29. Chetkowski, R. J. *et al.* Biologic effects of transdermal estradiol. *The New England journal of medicine* **314,** 1615–1620 (1986).

30. Mandel, F. P. *et al.* Biological effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. *The Journal of clinical endocrinology and metabolism* **57,** 133–139 (1983).
31. Melis, G. B., Fruzzetti, F., Paoletti, A. M., Carmassi, F. & Fioretti, P. Fibrinopeptide a plasma levels during low-estrogen oral contraceptive treatment. *Contraception* **30,** 575–583 (1984).
32. Barrett-Connor, E. & Bush, T. L. Estrogen and coronary heart disease in women. *JAMA : the journal of the American Medical Association* **265,** 1861–1867 (1991).
33. Manson, J. E. *et al.* Estrogen plus progestin and the risk of coronary heart disease. *The New England journal of medicine* **349,** 523–34 (2003).
34. Willett, W. C., Manson, J. E., Grodstein, F., Stampfer, M. J. & Colditz, G. A. Re: '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* **163,** 1067–8; author reply 1068–9 (2006).
35. Prentice, R. L. *et al.* Combined analysis of Women's Health Initiative observational and clinical trial data on postmenopausal hormone treatment and cardiovascular disease. *American journal of epidemiology* **163,** 589–99 (2006).
36. Grodstein, F., Manson, J. E. & Stampfer, M. J. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. *Journal of women's health* **15,** 35–44 (2006).
37. Prentice, R. L. *et al.* 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,** 404–14 (2005).
38. Lobo, R. A. Evaluation of Cardiovascular Event Rates With Hormone Therapy in Healthy, Early Postmenopausal Women: Results From 2 Large Clinical Trials. *Arch Intern Med* **164,** 482–484 (2004).
39. Hsia, J. *et al.* Conjugated Equine Estrogens and Coronary Heart Disease: The Women's Health Initiative. *Arch Intern Med* **166,** 357–365 (2006).
40. Cirillo, D. J. *et al.* Effect of estrogen therapy on gallbladder disease. *JAMA : the journal of the American Medical Association* **293,** 330–9 (2005).
41. Petitti, D. B., Sidney, S. & Perlman, J. A. Increased risk of cholecystectomy in users of supplemental estrogen. *Gastroenterology* **94,** 91–5 (1988).
42. Steinauer, J. E. *et al.* Postmenopausal hormone therapy: does it cause incontinence? *Obstetrics and gynecology* **106,** 940–5 (2005).
43. Grodstein, F., Lifford, K., Resnick, N. M. & Curhan, G. C. Postmenopausal hormone therapy and risk of developing urinary incontinence. *Obstetrics and gynecology* **103,** 254–60 (2004).
44. Hendrix, S. L. *et al.* Effects of estrogen with and without progestin on urinary incontinence. *JAMA : the journal of the American Medical Association* **293,** 935–48 (2005).
45. Wingo, P. A., Layde, P. M., Lee, N. C., Rubin, G. & Ory, H. W. The Risk of Breast Cancer in Postmenopausal Women Who Have Used Estrogen Replacement Therapy. *JAMA: The Journal of the American Medical Association* **257,** 209–215 (1987).
46. Grady, D. & Ernster, V. Invited Commentary: Does Postmenopausal Hormone Therapy Cause Breast Cancer? *American Journal of Epidemiology* **134,** 1396–1400 (1991).
47. Colditz, G. A. *et al.* The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *The New England journal of medicine* **332,** 1589–93 (1995).
48. Beral, V. 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,** 1047–1059 (1997).
49. Chen, W. Y. *et al.* Unopposed estrogen therapy and the risk of invasive breast cancer. *Archives of internal medicine* **166,** 1027–32 (2006).
50. Stefanick, M. L. *et al.* 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,** 1647–1657 (2006).
51. Chlebowski, R. T. *et al.* 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,** 3243–53 (2003).
52. Gann, P. H. & Morrow, M. Combined Hormone Therapy and Breast Cancer. *JAMA: The Journal of the American Medical Association* **289,** 3304–3306 (2003).

