A Randomized, Double-blind Placebo-controlled Multi-center Study of Identifying Neural Mechanisms of PTSD Symptom Reduction Induced by Combined Estrogen and Prolonged Exposure Therapy

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Sponsor

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A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTI-CENTER STUDY OF IDENTIFYING NEURAL MECHANISMS OF PTSD SYMPTOM REDUCTION INDUCED BY COMBINED ESTROGEN AND PROLONGED EXPOSURE THERAPY

Tool Revision History:

Version Number	Version Date	Summary of Revisions Made
.01	10 October 2019	Original approval
1.1	22 October 2019	Training Protocol changes, adding Bryana Schantz as study team member
1.2	2 December 2019	Clarify measures at each visit, clarify discontinuation procedures
1.3	5 December 2019	Remove 1-month follow-up visit and offer referrals earlier, at post-treatment assessment visit. Add three new study team members to the protocol (a 4th year medical student and two volunteers). Clarify exclusion criteria; specifically, detailing individual diagnoses that will be excluded
1.4	17 December 2019	Revised per IRB requests
1.5	16 January 2020	Fix mistake in Inclusion Criteria, and add Dylan Miller to the protocol
1.6	4 February 2020	Corrected visits for which patients take Estradiol to include PE session 6, added Adherence Questionnaires to Assessments Administered at PE Treatment Sessions (Table 2), added Early Withdrawal Form to Other Forms, and changed SAE language
1.7	27 February 2020	Updated criteria for additional PE sessions following study completion, added biphasic birth control to inclusion criteria, clarified procedures for experimental days 1 and 2, deleted 1-month follow-up to be consistent with rest of protocol, corrected amount of blood drawn, granted Emma Jennings EPIC access, clarified participation payment timing
1.8	15 April 2020	Added Sarah Boukezzi, PhD to protocol, and clarified language re: screening/baseline assessment and timing
1.9	30 June 2020	Updated inclusion/exclusion criteria as follows: added benzodiazepines to the exclusion criteria, removed right-handedness from inclusion criteria. Updated the version of the Life Events Checklist (LEC) used to the LEC-5 Extended version.
2.0	10 August 2020	Updated inclusion/exclusion criteria to remove peanut oil allergy. Updated study phase to Phase 3. Removed Rebecca Suzuki, Stephanie Wu, and Camille Johnson from study staff, and removed Rebecca Suzuki's EPIC access.
2.1	23 September 2020	Added state version of STAI (form Y1) to experimental days 1 and 2 both pre- and post-treatment (i.e, visits 2, 3, 10, & 11). Removed MINI from post-treatment assessment (visit 12) and 3-month follow-up (visit 13). Updated that electrodes will be placed on the fingers (not foot) to record skin conductance data in PE therapy sessions. Updated that electrodes will be placed on foot (not hand) for electric shocks on experimental days 1 and 2. Removed Sarah Boukezzi from study staff and

		discontinued her EPIC access. Added Zhenfu Wen to study staff.
2.2	8 October 2020	Granted EPIC access to Katie Eastburn, NP. Clarified number of subjects to be enrolled in training portion of study. Clarified randomization procedures. Added PDS-5 measure to baseline assessment, post-treatment assessment, 3-month follow-up, and 6-month follow-up.
2.3	21 October 2020	Added that participants who are deemed ineligible during or after the Baseline Assessment visit will be paid $10 for each hour they spent completing study procedures /assessments. Clarified that the lifetime/recent version of the CSSRS will be performed at the Baseline Assessment, but only the recent (i.e., past 1-3 months) version will be administered at the post-treatment assessment and 3- and 6-month follow-ups.
2.4	4 November 2020	Added NuvaRing birth control method to study inclusion criteria. Added specification to exclusion criteria on self-injurious behaviors.
2.5	21 December 2020	Added CAPS-5 Past Week version to assessment visits (baseline assessment, post-treatment assessment, and 3- and 6-month follow-ups). Removed CAPS-5 Past Month version from Post-treatment Assessment and 3-month follow-up. Added option to obtain participant consent electronically via REDCap. Added ClinCards as payment option. Clarified that forms can be filled out either in writing or electronically. Updated that the 4 additional PE sessions will be offered to eligible patients at the 3-month follow-up, not the post-treatment assessment.
2.6	23 March 2021	Added option for pre- and post-experimental day blood draws to occur on Day 2 instead of Day 1 if needed due to scheduling. Added option for any visit, except for pre- and post-experimental days to occur remotely via Webex if needed in special circumstances. Updated exclusion criteria to include neurological disorders and history of seizures or head trauma.
2.7	03 June 2021	Updated inclusion criteria to include the transdermal birth control patch. Granted EPIC access to Isabel Moallem, PhD. Added Nextdoor to the list of advertisement platforms. Updated the name of Dr. Simon's lab.
2.8	14 October 2021	Added additional recruitment methods; broadened inclusion criteria to include certain types of vaginal birth control ring (not only brand NuvaRing). Granted Epic access to Noor Nassar and Joseph Borelli.
2.9	07 December 2021	Created process regarding positive UTOX for THC during the Experimental/Scanning visits: Added saliva THC test as additional procedure to implement if participant has positive UTOX at the Experimental/Scanning visit. Added information about texting participants.
3.0	08 February 2022	Clarified use of updated NEO measure: NEO-FFI-3 form
3.1	14 April 2022	Updated list of team members who have access to EPIC results and clarified information for incidental findings.

		Added information about new recruitment source: BuildClinical.
3.2	09 June 2022	Added an exclusion criterion to improve subject screening. Included additional NYU MRI scanning location 159 E. 53rd Street location. Clarified that recruitment strategies will including contacting participants through the iConnect registry.
3.3	15 JULY 2022	Updated list of study staff who will have access to EPIC. Clarified that Adverse Events will be assessed at experimental visits (in addition to the clinical visits and follow-up assessment visits).
3.4	24 AUGUST 2022	Updated the list of personnel who will have access to Epic Updated section 8.8.2 to indicate that any study physicians will review AEs/SAEs and handle prescriptions.
3.5	11OCTOBER2022	Updated language to specify blue ink is also allowable. Update to list of staff with Epic access Updated payment distribution to increase compensation for completing the first visit. Removal of Payment Method Language
3.6	12 DECEMBER 2022	Edited inclusion criterion (CAPS score cutoff and including subPTSD) Update to staff list with Epic access Update to incidental finding language Clarified language regarding data deletion Update to Datacore recruitment practices
3.7	19JUNE2023	Initial submission to UTHealth Houston IRB
3.8	21AUG2023	Replaced Methodist Hospital with Baylor College of Medicine, as MRI scanning site. Revised compensation. Revised recruitment strategies. Added Labcorp, where blood will be drawn and processed.
3.9	11SEPT2023	Updated an exclusion criterion with UTHealth information
4.0	05OCT2023	Added to Recruitment strategies: Info/IRB number for BBSB recruitment registry. Edited Study Drug Label. Added information on blood draw.
4.1	31OCT2023	Added Jair C. Soares, MD, PhD to the study.
4.2	13DEC2023	Added estradiol test.
4.3	08JAN2024	Updated role of Dr. Mira Milad in the protocol. Added Quest Diagnostics as option for blood draw and processing.
4.4	03MAR2025	Added plan for sharing participant contact information with protocol HSC-MS-23-1106

Study Personnel

Principal Investigator:	Mohammed R. Milad, Ph.D. [redacted]
Additional Investigators:	Edna Foa, Ph.D. [redacted]
Key Study Personnel:	Mohammed R. Milad, PhD Principal Investigator Mira Milad, M.D. Physician Ronald E. Acierno, Ph.D. Investigator Isabel Moallem, Ph.D. Clinical Assessor Zhenfu Wen, Ph.D. Investigator Jair C. Soares, M.D., PhD. Physician
UTHealth Houston Study Number:	HSC-MS-23-0497
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Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation ("ICH") Guideline for Good Clinical Practice ("GCP") (sometimes referred to as "ICH-GCP" or "E6") will be applied only to the extent that it is compatible with FDA and DHHS regulations. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

Table of Contents

List of Abbreviations

ACTH	Adrenocorticotropic hormone
AE	Adverse Event/Adverse Experience
BOLD	Blood Oxygen Level Dependent
CFR	Code of Federal Regulations
CR	Conditioned Response
CRF	Case Report Form
CS	Conditioned Stimuli
CSOC	Clinical Study Oversight Committee
CTSA	Center for the Treatment and Study of Anxiety
CX	Conditioning Context
CV	Coefficient of Variance
dACC	Dorsal anterior cingulate cortex
DCC	Data Coordinating Center
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone Sulfate
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
E2	Estradiol
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
FFR	Federal Financial Report
fMRI	Functional magnetic resonance imaging
FWA	Federalwide Assurance
GAD	Generalized anxiety disorder
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LH	Luteinizing Hormone
MOP	Manual of Procedures
N	Number (typically refers to participants)

NIH	National Institutes of Health
OCs	Oral Contraceptives
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PHI	Personal Health Information
PI	Principal Investigator
PTSD	Post-Traumatic Stress Disorder
QA	Quality Assurance
QC	Quality Control
RF	Radiofrequency coil
ROI	Religion of interest
SAE	Serious Adverse Event/Serious Adverse Experience
SCR	Skin Conductance Response
SOP	Standard Operating Procedure
THC	Tetrahydrocannabinol, the main psychoactive compound in cannabis
UIC	University of Illinois at Chicago
US	Unconditioned stimulus
UTOX	Urine toxicology screen or urine drug screen
vmPFC	Ventromedial prefrontal cortex

Protocol Summary

Title	Estradiol as a Cognitive Enhancer for the Treatment of Post-Traumatic Stress Disorder in Women
Short Title	The influence of gonadal hormones on fear extinction
Brief Summary	The purpose of this research study is to determine if taking a pill of estradiol (E2) together with prolonged exposure (PE) therapy can improve this treatment outcome in women diagnosed with Post-Traumatic Stress Disorder (PTSD). 80 subjects will take part in this research study across UTHealth Houston and Penn (40 subjects at each site). Participants will be randomized into one of two groups, PE + E2 or PE + placebo. The study will include preliminary screening and baseline visits, experimental visits, and therapy visits over the course of six weeks. Two follow-up visits will take place at 3 and 6 months.
Phase	3
Objectives	Primary: To examine the effects of E2+PE on the extinction-induced psychophysiological responses (SCR), neural responses, and the information flow between the fear extinction nodes at post-PE sessions relative to pre-PE. Secondary: To examine the impact of E2 administration on PE efficacy at reducing PTSD symptom severity. Tertiary: To test the interaction between E2+PE induced psychophysiological and neuronal changes (pre-to-post) with the improvement of PTSD symptom severity.
Methodology	Randomized, double-blind, placebo-controlled trial
Endpoint	Primary study endpoint will be Blood Oxygen Level Dependent BOLD fMRI signals and Skin Conductance Response (SCR) during recall. The secondary study endpoint will be symptom severity.
Study Duration	3 years
Participant Duration	The initial study takes 6 weeks with follow-up visits at 3 and 6 months post-completion of the initial study.
Duration of IP administration	2 mg of Estradiol, as obtained from Estrace, to be administered once over the course of 5 sessions.
Population	Sample size is 80 enrolled subjects, 40 at UTHealth Houston, 40 at Penn. Participants will be females ages 18-45 with chronic (at least 1 month post-trauma) DSM-5 FULL PTSD diagnosis and a Clinician-Administered PTSD Scale for DSM-5 (CAPS) Past Month version score ≥20 OR chronic (at least 1 month post-trauma) subPTSD diagnosis and CAPS Past Month version score of ≥20 (subPTSD defined as: meeting criterion A, F, G, H, and clusters B, C, and at least 1 of the clusters D or E.). Trauma needs to fall under Criterion A. Participants will be on stable medications (for 3 or more months by the time of study entrance), outpatient, and will provide informed consent. For naturally cycling female subjects, stage of menstrual cycle will be ascertained by history, and by serological measures. For women on oral contraceptives, we will identify those using monophasic or biphasic of first, second, third or fourth generation with up to 35mcg of ethinyl estradiol.

