Protocol Title: The central nervous system effects of pharmacologically induced hypogonadotropic hypogonadism with and without estrogen and progesterone replacement.

Protocol Number: 92-M-0174

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Medical Advisory Investigator

Total requested accrual
0 Patients
150 Volunteers

Human Research Protections Program Investigator and Staff Training:

"Just in time" human subjects protection training courses are required for investigators and staff participating on this protocol: None

Project Uses Ionizing Radiation: ☒ No ☐ Yes (attach *RSC/RDSC documentation)*
☐ Medically-indicated only
☐ Research-related only
☐ Both

IND/IDE ☐ No ☒ Yes *(attach FDA documentation)*
Drug/Device/# depot Lupron 3.75 mg
Sponsor PJ Schmidt

Durable Power of Attorney ☒ No ☐ Yes
Multi-institutional Project ☒ No ☐ Yes

Institution_____FWA #_____
Date of IRB approval_____*(attach IRB documentation)*

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☐ No ☒ Yes
Agreement type and number____CTA 14-10_____Expiration Date__08/01/19_____

Confidential Disclosure Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent forms:
Standard 6 month: 9.3
MDE: 9.4
Estradiol Gel : 9.4

Standard 5 month: deactivated
Stress Reactivity DEX/CRH: deactivated
Estradiol suppository: deactivated

Precis:

Evidence suggests that the gonadal steroids may exert clinically significant effects on central nervous system function. For example, the menstrual cycle may influence the occurrence of seizures in some female epileptics and the performance on certain cognitive tests. Central nervous system effects of gonadal steroids have been inferred largely from changes in behavior occurring in association with presumed changes in gonadal steroids during the normal menstrual cycle, during the administration of ovarian hormones, or in a gender-specific context. These inferences are, by definition, indirect and associational in nature and further are incapable of disentangling the effects of hormones which are simultaneously present in women of reproductive age. This study is designed to address those problems by comparing measures during Lupron-induced hypogonadism with those during replacement with estrogen or progesterone. On the basis of prior findings from our group and from others, we will be asking the following questions: 1) Is the decreased r-CBF that we observed in the prefrontal cortex during the hypogonadal state confirmed in individual women using new imaging techniques; 2) Will variation in genotype (e.g., COMT val/met, BDNF val/met) confer differential sensitivity to ovarian steroids in brain circuitry and 3) Are the menstrual cycle phase-related changes in reward systems that we previously observed related to estradiol or progesterone actions within the brain (1). Additionally, this protocol will serve as a control study for protocol # 90-M-0088.

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1) Introduction

A. Gonadal steroids, sexual dimorphisms, brain, and behavior

For centuries medical observers have suggested that a special relationship exists between female reproductive function and/or dysfunction and disturbances in central nervous system activity. More recently a putative interaction between gonadal steroids, in particular estradiol and progesterone, and central nervous system function has been suggested to contribute to a number of observed epidemiologic, phenomenologic and treatment response characteristics in women compared to men. Studies have consistently identified a two-fold increased prevalence of depression in women compared with men (2-5). This increased prevalence has been observed in a variety of countries (4). A two- to three-fold increased prevalence of dysthymia and threefold increase in seasonal affective disorder (6) in women has also been noted (7), while bipolar illnesses is equi-prevalent in men and women (2, 8, 9) (and reviewed in (10)). Pre-pubertal depression prevalence rates are not higher in girls (11, 12), possibly reflecting ascertainment bias/reporting bias (depressed boys may be more likely to come to the attention of health care providers) or the possibility that pre-pubertal major depression is premonitory of bipolar illness (13). Women are more likely to present with anxiety, atypical symptoms, or somatic symptoms (6, 14-21); are more likely to report symptoms, particularly in self-ratings (6, 14, 19); are more likely to report antecedent stressful events (22, 23); manifest a more robust effect of stress on the likelihood of developing depression during adolescence (24); and display increased comorbidity of anxiety and eating disorders (25-28), thyroid disease (29, 30), and migraine headaches (31), as well as lower lifetime prevalence of substance abuse and dependence (16, 28, 32). Reported differences in treatment response characteristics in women compared with men include poor response to tricyclics (33-36) particularly in younger women

(34), superior response to SSRIs or MAOIs (37-39), and a greater likelihood of response to triiodothyronine (T3) augmentation (30, 40). The extent to which these differences reflect gender-related differences in pharmacokinetics (41-47) remains to be determined. Finally, while the prevalence of bipolar disorder is comparable in men and women, women are more likely to develop rapid cycling (10) and may be more susceptible to antidepressant-induced rapid cycling (48).

The epidemiologic observations described above are increasingly complemented by demonstrations of sexual dimorphisms in brain structure and physiology in humans. Structural and functional brain imaging studies, for example, have shown the following: 1) differences in functional organization of the brain, with brain activation response to rhyming task lateralized in men but not women (49); 2) gender specific decreases in regional brain volume (caudate in males and globus pallidus, putamen in females) during development (50); 3) increased neuronal density in the temporal cortex in women (51); 4) greater interhemispheric coordinated activation of brain regions in women (52); 5) larger volume hypothalamic nucleus (INAH 3) in men (53); 6) differences in both resting blood flow and the activation pattern accompanying self-induced mood change (54); 7) decreased 5-HT2 binding in the frontal, parietal, temporal, and singular cortices in women (55); 8) differences in whole brain serotonin synthesis (interpreted as decreased in women but possibly increased if corrected for plasma free tryptophan levels) (56); 9) higher and more symmetric cerebral blood flow in women (57-61); 10) greater asymmetry in the planum temporale in men (62); and 11) greater brain glucose metabolism (19%) in women (63, 64). The potential relevance of gonadal steroids in some of these differences has also been demonstrated with the same technologies. Recent brain imaging studies in asymptomatic women (i.e., women without PMD) confirm for the first time in humans that physiologic levels of

ovarian steroids have the capacity to modulate the neurocircuitry thought to be involved in both normal and pathological affective states. First, Berman, et al. performed cognition-activated O^{15}PET scans in women during conditions of GnRH agonist-induced hypogonadism and gonadal steroid replacement. They observed the elimination of Wisconsin Card Sort-activated regional cerebral blood flow (rCBF) in the dorsolateral prefrontal cortex as well as an attenuation of cortical activation in the inferior parietal lobule and posterior inferior temporal cortex (bilaterally) during GnRH agonist-induced hypogonadism (65). The characteristic pattern of cortical activation re-emerged during both estradiol and progesterone addback. Additionally, they observed a differential pattern of hippocampal activation with estradiol increasing and progesterone decreasing activation relative to hypogonadism. This was the first demonstration that ovarian steroids have activational effects on rCBF during cognitive stimulation in the brain regions

(i.e., PFC) implicated in disorders of affect and cognition. Recent observations also suggest that estrogen's modulatory effects on PFC function could be influenced by variation in catechol-O-methyltransferase gene (66). Second, Goldstein, et al. (67) observed an increase in amygdalar activity and arousal (as measured by fMRI and skin conductance, respectively) during the late follicular phase of the menstrual cycle (higher estradiol levels) compared to the early follicular phase (characterized by relatively low estradiol levels). Third, Protopopescu, et al. (68)employed an affective pictures task in an fMRI study and observed increased OFC activity (a region that in some studies exerts inhibitory control over amygdalar functioning) during the luteal compared with the follicular phase. Moreover, preliminary data from these same investigators in women with PMD (69) suggest a relative loss of OFC activity (decreased inhibition) in women with PMD during the luteal phase. Notwithstanding the caveat that

decreased cortical "activity" also could reflect more efficient or optimal function, these data suggest that a reduction in OFC inhibition of amygdalar function during the luteal phase is associated with PMD symptoms. Finally, Dreher, et al. (1), have initiated an event-related fMRI study of reward processing across the menstrual cycle in women with PMD and controls. The paradigm employed disentangles transient reward error prediction (PFC) from sustained response to reward uncertainty (ventral striatum). Preliminary data in the controls demonstrate, for the first time in humans, that ovarian steroids modulate reward system function, with increased follicular phase activation of the OFC and amygdala during reward anticipation and of the midbrain, striatum, and left ventrolateral PFC during reward delivery. New analytical approaches will allow for testing the hypothesis that the hormonally-induced alteration in function includes changes in interregional neural interactions. These findings then suggest that cognitive and affective information processes may serve as probes to identify candidate circuits for the mediation of gonadal steroid-dependent affective dysregulation. Additionally, neuroimaging studies in women suggest that ovarian steroids can influence many neural processes and systems relevant to PMD including arousal, stress-responsivity, and reward processing. The contribution of these and other effects of gonadal steroids to observed gender dimorphisms and reproductive-related mood disorders must, obviously, await further determination.