53. Li, C. I. *et al.* Relationship Between Long Durations and Different Regimens of Hormone Therapy and Risk of Breast Cancer. *JAMA: The Journal of the American Medical Association* **289,** 3254–3263 (2003).
54. Sturmer, T. & Manson, J. E. Estrogens and breast cancer: does timing really matter? *Journal of clinical epidemiology* **57,** 763–765 (2004).
55. Rossouw, J. E. *et al.* Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. (2002). at <http://escholarship.umassmed.edu/wfc_pp/401>
56. Anderson, G. L. *et al.* 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,** 1701–12 (2004).
57. Henzel, M. R. in *Reproductive endocrinology: physiology, pathophysiology and clinical management* 643–682 (W.B. Saunders Company, 1986).
58. First, M. B., Spitzer, R. L., Gibbon, M. & Williams, J. B. W. *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. (SCID-I/NP)*. (Biometrics Research, New York State Psychiatric Institute, 2002).
59. Watson, D., Clark, L. A. & Tellegen, A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* **54,** 1063–1070 (1988).
60. Hamilton, M. A Rating Scale for Depression. *J. Neurol. Neurosurg. Psychiatry* **23,** 56 (1960).
61. Watson, D. *et al.* Development and validation of the Inventory of Depression and Anxiety Symptoms (IDAS). *Psychol. Assess.* **19,** 253–268 (2007).
62. Watson, D. & Clark, L. A. *The Mood and Anxiety Symptoms Questionnaire.* (Southern Methodist University, 1991).
63. Pizzagalli, D. A. *et al.* Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *Am. J. Psychiatry* **166,** 702–10 (2009).
64. Greene, J.G. Constucting a standard climacteric scale. *Maturitas* **61**, 78-84 (2008).
65. Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., & Kupfer, D.J. The Pittsburge Sleep Quality Index (PSQI): A new instrument for psychiatric research and practice. *Psychiatry Research* **28**, 193-213 (1989).
66. Knutson, B., Adams, C. M., Fong, G. W. & Hommer, D. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J. Neurosci. Off. J. Soc. Neurosci.* **21,** RC159 (2001).
67. Dichter, G. S., Kozink, R. V., McClernon, F. J. & Smoski, M. J. Remitted major depression is characterized by reward network hyperactivation during reward anticipation and hypoactivation during reward outcomes. *J. Affect. Disord.* **136,** 1126–1134 (2012).
68. Knutson, B., Bhanji, J. P., Cooney, R. E., Atlas, L. Y. & Gotlib, I. H. Neural responses to monetary incentives in major depression. *Biol. Psychiatry* **63,** 686–92 (2008).
69. Pizzagalli, D.A., Jahn, A.L., O'Shea, J.P. Toward an objective characterization of an anhedonic phenotype: A signal-detection approach. *Biological Psychiatry*, **57**, 319-327 (2005).
70. Soules, M. R. *et al.* Stages of Reproductive Aging Workshop (STRAW). *J. Womens Health Gend. Based Med.* **10,** 843–848 (2001)

# Appendix A. Data and Safety Monitoring Plan

**Data and Safety Monitoring Plan**
**IRB Study** #18-2129
**Title of Study**: Effects of a Tissue Selective Estrogen Complex on Depression and the Neural Reward System in The Perimenopause (Duavee)
**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

Despite decades of clinical research, affective disorders continue to affect 20.9 million Americans each year and remain the leading cause of disability worldwide. Women are twice as likely as men to experience depression. Depressive symptoms are particularly common during periods of hormone fluctuation, including the transition to menopause. The proposed research will help identify the effects of estrogen on brain function and mood during the menopause transition. This research will provide important information for 1) understanding the triggering of and susceptibility to perimenopausal depression, 2) identifying women for whom hormone replacement therapy is likely to have beneficial effects, and 3) developing new medications for depression in women.

Using neuroimaging, we will study the effects of tissue selective estrogen complexes (TSECs) on mood and brain function in perimenopausal women with depression. Participants will include 20 unmedicated, naturally menopausal women who are diagnosed with major depressive disorder associated with menstrual cycle irregularity (n=20). An initial interview will confirm participants' presence of depression, and current perimenopausal status as well as determine any exclusionary criteria.

Inclusion Criteria.

1. Perimenopause Status: We will employ the Stages of Reproductive Aging Workshop (STRAW) criteria 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). Women who meet these criteria will not require an FSH level. Women who have taken oral contraceptives continuously for relief of perimenopausal symptoms and women who have had hysterectomies (with ovaries remaining intact) will be exempt from our LMP criteria, and their perimenopausal status will be determined by FSH level instead (FSH ≥ 14). Because extremes of body weight (BMI < 18 or > 35 kg/m2) or a history of chronic menstrual cycle irregularity can contribute to inaccurate reproductive staging, 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)

Exclusion Criteria. Patients will not be permitted to enter this protocol if they have important clinical or laboratory abnormalities including 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. LMP 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 onset 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. current cigarette smoking;
26. current suicidal ideation, mania, psychosis, or alcohol/drug dependence;
27. past suicide attempts, mania, alcohol/drug dependence, or psychotic episodes;
28. chronic depression (i.e., episode(s) lasting 3+ years);
29. depressive episode(s) within 2 years of enrollment;
30. self-reported claustrophobia
31. Allergies to red or yellow dye.