Study Sites	UTHealth Houston [redacted] [redacted] University of Pennsylvania Perelman School of Medicine [redacted]
Number of participants	80 participants expected to be enrolled across 2 sites
Description of Study Agent/Procedure	Study drug will consist of one 2.0 mg dose of estradiol, (obtained from Estrace® Tablets, 2.0 mg estradiol tablets, USP, Warner Chilcott [now Allergan, as Warner Chilcott was acquired by Allergan]) taken orally.
Reference Therapy	Placebo
Key Procedures	Pregnancy Test, Urine Toxicology Screen, fMRI scan, Blood Draw, Estradiol/Placebo Dosage
Statistical Analysis	For all aims, analyses of SCR will test for group x condition interactions for the different groups in each aim with respect to CS+E vs. CS+U. With respect to the fMRI data, the initial analysis will involve assessing for a main effect of group (E vs. Plc), post treatment using CS+E vs. CS+U contrast. In addition, we will compare post treatment brain activations vs. pre-treatment within each group as well as the interactions between time and group also using the same imaging contrast.

Schematic of Study Design

Process Diagram

Visit 0: Telephone Screening

- Local advertising efforts instruct prospective participants to complete a screener
- The Research Assistant/study team member at UTHealth Houston calls and screens individuals for eligibility based off of the screener. Such screening is routine at UTHealth Houston and requires about 30 minutes.
- Callers who pass the screen will be scheduled for an in person consent (or consented online via Redcap) and clinical assessment where their final eligibility for the study will be determined.

Visit 1: Screening/Baseline Assessment

- Total n=40
- Obtain informed consent
- Screen potential subjects by inclusion and exclusion criteria
- Assessments Administered: CAPS-5 Past Month and Past Week versions, Demographics Form, Day to Day Form, MINI, Pregnancy Test, Urine Toxicology Screen, Edinburgh Handedness Survey, LEC-5 Extended, Safety Assessments, CGI-S, CTQ, BDI-II, Beck Anxiety Inventory, STAI, ASI, NEO-Short Form, PSQI, Q-LES-QSF, PDS-5
- Assessments Administered by IRB-approved Medical Professional (MD or NP): Gynecological History Form, General Health Form, Prior/Concomitant Medications
- This IRB-approved medical professional will also be available to answer any questions about the medication should they arise during the informed consent process.
- The clinical assessment will be audiotaped

Visit 2: Experimental Day 1

- The participant will undergo the experiment in an experimental room with psychophysiological instruments at UTHealth Houston
- Habituation, conditioning, and extinction learning procedures will be performed
- Conditioning procedures: subjects will view several presentations of pictorial stimuli and receive a shock
- Assessments Administered: Blood draw (4.5ml) conducted to determine level of hormones of interest; STAI (state version / form Y1), Shock Questionnaire, Pregnancy Test, Urine Toxicology Screen, Saliva THC test (if UTOX positive for THC) (THC is the main psychoactive compound in cannabis)

Visit 3: Experimental Day 2

- Extinction recall and renewal procedure in the scanner at Baylor College of Medicine: subjects will view several presentations of similar randomized pictorial stimuli, but with no shock
- Assessments Administered: STAI (state version / form Y1), Shock Questionnaire, Pregnancy Test, Urine Toxicology Screen, Saliva THC test (if UTOX positive for THC), (Blood draw could occur on this day instead of Visit 2 if needed due to scheduling)
- Subjects randomized: Estrogen (n=20), Placebo (n=20)

Visits 4-9: PE Sessions 1-6

- Treatment Session 1: General overview of treatment and rationale for exposure. A medical professional (MD or NP) will prescribe and provide the patient with the study medication bottle, confirm the labelling and review the instructions with the participant.
- Blood draw (4.5ml) conducted to determine level of hormones of interest at visits 5 and 9 (PE sessions 2 and 6)
- 2 mg Estradiol or placebo will be taken by participant via a pill form 5-6 hours before sessions 2-6 of PE treatment
- Sessions 2-6: 60 minute sessions of imaginal exposure and processing as well as in vivo exposure homework

- Assessments Administered: PDS-5, PTCI, BDI-II, Skin conductance, Safety Assessments
- All PE sessions are videotaped

Visit 10: Experimental Day 1

- The participant will undergo the experiment in an experimental room with psychophysiological instruments at UTHealth Houston
- Habituation, conditioning, and extinction learning procedures will be performed
- Conditioning procedures: subjects will view several presentations of pictorial stimuli and receive a shock
- Assessments Administered: Blood draw (4.5ml) conducted to determine level of hormones of interest, STAI (state version / form Y1), Shock Questionnaire, Pregnancy Test, Urine Toxicology Screen, Saliva THC test (if UTOX positive for THC)

Visit 11: Experimental Day 2

- Extinction recall and renewal procedure in the scanner at Baylor College of Medicine: subjects will view several presentations of similar randomized pictorial stimuli, but with no shock
- Assessments Administered: STAI (state version / form Y1), Shock Questionnaire, Pregnancy Test, Urine Toxicology Screen, Saliva THC test (if UTOX positive for THC), (Blood draw could occur on this day instead of Visit 10 if needed due to scheduling)

Visit 12: Post Treatment Assessment

- An independent evaluator (IE) will administer the CAPS-5 Past Week version immediately after therapy. This visit should occur within a week of Visit 11. Visit 12 can occur on the same day as Visit 11.
- Any participants who ask for referrals or are deemed clinically in need of additional care indicated by the clinician for any reason will be provided with them at this visit.
- Assessments Administered: CAPS-5 Past Week version, Day to Day Form, Safety Assessments, CGI-S, CTQ, BDI-II, Beck Anxiety Inventory, STAI, ASI, NEO-Short Form, PSQI, Q-LES-QSF, PDS-5

Visits 13 & 14: 3 & 6-month Follow-Ups

- At the 3-month follow-up visit, participants whose pre-treatment to 3-month follow-up CAPS-5 Past Week score has not improved by at least 30% and whose CAPS-5 Past Week score is 26 or greater at the 3-month follow-up visit will be offered additional PE treatment if they wish. Specifically, participants will have the option to receive up to 4 additional sessions of PE delivered for an hour a week (10 total sessions of PE).
- All participants regardless of scores interested in additional treatment after their study participation will be offered assistance with clinical referrals, as clinically appropriate.
- Assessments Administered: CAPS-5 Past Week versions, Additional Treatment Inventory (ATI), BDI-II, Day to Day Form, Safety Assessments, CGI-S, CTQ, BDI-II, Beck Anxiety Inventory, STAI, ASI, NEO-Short Form, PSQI, Q-LES-QSF, PDS-5. The MINI and CAPS-5 Past Month will be administered at Visit 14 (6-month Follow-Up) only.

1 Key Roles

Dr. Mohammed R. Milad will be the principal investigator on this study. He will oversee the implementation of the fMRI experiments, data acquisition and analyses. Dr. Milad will also be in communication with the MRI Site at UTHealth Houston for the duration of the study to discuss the implementation of the experiments, monitor safe implementation of the paradigm, and review and document any adverse events during that part of the study. Dr. Jair C. Soares and/or Dr. Mira Milad will be the clinically responsible physician investigator clinical trialists for this treatment trial, and the clinical trial diagnostic, treatment and safety monitoring will occur within the clinical research program at UTHealth Houston. Drs. Mohammed R. Milad, Mira Milad, and Jair C. Soares will work very closely to coordinate the experimental imaging and clinical trial aspects of this translational protocol, and Drs. Mira Milad and Jair C. Soares will support Dr. Mohammed R. Milad in reporting requirements.

Mohammed R. Milad, Ph.D.
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University of Pennsylvania Study Site (subcontract)
Edna Foa, Ph.D. (PI at U Penn)
[redacted]

2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

2.1.1 Introduction to PTSD and the Relevance of Fear Learning and Extinction

PTSD is a common chronic condition associated with considerable morbidity and mortality. Approximately 10% of the US adult population has had a lifetime PTSD (Kessler, 2012). The prevalence rates for women are significantly higher than for men, with an estimated 4% of the women in the United States reporting current clinically significant PTSD symptoms (McLean, 2011). Natural recovery from PTSD usually occurs within the first three months, but studies have found rates of 11-38% of significantly interfering PTSD symptoms in women up to 6 years after a traumatic event (Steenkamp, 2012; Arnberg, 2013).

In human fear conditioning paradigms, a neutral cue such as a blue square, is paired with the presentation of an aversive cue, such as a mild electric shock. The subjects eventually express conditioned fear responses, such as higher skin conductance responses and changes in heart rate when the conditioned cue is presented. The subsequent presentation of the conditioned stimulus in the absence of the aversive one (i.e., presenting the blue square without the shock) leads to fear extinction learning (Milad, 2012). Milad and colleagues, along with several other labs, have identified a network of brain regions involved in the acquisition and consolidation of both fear and extinction memories (Graham, 2011). The amygdala, insular cortex, and dorsal anterior cingulate cortex (dACC) typically show increased activation during the fear learning phase. As extinction learning takes place, the ventromedial prefrontal cortex (vmPFC) exhibits increased activation, suggesting that it may be involved in the encoding and/or consolidation of the fear extinction memory. After a

delay (typically 24 hours later), successful recall of the extinction memory trace has been associated with increased activations of the vmPFC and the hippocampus (Graham, 2011; Milad, 2007; Phelps, 2004; Kalisch, 2006; Pitman, 2012). Milad et al. (2009) have shown that the delayed extinction memory recall is impaired in PTSD as measured by SCR. Importantly, this SCR deficit during recall has been associated with dysfunctional activation patterns of the fear extinction network in this patient population. PTSD patients were able to form associations between neutral stimuli and aversive stimuli and were able to learn to extinguish the conditioned fear responses during extinction learning. After a 24-hour delay, however, the PTSD patients showed very high conditioned fear responses to the extinguished cues, despite the apparent successful extinction training. These data suggest that the consolidation or retrieval of extinction or safety memories may be impaired in PTSD patients. Moreover, this failure to store and express the extinction memory was associated with altered functioning of the vmPFC, dACC, amygdala, and hippocampus in those patients (Milad, 2009a).

2.1.2 Prolonged exposure, Estrogen and Extinction Learning

Prolonged exposure (PE) is a treatment for PTSD that has the most evidence for its efficacy (McLean, 2013; Powers, 2010; Foa, 2013; Foa, 2007). Exposure-based treatments for anxiety related disorders including PE have cogent parallels with fear extinction paradigms (Foa, 2000; Gillihan, Foa, 2011). PE involves confrontation with safe but feared situations, objects, and memories, which result in fear reduction to the formerly feared stimuli. This fear reduction is thought to be a result of an active process in the brain, leading to the formation of a new memory (safety memory), which updates or modifies the original fear memories that caused the expression of PTSD symptoms (Bouton, 2004). Notably, while most patients benefit from PE, some remain symptomatic. Given the parallel between PE and fear extinction, neuroscience laboratories that are in search of pharmacological agents to strengthen extinction memory consolidation have used fear conditioning paradigms to test the effectiveness of their interventions.

2.2 Name and Description of the Investigational Agent

Study drug will consist of either one 2.0 mg dose of estradiol, (obtained from Estrace® Tablets, 2.0 mg estradiol tablets, USP, Warner Chilcott [now Allergan, as Warner Chilcott was acquired by Allergan]) orally or look-alike placebo capsules. These doses was selected based on studies of micronized estradiol in the literature suggesting that peak plasma levels are reached 5-6 hr post-administration.