B. Mechanism by which gonadal steroids may alter mood and behavior

Animal studies have identified that gonadal steroids influence several of the neuroregulatory systems thought to be involved in both the development of affective disorders and the putative mechanisms underlying the efficacy of antidepressant or convulsive therapies.

The manifold interaction between gonadotropins/gonadal hormones and neurotransmitters/neuromodulators (70-74) suggest a neurobiological basis for the effect of changes in reproductive endocrine function, such as the menopause, on mood and behavior. For example, estrogen receptors (ER) (both α and β types of the ER) have been identified in several brain regions: in the preoptic area and the arcuate nucleus-median eminence region, where gonadotropin releasing hormone (GnRH), dopamine, and B-endorphin neurons are concentrated; and in extra-hypothalamic sites, including the interstitial nucleus of the striae terminalis, medial amygdaloid nucleus, forebrain, and brainstem (75, 76). Gonadal steroids have been shown to play a role in all stages of neural development, including neurogenesis, synaptogenesis, neural migration, growth, differentiation, survival, and death (77). These effects occur largely as a consequence of the ability of gonadal steroids to modulate genomic transcription. As transcriptional regulators, the receptors for gonadal steroids direct or modulate the synthesis of the synthetic and metabolic enzymes as well as receptor proteins for many neurotransmitters and neuropeptides (78). They additionally influence the levels of several critical enzymes involved in signal transduction. These actions permit gonadal steroids to influence all aspects of neurotransmitter activity.

Estrogen's role in mood regulation is not only suggested by its widespread actions on neurotransmitter system function but also by certain neuroregulatory actions shared by both estrogen and traditional therapies for depression (i.e., antidepressants, ECT). In some, but not all (79), experimental paradigms, estradiol has been observed to inhibit SERT mRNA (80) and decrease activity at $5HT_{1A}$ receptors (81, 82), consistent with some reported actions of antidepressants on serotonergic system function. Moreover, in one study estradiol replacement facilitated imipramine-induced downregulation of 5HT2 receptors in the rat frontal cortex (83).

Additionally, several candidate neural signaling systems have also been identified as potential mediators of the therapeutic actions of antidepressants and ECT (e.g., guanine nucleotide-stimulated adenylyl cyclase and c-AMP dependent protein kinase (PKA) (84-87)), based on observations that these systems are modulated by a range of therapies effective in depression (e.g., serotonergic and noradrenergic agents and ECT) and exhibit a pattern of change consistent with the long term effects of antidepressants on mood (88). Similarly, estradiol has been reported to influence many of these same neuroregulatory processes. Specifically, ovariectomy has been reported to decrease, and estradiol increase, BDNF levels in the forebrain and hippocampus (89, 90). Estrogen has also been reported to increase CREB activity (91, 92) in rat brain and, additionally, to increase trkA (93). In contrast, an estradiol-induced decrease in BDNF has been reported to mediate estradiol's regulation of dendritic spine formation in hippocampal neurons (94). It is also of interest that interactions between glucocorticoid and estrogen response elements and their receptors suggest additional ways by which gonadal steroids may modulate neural activity (95). For example, estrogen (ER) and glucocorticoid receptors (GR) compete for CREB binding protein (CBP) and glucocorticoid receptor interacting protein (GRIP), with the relative amounts of these receptors increasing (ER) or decreasing (GR) transcription at the AP-1 site (96, 97).

Gonadal steroids may also exert important regulatory effects on the hypothalamic-pituitary-adrenal axis. Studies in animals indicate that estrogen administration decreases glucocorticoid receptor mRNA production in the thymus (98) and the pituitary (99-101), and enhances hypothalamic-pituitary adrenal (HPA) responses to stress (102-105). Moreover, evidence from animal studies further suggests that gonadal steroids may influence the serotonergic regulation of the HPA axis by altering the function of the 5-HT_{1a} and 5-HT_2 receptor systems in the cortex and

hippocampus (106-108). In humans, gender dimorphisms in the physiologic responses to stress (including HPA axis function) have been reported (109-115); however, the relationship between gonadal steroid hormones and HPA axis activity has not been systematically examined in humans.

Our studies in women using an exercise stressor in the context of GnRH agonist-induced hypogonadism with and without estrogen and progesterone replacement, indicate that progesterone is a more important influence on the stress response than is estrogen (116). Further, in our menstrual cycle studies using the exercise stressor, we have found that 1) there is a decreased cortisol and ACTH response to stress during the follicular as opposed to the luteal phase of the cycle (117) and 2) that women with a history of PMD fail to demonstrate this follicular phase blunting of the axis (116). What accounts for the differential response to stress in women with PMD? Since we know that women with PMD respond differentially to gonadal steroids, such that they (but not controls) display mood dysregulation in this GnRH paradigm, one possibility is that the differences in HPA axis function that we observed in women with PMD may again represent a differential (abnormal) response to either estrogen or progesterone. To test this hypothesis, we will perform the exercise paradigm in patients (protocol # 90-M-0088) and control women under conditions of GnRH agonist-induced hypogonadism with and without estrogen and progesterone replacement. Protocol # 92-M-0174 provides a unique opportunity to investigate the effects of ovarian steroids on the regulation of HPA axis function in these women.

Despite the possible effects of gonadal steroids on these neural systems, one cannot easily infer the mechanism underlying the observed psychotropic effects of these compounds. The extent to which gonadal hormones, gonadotropins, catecholamines, and neuropeptides may

interact in the development of reproductive endocrine-related mood and behavioral disorders is purely a matter for speculation but may potentially elucidate relevant mechanisms in the pathophysiology of mood disorders.

Previous studies infer the effects of gonadal steroids on CNS activity in humans from the occurrence of changes in CNS function in association with presumed changes in gonadal steroids during the menstrual cycle or during the administration of ovarian hormones (e.g., post menopausal estrogen replacement), or in the context of gender specific behaviors. As noted in the precis these methodologies suffer not only from their indirect and associational nature but as well from both their inability to disentangle the effects of hormones which are simultaneously present and the effects of aging and menopausal status on CNS function. In this study we propose to evaluate the selective effects of the gonadal steroids under three pharmacologically controlled conditions, specifically during GnRH agonist-induced hypogonadism, GnRH agonist-induced hypogonadism with estrogen replacement, and GnRH agonist-induced hypogonadism with progesterone replacement. Further, the mood and behavioral effects observed in subjects participating in this protocol will serve to control for our observations in protocol # 90-M-0088.

C. <u>Observations since Protocol Commencement</u>:

To date results of this protocol suggest the following: 1) the efficacy of GnRH agonist induced ovarian suppression compared to placebo in women with PMD; 2) the re-emergence of clinically significant dysphoria induced by both E2 and P4 (but not placebo) in women with PMD but not controls; 3) the absence of a significant effect of GnRH agonist induced hypogonadism, with or without E2 and P4 replacement, on the self-reports of cognitive abilities or neuropsychological test scores in both patients and controls (with the exception of significantly improved performance in motor dexterity tasks during progesterone compared to

other hormone conditions); 4) the elimination of Wisconsin Card Sort Test-activated regional cerebral blood flow (CBF) in the dorso-lateral prefrontal cortex during Lupron induced hypogonadism ($p > 0.2$) and the re-emergence of the characteristic pattern of activated CBF during E2 or P4 addback in both patients and controls; 5) both the increase (E2) and decrease (P4) in N-back-activated regional CBF in the hippocampus; 6) the altered pattern of correlative relationships between the prefrontal cortex and hippocampus during hypogonadism compared to estradiol replacement; 7) the significant increase in m-CPP stimulated plasma prolactin secretion during P4 hippocampus; 8) the significant increase in exercise-induced cortisol, ACTH, and AVP secretion during P4 addback compared to hypogonadism; and 9) the inability of estradiol suppositories at doses of 100 micrograms administered twice a day to maintain plasma estradiol levels between 80-120 pg/ml in women receiving Lupron during a 9 week pilot trial.

D. Medication to be Employed in this Protocol:

1. GnRH Agonists

GnRH is produced by the hypothalamus and causes the anterior pituitary to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). Leuprolide acetate (Lupron) is a synthetic nonapeptide that functions as an agonist that is 80 to 100 times more potent than synthetic native GnRH in inducing the release of LH (118, 119). Depot Lupron is a GnRH agonist that, administered monthly, results in a reversible cessation of pituitary ovarian axis function; i.e., both the gonadotropin and ovarian steroid secretion are effectively prevented. In the absence of ovarian activity, the effects of exogenously administered estrogen or progesterone on the CNS can be evaluated in isolation and free of the obfuscating effects of the usual changes in gonadal steroids. During the first week of Lupron therapy, there is an initial increase in the pituitary release of LH and FSH leading to a transient increase in levels of the gonadal steroids

estrone and estradiol in premenopausal females. However, with long-term administration of Lupron, there is a subsequent decrease in the number of GnRH receptors and an inhibition of pituitary elaboration of gonadotropins leading to levels of estrogens similar to those observed in post-menopausal women. These decreases in gonadal steroid levels occur within two to four weeks after initiation of treatment. Following a single Lupron depot injection (3.75 mg) to patients, mean peak leuprolide acetate plasma concentration was almost 20 ng/ml after four hours and .36 ng/ml at four weeks, levels associated with effective gonadotropin suppression. The plasma half-life of depot Lupron is not known; however, the half-life in the non-depot form is approximately three hours.