*all reported prescription medications will be reviewed and cleared by a study physician prior to a participant's enrollment;
**women who have recently taken oral contraceptives continuously for relief of perimenopausal symptoms will be exempt from LMP criteria, and instead, the presence of menstrual irregularity prior to the use of oral contraceptives and elevated FSH will be used to determine their perimenopausal status;
***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. There is no evidence for first degree relative with history of MI/CVA as a contraindication for short-term ERT, and in our first 6 months of recruitment we have come to find this criterion superfluous for our purposes. Per the Endocrine Society Scientific Statement, thrombosis risk in HRT users is substantially increased with oral HRT administration and obesity; neither of which applies to our participants. Further, the American College of Obstetricians and Gynecologists does not list family history of MI/CVA as a contraindication for HRT. In our recruitment to date we have assessed many women with family history of MI/CVA who are healthy and otherwise appropriate for the study, and we do not want to continue to exclude them unnecessarily.
Santen, R. J., Allred, D. C., Ardoin, S. P., Archer, D. F., Boyd, N., Braunstein, G. D., ... Endocrine Society. (2010). Postmenopausal hormone therapy: an Endocrine Society scientific statement. The Journal of Clinical Endocrinology and Metabolism, 95(7 Suppl 1), s1–s66. ,
ACOG Practice Bulletin No. 141: management of menopausal symptoms. (2014). Obstetrics and Gynecology, 123(1), 202–216.

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.

The estimated level of risk to subjects for this protocol is level 2: minor increase over minimal risk.

**Potential Risks to Human Subjects and Minimization of those Risks**

**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 depressive symptoms and associated psychopathology. However, we have conducted several studies assessing 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. Kilborn and Labbe estimated that from 5-10% of patients become claustrophobic during clinical MRI scans. Murphy and colleagues reported that 14.3% of 949 patients undergoing MRI testing in their hospital required sedation to tolerate the procedure. The numbers reported in these studies are not representative of our experience at BIAC 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.

**Duavee Side Effects**

We do not expect any adverse side effects associated with the hormonal manipulations outlined in this protocol for the following reasons: We will be administering estrogen paired with selective estrogen receptor modulators (SERMs). SERMs are synthetic non-steroidal agents that have estrogen agonist and antagonist effects in different tissues, to block the negative effects of estrogen on non-target tissues, including the reproductive organs and cardiovascular system, while allowing estrogen to exert effects on target tissues like the brain, and thus decreasing risk for side effects.

Duavee: Nausea is the most common side effect of TSEC 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. Abdominal pain, hot flashes, breast engorgement, endometrial hyperplasia and bleeding are also common side effects of TSEC administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of TSECs; however, at the dosage and for the duration of estrogen administration in this protocol this risk is small.

The relationship between estrogens, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation. 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 SERMS 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. There is an increased risk of thromboembolism in women receiving non-contraceptive estrogen therapy. Additionally, some but not all studies report an increase in risk of stroke, 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. 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. In contrast, recent randomized controlled trials in older postmenopausal women (e.g., Women’s Health Initiative [WHI]) report an increased risk of cardiovascular disease. 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. Additionally, the increased risk of CHD was observed in older but not younger perimenopausal women. 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. Estrogen therapy also may increase the risk of urinary incontinence in older postmenopausal women. Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use, similar to the risk expected if the onset of menopause was delayed for a comparablelength of time. Due to the publicity surrounding the cancellation of the treatment arm of the Women’s Health Initiative study 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 (estradiol) 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. Conjugated estrogens, the form of estrogen that we use in this study, is administered with bazedoxifene, a SERM, that helps to prevent 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. 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.

**Potential Benefits to Human Subjects**

There is evidence suggesting that estrogens may serve as a therapeutic option for affective disorders in perimenopausal women. Thereby women who participate in the study may find that the estrogen therapy yields an improvement in symptoms related to mood or physical symptoms, and will have the option to continue hormone replacement after the study concludes. If a participant is interested in learning whether she demonstrated a differential susceptibility to the hormone replacement used in this study, she will be welcome to review the mood and physical symptoms that she experienced during 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 will have 80% power to detect a large effect size (.7) in 20 women. Our recently completed study of the effects of transdermal estradiol on mood symptoms in the same population showed a reduction in depressive symptoms with the PM-MDD group from pre-treatment to post-treatment ($t=4.3$, $p<0.001$ Cohen's $d=1.15$). We anticipate the TSEC (the active ingredient of which is estrogen) to have similar effects on mood symptoms, and thus, the sample size of 20 is a conservative estimate of the number of subject we will need to detect a statistically significant effect.