2.2.1 Preclinical Data

Female rats as well as women who undergo extinction training while their estrogen (E) levels are naturally high show significantly lower fear during the extinction recall test, indicating enhanced extinction memory consolidation (Milad, 2009b; Milad, 2010; Zeidan, 2011). Estrogens are female sex hormones that are primarily produced by the ovaries and include estrone (E1), estradiol (E2) and estriol (E3). E2 is the predominant and most potent circulating estrogen produced during the reproductive years in non-pregnant women and is commonly prescribed in pill and transdermal form to treat postmenopausal symptoms. Henceforth, when we use the term estradiol (E2), we are referring to the exogenously administered estradiol and when we use the term estrogen (E), we are referring more broadly to all estrogens, still noting E2 as the most potent one. Elevated E levels significantly increased activation of brain regions associated with fear extinction in both women and female rats, including the vmPFC (IL in rodents) along with other brain regions such as the hippocampus in humans (Zeidan, 2011). Notably, women using oral contraceptives (OC), which induce tonically low E levels, exhibit extinction memory deficits (Graham, 2013). Women diagnosed with PTSD exhibit increased fear acquisition compared to men with PTSD (Inslicht, 2013). Moreover, low levels of estrogen in women diagnosed with PTSD are associated with impaired fear extinction and more severe PTSD symptoms (Glover, 2012). Taken together, these facts not only support the epidemiological data showing higher levels of PTSD in women but also implicate low E levels in PTSD pathophysiology, suggesting a novel potential target for PTSD prevention and intervention.

2.2.2 Clinical Data to Date

In humans, neuroimaging studies have illustrated differential, sex-specific activity in several limbic regions, including the vmPFC, during the processing of emotional stimuli. These sex-specific responses are modulated by natural variance in gonadal hormones during the menstrual cycle. These data indicate that gonadal hormones could regulate fear extinction and thereby potentially account for sex-differences in the prevalence of anxiety disorders.

In humans, we recently conducted a study examining the psychophysiological responses of fear extinction and extinction recall in healthy males vs. females in two different phases of their menstrual cycle: mid follicular, where estrogen levels are highest, and early follicular, where estrogen levels are at their lowest. We observed that males show higher conditioned responses relative to females. Moreover, we observed that males and females in early follicular phase expressed similar levels of extinction memory whereas females tested during late follicular phase showed relatively impaired extinction memory recall (Milad et al., 2006). However, in the above pilot data, we did not obtain blood samples to specifically measure the levels of gonadal hormones at test. In addition, other phases of the menstrual cycle, early and late luteal phases, were not tested. These latter phases have different hormonal characteristics that may have different effects on fear conditioning and extinction. Rising levels of both estrogen and progesterone characterize early luteal phase, where progesterone levels tend to be slightly higher than those of estrogen. In the late luteal phase, both estrogen and progesterone levels are declining. See figure 1 below for graphical representation of the levels of estrogen and progesterone at the four different phases of the menstrual cycle.

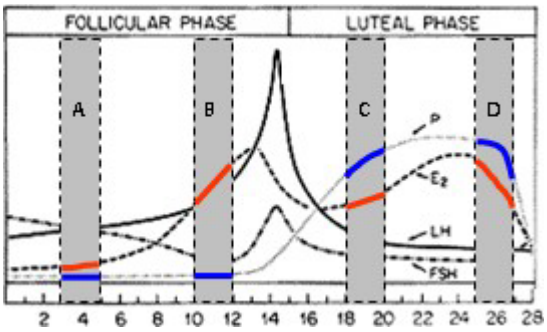

Figure 1. The levels of estrogen and progesterone during the four different phases of the menstrual cycle.

2.2.3 Dose Rationale

2.0 mg Estradiol tablets or pill placebo will be administered orally 5-6 hours prior to each PE treatment session.

2.3 Rationale

In a double-blind placebo-controlled study, we recently found that a single administration of 2mg of estradiol (E2) (which is an active form of Estrogen) to healthy women before extinction learning significantly enhanced extinction memory recall when tested the next day. Naturally cycling women participated in a 3-day paradigm while their endogenous estradiol levels were low (early follicular phase of their menstrual cycle). On day 1, they underwent a fear conditioning procedure without hormonal manipulations. On day 2, women received either a pill of E2 (2mg) or placebo prior to extinction training. Extinction recall was then assessed on day 3 in a drug-free state. On day 2, extinction was successful for both groups and no between-group differences were noted during this phase. On day 3, however, the percent of fear recovered in women who have received E2 was significantly lower compared to women receiving placebo. Thus, a single pill of E2 administered before extinction learning significantly enhanced consolidation of the extinction memory trace in healthy naturally cycling women.

We will recruit women with PTSD symptoms and who are taking low-dose monophasic or biphasic oral contraceptives. All participants will receive 6 Prolonged Exposure (PE) sessions (2 sessions/week for 3 weeks). During the last 5 of the 6 PE sessions, participants will receive either PE combined with 2mg of E2 or PE combined with Placebo. Note that the typical PE usually involved 10-12 sessions. In this case, we have decided to use a suboptimal level of PE to avoid ‘floor’ effects and thus be able to examine if E2 used in conjunction with PE will accelerate PTSD symptom reduction relative to Placebo given in conjunction with PE. All women will undergo a 2 day-fear conditioning and extinction paradigm at baseline and after having completed the 6 PE sessions. They will then receive follow up assessments at 1, 3 and 6 months.

In the current study, we propose to examine how 2 mg doses of estrogen might modulate the neural circuitry of fear extinction in conjunction with Prolonged Exposure therapy. Delineating how sex and gonadal

hormones modulate fear extinction could set the stage for the development of novel and sex-specific treatments for anxiety disorders, specifically PTSD.

2.4 Potential Risks & Benefits

2.4.1 Known Potential Risks

Risks are as follows: a) Participants could develop mild to moderate emotional discomfort or frustration associated with psychiatric interviewing or filling out questionnaires; b) participants may experience subjective distress during treatment; c) potential side effects of E2 include headache, breast pain, irregular vaginal bleeding, stomach cramps, nausea, or hair loss. These side effects, however, are documented with the long-term use of E2 and there are no known side effects of short-term use of E2 as proposed in this application; e) noise from the fMRI machine can cause discomfort. Participants will be wearing earplugs to minimize this discomfort and they can stop the study at any time; f) the electrical shock will be applied to the foot and is uncomfortable but not painful but may cause discomfort in the participants; g) participants may find blood withdrawal at different time points discomforting or slightly painful; h) the association between the electric shocks and the pictures to be presented during the fear conditioning and extinction paradigm might induce some anxiety when viewing the pictures in anticipation of the shock.

It is anticipated that subjects with derive benefit from receiving well-delivered treatment for PTSD. The findings from these studies will help to advance psychiatric research generally, and knowledge of the role of estrogen in augmenting existing treatments for PTSD. Results of this study will also help us to identify important information regarding fear extinction and learning, which could advance clinical knowledge of anxiety disorders. By addressing these important questions, the findings from the proposed study will help shape further research on fear extinction, thereby improving our understanding of fear learning.

2.4.2 Known Potential Benefits

Participants may not directly benefit as a result of participating in this study. Participants may benefit from receiving careful diagnostic assessment, clinical monitoring and well-delivered first line evidence based targeted psychotherapy treatment for PTSD. Participants may also benefit from knowing that they have contributed to the scientific understanding of the role of estrogen in augmenting existing treatments for PTSD and that this may help other women with similar problems. The data to be ascertained will contribute to enhancing our knowledge of PTSD psychopathology and the neural mechanisms associated with treatment outcomes in PTSD.

3 Objectives and Purpose

In this proposal we will examine the mechanisms by which estradiol (E2) administration in conjunction with Prolonged Exposure therapy (PE) could enhance extinction recall and activation of the extinction network in individuals with significant PTSD symptoms; and to examine how such an enhancement may be associated with PTSD symptom reduction

3.1 Primary Objective

The primary objective is to delineate the influence of exogenous administration of estrogen on conditioned responses during extinction recall in women with PTSD.

Specific Aim 1.1: To examine the effects of E2+PE on the extinction-induced psychophysiological responses (SCR), neural responses, and the information flow between the fear extinction nodes at post-PE sessions relative to pre-PE.

Hypothesis 1.1: During extinction recall, both groups will show lower SCR at post- relative to pre-PE. A general improvement is expected over time in both groups given the anticipated benefits of PE alone. However, we expect a significant Time x Group interaction, where the Estradiol-treated group should exhibit significantly lower fear levels (indexed via SCR) at the follow-up assessment relative to the Placebo group. 2) For both groups, extinction recall-induced activations will be higher in brain regions associated with extinction learning and recall (i.e. vmPFC and hippocampus) and lower in regions associated with fear expression (i.e. dACC and amygdala) at post- relative to pre-treatment. Note that a general improvement from pre to post is expected in both groups, given the anticipated benefits of PE alone. We do expect, however, a significant

Time x Group interaction, with most pronounced effects at post-PE in the estradiol-treated group. 3) Information flow between the extinction nodes will improve (indexed by DCM models), with stronger effects in E2+PE after treatment.

Specific Aim 2: To examine the impact of E2 administration on PE efficacy at reducing PTSD symptom severity.

Hypothesis 2: PTSD symptom severity will be significantly lower in the E2+PE group relative to the Placebo+PE group following acute treatment, as well as at the 3- and 6-month follow-up assessments.

Specific Aim 3: To test the interaction between E2+PE induced psychophysiological and neuronal changes (pre-to-post) with the improvement of PTSD symptom severity.

Hypothesis 3: PTSD symptom reduction will correlate with BOLD and SCR changes observed during extinction recall following PE.

4 Study Design and Endpoints

Section 4 will provide a brief overview of the phases of the clinical protocol (screening, baseline, experimental days). More details on procedures occurring during each phase will be included in subsequent sections of the protocol.

Therapist Training Procedures:
In addition, prior to beginning work with randomized study participants as an approved study therapist, all study therapists at UTHealth Houston will undergo a training procedure with a study participant recruited to participate in this training phase of the study. The details of this are outlined in 7.3.1. and a separate consent form explaining the training procedures with the treatment but not imaging components of the study is included.

4.1 Description of Study Design

This is a randomized, double-blind, placebo-controlled trial where 80 participants will undergo fear conditioning, fear extinction, and fear recall experiments, fMRI scans, as well as receive Prolonged Exposure treatment. Participants will be randomly assigned to additionally receive a pill placebo or 2 mg Estradiol.

The study includes a) Psychiatric intake evaluation to determine study eligibility, b) 2 consecutive day visits that occur at visits 2 and 3 and again at visits 10 and 11 with fMRI scanning on the second day, c) prolonged exposure therapy sessions. The 4 consecutive visits include roughly 1 hour fMRI scans, as well as fear conditioning through a mild shock stimulus from a battery-powered stimulator, the Biopac STM100C Stimulator, and the use of psychophysiological measures (SCR). There are clinical follow up assessments at 3 and 6 months post treatment.

4.2 Study Endpoints

4.2.1 Primary Study Endpoints

Primary study endpoint will be Blood Oxygen Level Dependent BOLD fMRI signals and Skin Conductance Response (SCR) during recall.

4.2.2 Secondary Study Endpoints

The secondary study endpoint will be the evaluation of PTSD symptom severity using DSM-5 PTSD symptoms measured with the Clinician-Administered PTSD Scale for DSM-5 (CAPS) Past Week version at the Post-treatment assessment. In addition to the experimental primary and secondary end points, there will be primary safety endpoints. Subjects will not receive any stimulation beyond what they consider highly annoying to them. Subjects will be thoroughly screened prior to starting the fMRI component of the study to make sure that they have no metal implants of any kind that would be considered a hazard to their health in the fMRI. We will use a metal detector to screen all subjects and make sure that they carry no metals to the

scanner room. Pregnancy tests will be done for all female participants with child-bearing potential prior to the initiation of the experiment to rule out pregnancy. If pregnancy is suspected, subjects will not be allowed to participate in the study. If any excess movement by the subjects is observed (which could be indicative of discomfort), the study staff will check with the subjects and inquire whether they would like to stop the experiment. After estrogen administration, all subjects will be monitored for any adverse events. This risk, however, is low given that estrogen is a commonly used agent with known side effect profiles, a component of many oral and non-oral birth controls and given only for 5 total 2mg/ dose twice weekly doses in the study. Reasons for study discontinuation for any participants who stop the treatments or study prematurely will also be recorded and reported.