2. Estradiol

The administration of 17β-estradiol via a transdermal system was chosen for this study because it has several relevant advantages over oral preparations (120, 121). First, it delivers the primary ovarian estrogen, estradiol, into the circulation at a constant rate and results in sustained and easily measurable plasma levels of estradiol and in estrone/estradiol ratios less than one (as seen in the pre-climacteric period of life) (122). Second, it delivers sufficient estradiol into the circulation to raise estradiol plasma concentrations to levels similar to those of women in the early follicular to mid-follicular phases of the menstrual cycle (120, 123-125), levels reported by some investigators as the minimum necessary for the relief of menopausal symptoms, particularly hot flushes (123). In contrast to oral estrogen preparations, both transdermal and transvaginal systems avoid first-pass effects of hepatic metabolism, and, therefore, have less impact on hepatic protein synthesis including clotting factors, C-reactive protein and renin substrate. An increase in renin substrate has been suggested to accentuate or initiate the development of high blood pressure in susceptible women with other predisposing factors (126)

and has been implicated as a factor in the association of hypertension with the administration of oral conjugated estrogen (127). In our experience with the transdermal estradiol patch in over 100 women in several protocols, it has been well tolerated and we have observed no unexpected adverse effects. With the application of the estrogen patch every three days, patient compliance has been reported to be excellent, and only occasional local irritation has been observed (123). Similarly, a transvaginal system has been employed to deliver progesterone in this protocol without expected adverse events (as detailed below).

Formulation of Estradiol employed in this protocol:

1. From 1972 until September 2013 – transdermal estradiol patches were employed at a dose of 100 micrograms per day. However, due to the unavailability of matched placebo patches and the prohibitive costs of manufacturing the small quantities of placebo patches needed for this protocol we ceased the employment of estradiol skin patches in this and related protocols (i.e., 90-M-0088, 05-M-0059).
2. Between September 2013 and May 2014 – we performed a pilot study lasting approximately two months in which we evaluated estradiol vaginal suppositories (100 micrograms once to twice daily) as a substitute for the transdermal estradiol patches. However, we could not achieve the desired plasma concentrations consistently in women and, therefore, we could not employ vaginal estradiol administration in this protocol.
3. Between July 2014 and June 2016 – we performed a pilot study lasting approximately two months in which we evaluated estradiol transdermal skin gel (.06%) three to four applications per day (.75 to 3.0 mg estradiol per day) as a substitute for the transdermal estradiol patches. However, we could not achieve the desired plasma concentrations of

estradiol consistently in women and, therefore, we could not employ .06% estradiol gel administration in this protocol.

4. Amendment: we intend to conduct a pilot trial of a higher dose of estradiol gel (.36% estradiol in hydro alcoholic base) at doses of 6 to 9 mg of estradiol per day (2-3 applications per day)

3. Progesterone

In this protocol progesterone 200 mg will be administered twice daily. Progesterone is widely prescribed in gynecologic settings with proved indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea and precocious puberty (128). In our experience with the use of progesterone vaginal suppositories in over 80 women in several protocols, they have been well tolerated and we have observed no unexpected adverse events. Mild to moderate perineal irritation has been experienced rarely and when it occurs it is responsive to barrier creams such as vaseline.

Estrogel .36% pilot study only: we will prescribe one of three forms of progestin to induce endometrial shedding and menstruation after women complete the four weeks of unopposed estradiol administration (Estrogel .36%). The decision to choose a specific form of progestin will be made by the PI and will depend on the availability of these progestins in the NIH CC Pharmacy. Thus, we will prescribe one of the following forms of progestin: progesterone by suppository at a dose of 200 mg twice a day for one week (as approved in the original protocol), or oral progesterone or oral medroxyprogesterone acetate (Provera) at standard clinical doses indicated for the induction of menses after unopposed estradiol exposure in healthy women.

Finally, administration of depot Lupron up to a maximum of six monthly injections with and without the concurrent administration of the estraderm patch (alone for four weeks and in

combination with progesterone suppositories for one week) and progesterone suppositories (for five weeks) has been approved by the NIMH IRB for administration to patients with menstrual-related mood disorder under NIMH protocol # 90-M-0088.

2) Study Objectives:

The objectives of this study are to examine the effects of hypogonadism with and without physiologic replacement of estradiol and progesterone on measures of brain function and behavior. The use of GnRH agonists to suppress ovarian function (hypogonadism) permits us to examine the effects of estradiol and progesterone on brain function separately, and to standardize the exposure to these hormones across individual women and in our comparison in women with premenstrual dysphoria (PMD), who participate in an identical companion protocol (90-M-0088). The outcome measures currently employed in this study are as follows: 1) brain imaging with 0^{15} PET and fMRI to examine brain activation and connectivity and the impact of genomic variation on these neuroimaging measures; 2) HPA testing with Dex/CRH stimulation; 3) metabolomic profile of neurosteroid and neuroactive steroid metabolites, and 3) cell culture studies (lymphoid and IPCs) to examine cellular responses to exposures to ovarian steroids in controls and women with PMD (90-M-0088).

3) Subjects:

a) Description of study populations

Healthy asymptomatic premenopausal women with no current Axis I diagnosis and no evidence of menstrual-related mood or behavioral disturbances. The targeted accrual of 150 women reflects the use of this protocol to examine specific goals that have developed over the course of this protocol.

b) Inclusion criteria

Volunteers participating in this study will be women meeting the following criteria:

- between the ages of 18 and 50 years,
- not pregnant,
- in good medical health,
- medication free,
- no history of menstrual-related mood or behavioral disturbances.

Additionally, we will recruit a subsample of 20 asymptomatic women who will meet all inclusion and exclusion criteria in this protocol except they will have a history of a past major depressive episode.

Finally, a sample of 10 women who meet all the inclusion and exclusion criteria listed above for this protocol will be recruited to establish the dose range of transdermal .36% estrogen gel for this and related protocol (i.e., 90-M-0088).

c) Exclusion criteria

The following conditions will constitute contraindications to treatment with hormonal therapy and will preclude a subject's participation in this protocol:

- Current Axis I psychiatric diagnosis (with the exception of those women with a past major depression who will be studied in this protocol);
- History consistent with endometriosis;
- Diagnosis of ill-defined, obscure pelvic lesions, particularly undiagnosed ovarian enlargement;
- Hepatic disease as manifested by abnormal liver function tests;
- History of mammary carcinoma;
- History of pulmonary embolism or phlebothrombosis;

- Undiagnosed vaginal bleeding;
- Porphyria;
- Diabetes mellitus;
- History of malignant melanoma;
- Cholecystitis or pancreatitis;
- Cardiovascular or renal disease;
- Pregnancy.
- Any woman meeting the Stages of Reproductive Aging Workshop Criteria (STRAW) for the perimenopause (129). Specifically, we will exclude any woman with an elevated plasma FSH level ($\geq$ 14 IU/L) and with menstrual cycle variability of > 7 days different from their normal cycle length.

NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.

Subjects taking birth control pills will be excluded from the study. Subjects taking diuretics, prostaglandin inhibitors, or pyridoxine (putative treatments for MRMD) will similarly be excluded from the study, as will patients taking psychotropic agents (e.g., lithium carbonate, tricyclic antidepressants). All subjects will be required to use non-hormonal forms of birth control (e.g., barrier methods) to avoid pregnancy during this study. Finally, participants who have an active condition that places them at an increased risk for osteoporosis (see Table I) will be excluded from this protocol.

4) Study Design and Methods:

a) Study overview

This is an out-patient study lasting approximately 5-7 months. Subjects will be asked to make visits to the clinic every one to two weeks. Each visit will last up to two hours. In addition, some subjects will complete additional procedures such as PET or fMRI scans for which there are separate consents.

b) Recruitment

Recruitment Screening Methods

Participants will be sought by advertising for women who do not experience menstrually-related mood disturbances.

- Flyers will be produced with tear-offs. Posters will not have tear-offs.
- Flyers/poster will be used in color as submitted, or may be printed in black and white. Color changes to recruitment material will be made proportionately throughout and will not be used to change the emphasis of the material.
- The size of the flyers/posters/ads may vary, but all parts, including fonts and pictures, will be changed proportionately. Disproportionate changes in size will not be used to change the emphasis of the material.
- The size of the flyers/posters may vary, but all parts, including fonts and pictures, will be changed proportionately to the rest of that flyer. Disproportionate changes in size will not be used to change the emphasis of the flyer.
- Flyers/posters may be posted on bulletin boards on the NIH campus, and at coffee shops, grocery stores, bookstores, libraries, fitness centers, community centers, or placed in venues, such as at advocacy group offices, in doctor's office waiting rooms, and retail establishments with approval of the venue or in accord with their policy.