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 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 Hamilton Rating Scale for Depression (HRSD) at each study visit. If suicidal thoughts or severe mood symptoms are observed at any period in the protocol, then the HRSD will be administered on consecutive days for three days. Anyone scoring > 20 on the HRSD or 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 receive conjugated estrogens/bazedoxifene (Duavee) tablets (0.45 µg, 20 mg) for 3 weeks. fMRI sessions (described below) will take place at baseline and at the end of the third week of Duavee administration. In the first study visit, women will have a basic lab panel and serum pregnancy test performed. Prior to Duavee administration 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 Duavee administration to assess blood levels of conjugated estrogens/bazedoxifene and mood symptoms.

**Clinical Assessments.** The SCID will be administered at baseline to determine study eligibility by Dr. Schiller or her trained study staff. The following standard measures will be administered at baseline, both fMRI sessions, and weekly clinic visits: The Positive and Negative Affect Schedule 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) 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) 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) 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 depression.
The Greene Climacteric Scale (GCS) 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) is a 9-item questionnaire that measures the quality and sleep patterns in older adults.
The Hormone History Questionnaire is a 19-item inventory that assesses women's mental and physical health and was created for the purpose of this study.
The Trauma History Questionnaire is a 24-item measure that examines participant's experiences with potentially serious or traumatic life events.

**fMRI Task and Protocol.** The Monetary Incentive Delay (MID) Task engages reward circuitry during monetary incentive anticipation and outcomes. Each of two event-related MID runs consists of 90 6-second trials during which women will be presented with a cue shape, a fixation crosshair (for a variable duration), the target, and performance (win/loss/neutral) feedback. Cues indicate whether it is an incentive (gain, loss) or non-incentive trial. In incentive trials, women can either gain or lose money by pressing a button during target presentation, and difficulty is based on individual reaction times. This task elicits reliable frontostriatal dysregulation in people with remitted MDD[8]. Resting state scans will also be conducted.

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 outcomes, although the anticipation of wins will also be explored.

Probabilistic Reward Task (PRT) will be conducted outisde of the scanner. This task allows us to test the additional hypothesis that perimneopausal 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.

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

| **Assessments** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Study visits | X | X | X | X | X | X |
| Consent | X | | | | | |
| OBGYN Exam | | X | | | | |
| fMRI | | | X | | | X |
| PRT | | | X | | | X |
| Venipuncture | | | X | X | X | X |
| SCID | X | | | | | |
| SNAP | X | | X | X | X | X |
| HAM-D | X | | X | X | X | X |
| IDAS | X | | X | X | X | X |
| GCS | X | | X | X | X | X |
| PSQI | | | X | | | X |
| HHQ | | | | | | X |
| THQ | | | | | | X |
| Duavee Rx Provided | | | X | X | X | |
| Payment | $100 | $30 | $150 | $35 | $35 | $150 |

b. Statistical analysis strategy:

**Aim 1: Determine the extent to which TSEC reduces anhedonia and other depressive symptoms in PM-MDD.** Anhedonia and other depressive symptoms will be assessed at baseline and following 3 weeks of TSEC administration.

Null hypothesis ($H_0$): TSEC has no salutary effect on depression.

Alternative hypothesis ($H_1$): TSEC reduces depression as evidenced by 1) a statistically significant decrease in average MASQ Anhedonia scale score following 3 weeks of treatment, and 2) a clinically significant increase of 1 SD in MASQ Anhedonia scale score.

We will test this hypothesis using a paired t-test of MASQ Anhedonia scores at visit 1 (pre-treatment) and visit 6 (post-treatment).

**Aim 2 (Exploratory): Quantify the effect of TSEC on the neural reward system in PM-MDD.** We will use fMRI at baseline and following TSEC treatment in PM-MDD to probe

frontostriatal reward circuitry. We also will compare the effects of TSEC and ERT in PM-MDD.

Null hypothesis ($H_0$): TSEC has no meaningful effect on activity of the neural reward circuit in response to rewards.

Alternative hypothesis ($H_1$): TSEC reduces increases neural responsiveness to reward in the frontostriatal reward circuitry as evidenced by increased activation of the caudate, putamen, and nucleus accumbens in PM-MDD in response to reward following TSEC administration (compared with baseline).

We will test this hypothesis using a paired t-test to compare activity in 3 regions of interest (ROIs)—caudate, putamen, and nucleus accumbens—at the pretreatment and posttreatment scans.

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.

Santen, R. J., Allred, D. C., Ardoin, S. P., Archer, D. F., Boyd, N., Braunstein, G. D., ... Endocrine Society. (2010). Postmenopausal hormone therapy: an Endocrine Society scientific statement. The Journal of Clinical Endocrinology and Metabolism, 95(7 Suppl 1), s1–s66. ,
ACOG Practice Bulletin No. 141: management of menopausal symptoms. (2014). Obstetrics and Gynecology, 123(1), 202–216.