5 Study Enrollment and Withdrawal

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Female, 18-45 years of age
2. Chronic (at least one month post-trauma) DSM-5 FULL PTSD diagnosis **OR subPTSD** diagnosis (subPTSD defined as: meeting criterion A, F, G, H, and clusters B, C, and at least 1 of the clusters D or E.)
3. CAPS-5 Past Month score ≥ 20
4. Criterion A traumatic event
5. Stable medications for 3 or more months by the time of study entrance (with the exception of benzodiazepines)
6. Women on oral contraceptives, specifically those using monophasic or biphasic of first, second, third or fourth generation with up to 35mcg of ethinyl estradiol; OR using etonogestrel / ethinyl estradiol 0.120mg/0.015mg per day vaginal ring birth control; OR using the norelgestromin / ethinyl estradiol 0.150mg/0.035mg per day transdermal patch birth control.
7. Willing and able to provide informed consent

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Diagnosis of bipolar I disorder with a past year manic episode
2. Diagnosis of a psychotic disorder or psychotic symptoms that would interfere with the ability to focus on posttraumatic stress disorder (PTSD) in clinic, as determined by clinical judgment.
3. Diagnosis of moderate or severe substance use disorder that would interfere with the ability to focus on posttraumatic stress disorder (PTSD) in clinic, as determined by clinical judgment.
4. Cognitive impairment that would interfere with the ability to focus on posttraumatic stress disorder (PTSD) in clinic, as determined by clinical judgment.
5. History of neurological disease (that involves the brain), seizure, or significant head trauma (i.e., extended loss of consciousness, neurological sequelae, or known structural brain lesion).
6. Suicidal ideation with imminent risk that warrants a higher level of care.
7. Concurrent trauma focused psychotherapy
8. Pregnancy (to be ruled out by urine ß-HCG).
9. Metallic implants or devices contraindicating magnetic resonance imaging by interfering with patient safety or fMRI data collection. Cases will be cleared by the Principal Investigator and/or Baylor College of Medicine (Imaging).
10. History of breast cancer or hormone-responsive cancer.
11. Use of benzodiazepines
12. Self-injurious behavior that involves suicidal intent, requires medical attention, or occurs daily.
13. High risk of adverse emotional or behavioral reaction, and/or an inability to understand study procedures or the informed consent process, based on investigator/clinician clinical evaluation (e.g., evidence of serious personality disorder, antisocial behavior, serious current stressors, lack of meaningful social support)

5.3 Vulnerable Subjects

This study does not involve the enrollment of pregnant women, prisoners, employees, people with cognitive impairment or any decisional incapacity, fetuses, neonates or elderly or children under the age of 18.

Confidentiality for all study participants will be strictly adhered to. Only group level de-identified outcomes will be shared outside the study team and UTHealth Houston approved functions with access to clinical research participant level data (e.g. IRB, UTHealth Houston research pharmacy). All participants will undergo formal informed consenting in person (or online via Redcap) with an IRB approved clinical study investigator with as much time for questions as needed.

5.4 Strategies for Recruitment and Retention

Subjects will be recruited from the local community via advertisement as well as social media such as: Craigslist, Reddit, Instagram, LinkedIn, Twitter, Facebook, Research Match, and Nextdoor, as well as UTHealth Houston‘s MyChart, and BuildClinical. Research Match is a nonprofit program funded by the NIH that connects people interested in participating in research studies with applicable studies. It will specify location, the study’s inclusion criteria, and be linked with the approved prescreen. BuildClinical is a data-driven software platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. They have worked with IRBs in the US to ensure they adhere to all the appropriate guidelines and procedures. They utilize study-specific advertisements to engage participants on digital platforms such as Facebook, Google, WebMD, etc., and redirect them to a study-specific landing page should they click it. On the landing page, the person can complete an online pre-screen questionnaire that gets routed into BuildClinical's platform. BuildClinical's Secure Socket Layer (SSL) software, which encrypts all inputted information, keeps subject information private and HIPAA compliant. Their backend servers are stored in the USA at some of the most secure data centers in the world.

The study team will work with the UTHealth Houston communications department to create a webpage describing our research study on the websites that the institution uses to recruit participants for studies and will work with UTHealth Houston‘s MyChart to recruit from UTHealth Houston patients. In addition, we will post flyers at UTHealth locations, show ads on the internal UTHealth Houston tv monitors, and use UTHealth Houston’s website and newsletters.

Subjects will also be recruited from the public through local media advertisements and UTHealth Houston clinical sites, including Behavioral Sciences Research Institute registry/recruitment (BSRI). We will approach patients in clinic, via posted flyers in the outpatient buildings, post signs on campus, and we will also recruit from the Department of Psychiatry Recruitment Registry: HSC-MS-23-0768. Flyers will be posted at local university campuses, hospitals, and community clinics. Local advertising efforts will instruct prospective participants to complete a brief online confidential screening questionnaire. The Research Assistant, or study team member, at UTHealth Houston will then call and screen individuals for eligibility based on results of the screening questionnaire. Individuals who contact our centers will be screened initially by phone for interest in the proposed research, as well as general diagnostic and study eligibility, and interest and availability for research study participation. Such screening is routine at UTHealth Houston and requires about 30 minutes. Callers who meet initial potential eligibility based on the phone screen will be scheduled for an in-person assessment with a study clinician. At this visit they will review and sign the informed consent form (if not already consented online via Redcap), and assessments will occur to determine their final eligibility for the study.

There is a plan for sharing contact information of the previously consented participants interested in future research studies. We will share a list of participant contact information (name, email and phone number) to Dr. Li’s team through a secure process (i.e., OneDrive) for the study HSC-MS-23-1106.

Communicating with the Research Team: We added the following information to our informed consent, regarding texting research participants and give participants the option to opt in or out of receiving text communications from the research team.

Researchers may need to communicate with you about information relevant to the research study. The research team will usually contact you for these purposes by phone, but if you have given the Researchers your email address and mobile/cell phone number and permission to send a text message, the research team may contact you that way. When the research team sends email messages that include identifiable health information, they will use encrypted messaging (e.g., SendSafe). When the research team uses texting over mobile/cell phones there is no way to encrypt the message. This means that information you send or receive by text message is unencrypted and could be intercepted or viewed by an unintended recipient, or by your mobile/cell phone provider or carrier. Therefore, text messages carry security and privacy risks.

Below are some important points about texting in this research study.

- Text messages are not encrypted, and therefore are unsecure and may result in a breach of your confidentiality.
- You will be responsible for all fees charged by your carrier's service plan for text messaging. This research study and UTHealth Houston will not cover the cost related to any increased charges, data usage against plan limits or changes to data fees from the research texts.
- Text messages will only be read during regular business hours. However, if you have a scheduled visit outside of business hours, you may receive a text in relation to this visit outside of regular business hours.
- Text messaging should not be used in case of an emergency. If you experience a medical emergency, call 911 or go to the nearest hospital emergency department.
- You may decide to not send or receive text messages with staff associated with this research study at any time. You can do this in person or by sending the research number a text message that says "Stop Research Text."
- Your agreement applies to this research study only. Agreeing to other texts from UTHealth Houston, for example appointment reminders, is a separate process. Opting out of other texts from UTHealth Houston is a separate process as well.

5.4.1 Use of MyChart for Recruitment Purposes

The study will also use UTHealth Houston's MyChart to identify subjects. Data gathered will be used to identify potentially eligible subjects who did not opt out of being contacted for research. We will also use this data to gain contact information about the patient.
A research coordinator, or study team member, will contact potential participants identified through MyChart. Any recruitment information containing sensitive information will be sent by secure email (e.g., Send Safe) email or will be done through MyChart. Once contact is made, approved recruitment language will be used to communicate the reason for contact and subjects will be asked if they are interested in participating in this specific study. If the potential subjects agree, the study team will provide the potential participants with information regarding the next steps for participation. If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact the UTHealth Houston department/contact that is responsible for processing such requests.

5.5 Duration of Study Participation

We will ask participants to make 14 visits to our facilities at UTHealth Houston. After the first assessment visit, participants will go through the 2-day visit for the emotional learning task. Following this, the participants will go through 6 therapy sessions over 3 weeks: 2 sessions per week. On visits 10 and 11 participants will go through the emotional learning task again and complete clinical outcomes assessments. Participants will return at 3 and 6 months for follow ups.

5.6 Total Number of Participants and Sites

Recruitment will end when approximately 80 subjects complete the screening, meet eligibility criteria and are randomized. It is expected that approximately 80 subjects will be randomized between both UTHealth Houston and Penn.

5.7 Participant Withdrawal or Termination

5.7.1 Reasons for Withdrawal or Termination

Participants are informed they are free to withdraw from participation in the study at any time upon request. Patients' participation in the treatment trial may be discontinued at any time because of serious medication side effects or if continued participation is considered a danger to their welfare. Specific reasons for treatment discontinuation include: 1) Serious adverse event; 2) Extreme laboratory values confirmed with a repeat test; 3) discontinuation would be in the patient's best interest; 4) Participant no longer wants to continue.

5.7.2 Handling of Participant Withdrawals or Termination

Participants deemed to be candidates for discontinuation will be reviewed by the principal investigator and other relevant project personnel (e.g., study psychiatrist or study treatment provider). Patients who require discontinuation of study treatments for any reason will be scheduled for a final evaluation and given appropriate treatment referrals. The reason patients are discontinued from the clinical trial will be clearly specified.

6 Study Drug and Procedural Intervention

6.1 Study Agent(s) and Control Description

Study drug will consist of either one 2.0 mg dose of estradiol (obtained from Estrace® Tablets, 2.0 mg estradiol tablets, USP, Warner Chilcott [now Allergan, as Warner Chilcott was acquired by Allergan]) orally or look-alike placebo capsules. The IND number is 135357; this study has received an IND Exemption from the FDA given that our use of Estrace has met all 5 IND regulations set forth by the FDA (please see FDA letter included in the IRB application for details).

6.1.1 Acquisition

UTHealth Houston Investigational Pharmacy will dispense study medication in patient-specific kits. The research assistant assigned to this study will pick up the medication from the pharmacy with the study psychiatrist or nurse, will account for all study medication received. Any damaged or unusable study pills in a given shipment (active drug or placebo) will be documented in the study files. Study medication will be provided to the participants only by approved study clinicians who will review the medication prescription, instructions and answer any questions with the participant. The investigator will notify the pharmacy of any damaged or unusable study medication received.

6.1.2 Formulation, Appearance, Packaging, and Labeling

The Estradiol 2mg tablets and Placebo tablets will be prepared by a compounding pharmacy at both sites so the active and placebo pills are indistinguishable from one another in terms of appearance of the capsules. This will permit the appropriate concealment of the intervention from participants and study personnel alike, thereby enabling the double-blind. The capsules will be placed into a sealed plastic container and labeled clearly with the contents (drug, strength, quantity).

At the time of dispense, five capsules will be placed into an Amber vial with a child-resistant, tightly sealing screw-cap. The vial will be labeled as follows:

Caution: New Drug Limited by Federal (United States) Law to Investigational Use Only	**UTHealth Houston -Investigational Pharm** [redacted] RX # XXXXXXXXX Pt#: ###### Date:_____ Dr._____ PATIENT NAME TAKE ONE CAPSULE BY MOUTH ONCE AS INSTRUCTED **ESTRADIOL 2MG OR PLACEBO CAPSULES** Quantity: 5 Capsules Refills: 0 Do Not Use After: MM/DD/YYYY PROTOCOL: HSC-MS-23-0497

6.1.3 Product Storage and Stability

Medication will be managed and stored in a double-locked medication closet at UTHealth Houston's Investigational Pharmacy. Study medication will be stored at ambient temperature. Temperature is monitored in the Investigational pharmacy daily. It remains at ambient temperature at the study site.