- Flyers/posters may be made available at outreach exhibits, speaking engagements, support group meetings, parenting groups, professional meetings, and association/trade meetings with approval of the venue or in accord with their policy.
- Flyers/posters may be given directly to persons requesting study information.
- Flyers/posters may be posted electronically on websites such as such as NIH or NIMH websites, advocacy/parenting group websites such as PTAs, athletic groups or neighborhood groups, publications' websites such as Washington Parent, or Gazette.
- Flyers/posters may be sent electronically to persons requesting study information
- Flyers/posters may be sent electronically to listserv administrators. SBE will not post directly to listservs. Rather, an email with the material attached, including the following statement will be sent to the administrator of the listserv:

  You are receiving the email because your email address is included on this listserv. The purpose of this message is to inform you of an NIMH study that is recruiting subjects. The administrator of this listserv has permitted its use for this distribution.

  The administrator has the option of sharing the information with their list. Examples of types of listservs to be contacted include professional groups, parenting/ family, school, sport groups, women's health and fitness. SBE will retain copies of all correspondence with the administrator of each listserv and submit as requested to the IRB.

- IRB-approved advertisements may be placed in local and national print publications of newspapers, magazines and support or health care organizations, such as The Washington Post, The Express, Washington Parent, Gazette, Washingtonian, Washington Jewish Week and others.
- NIH Record announcements will appear as text in the NIH Record.

c) Screening

Subjects are initially screened for this protocol via participation in screening protocol 81-M-0126. All subjects will have a two month baseline screening period during which mood and behavioral ratings will be obtained. The absence of menstrual-related mood disorders will be prospectively confirmed during a two month period prior to the study entry when subjects will complete daily visual analogue rating scales monitoring both mood and behavior as outlined in NIMH protocol # 81-M-0126.

The Structured Clinical Interview for DSM-IV (130) will be administered to controls prior to study entry. Any control with a current or past axis I psychiatric diagnosis will be excluded from participating in this protocol (except in the subgroup of asymptomatic women with a past major depressive episode who participate in this protocol).

Prior to treatment, a complete physical and neurological examination will have been performed and the following routine laboratory data obtained:

A. Blood

Complete blood count; thyroid function tests; cortisol; renal function tests, such as BUN and creatinine; electrolytes; glucose; liver function tests, B-hCG for pregnancy test.

B. Urine

Routine urinalysis.

GnRH agonist will not be administered to any subject with significant clinical or laboratory abnormalities.

Additional tests.

Results of Pap smear performed not longer than 12 months prior to onset of treatment will be obtained.

d) Study Procedures

Medications:

Following the baseline screening period, subjects will be administered 3.75 mg of GnRH agonist via intramuscular injection on a monthly basis in our clinic for a maximum of 24 weeks (see Figure I).

After week eight of GnRH agonist treatment participants will be randomized to either Group # 1 or # 2 and receive, in a sequential fashion, estradiol and progesterone as follows. The subjects who elect to participate in the brain imaging studies will receive three months of Lupron alone (rather than two) to permit their data to be compared with those from protocol # 90-M-0088. Consequently, subjects in the brain imaging studies will be randomized to Groups 1 or 2 after week 12 of Lupron alone.

Group # 1: (Figure 1) While continuing on the GnRH agonist, subjects will be administered 0.1 mg/day of 17ß-estradiol once or twice a day via vaginal suppository for a period of five weeks. After the fourth week, progesterone suppositories (200 mg BID) will be added to provide progesterone withdrawal induced shedding of the endometrium and menses in order to prevent any effects on the endometrium of prolonged exposure to unopposed estrogen. After the four weeks of estradiol administration and the one week of combined estradiol and progesterone, there will be a two week washout period when neither estradiol nor progesterone will be given.

Subjects will then be administered progesterone suppositories (200 mg) twice daily for a period of five weeks after which no progesterone will be taken.

Group # 2: Group # 2 is identical to Group # 1 except that the order of estradiol and progesterone treatments will be reversed: progesterone suppositories (200 mg) will be administered twice daily for the first five weeks, and after a two week washout period estradiol suppositories will be administered for five weeks and in combination with progesterone during the fifth week.

Symptom Ratings:

Subjects will fill out the following symptom rating scales: (1) Self-ratings: daily ratings include a visual analogue scale (VAS) measuring the reported severity of 15 mood and behavioral symptoms, and the Daily Rating Form (131) (modified to include selected items from the DRSP (132)), which measures the severity of 15 mood and behavioral symptoms and functional impairment; biweekly clinic ratings include the Beck Depression Inventory (BDI) (133), the rating scale for Premenstrual Tension Syndrome (PMTS) – self (134), a self rating scale measuring the severity of mood and behavior symptoms, and a side effect checklist; the Derogatis Inventory of Sexual Function (135), which measures sexual interest and function, will be completed at baseline and during weeks 10-12, 14-16, and 21-23; (2) Observer ratings: the PMTS-rater form (134) will be completed during biweekly clinic visits. Symptom ratings, diagnostic interviews, and other questionnaires will be collected through an online system using a subject-specific log in and password to protect confidentiality (see Appendix I). Both participants and investigators will input data into CTDB/CTSS. Participant entered data will be reviewed during the clinic visit.

Blood Samples - After the administration of the GnRH agonist, in addition to the completion of daily mood and behavioral rating scales, blood samples (50 ml) will be drawn at

each visit, if possible, for the duration of the study (20-24 weeks). Total blood volume will be 550-660 ml. All blood sampling will be obtained in the following fashion. Subjects will arrive at the 4th or 9th floor outpatient clinic between 7:30 and 9:00 a.m.; they will be seated or placed at bed rest, a scalp vein needle will be placed in the forearm or hand via venipuncture, and approximately 50 ml of blood will be drawn. This blood will then be analyzed for several measures such as gonadotropins, estradiol, progesterone, (and its neurosteroid metabolites). Additionally, blood samples and skin biopsies (in combination with those obtained in protocol # 90-M-0088) will be analyzed for hormone and metabolic measures (as well as cellular function of the induced pluripotent cells from the skin fibroblasts taken by biopsy or peripheral blood T lymphocytes) that could distinguish either the symptomatic state in women with PMD or women with from those without PMD. In order to assess the effects of suppression of the hypothalamic pituitary gonadal axis and the re-introduction of (exogenous) estradiol and/or progesterone on cognitive function, subjects will be administered a series of cognitive test batteries at baseline, during the second month of depot Lupron treatment, and during the third weeks of both E2 and progesterone replacement. Further, subjects will be approached for their consent to participate in a number of related protocols attempting to assess and investigate the central nervous system effects of hypothalamic pituitary ovarian axis suppression as well as the effects of selective re-introduction of estradiol and/or progesterone. Related projects will include measures of regional cerebral blood flow, O^{15} water PET, BOLD signal (protocol # 90-CC-0014), lymphoblastoid cells, and induced-pluripotent cells.

Additional Procedures:

A. Skin Fibroblasts and Peripheral Blood Cells for Induced Pluripotent Cell Studies

We wish to perform skin biopsies and collect an additional sample of venous blood in

women who have participated (or who currently are participating) in protocols 90-M-0088, titled "The treatment of menstrually-related mood disorders with the gonadotropin releasing hormone (GnRH) agonist, depot leuprolide acetate (Lupron)," and 92-M-0174, titled "The central nervous system effects of pharmacologically induced hypogonadotropic hypogonadism with and without estrogen and progesterone replacement." Previous findings from these protocols have demonstrated a differential behavioral response (i.e., a recurrence of typical mood and behavioral symptoms experienced premenstrually) to physiologic levels of the ovarian steroids estradiol and progesterone in women with premenstrual dysphoria (PMD) compared with asymptomatic controls. The observed differential response appears to be tissue specific (i.e., neuronal) since the effects of ovarian steroids at several other target tissues does not differ in women with and without PMD (e.g., endometrium, pituitary, breast). The biological basis for this differential behavioral response to ostensibly the same physiologic stimulus is unclear; however, it is well established that tissue specificity in the response to sex steroids is mediated by factors at the level of cellular function. Receptors for both estradiol and progesterone are widely distributed throughout the brain, and recent neuroimaging studies have demonstrated that both estradiol and progesterone regulate the activation patterns in brain regions involved with the processes of affective adaptation and stress responsivity.