6.1.4 Preparation

The study drug is prepared and dispensed from UTHealth Houston's BBSB Pharmacy.

6.1.5 Dosing and Administration

A single dose of estradiol 2mg or placebo will be taken at home by the study participant 5-6 hours before each of 5 PE treatment sessions (sessions 2 to 6).

The study medication, 5 pills, will be given to the participant to take home after the first PE session by an approved study clinician (MD or NP) who will confirm the labelling and review the instructions with the participant. The subject will be instructed and reminded through a text or phone call to take the medication 5 hours before the time of each of their next 5 therapy sessions.

6.1.6 Route of Administration

The study medication will be taken orally.

6.1.7 Duration of Therapy

Subjects will take one pill 5-6 hours before each PE session 2-6, which will take place over the course of approx. 3 weeks. Typically, a minimum of 8-12 PE sessions are required for the treatment of PTSD. In our study design, we aim to examine the effectiveness of only 6 PE sessions that would be strengthened by the administration of estradiol. The interaction between exposure/extinction learning that occurs while estradiol is elevated (due to the pill) during 5 PE sessions is predicted to be sufficient to induce a significant reduction in PTSD symptoms in the active drug group compared to placebo.

Primary analyses will be based on a modified intent to treat sample including all participants who took at least one dose of the study medication.

6.1.8 Tracking of Dose

Medication records will be completed on a regular basis with the following information: date administered, amount administered and to which subjects, product remaining, product damaged/destroyed and date of damage/destruction, product returned and date of return, etc. A standard form will be utilized to document this information throughout the study period.

6.2 Study Agent Accountability Procedures

The Pharmacy will destroy remaining study medication, after the study research assistant, under the direction of the study nurse and/or physician, returns it to them from this site.

At the completion of the study, there will be a final reconciliation of drug dispensed, shipped, consumed, 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.

7 Study Procedures and Schedule

The screening/baseline assessment must be completed within 4 weeks of the first experimental days. If more than 4 weeks have passed since the screening/baseline assessment, the study PI/clinician will advise if participant will be rescreened during a separate visit and/or the CAPS will be repeated at Experimental Day 1.

The 2-day experimental visits must occur on 2 consecutive days. All study treatment visits can occur within a window of 7 +/- 5 days from the previous visit.

Randomization to study medication will occur after the participant has come for Visit 3, and the participant will receive the medication and instructions for its use at visit 4 (the first PE session) so they can initiate medication at home 5 hours prior to visit 5 (the second PE session)

In order to have the study intervention and assessments be as complete as possible, any visit, with the exception of the pre- and post-experimental days, may be conducted remotely via Webex if needed at the discretion of the research team, such as in the circumstance of weather or a possible participant COVID-19 exposure. Webex will only be utilized if the research team has determined that the participant will be able to tolerate remote exposures safely.

7.1 Study Procedures/Evaluations

7.1.1 Screening/Baseline Phase

Subjects will complete a pre-screening survey on REDCap. The link to the initial REDCap survey will be included in our advertisements and potential participants will be screened out if they meet for any exclusion criteria. Once participants get through this survey, they will be contacted by a research coordinator to schedule the initial phone screen. An initial phone contact will be arranged with the study coordinator, or study team member, at UTHealth Houston based off of the initial pre-screening survey. During the initial contact, entry criteria will be reviewed and the study procedures will be outlined. Female subjects will be asked regarding their use of oral contraceptives as well as initial approved phone screening questions. Those women using oral contraceptives will be asked what kind to initially check if they are using 1st, 2nd, 3rd, or 4th generation, monophasic or biphasic oral contraceptives containing up to 35mcg of ethinyl estradiol as part of the phone screen (to be confirmed by the in-person screening clinician). For candidates who are interested in participating and appear to be potentially appropriate for enrollment, an onsite visit at UTHealth Houston for formal consenting (or consenting will be completed online via Redcap), and in person psychiatric assessment with an IRB approved study clinical evaluator will be arranged.

All participants will meet with a study clinician for consent (or complete consent online via Redcap) prior to the baseline in person screening evaluation and baseline assessments, consisting of a diagnostic interview and completion of the battery of self-report measures. The study clinical rater will describe the study in detail (i.e., the nature of the treatments and random assignment, duration of the treatment, fMRI component, payments for assessments, measurements collected, and follow-up procedures) and answer any questions. After signing the consent form, the participant will complete the clinician rated assessments and eligible participants will complete the self-report forms.

Participants who are deemed ineligible after baseline screening will be offered assistance with clinical referrals, as clinically appropriate. Individuals who have consented and are found ineligible after baseline screening, will have all identifiers related to their clinical screening maintained, unless the participant requests for such identifiers to be destroyed.

The initial evaluation will be conducted by a masters or doctorate-level clinician (or Independent Evaluator-IE) who is trained in psychiatric assessment. All study staff with the exception of the research pharmacy will be blind to medication treatment assignment. During the evaluation, the clinician or IE will interview the participant to assess current and lifetime disorders that meet exclusion criteria using the MINI, and to determine whether the participant meets the cutoff score using the CAPS-5 Past Month version. All efforts will be made to have the same independent evaluator assess the same patient throughout the course of the study when possible. Audio consent will be obtained to have all assessments audiotaped for reliability purposes.

If a participant does not meet PTSD/subPTSD inclusion criteria, the clinician or IE may offer to repeat the CAPS-5 Past Month assessment one week or more following the original baseline assessment visit. This will be offered at the clinician's discretion for participants whose scores are close to the cutoff score because symptoms may vary with time and/or changed circumstances. Participants will not be told the specific reason

for repeating the CAPS, but rather that further assessment is needed to determine eligibility. All baseline assessment measures must still be completed within 4 weeks of the initial experimental days.
Participants will also complete a pregnancy test before each scan to ensure safety of fMRI scanning and will be scheduled to complete the first (of a total of two) fMRI paradigm (see below) and the treatment sessions.

7.1.2 Experimental, Treatment, and Follow-up Days

Experimental Procedure: Fear conditioning and extinction paradigm
To assess the effects of the combined E2 and PE on the neurobiology of fear regulation, all participants will undergo our 2 day-fear conditioning and extinction paradigm before the commencement of PE and after the completion of the 6 PE sessions with double blind E2 or pill placebo. Psychophysiological experiments will be performed either in an experimental room or in the scanner.

Prior to the conditionability procedure, the subject will be reminded that a mild electric unconditioned stimulus (US) will be used and that if necessary she will be free to terminate the experiment at any time. Stimulating and recording electrodes will be attached. The stimulating electrodes will be attached to only one foot. Prior to setting the US level, the experimenter will give the following instructions: "For this experiment, you will set your own level of electric stimulation. You should choose a level that is highly annoying but not painful. I will start the stimulation at a very low level and gradually increase the level until you say ‘stop’. The level that you set will then be used throughout the remainder of the experiment." After the US level is established, the experiment will begin.

Prior to the initiation of the experiment, subjects will view pictures of two rooms presented on a monitor, both of which differ in color and content (e.g., an office and a conference room). The conditioned stimuli (CSs) will be three different cues in the room (e.g. a light that differs in color: yellow, blue and red). Two of these lights will be followed by the US at a partial reinforcement rate of 62.5% (CS+s). The third CS will never be followed by the US. The CS+s, CS- and the US will only be presented in one context (conditioning context; CX+). The other context will be used for extinction training (CX-). Neither CS+ presented in the context of the CX-, nor a CS- presented in either context, will ever be paired with the US. The experiment will be composed of five sequential phases. The initial Habituation phase will consist of 4 presentations of the to-be CS+ (i.e. blue), 4 presentations of the other to-be-CS+ (i.e. red) and 4 presentations of the to-be-CS- (i.e. yellow) in pseudo-random order (there will be no more than two consecutive presentations of the same type), all in the context of the CX+. Each CS will last 6 sec. The inter-trial-interval will be 15 + 3 sec., determined at random.

Immediately after will be the Conditioning phase, which will consist of presentations of each stimulus type in pseudo-random order, again all in the context of the CX+. At the offset of 5 out of 8 trials CS+ (but not CS-); a 500-msec. electrical current US will be administered. There will be a total of 8 CS+ (blue), 8 CS+ (red) and 16 CS- (yellow) trials during the conditioning phase. After a rest period, the Extinction phase will begin. During extinction, the CS- and only one of the CS+s (Extinguished CS+, CS+E) will be presented in the context of the CX-, and no US will be delivered. There will be a total of 16 CS+E trials and 16 CS- trials.

One day after extinction training, the subject will return for the Extinction Recall phase (extinction memory test). During this test, the CS+, CS-, and the Unextinguished CS+ (CS+U) will be presented. There will be 8 CS+E, 8 CS+U, and 16 CS- trials presented during this phase in the extinction context (CX-). Following a brief rest period, the Renewal phase will be administered. The Renewal phase will be identical to the Extinction Recall phase except that all stimuli will be presented in the conditioning context (CX+). No shocks will be delivered during either phase during the retention test.

Treatment with Prolonged Exposure combined with E2 or placebo:
To test the hypothesis that E2 will augment the efficacy of PE in reducing PTSD symptoms, we propose to conduct a double-blind placebo controlled study in which we will administer E2 or placebo to women with significant PTSD symptoms who undergo PE. 2mg of synthetic estradiol (Estrace) will be administered 5 hours prior to each of the 5 PE therapy sessions in which imaginal exposure will be administered (PE sessions 2 to 6). An automatic reminder will be sent to the participant when medication has to be taken in order to increase compliance. After the first introductory PE session, 5 imaginal exposure sessions will occur, such that there will be 2 sessions per week conducted for a total of 6 sessions over 3 weeks (more details in

the next section). The timing of the E2 administration was selected using our preliminary data that was based on the pharmacokinetics obtained from a recent publication. These data show that peak estrogen levels are achieved approximately 6 hours after ingestion (therapy session would be ending or about to end). This timing would allow for maximum estrogen levels to be present to strengthen the consolidation of the safety memory acquired during the therapy.

Therapy Sessions and Assessment During Treatment:
Session 1. This session provides an overview of the PE treatment and a general rationale for exposure, along with the proposed mechanism of action of estrogen on the exposure process. The therapist (same for all sessions) gathers information about the patient's symptoms, details of the trauma, history of previous trauma, and functioning, and provides an explanation of PTSD and discusses common reactions to trauma and the rationale for treatment. In addition, the therapist will construct an in vivo hierarchy with the patient, which is why this session is extended to 120 minutes. The study medication, 5 pills, will be given to the participant to take home after the first PE session by an approved study clinician (MD or NP) who will confirm the labelling and review the instructions with the participant.

Sessions 2-5. These sessions are 60 minutes long and consist of imaginal exposure. Specifically, the patient is instructed to imagine the trauma and recount it aloud for about 25-30 minutes. In the following 15 minutes, the patient processes her reactions to revisiting the traumatic event by discussing related thoughts and feelings. The session ends with in vivo exposure homework to be completed that same day as the session.

Session 6. In this last session, imaginal exposure is conducted one last time for the same duration as previous sessions. The therapist and patient review treatment progress and discuss applications of treatment principles to daily life.

Participants will complete the PDS-5 and BDI-II at each session and the PTCI at baseline, Visit 5, 7, and 9. A non-invasive skin conductance monitor (purchased from Mindfield eSense Skin Response) will be used to measure skin conductance throughout the imaginal exposure sessions. Electrodes will be placed on the fingers of the participant and will be connected to an iPad to record the skin conductance throughout each PE session. This will not cause discomfort or any additional risk to the subject.

Post-treatment Assessment:
Lastly, an independent evaluator, blind to treatment assignment, will assess PTSD severity after therapy (Visit 12) (following same procedures as before therapy (Visit 1), and at 3 and 6 month follow-up visits) using the CAPS-5 Past Week version to assess whether changes in symptom severity are stable over time, and to detect any effects of the experimental manipulation on reduction of fear.

Follow-Up Assessments (3 and 6 months post-treatment):
These assessments will be identical to the post-treatment clinical and self-rated questionnaire assessments, and the 6-month follow-up will also include the CAPS-5 Past Month assessment. There is no additional experimental conditioning, fMRI or pregnancy test in follow up. In addition, participants will be asked to report any psychological, psychiatric and medical treatment they received since the last assessment. This information will be recorded on the Additional Treatment Inventory (ATI). Each follow up visit will include a clinical safety assessment for suicidality, and consider the potential need for clinical referrals, as above.

Participants who do not improve at least 30% from the pre-treatment to 3-month follow-up CAPS-5 Past Week score and who have a CAPS-5 Past Week score of 26 or greater at the 3-month follow-up assessment will be offered additional PE treatment at UTHealth Houston if they wish. Specifically, participants will have the option to receive up to 4 additional sessions of PE delivered for an hour a week (for a total number of 10 sessions of PE) immediately following the 3-month follow-up, consistent with prior study designs utilizing PE and to mimic real world clinical practice where patients receive a variable number of sessions of PE. The need for clinical referrals will be discussed and assessed with all participants by the study clinician regardless of endpoint improvement scores, and referrals made when indicated.

7.1.3 Allocation to Interventional Group

Participant randomization will be done by the UTHealth Houston Investigational pharmacy, from which we will be obtaining Estradiol and placebo. We will employ block randomization (with blocks size TBD to retain the blind), and randomization will be stratified by severity score at baseline.

7.2 Study Evaluations and Measurements

7.2.1 Baseline Evaluation

Baseline evaluations including:

- Trauma history
- Medical (including gynecological), psychiatric, and neurological history
- Characteristics (age, gender, race)
- Drug and medication use
- Life history such as any stressful life events
- Information regarding the subject's OCP use and associated menstrual cycle

7.2.2 Laboratory Evaluations

Approximately 18ml of blood will be drawn from each participant over the course of the study (see Table 1 below) using venipuncture. Hormone measures examined will include gonadal (total estrogens, estradiol and progesterone), hormones. The blood samples will be drawn and processed at LabCorp or Quest Diagnostics. If needed, due to scheduling constraints, blood may also be drawn by phlebotomist at UTHealth Houston and then processed by LabCorp/Quest Diagnostics. There are no plans to store any samples for prospective processing.

7.2.3 Urine Toxicology Screen and Saliva THC Test

If, on the day of any scanning visit, the participant's UTOX is positive for THC (and negative for all other subtances), a saliva THC test will be administered in order to determine recency of THC use. If saliva THC test is negative; the scan will proceed as scheduled. If the saliva test is positive; the scan will be rescheduled. Saliva THC tests are rapid tests, with low participant burden, that can indicate recent THC use.

7.2.4 Pregnancy Testing

A urine pregnancy test will be performed for all female subjects. See section 7.2.5 for more detail.

7.2.5 fMRI Scans

Research subjects will undergo a 3T MRI scan during the second of the two experimental days at baseline and again at treatment endpoint. The MRI does include the use of experimental pulse sequences and/or RF coils. Some of the pulse sequences and/or RF coils are not FDA approved but are considered to pose no more than minimal risk.

Pregnancy Clause -- Although there are no known risks related to MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no possible benefit from participating in this protocol for a pregnant woman, we will exclude pregnant women. A negative urine pregnancy test will be required before a woman of child-bearing potential can participate in this study, and all participants will be on an oral contraceptive. Should a pregnancy be identified, a separate modification request including information on whether or not the pregnancy will be followed will occur.

7.2.6 Other Evaluations, Measures

Clinician Rated Measures

- MINI International Neuropsychiatric Interview for DSM-5 (MINI; Hergueta, Baker, & Dunbar, 1998)
- Clinician-Administered PTSD Scale for DSM-5 Past Month and Past Week versions (CAPS)
- Medical health: the general health form

- Gynecological/Menstrual history form
- Columbia Suicide Severity Rating Scale (C-SSRS; Posner et al., 2007)
- Clinical Global Impressions Severity and Improvement Scale (CGI-S; Guy 1976)
- Life Events Checklist Extended (LEC-5 Extended; Weathers et al., 2013)

Self-Rated Measures

- The Beck Depression Inventory (BDI-II: Beck et al., 1961) to quantify depressive symptoms
- The Beck Anxiety Inventory (Beck and Steer, 1990) to quantify general anxiety symptoms
- The Speilberger State Trait Anxiety Inventory (STAI: Spielberger, 1983) to quantify trait aspects of anxiety
- The Anxiety Sensitivity Index (ASI)
- Shock Expectancy Questionnaire
- Day-to-Day Experiences Form
- NEO-FFI-3-Short form (to assess big five dimensions of personality)
- Pittsburg Sleep Quality Index (PSQI)
- PTSD Diagnostic Scale for DSM-5 (PDS-5)
- Posttraumatic Cognitions Inventory (PTCI)
- Childhood Trauma Questionnaire (CTQ)
- Quality of Life Enjoyment and Satisfaction Questionnaire Short Form (Q-LES-Q-SF)

Safety Assessments:
All clinical visits and follow up assessments and experimental visits will include the following:

- Adverse Events (AE): clinicians/study team will track any adverse events that patients have experienced since their last visit. AEs will be documented and reviewed at all study visits.
- Concomitant Medications and Therapy: The study clinician will record the use of both study medication and concomitant medications on the Concomitant Medication log at each study visit. In addition any other psychosocial treatment will be recorded.
- Suicide Checklist: A modified CSSRS derived checklist to enable the clinician to assess, track and record any suicidal ideation, intent or behavior as part of the safety assessment.

Other Forms

- Demographics Form
- Edinburgh Handedness Survey
- Shock Expectancy Questionnaire
- Prior/Concomitant Medications Form
- Early Withdrawal Form

All study assessments and measures can be completed as written forms, filled out electronically using fillable PDFs, or completed by participants as surveys via the REDCap database system.

7.3 Study Schedule

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Study Phase	Phone Screen	Screening/ Baseline Assessment	Experimental Days (I)		PE Session 1	PE Sessions 2-6	Experimental Days (II)		Post-Treatment Assessment	Follow-ups
Visit Number	**n/a**	**1**	**2**	**3**	**4**	**5-9**	**10**	**11**	**12**	**13-14**
Informed Consent		X								
Review Inclusion/Exclusion Criteria	X	X								
Demographics/Medical History		X								

Pregnancy Test		X	X	X			X	X		
Urine Toxicology Screen, Saliva THC test (if positive UTOX for THC on experimental/scanning days)		X	X	X			X	X		
Concomitant Medications and treatments	X	X			X	X			X	X
Blood Draw			X (or Visit 3)			X (at visit 5 and 9)	X (or Visit 11)			
Randomization				X						
Psychiatric evaluation measures		X	X	X	X	X	X	X	X	X
Estradiol/Placebo dosage						X				
Adverse Event / Unanticipated Problems Assessment		X	X	X	X	X	X	X	X	X
Habituation/Extinction Procedures			X				X			
Recall/Renewal Procedures				X				X		
PE Treatment Procedures					X	X				
Resting Skin Conductance					X	X				

TABLE 2: Schedule and list of evaluations and self-reports completed by participants.

Assessments Administered at Visit 1	**Assessments Administered at PE Treatment Sessions**	**Assessments Administered at Experimental Visits**	**Assessments Administered at Post Treatment and Follow-ups**
Clinician Administered Forms: LEC-5 Extended, MINI, CAPS-5 Past Month and Past Week versions, CGI-S, CSSRS (lifetime and recent)	Psychiatric Evaluation Measures: PDS-5, PTCI (PE Session 2, 4, and 6), BDI-II, Safety Assessments, Adherence Questionnaires	Shock Questionnaire, Speilberger State Trait Anxiety Inventory (STAI; state version / form Y1 only), AE Form	Clinician Administered Forms: CAPS-5 Past Week version, CAPS-5 Past Month version at 6-month follow-up only, MINI at 6-month f/u only, CGI-S, CSSRS (recent)
Psychiatric Evaluation Measures: CTQ, Beck Depression Inventory (BDI-II), Beck Anxiety Inventory, Speilberger State Trait Anxiety Inventory (STAI), Anxiety Sensitivity Index (ASI), Day-to-Day Experiences Form, NEO-Short Form, PSQI, PTCI, Quality of Life Enjoyment and Satisfaction Questionnaire Short Form (Q-LES-QSF), PDS-5		Pregnancy test, Urine Toxicology Test, Saliva THC Test (if UTOX is positive for THC)	Psychiatric Evaluation Measures: CTQ, Beck Depression Inventory (BDI-II), Beck Anxiety Inventory, Speilberger State Trait Anxiety Inventory (STAI), Anxiety Sensitivity Index (ASI), Day-to-Day Experiences Form, NEO-Short Form, PSQI, PTCI, Quality of Life Enjoyment and Satisfaction Questionnaire Short Form (Q-LES-QSF), PDS-5
MD/NP Administered Forms: Medical History/General Health Form, Gynecological History Form, Safety Assessments			MD/NP Administered Forms: Safety Assessments, Additional Treatment Inventory
Other Self-Rated Patient Forms: Edinburgh Handedness Survey, Demographics Form			
Pregnancy Test, Urine Toxicology Test			

7.3.1 Procedures for Training of Clinicians in Prolonged Exposure (PE) Therapy

Training Specific Aim: To train psychotherapists who will be involved in the main study to administer Prolonged Exposure (PE) therapy for posttraumatic stress disorder (PTSD) prior to the initiation of the main study.

Training Enrollment: Each clinician performing PE therapy on the study needs to pass on performance for 6 sessions of PE therapy for 2 eligible participants in order to receive their PE certification. If new clinicians are hired onto the study, more training participants will be recruited in order to certify these clinicians. We estimate this will take 2-3 participants treated per trainee. We also estimate a 50% screen fail rate on eligibility. Thus, we estimate that in total 25 participants will be enrolled in order to train 5 clinicians over the course of the study. These participants will only be included for training purposes and their data and participation will not contribute to the total participants included, or the analyses in the main part of the study

Consent for Training Participation: Participants will undergo a specific consent for the training protocol included in this submission. This consent will clarify the training aspects of the PE training and will not include the study medication or imaging components of the study (which will not be delivered to training participants). All other questionnaire assessments will be identical.

Recruitment and screening procedures, safety monitoring and post treatment referrals as needed for training participants will be identical to the procedures outlined in the main trial. Screening procedures will identify adults with a primary diagnosis of PTSD for whom treatment with PE is appropriate with the same entry criteria with the exception of concerns related to undergoing MRIs (e.g. metal implants) or testing estrogen. Thus the entry criteria for training participants is as follows:

Training Participant Inclusion Criteria

- Male or Female, 18-70 years of age
- Criterion A traumatic event
- At least one month post-trauma DSM-5 PTSD symptoms
- PDS-5 score ≥ 28
- Stable medications (for 3 or more months by the time of study entrance), with the exception of benzodiazepines). Benzodiazepine use will be excluded.
- Provide informed consent

Training Participant Exclusion Criteria

- Other primary diagnoses (e.g., pervasive developmental disorders, psychotic disorder, thought disorder, or presence of other disorder that is primary relative to PTSD symptoms)
- Imminent suicide risk in need of a higher level of care
- Concurrent trauma focused psychotherapy
- Pregnancy (to be ruled out by urine ß-HCG).

Training procedures: Participants will receive the identical PE intervention described above with therapists being trained as approved study therapists. In addition, they will consent to videotapes of their therapy and assessment sessions being used for training purposes to certify the study therapist, as below.