We hypothesize that the capacity for phenotypic differences between women with and those without PMD will be preserved in the cellular function of induced pluripotential cells (IPCs). Although the phenotypic difference in response to sex steroids is tissue specific, the latent capacity for differential expression in response to steroids should be preserved in IPCs and expressed in the differentiation of the IPCs into neural elements. Thus, we plan to obtain fibroblasts from skin biopsies and T-lymphocytes from peripheral blood (136) in order to convert

these cells into IPCs to establish neuronal and glial cell lines in both women with PMD and controls. This study will be done in collaboration with Dr. David Goldman at the NIAAA. The goal of this project will be to develop neurons and glial cells from participants and to characterize their gene expression patterns, morphological properties, and cell signaling pathways in response to exposures to physiologic levels of estradiol and progesterone. These experiments, therefore, will allow us to evaluate the nature of the differential behavioral response by examining protein expression and changes in cellular behaviors associated with the exposure to physiologic levels of either estradiol or progesterone across the two different behavioral phenotypes. The two behavioral phenotypes will be constituted by those women with PMD whose symptoms remit during ovarian suppression and whose symptoms recur during physiologic addback of either estradiol or progesterone. This assures the phenotypic fidelity of the subjects and avoids the false positives that would otherwise be generated if diagnoses are established solely on the basis of baseline behavioral ratings. The selection of each woman will occur after completion of the six month study to ensure both the proper characterization of the behavioral phenotype and to include comparable numbers of women in whom symptoms recur after estradiol and progesterone exposure. To recruit sufficient numbers of women in each group we will contact women who have previously participated in these protocols, have met our baseline criteria for PMD or asymptomatic controls, and have further demonstrated either the elimination of symptoms during GnRH agonist and return of symptoms upon re-exposure to estradiol or progesterone (PMD) or the absence of negative behavioral symptoms during both GnRH agonist and ovarian steroid addback phases (controls). Each of these women who is interested in participating will be re-consented for this study.

We hypothesize that the skin biopsy of the fibroblasts and the T-lymphocytes obtained

from women with PMD and those from asymptomatic controls can be converted into IPCs and differentiated into model neuronal and glial cell lines. The production of these pluripotential cell lines will retain genetic (or epigenetic) variations that will help us understand the differential behavioral response at a cellular level in this condition.

Women who participate in these protocols will be approached to obtain consent for the skin biopsies as part of the core consent documents. Additionally, we wish to contact women who have previously participated in this study in order to obtain their consent to perform a skin biopsy. Previous participants will be asked to provide consent using the new consent form accompanying this amendment. All women will be asked to provide a skin sample to study functional genomics of neuronal and glial precursors. Skin biopsies will be performed using an instrument that cuts out one or two 4 mm cylindrical pieces of skin. These biopsies are performed under local anesthesia (2% Lidocaine with or without epinephrine). The level of discomfort associated with the anesthesia is analogous to a PPD skin test for tuberculosis (TB). The biopsy itself is painless. This type of biopsy site usually heals by secondary intention and is covered with a steri-strip type bandage. If necessary, it will be closed with 1 or 2 non-absorbable sutures. These sutures can be removed by the investigator or his/her assistant, in about 7 to 10 days. No more than two biopsies will be taken at any one time. Samples derived from biopsies may be utilized for procedures such as special stains, immunohistochemistry, direct immunofluorescence or immunoelectron-microscopy for localization of immune deposits, PCR, or establishment of cell cultures. In our protocol, the samples will be used to isolate fibroblasts to grow a pluripotent cell lines.

If we are unable to produce cell lines from the initial skin sample obtained then participants will be re-contacted to allow us to take additional samples from a different area of the skin. The

repeat sample will be obtained under the same conditions as those employed to obtain the first biopsy but from a different area of the body. Risks and potential adverse reactions from a second skin sample should be similar to those of the initial sample.

B. Procedures for Sample to Determine Appropriate Dose of Transdermal Estradiol Gel:

We wish to identify the lowest daily dose of transdermal estradiol gel to achieve plasma estradiol levels between 80 and 120 pg/ml; blood levels of estradiol comparable to those obtained with the previously employed transdermal system in this protocol. We do not expect sustained plasma estradiol levels above this range. Nonetheless, the normal follicular phase range of plasma estradiol is between 60 and 300 pg/ml and it is possible that a single high level of estradiol will be observed depending on how soon after gel application blood samples are obtained. Thus, we will recruit up to 10 women who meet the protocol's selection criteria. Each woman will be administered Lupron for two months only. After the first month of Lupron treatment when ovarian suppression has been achieved, we will administer 2-3 applications (approximately 6.0– 9.0 mg estradiol gel (open label)) once a day for four weeks. Estradiol gel (EstroGel® 0.36% estradiol in a hydroalcoholic absorptive gel base; Ascend Therapeutics, Herndon, VA) is dispensed in a controlled release pump bottle calibrated to provide 1 ml of gel containing 3.0 mg of estradiol per pump. The colorless, odorless, and fast drying gel is applied to the skin over the arms, shoulders and outer thighs (as needed) once each day (usually in the morning). Plasma estradiol levels after use of 3.0 and 6.0 mg daily doses (i.e., 1 and 2 pumps respectively) for 7 days in men were approximately 86 and 125 pg/ml, respectively (137). Thus, we anticipate that plasma estradiol levels should reach approximately 100 pg/ml with the two to three applications proposed in this pilot study. Blood samples will be obtained at weekly clinic visits to evaluate whether this dose administered once a day is sufficient to achieve the plasma

estradiol levels targeted in this study (i.e., 80-120 pg/ml). If after one week of two applications of estradiol gel once a day, we observe that this dose schedule is insufficient to consistently obtain the desired blood levels, we will increase the dose to three applications (9.0 mg) each day for the next three weeks. Alternately, if adequate blood levels are observed after the two applications once a day regimen then women will continue at this dose for the remaining three weeks to evaluate the stability of plasma estradiol levels with the two applications each day regimen. We do not anticipate needing a dose greater than three applications each day. After the four weeks of estradiol treatment, all women will receive one week of progesterone suppositories (200 mg twice a day) to precipitate a progesterone withdrawal-induced menses. Upon completing this evaluation, we will provide the data to the FDA to modify the IND under which this protocol and its companion protocol (90-M-0088) are conducted. The duration of Lupron treatment is a third the length of time in which Lupron is administered in the regular six month protocol and the duration of estradiol treatment is the same as that approved in the original protocol. Women participating in this component of the protocol will not be asked to participate in any of the additional procedures employed in the six month protocol (i.e., brain imaging, IPCs, or metabolomics).

Blood Samples for this component of the protocol (10 ml) will be drawn at each visit, if possible, for the duration of the study (8 weeks). Total blood volume will be 90 ml. All blood sampling will be obtained as described in the core protocol. This blood will then be analyzed for gonadotropins and estradiol.

e) End of Participation

When available, the results of this study will be discussed with each participant. Controls

will be discharged from this protocol with follow-up as needed by their private healthcare provider.

5) Management of Data and Samples:

a. Storage

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. DNA samples and cell lines will be stored in locked freezers in David Goldman's laboratory (NIAAA) on Fischer's Lane in Rockville. Only study investigators will have access to the samples and data. Upon the completion of the study, blood samples, DNA and cell lines will be retained. Any loss or destruction of samples will be reported to the IRB.

Data from structured diagnostic interviews and symptom ratings are kept in secure research files and electronically on the Branch server space or within the CTDB database. Access to these research files is only available to study investigators. Symptom ratings, diagnostic interviews, and other questionnaires will be collected through an online system using a subject-specific log in and password to protect confidentiality (see Appendix).

b. Data and sample sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) policy. The sharing of Genomic data according to the GDS policy is covered under the screening protocol 81-M-0126. Data and samples may be shared with dbGaP, collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or

unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6) Additional Considerations:

Estradiol gel (EstroGel® 0.36% estradiol in a hydroalcoholic absorptive gel base) is manufactured by Ascend Therapeutics, Herndon, VA. Progesterone suppositories will be manufactured and supplied by the NIH Pharmacy.