TABLE 3: SCHEDULE OF TRAINING STUDY PROCEDURES

Study Phase	Screening	Baseline Assess	PE Session 1	PE Sessions 2-6
Visit Number	**n/a**	**1**	**2**	**3-7**
Informed Consent/Assent		X		
Review Inclusion/Exclusion Criteria	X	X		
Demographics/Medical History		X		

Pregnancy Test		X		
Urine Toxicology Screen		X		
Prior/Concomitant Medications	X	X		
Psychiatric evaluation measures		X		
Adverse Event / Unanticipated Problems Assessment		X	X	X
PE Treatment Procedures			X	X

Training protocol risks and discomforts are limited to those associated with assessments and PE therapy, with the addition of potential discomfort having sessions videotaped and reviewed by trainers.

Benefits include that training participants undergo expert diagnostic assessments and receive a gold standard psychotherapy treatment of PTSD at no cost. Prolonged Exposure is an evidence based first line treatment designed to reduce symptoms of PTSD.

Participants will receive free, evidence-based, first-line treatment for PTSD as part of the training study. Monetary compensation in the amount of $100 will be provided to compensate for their time and any travel expenses. This amount will be given to participants upon completion of the PE therapy sessions.

Recording of Therapy Sessions:
All therapy sessions will be digitally video-recorded. This will allow us to 1) provide supervision for the clinicians, and 2) monitor the treatment and assessment information to be sure that we can evaluate whether the sessions are carried out according to the therapy guidelines. These recordings will be kept on an electronically secure, password protected hard drive in a locked room for no longer than 5 years. We will provide participants with a separate video consent form to review and complete.

People who will watch the recordings include the participant's study therapist and study-approved supervisors at UTHealth Houston and the University of Pennsylvania. Other study clinicians and/or people who are learning the study protocol may also hear some segments of the recording. Videos will be accessed only through secure channels that give access for viewing within the UTHealth Houston firewall.

Post-Endpoint:
Following the end of the treatment phase, patients interested in further treatment for residual PTSD symptoms will be given appropriate clinical referrals.

7.3.2 Standard of Care Study Procedures

All procedures will be specific to the study protocol.

7.4 Concomitant Medications, Treatments, and Procedures

See Inclusion/Exclusion Criteria above.
All concomitant prescription medications taken during study participation will be recorded on the case report forms (CRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and non-prescription medications.
In addition, all psychosocial interventions will be recorded.

7.5 Justification for Sensitive Procedures

Assessment of trauma is critical to the study aims and needed for the diagnosis and treatment of PTSD with prolonged exposure. Details of trauma will be asked only by trained approved study assessors and clinical providers, and all clinical study staff will be supervised by [study physician] at UTHealth Houston. See procedures to minimize risk below.

The use of placebo in our experiments is very important to reduce experimental bias. All participants will be receiving an active evidence-based treatment for PTSD, PE, in addition to the study medication, and non-responders with significant PTSD symptoms at the 3-month follow-up timepoint (who did not improve at least 30% in their CAPS-5 Past Week score from pre-treatment to the 3-month follow-up and have a CAPS-5 Past Week score of 26 or greater post-treatment) will be offered additional PE sessions after the 3-month follow-up visit.

7.5.1 Precautionary Medications, Treatments, and Procedures

The serum peak concentration of 2mg doses 1-2X/week of estradiol is expected to result in levels that are lower than that reached during ovulation in naturally cycling women (which is on average 400pg/ml). Therefore, the dose we are using will induce E2 levels that still fall within a normal physiological range for women of reproductive age. Thus, we do not anticipate any medical complications or concerning drug interactions from administration of the dose chosen in our study.

8 Assessment of Safety

8.1 Specification of Safety Parameters

Procedures to Minimize Risk. Participants' reactions to study assessments and treatment will be closely monitored, and negative reactions will be addressed therapeutically. Participants could develop mild to moderate emotional discomfort or frustration associated with psychiatric interviewing or filling out questionnaires. Participants will be allowed any breaks in the testing that are needed. Additionally, the Intake Assessment can be spaced over visits, if needed.

Risks associated with PE therapy are mild to moderate discomfort when exposed to anxiety-provoking images and situations in treatment sessions and at home for homework and discomfort about being in treatment for victims of a traumatic event. The exposure sessions of PE are intended to elicit some emotional reactions (fear/anxiety) in order to engage the client in processing the traumatic memories. All exposures will be carried out with the full knowledge and consent of the client. Exposure therapy has produced considerable benefit for thousands of participants with anxiety disorders. While some participants fail to benefit from this therapy, there are only a handful of reports of negative side effects from it. None of the participants in our past or most recent treatment studies, sustained prolonged negative reactions to exposure. Potential side effects from PE (e.g., exacerbation of PTSD and associated symptoms) will be addressed by reassuring the client if necessary that these are temporary and are often associated with improvement. The therapists will also be trained to titrate response to exposure. In vivo exposure homework assignments will be gradual, beginning with situations that elicit moderate anxiety and progressing to increasingly anxiety-provoking situations. Participants will be informed by their therapist that they may call him/her between sessions should they experience crisis or are distressed enough to need psychological support.

In addition, any participants who have not improved at least 30% from their pre-treatment the 3-month follow-up CAPS-5 Past Week score and have a CAPS-5 Past Week score of 26 or greater at the 3-month follow-up will be offered additional treatment if they wish. Participants will then have the option to receive up to 4 additional sessions of PE delivered for an hour a week (for a total number of 10 sessions of PE) immediately following the 3-month follow-up visit. All participants will receive safety assessments throughout the study and at 3 and 6 month follow ups, and will be assisted with clinical referrals if desired and/or indicated.

Monitoring of Mental Status and Mood. Subjects' mental status will be closely monitored throughout the course of the study. As previously stated, it is not uncommon for treatment of trauma-related symptoms to be associated with temporary exacerbation in participants' symptoms of PTSD and depression. However, some participants may experience symptom exacerbations or psychiatric emergencies that require treatment beyond that provided by the study. In all cases, the client's mental status will be the utmost priority and immediate action will be taken to provide appropriate care. The intake evaluation for the study will include the Columbia-Suicide Severity Rating Scale to adequately assess suicide risk before study enrollment, and study therapists will routinely evaluate mood over the course of treatment, including the presence or absence of suicidal thoughts (using the BDI-II followed by clinical interview with a modified suicide check list at each

visit), and if any suicidal thoughts, plans or actions are present, these will be dealt with using the detailed plan outlined in Protection against Suicidality in the section immediately below.

Management of Suicide Risk
Several strategies will be implemented to assist in early identification and appropriate management of suicide risk. This protocol will utilize the Columbia Suicide Scale (C-SSRS) at screening and a suicide check list at each clinical and psychiatric assessment follow up visit to routinely assess suicidal ideation, impulses and behaviors. In addition, the BDI will be completed and reviewed by the study therapist who will be working closely with each participant.

Further the primary clinical supervisors on the project a study clinician serving as her designate will be on call and available to discuss urgent and emergent clinical issues. Study therapist training will include identification of signs of depression and suicidality in patients. As noted above, clinicians will assess mood symptoms and suicidality on an ongoing basis and will specifically inquire about it at every visit.

Participants deemed to be at acute risk for suicide based on the C-SSRS, checklist or clinical assessment will be referred for emergency care (e.g., hospital emergency room or urgent care) and, if necessary, transferred to an appropriate level of clinical care (i.e., inpatient hospitalization) until stabilized. Once stable, the need for study treatment termination will be determined on a case by case basis in coordination with the care team, site PI, and the study PI. However, no prohibition against necessary treatment will be in place, if needed.

Safe Use of Estrogen. Common side effects of chronic administration of this drug include headache, breast pain, irregular vaginal bleeding, stomach cramps, nausea, or hair loss. However, there are no known side effects of acute single doses of estrogen, as it is the case in the current application. Importantly, all women will already be using oral contraceptives. A total of 5 doses of 2mg each over 3 weeks will be administered for this study. These acute E2 doses do not pose a significant risk for the participants. In fact, OC use plus these additional exogenous estrogen doses act through a negative feedback loop. Therefore, pregnancy is even less likely than with OCs alone, as is spotting. The exogenously administered E2 will mimic estrogen levels that are observed during ovulation in naturally cycling women. Importantly, at each appointment, participants will be asked how they are feeling and if they had encountered any of the potential side effects. If that is the case, participants will discuss with the MD in charge of the study and a clinical decision will be made whether or not the subject should stop her participation in the study.

Minimizing risk during fMRI. No subjects will have metallic implants contraindicating MRI. All female subjects will have pregnancy ruled out via a urine pregnancy (beta-HCG) test. Subjects may ask to have a scan stopped and discontinue participation in the study at any time. Every effort will be made to reassure the patient and minimize any discomforts while in the fMRI scanner. Subjects will be able to converse with a staff member via a microphone and speaker system throughout. To reduce the discomfort caused by the MRI loud noises, subjects will wear earphones to enable presentation of auditory stimuli while minimizing ambient scanner noise.

Participant Study Discontinuation
Participants are free to discontinue study treatments or assessments at any time.

An individual patient's participation in the treatment trial may be discontinued at any time because of serious medication and/or therapy side effects or if continued participation is considered a danger to their welfare.

Patients who require discontinuation of both of the study treatments (PE and double blind Estrace) for any reason will be scheduled for a final evaluation and given appropriate treatment referrals. The reason the patients are discontinued from the clinical trial and any referrals made will be clearly specified.

8.1.1 Definition of Adverse Events (AE)

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

8.1.2 Definition of Serious Adverse Events (SAE)

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**.

8.1.3 Definition of Unanticipated Problems (UP)

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)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

8.2 Classification of an Adverse Event

8.2.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 Relationship to Study Agent

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.2.3 Expectedness

Study clinicians will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study intervention (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution, as appropriate.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained or addressed. At the last scheduled visit, the investigator should instruct each subject

to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

The investigator should notify the study sponsor, IRB and DSMB of any death or serious adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

8.4 Reporting Procedures – Notifying the IRB

All AEs will be reported to the IRB in the yearly continuing review process. SAEs that are at least probably related to the study and unexpected will be reported to the IRB and DSMB within 72 hours of the study clinician/PI learning of the SAE.

SAE and UP reporting to the NIH sponsor will follow NIMH reporting guidelines (ie deaths as soon as possible but within 5 days of PI learning, related SAEs and UPs within 10 days of PI learning: https://www.nimh.nih.gov/funding/clinical-research/nimh-reportable-events-policy.shtml).

Reports will be completed by a study staff member.

8.4.1 Unanticipated Problem Reporting

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report any UPs to their IRB and the study PI who will be responsible for reporting to the DSMB and to the NIH. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are study related SAEs will be reported to the IRB and to the DCC/study sponsor within 72 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB, DSMB and NIH at regular reporting or continuing review.

8.4.2 Reporting of Pregnancy

Pregnancy tests will be done for all female participants with child-bearing potential prior to the initiation of the experiment to rule out pregnancy. If pregnancy is suspected, subjects will not be allowed to participate in the study. Further, participants will all be on oral contraceptives, making pregnancy unlikely.

Although there are no known risks related to MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no possible benefit from adding estrogen to treatment as being tested in this protocol for a pregnant woman and potential risks with MRI, we will exclude pregnant women. A negative urine pregnancy test will be required before a woman of child-bearing potential can participate in this study. If the subject is post-menopausal for at least one year or has undergone a hysterectomy, the subject will not be required to undergo a urine pregnancy test prior to obtaining the MRI scan. Should a pregnancy be identified after initiation of study drug, study medication will be discontinued and this will be reported to the IRB, DSMB and NIH in regular reporting with a separate IRB event report including information on how this may alter study treatment and follow up will occur.