7) Risks and Discomforts:

A. Effects of Medications Administered in this Protocol

Lupron

The most frequent adverse effect of Lupron is hot flushes (flashes) reportedly occurring in 4-89% of patients receiving the drug. Lupron-induced hot flushes have ranged in severity from occasional mild flushing to frequent sweating. In general, episodes of flushing persist with continued therapy in most patients receiving Lupron. In a recently completed study of 400 women of reproductive age with either uterine fibroids or endometriosis who each received 3.75 mg depot Lupron every month for a period of six months, the most common side effects

reported to occur were as follows: 1) hot flashes of mild to moderate intensity (89%), 2) headache (22%), 3) nervousness or irritability (11%), and 4) insomnia (10%). Local irritation at the injection site was complained of in less than 10% of the patients in this sample, and there was a mean decrease in bone density, as measured by bone densitometry, of 3.4 to 4.0%, which totally reversed after the medication had been discontinued for six months. Approximately ten patients of the original sample of 400 found the side effects to be severe enough to discontinue therapy. Regular menstrual cycle function returned within two months following the last injection of depot Lupron (Tapp Pharmaceuticals, personal communication). Blurred vision, lethargy, memory disorder, and numbness have been reported in less than 3% of patients receiving the drug. Thrombophlebitis, phlebitis and/or pulmonary embolism, and congestive heart failure have occurred rarely in patients receiving Lupron, but a causal relationship to the drug has not been established. Adverse GI effects occurring in 2% or more of patients receiving Lupron include nausea and/or vomiting, constipation, and anorexia. Diarrhea and a sour or unusual taste in the mouth have been reported less frequently. Other adverse effects of Lupron occurring in less than 3% of patients include decreased hematocrit and hemoglobin concentration, fatigue, fever, facial swelling, rash, hives, hair loss, and itching. Limited information is available on the acute toxicity of Lupron. Following subcutaneous administration of Lupron in rats at dosages 250-500 times the usual human dosage, dyspnea, decreased activity, and local irritation at the injection site were observed; however, there is no evidence to date that overdosage in humans produces similar adverse effects. Lupron dosages up to 20 mg daily for up to two years have not produced unusual adverse effects in humans. There has been one report of an anaphylactic reaction in a patient following administration of a GnRH agonist. Recent longitudinal follow-up studies of girls and boys receiving GnRH agonists as a treatment for

precocious puberty report the development of normal reproductive function and fertility (138-140). The FDA recently published a caution about the effects of GnRH agonists in elderly males; however, the safe use of these agents in younger adults and children (141) was not questioned in the FDA statement.

Estradiol

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

The relationship between estrogen, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation (142). Numerous retrospective case control studies published since 1975 have indicated that post menopausal exposure to unopposed estrogens for more than one year results in a two to 12 fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen therapy appears to decrease the risk of endometrial hyperplasia and endometrial cancer to equal or below that of women receiving no hormonal treatment. Recent studies suggest that the optimal regimen to prevent hyperplasia and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progestin treatment each month when estrogens are administered (143). There is an increase in thromboembolism and stroke in

women receiving estrogen therapy (144-151) (and, possibly, ovarian cancer (152) and lung cancer (153, 154)); however, these complications are unlikely at the dose and duration of estrogen therapy employed in this protocol and in the younger age of the women participating in this trial. One study (123) reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use (143, 155, 156). Additionally, recent studies (151, 157, 158) observed that an increased risk of venous thromboembolism was associated with oral but not transdermal estrogen compared with nonusers (in one study (157) odds ratios = .42 [95% CI, 1.5 to 11.6] and 0.9 [95% CI, 0.4 to 2.1] respectively). The mechanism underlying this observed difference in the risk of thromboembolism is not known; however, it has been suggested to reflect a lower activation of hepatic metabolism by the transdermal route of administration due to a smaller first past effect. Blood pressure, on average, appears to be unaffected by estrogen therapy, although both increases and decreases have been reported. Post menopausal estrogen therapy has been observed to increase the relative risk of cardiovascular disease in some (148, 159) but not all studies (160-162). Indeed recent analyses of the Women's Health Initiative demonstrate that the adverse effects of estrogen therapy on cardiovascular outcomes were largely confined to older women compared with younger perimenopausal women (150, 163-171). 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 (172-174). Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use (150, 175-187),

similar to the risk expected if the onset of menopause was delayed for a comparable length of time.

Due to the publicity surrounding the cancellation of the treatment arm of the Women's Health Initiative study (188) that involved the administration of combined conjugated estrogens and medroxyprogesterone acetate (Prempro), we have included the following statement in the consent documents:

Adverse Events Related to Combined Hormone Replacement and the Results of the Women's Health Initiative (WHI):

The WHI study demonstrated that continuous administration of one form of estrogen (conjugated estrogens) in combination with one form of progesterone (medroxyprogesterone acetate) is associated with an increased risk of dementia, heart attacks, stroke, blood clots, and breast cancer. Estradiol, the form of estrogen that we use in this study, is administered as a sole agent (with the exception of one week's combination with progesterone) and, consequently, we do not expect that it will pose the increased risks observed with the chronic combination of the conjugated estrogens and medroxyprogesterone administered in the WHI study. Indeed, while the estrogen alone arm of the WHI trial was shown to be associated with an increased risk of stroke, no increased risk of either heart disease or breast cancer was observed (188, 189).

Estrogens may precipitate migraine headaches, and depression has also been reported to occur with the use of estrogens. In general, considering the dose and duration of treatment that we propose to use in this protocol, the risk of developing such side effects is negligible.

Progesterone

Progesterone and the synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty

(128). Side effects reported in women taking progestins are uncommon but may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression of mood, easy fatigue, lack of initiative, and chloasma. Since progestins are often used in women with antecedent menstrual irregularity, it is not clear whether the breakthrough bleeding represents an effect of the medication or refractoriness to treatment. In the large majority of patients, menstruation occurs predictably following withdrawal of progestins and is usually more regular than in spontaneous cycles.

For the sake of completeness, it is also notable that side effects have been observed in women receiving combined oral contraceptives, including nausea, breast soreness, vaginal discharge, fluid retention, hypertension, and clotting abnormalities, which have been associated with the estrogen component of the oral contraceptive. Thromboembolic disorders including thrombophlebitis, pulmonary embolism, and cerebral and coronary thrombosis appear to occur with greater frequency in women taking oral contraceptives. While the increased incidence of these disorders has been associated with the estrogen component of the oral contraceptives, it is now believed that the progestogen component may, to a lesser extent, contribute to the increased risk. There are relatively few reports associating oral contraceptives with the development of carcinomas (vaginal, uterine, hepatic, and mammary) despite the vast use of these agents, although this may reflect the latent period needed for cellular transformation. Finally, several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies.

B. Effects of Additional Procedures

Skin Biopsies

The risk of skin biopsy may be slight bleeding or infection. To avoid these complications a sterile dressing will be applied for 7-10 days until the scar is well formed. On occasion, the wound may be closed with 1 or 2 non-absorbable sutures. Acquiring skin fibroblasts from voluntary subjects is minimally invasive. Skin biopsy procedures can be considered as minimal risk since the risk is not greater than blood draws, a procedure already included in the protocol. The level of discomfort associated with the anesthesia is analogous to a PPD skin test for TB and the biopsy itself is painless.

Blood Drawing

One discomfort of this study may occur due to the venipuncture and multiple blood sampling. Total blood withdrawal (550-660 ml) falls within NIH guidelines (550 ml. per eight week period during this 20-24 month study).

8) Subject Safety Monitoring:

Subjects are evaluated by one of the associate investigators. Medical history and physical assessments occur at each clinic visit including interviews, symptom assessments, vital signs, and laboratory testing when clinically indicated. After completing the study, subjects will either be discharged from this protocol with a referral to the community, or will be referred to other NIMH studies.

Patients will be warned not to become pregnant during the study and will be advised to employ barrier contraceptive methods.

9) Outcome Measures:

The outcome measures are as follows: 1) brain imaging measures including O^{15} PET and fMRI (performed as part of NIMH protocol 90-M-0014); 2) Dex/CRH stimulation tests; 3) metabolomic profile; 4) DNA for genotyping (performed under protocol 81-M-0126; and

5) cell culture studies (lymphoid and IPCs). Additionally, we will employ the symptom ratings (described above) to evaluate the effects of GnRH-agonist-induced hypogonadism and estradiol and progesterone replacement on measures of mood and behavior.

10) Statistical Analysis:

In this protocol we originally proposed using 30 subjects. Calculations of the minimum sample size that will provide statistical significance between the three different hormonal states (hypogonadal, hypogonadal plus estrogen, and hypogonadal plus progesterone) with a power of 80% were performed using the results obtained in the Hampson and Kimura study (190). Additionally, we supplemented this analysis with our own preliminary neuropsychological testing results from protocol # 90-M-0088. In the Hampson and Kimura article standardized differences of 0.6, 0.7, and 0.5, were calculated for the finger tapping, Purdue Pegboard, and the manual sequence box, respectively. The standardized difference obtained from five women who have been tested during the hypogonadal and during the hypogonadal plus estrogen phase of protocol # 90-M-0088 results in a standardized difference of .6. Thus, the originally calculated sample size required to provide 80% power at an alpha of 0.05 with the standardized difference obtained from these two investigations was approximately 30 subjects.

Amendments to Sample Size

Brain Imaging

On the basis of our prior work (65), and as described in protocol # 90-M-0014 we anticipate that the 20 subjects recruited for the HPA axis studies will be ample for showing differences in rCBF.

Skin Biopsies

To recruit sufficient numbers of women in each group we will contact women who have

previously participated in these protocols, have met our baseline criteria for PMD or asymptomatic controls, and have further demonstrated either the elimination of symptoms during GnRH agonist and return of symptoms upon re-exposure to estradiol or progesterone (PMD) or the absence of negative behavioral symptoms during both GnRH agonist and ovarian steroid addback phases (controls). Each of these women who is interested in participating will be re-consented for this study.

11) Human Subjects Protection:

a. **Subject selection**

All subjects must meet the inclusion and exclusion criteria listed in Section 6. We will select physically healthy adult female individuals. The proportion of ethnic minorities (vs. Caucasians) in the total sample, will be approximately consistent with the overall U.S. population proportions.

We are limiting the upper age range of the women in this study to 50 years of age to match the age ranges with those in the existing companion protocols, 90-M-0088 and 05-M-0059. Women over the age of 50 may have age-related changes in brain function, HPA axis function, or reproductive hormone secretion, which could have confounding effects on the outcome measures in this and the companion protocols.

b. Justification for exclusion of children

We will exclude children or minors because the study population is menstruating premenopausal women.

c. Justification for exclusion of other vulnerable subjects

We will exclude subjects that are unable to provide their own consent given the length of the protocol and associated risks.

d. Justification of sensitive procedures N/A

e. Safeguards for vulnerable populations

Protections for NIH employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff.

f. Qualifications of investigators

Peter J. Schmidt, M.D., is an investigator with the Section on Behavioral Endocrinology, NIMH. He has over 20 years experience performing studies that examine the effects of reproductive hormones on mood and behavior in women with reproductive endocrine-related mood disorders. He will be allowed to obtain consent.

David R. Rubinow, M.D., is a special volunteer and collaborator within the Section on Behavioral Endocrinology, NIMH. He has over 30 years experience in reproductive endocrinology and psychiatry. He will not be involved in obtaining consent nor will he have access to PII.

Lynnette K. Nieman, M.D., is a senior investigator in the Reproductive Biology and Medicine Branch, NICHD and has extensive experience with clinical research studies in endocrinology and reproductive biology. She will not be involved in obtaining consent.

Karen F. Berman, M.D., is a senior investigator within the Section on Integrative Neuroimaging, CDBD, NIMH. Dr. Berman has 25 years of experience in neuroimaging. She will not be involved in obtaining consent.

Pedro E. Martinez, M.D., is a staff clinician within the Section on Behavioral Endocrinology, NIMH and has performed endocrine studies examining the effects of aging and reproductive hormones on mood and behavior in both adults and children. He will be allowed to obtain consent.

Shau-Ming Wei, PhD has an extensive experience in experimental design, and she has designed and implemented both behavioral and neuroimaging experiments over the past several years. Her graduate training at the NIH/Brown Program included collaborative neuroimaging studies on the effects of genotype and reproductive hormones on brain function. She has worked with our program for more than ten years (as part of our ongoing collaborations with Dr. Karen Berman's group), first as a doctoral candidate, then as a postdoctoral fellow. Currently, she is a research fellow within our Branch and in the future will be offered a position as a staff scientist. Over the last decade Dr. Wei has worked closely with our clinical program and this protocol involving the administration of a GnRH agonist with and without ovarian steroid hormone replacement. She will be involved in imaging data collection and analysis, and manuscript preparation. She will not obtain informed consent.

Qualifications of telephone interviewers:

Screenings are performed by Annie Shellswick, who is a licensed social worker in our group, and who is in charge of our recruitment and outreach activities. Personally identifiable information is not obtained in callers who are determined to be ineligible, and the screening forms are not retained in the research records.

12) Anticipated Benefits:

There are no direct benefits to participants in this protocol. This protocol allows us to selectively examine the central nervous system effects of the gonadal steroids and, thus, provides

the opportunity to explore the linkages between changes in mood and behavior and gonadal steroid activity. Further, as a control for protocol # 90-M-0088 this study will extend our understanding of potential biological mechanisms underlying menstrual-related mood disorders and offers the possibility of uncovering some etiopathogenic mechanisms involved in these and related mood disorders.

13) Classification of Risk:

The overall risks for this study are more than minimal.

There are low risks to individual subjects in the use of medication and procedures under the conditions stated in this protocol.

14) Consent Documentation and Process:

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent in section 12 above, will obtain informed consent.

b. Consent procedures

Each patient will receive a verbal and written explanation of the purpose, procedures, and potential hazards of this project. A record of the communication of this information and of the consent to participate in this study will be placed in the medical record. The right of the subjects to withdraw from the study or to refuse any procedure will be made clear. Any patient whose side effects become excessive during either the GnRH alone or estrogen/progesterone replacement phases will be offered the option to withdraw without completing the five or six month trial. Confidentiality of patients will be assured according to the laws of the State of Maryland. In case of published data resulting from the study, care will be taken to protect the anonymity of patients.

c. Consent documents

Each consent contains all required elements. There are 3 consent forms used for this protocol: Standard (6 months), Estradiol gel (pilot study), and MDE (major depressive episode).

15) Data and Safety Monitoring

a) Data and safety monitor

The PI will serve as the data and safety monitoring official.

b) Data and safety monitoring plan

As we are administering doses of estradiol and progesterone designed to produce physiologic levels, we expect - and have seen - no unexpected adverse events. Similarly, we do not anticipate unexpected serious adverse events with the dose of Lupron that we propose to use. Nonetheless, we see subjects every one to two weeks during their clinic visit and advise them in the consent form that if they experience side effects, they should notify the investigator immediately. Any adverse events will be reported as per NIH policy. The PI will review data and safety parameters at least annually. The PI will document the data and safety review in the research records and at the time of continuing review.

c) Criteria for stopping the study or suspending enrollment or procedures

The study will be stopped if the estradiol suppositories become unavailable or if new findings emerge that indicate it would not be safe to continue the study. The study will be stopped if there is any Serious Adverse Event related to the research.

The PI and IRB will determine if changes are needed for the research to continue or if it will be closed.

16) Quality Assurance:

A. Quality assurance monitor

Quality assurance will be monitored by the PI, the research team and the NIMH Office of Regulatory Oversight (ORO).

B. Quality assurance plan

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the ORO SOP based on the study level of risk. Results of ORO audits are provided to the PI, The Clinical Director and the CNS IRB. As an IND study, this protocol will be subject to GCP audits at study initiation and after the first enrolled subject. Timing of subsequent review will be established by ORO but no less frequent than every other year.

17) Reporting of Unanticipated problems, adverse events and protocol deviations:

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

Serious unanticipated problems, serious adverse events (including deaths) that are not unanticipated problems, and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event, unless immediate reporting is waived for specific serious adverse events as noted below. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

18) Alternatives to Participation:

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

19) Privacy

All research activities will be conducted in as private a setting as possible.

20) Confidentiality

a. For research data and investigator medical records

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers.

No sensitive information is collected, so employee information will be treated the same as all other participants.

b. For stored samples

Samples will be kept in locked storage. Only study investigators will have access to the samples and data. Samples and data will be stored using codes that we assign. DNA samples and cell lines will be stored in locked freezers in David Goldman's laboratory (NIAAA) on Fischer's Lane in Rockville. Only study investigators will have access to the samples and data.

c. Special precautions: N/A

21) Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts-of-interest to report.

22) Technology Transfer

There is an active CTA with Besins Healthcare which was executed on 08/01/14 and expires on 08/01/19. Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. DNA samples and cell lines will be stored in locked freezers in David Goldman's laboratory (NIAAA) on Fischer's Lane in Rockville. Only study investigators will have access to the samples and data.

23) Research and Travel Compensation:

Each volunteer will be compensated according to the following schedule:

Core Protocol	
Initial evaluation, physical exam (2 hours)	100.00
Screening phase	100.00
Clinic visits (weekly) 2 hour x 13	390.00
Multiple venipuncture x 13	260.00
Psychological testing x 13 (at each clinic visit)	100.00
Symptom rating scales x 6 months	150.00
Investigational drugs	
a) Depot Lupron injections 100.00 x 6	600.00
b) Vaginal suppositories/transdermal patch daily x 3 months	800.00
Total	2500.00

Dose-finding protocol for estradiol gel:	
Initial evaluation, physical exam (2 hours)	100.00
Screening phase	100.00
Clinic visits (weekly) 2 hour x 7	210.00

Multiple venipuncture x 9	180.00
Symptom rating scales x 2 months	50.00
Investigational drugs :	
a) Depot Lupron injections 100.00 x 2	200.00
b) Estradiol gel application daily x 1 month	60.00
Total	900.00

Reimbursement of travel and subsistence will be offered consistent with NIH guidelines. Compensation will be prorated for parts completed if subjects do not complete the study. No escort fee will be provided.

Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Additional Procedures

Skin biopsies	$100 per biopsy

Blood Drawing Schedules:

(A) Core Protocol (24 weeks) –660 ml (six months)

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Flow Sheet:

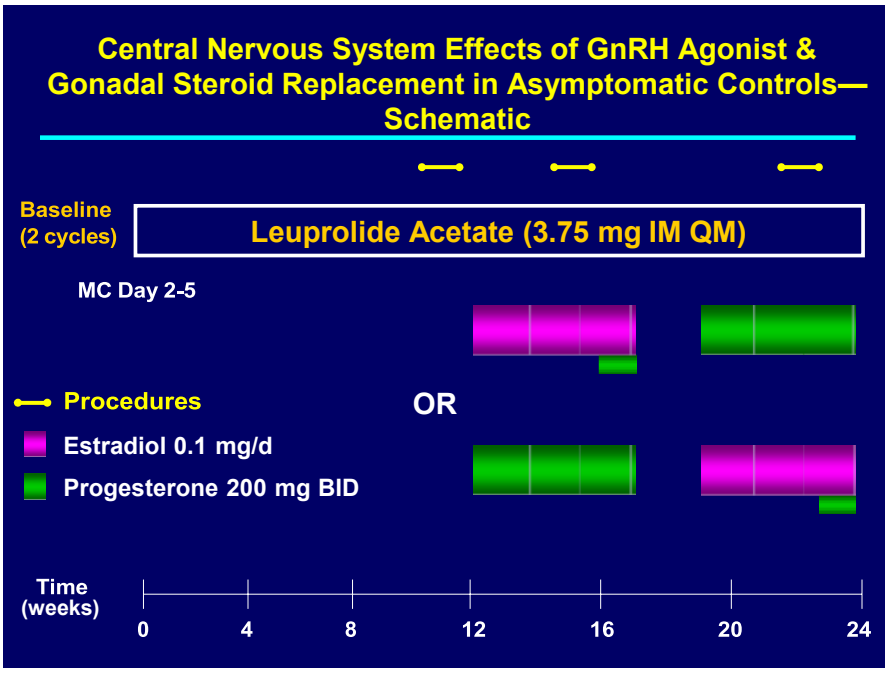
Central Nervous System Effects of GnRH Agonist & Gonadal Steroid Replacement in Asymptomatic Controls—Schematic
Baseline (2 cycles)
Leuprolide Acetate (3.75 mg IM QM)
MC Day 2-5
Procedures
Estradiol 0.1 mg/d
Progesterone 200 mg BID
OR
Time (weeks)
0
4
8
12
16
20
24

Table I

Conditions Associated with Increased Risk for Osteoporosis

AIDS/HIV	Hyperparathyroidism	Multiple Sclerosis
Amyloidosis	Hypogonadism (primary or secondary)	Multiple Myeloma
Ankylosing Spondylitis	Hypophosphatasia	Pernicious Anemia
COPD	Hypophosphatemia	Rheumatoid Arthritis
Congenital Porphyria	Idiopathic Scoliosis	Severe Liver Disease, esp PBC
Cushings Syndrome	Inadequate Diet	Spinal Cord Injury
Eating Disorders	Inflammatory Bowel Disease	Sprue
Female Athlete Triad	Insulin-Dependent Diabetes	Stroke/CVA
Gaucher's Disease	Lymphoma/Leukemia	Thallasemia
Hemochromotosis	Malabsorption Syndromes	Thyrotoxicosis
Hemophilia	Mastocytosis	Tumor

Adapted from National Osteoporosis Foundation Physician's Guide

Clinical Trials Database - Security Overview

There are multiple aspects to the security framework for the Clinical Trials Database (CTDB) and Clinical Trials Survey System. The following features allow for the safe and secure collection of research variables:

- **Application Firewall**- The NICHD has recently upgraded their application firewall which protects both the front end web server and back end database server for the CTDB. Strict policies are in place which control exactly who has pre-defined, limited access to the application. This firewall is a state-of-the-art hardware solution which blocks access to everyone but authorized users for the CTDB system.
- **Data Encryption and SSL Certificates** - The CTDB system makes use of military grade encryption both for the session and the data storage. The CTSS collects de-identified self-reported data. Both systems protect information from interception by encrypting the data flow using SSL with a 1024 bit signed certificate. The entire communication session from the time a client requests a connection to the system to the time a user logs out is encrypted using a certificate from an industry recognized vendor. The result is a secure communications channel for our partners, providing data confidentiality and integrity. In further, the CTDB program encrypts data stored within the system, thereby providing an additional layer or security for the sensitive CTDB clinical data.
- **CTSS HIPAA Requirements** - Below is the list of identifiers to be removed for the de-identification of health information under HIPAA. This is found in 45 C.F.R. 164.514(a),(b)&(c)

  (A) Names;
  (B) All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of the Census:
  (1) The geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and
  (2) The initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
  (C) All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89

and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older;
(D) Telephone numbers;
(E) Fax numbers;
(F) Electronic mail addresses;
(G) Social security numbers;
(H) Medical record numbers;
(I) Health plan beneficiary numbers;
(J) Account numbers;
(K) Certificate/license numbers;
(L) Vehicle identifiers and serial numbers, including license plate numbers;
(M) Device identifiers and serial numbers;
(N) Web Universal Resource Locators (URLs);
(O) Internet Protocol (IP) address numbers;
(P) Biometric identifiers, including finger and voice prints;
(Q) Full face photographic images and any comparable images; and
(R) Any other unique identifying number, characteristic, or code, except as permitted by paragraph (c) of this section

- **Logical Access Controls (Role/Privileges)** – Logical access controls are in placed using role based security for database access and application account access. Security controls are in place to detect unauthorized access attempts. The application is further protected by the NICHD firewall and NIH firewall. Inactive user accounts are monitored and removed when not needed, and users are disconnected after a specific period of inactivity. Encryption is used (1024bit SSL key) and data is HIPPA compliant. Access is monitored and apparent security violations are investigated when identified. Insecure protocols are disabled on all application servers. Guest and anonymous accounts/access is disabled.
- **Audit Trails** – Activity involving access to and modification of sensitive or critical files is logged and monitored for possible security violations. Access to these audit trails is strictly controlled and can be used to support after-the-fact investigations of how, when and why normal operations ceased should this occur. Off-line storage of audit logs is retained for a period of at least 1 year. Suspicious activity is investigated and appropriate action is taken when warranted.
- **Physical and Environmental Protection** – The servers are physically located in a secured NIH data center with controlled limited access. All work products from the system including Data backup tapes are rotated to off-site storage with must be authorize and are recorded. All visitors to sensitive areas are escorted with entry codes changed periodically. Fire prevention

and suppression devices are installed and in working condition. All heating and air-cooling systems are periodically checked to ensure proper working condition.

- **Production Input and Output Control** – Audit trails are in place to record data changes. Only authorized system administrators are allowed access to this data, as well as any data backup tapes. Damaged media is sanitized or destroyed, and any hardcopy media is shredded when no longer needed.
- **Contingency Planning** – All critical data files, database files and web server files have been identified. A regularly scheduled data backup solution is in place with identified resources supporting critical operations. A comprehensive contingency plan has been developed and documented. This plan has been approved by key affected parties. The Contingency plan/Disaster recovery plan is regularly tested and adjusted as appropriate.
- **Hardware and Software System Maintenance** – Access is limited to the hardware and software infrastructure. Restrictions are in place as to who performs maintenance activities. Procedures are in place to monitor the use of system resources. All new and revised hardware and software are tested and approved before implementation. All system and application components are tested, documented and approved prior to promotion to production environment. Detailed system specification as prepared and reviewed by management. A version control system is in place for all key application and operating system files. The systems are actively managed to monitor and reduce vulnerabilities with unnecessary services eliminated.

Strategic framework – The tactical security framework provides a mechanism whereby the Clinical Trials support personnel provide day-to-day operational support activities for the regular maintenance of the CTDB system. These initiatives incorporate application and software security. The CTDB application was designed using the latest Java technology. This allows the adaptation of the application to ever-changing business rules within the application. An Oracle 10g relational database provides the repository for the clinical data. The use of a robust, industry standard relational database provides a modular architecture design of CTDB which allows for the CTDB program to assign role based security to the participants in the system. This allows roles to be defined and implemented for different users- such as investigators, study participants, report writes, etc- in order to secure database access and the application data stored within the system. This implies that the NICHD CTDB partners can implement custom roles and maintain their own clinical data with a high level of confidence that the data will not be compromised nor shared with non-participants. As technology evolves, this attention to the strategic framework allows us to address individual software components and target them for enhancements or upgrades all while maintaining the integrity and confidentiality of the CTDB system. Another

example of this strategic framework is the reporting interface. The modular design of CTDB allows provides the ability to upgrade to Cognos reporting with the result being more detailed comprehensive reporting abilities. The net result is a feature-enhanced system while maintain the strict security framework of the system.