8.5 Reporting Procedures – Notifying the Study Sponsor

Any study-related unanticipated problem posing risk to subjects or others, and any type of serious adverse event, will be reported to the NIH sponsor and will follow NIMH reporting guidelines (ie deaths as soon as possible but within 5 days of PI learning, related SAEs and UPs within 10 days of PI learning: https://www.nimh.nih.gov/funding/clinical-research/nimh-reportable-events-policy.shtml).

To report such events, a Serious Adverse Event (SAE) form will be completed by the investigator which will be shared with the NIMH program officer (as well as the IRB and DSMB). The investigator will keep a copy of this SAE form on file at the study site.

The investigator will provide any 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 Serious Adverse Event form, 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 IRB, DSMB and NIMH PO.

8.6 Reporting Procedures – Participating Investigators

Investigators at the second study site, University of Pennsylvania, who are not UTHealth Houston faculty or affiliated with an UTHealth Houston research site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.

8.7 Study Halting Rules

Estrace is a safe widely used medication and it is being used at low dose with an evidence based trauma therapy in a population of women aged 18 to 45. Extensive safety data is available for this population for each intervention alone. Thus, the need for halting of the study itself is not anticipated. Nonetheless, the DSMB will review study continuation at yearly DSMB meetings considering study progress with guidelines included in the DSMP. Should any new concerns about the use of estrogen at this dose in women age 18 to 45 emerge they will be reported to the DSMB and IRB for consideration.

Nonetheless, this study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the necessary parties including investigators, the NIH, the FDA, and/or other regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality is addressed and satisfy the sponsor, IRB and/or FDA.

8.8 Safety Oversight

8.8.1 Data and Safety Monitoring Plan

The investigator will allocate adequate time for monitoring activities. The Investigator will also ensure that any compliance or quality assurance reviewers are given access to all the above noted study-related documents and study related facilities at UTHealth Houston if assigned. A detailed strategy outline can be found in the attached Data and Safety Monitoring Plan.

8.8.2 Data Safety Monitoring Board

Consistent with NIMH guidelines on data and safety monitoring, in advance of trial inception we will establish a DSMB. Details on DSMB role, composition, and operating procedures can be found in the attached Data and Safety Monitoring Plan. Clinical Monitoring. Dr. Milad will serve as Principal Investigator (PI) of the study. It is the responsibility of the PI to oversee the safety of the study at UTHealth Houston.

Imaging procedures will be conducted either at UTHealth Houston. The investigators for this project are not trained to perform radiological diagnosis, and the MRI scans performed in this study are not designed to find abnormalities and are being conducted only for research purposes. If the investigators notice a finding on an MRI scan that is of clinical significance and medically actionable, the scan will be shared with a licensed radiologist for further evaluation. Should this occur, a neuroradiologist would be consulted and the MRI scan will be sent to the UTHealth Houston. If the neuroradiologist thinks that further investigation of the finding is called for, the participant will be contacted about the finding and next steps. A copy of the radiology report will also be provided to the participant for their records. The decision as to whether to proceed with further examination or treatment lies solely with the participant and their physician. The images collected in this study do not comprise a proper clinical MRI study these images will not be made available for diagnostic purposes.

For this study, Dr. Soares and/or Dr. Mira Milad, licensed physicians, or a delegate (MD or NP) will be responsible for the prescription administration and all procedures related to the study treatments. All participants will be monitored for any adverse events related to Estrogen use and PE by an approved study physician, nurse practitioner, and psychologists involved in the study. A study Physician will be in charge of managing AEs, SAEs, and unanticipated problems related to study treatment and psychiatric risks to participants. The psychotherapy will be delivered by approved study clinicians but all medication related procedures will be overseen by IRB-approved, licensed MD or NP staff. Dr. Mira Milad and Dr. Soares will work with the PI Dr. Mohammed Milad to support all necessary reporting.

Dr. Mohammed R. Milad serves as the overall PI of the proposal. He will oversee the implementation of fMRI-related tasks and aspects of the project and the fMRI data acquisition before and after treatment. Dr. Milad will monitor the safe implementation of the protocol at the center for brain imaging. Dr. Jair C. Soares and/or Dr. Mira Milad serve as the overseeing, licensed physician(s). They or their delegates will be responsible for the prescription administration and all procedures related to the study medication. The delegates will consult with Dr. Soares/Dr. Mira Milad as needed/indicated. Clinical safety will be monitored by the psychologists, psychiatrists, and nurse, and all will consult with Dr. Soares/Dr. Mira Milad as needed/indicated. The psychotherapy will be delivered by approved study clinicians but all medication related procedures will be overseen by IRB-approved, licensed MD or NP staff. The PI and study clinicians will meet as needed to discuss recruitment and retention, data acquisition and management and all matters related to the prescription and therapy. They will review any unanticipated emerging problems or serious adverse events in real time with each other or a delegated, responsible, licensed, IRB-approved medical clinician. The PI will be responsible for reporting to the IRB and DSMB.

9 Statistical Considerations

9.1 Statistical and Analytical Plans (SAP)

The data to be gathered from this study is multimodal: neurobiological responses (fMRI) before and after therapy, psychophysiological data obtained before, during, and after therapy, self-assessment questionnaires, and clinical data pertaining to PTSD symptoms ascertained before and after therapy. Our analytic approach will examine the impact of using estradiol as an adjunct to PE on all of these data. The major focus of the analytic approach will be to examine change in each of the above noted metrics before and after treatment and how the use of estradiol may have improved/changed these multimodal data.

9.2 Statistical Hypotheses

Hypothesis 1: Compared to the placebo-PE group (Plc+PE): 1) the E2+PE group will exhibit faster reduction of SCR between PE sessions, and 2) after 5 E2+PE sessions, the extinction recall deficit indexed by elevated SCR during extinction retention test will be rescued (i.e., SCR in the E2+PE group will be significantly lower

relative to the Plc+PE group). 2) Extinction-induced activations will be higher in the vmPFC and lower in the dACC and amygdala in post relative to pre E2+PE treatment. When comparing E2+PE vs. Plc+PE, similar differences are expected, but to a lesser degree given the anticipated benefits of PE alone.

Hypotheses 2: PTSD symptom severity will be significantly lower in the E+PE group relative to the Plc+PE group following treatment, as well as at the 3 and 6-month follow-up assessments.

Hypothesis 3: The degree of PTSD symptom reduction post- compared to pre-PE will be associated with BOLD changes in the fear extinction network and reduction in SCR during the extinction recall test after PE. The magnitude of BOLD and SCR changes will be significantly larger in the E2+PE group compared to the Plc+PE group.

9.3 Analysis Datasets

The proposed experiments in this project are aimed to examine the effects of E2+PE on the extinction-induced SCR and neural responses at post-PE sessions relative to pre-PE. SCR will be recorded as a psychophysiological index of fear extinction and extinction recall. We will conduct standard fMRI analyses to examine information flow changes in the extinction network. These data will test hypothesis 1 stated above.

Another objective of the project is to examine the impact of E2 administration on PE efficacy at reducing PTSD symptom severity. Here, we will use all clinical data (CAPS) before and after treatment as well as 3, 6 months follow up data. These data will be used to test hypothesis 2 stated above.

The combined data sets will then be used to test hypothesis 3 above.

Primary analyses will be based on a modified intent to treat sample including all participants who took at least one dose of the study medication.

9.4 Description of Statistical Methods

9.4.1 General Approach

Statistical Methods

SCR Analyses

For all experimental phase, SCR will be computed for each trial by subtracting the mean skin conductance level observed during the last two seconds of context presentation from the maximal skin conductance level reached during CS presentation. All SCR values will be square-root transformed prior to any statistical analyses. To evaluate extinction recall, an extinction retention index (ERI) will be computed for each individual using the following formula: 100 – (mean SCR to the first 4 CS+E trials during recall / maximum SCR reached during conditioning for this same cue) * 100.

Functional MRI analysis

The specific emphasis of the fMRI analysis will be on the extinction recall phase (first four trials of the CS+E vs. first four trials of the CS+U contrast). This contrast has been chosen given the literature demonstrating a significant deficit of PTSD patients during that specific phase of the experimental protocol. BOLD signal will be extracted from the four ROIs (amygdala, dACC, vmPFC and hippocampus). For each of these ROIs, we will use mixed model ANOVAs with time (pre vs. post PE) and group (E2+PE vs. Plc+PE) to investigate main effect of each of these variables as well as group by time interaction. Whole-brain analyses will also be conducted as supplemental analyses for the sake of completeness. We will also conduct regression analyses comparing the change in BOLD responses in each of our ROIs from post- to pre-treatment with PTSD symptom reduction in each of the two groups (E2+PE and Plc+PE).

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

Details of the analyses for the primary efficacy endpoints are provided above in sections 10.1, 10.2, and 10.3.

9.4.3 Analysis of the Secondary Endpoint(s)

Details of the analyses for the secondary efficacy endpoints are provided above in sections 10.1, 10.2, and 10.3.

9.4.4 Safety Analyses

See Protection against risks and safety assessments under procedures above.

Participants' reactions to study assessments and treatment will be closely monitored, and negative reactions will be addressed therapeutically. Participants could develop mild to moderate emotional discomfort or frustration associated with psychiatric interviewing or filling out questionnaires. Participants will be allowed any breaks in the testing that are needed. Any events related to safety during assessment period will be documented as they occur.

PE is considered very safe and therefore risks associated with its delivery are mild to moderate. Patients might experience discomfort when exposed to anxiety-provoking images and situations in treatment sessions and at home for homework and discomfort about being in treatment for victims of a traumatic event. Based on our extensive experience in PE, only a handful report negative side effects from it. None of the participants in our past or most recent treatment studies, sustained prolonged negative reactions to exposure. Any negative events associated with PE will documented as they occur.

Regarding safety analyses associated with Estradiol use, common side effects of chronic administration of this drug include headache, breast pain, irregular vaginal bleeding, stomach cramps, nausea, or hair loss. As noted above, there are no known side effects of acute single doses of estrogen, as it is the case in the current application. All of the women to be recruited will already be using oral contraceptives. A total of 5 doses over 3 weeks will be administered for this study. These acute E2 doses do not pose a significant risk for the participants. To assess for safety, at each PE appointment, participants will be asked how they are feeling and if they had encountered any of the potential side effects. If that is the case, these will be documented and reported. An MD from our study staff will discuss and review the symptoms with the participants and a clinical decision will be made whether or not the subject should stop her participation in the study.

9.4.5 Adherence and Retention Analyses

The adherence to the protocol will be noted based on the absence or decision made by participants not complete the study procedures during the 6 PE sessions. Adherence to medication consumption will be assessed based on the subjective reports of the participants. Note that while blood samples will be collected to measure hormonal levels in our participants, these cannot be used to analyze adherence given that some participants will be using the placebo pills and thus no change in serum estradiol would be expected.

9.4.6 Baseline Descriptive Statistics

We will perform descriptive statistics prior to testing our hypotheses. These statistics will include means (or frequencies and proportions for categorical variables), standard deviations, skewness and kurtosis, and odds-ratios. Variables with distributions violating assumptions of normality will be transformed. If this fails to improve the normality of the distribution, distribution-free statistics will be employed. This study has baseline, post-treatment, and two follow-up assessments, creating clustered data due to repeated measures. Thus, we will use established methods for such study designs to examine treatment differences, including the two most popular approaches for longitudinal data modeling: the generalized estimating equations (GEE) and linear mixed-effects model approach (LMM). We will apply these models for both the intent-to-treat and completer analyses. Inference based on GEE or LMM is valid provided that missing data are non-informative or "missingness" does not depend on the value of the unobserved outcome. Although missing data in many studies in mental health research are non-informative, we will perform sensitivity analyses to ensure that this is the case. In particular, we will entertain both parametric and semi-parametric models that assume some missingness mechanism and compare estimates obtained from such models with those from GEE and LMM. Such analyses will inform us whether the non-informative missingness assumption is violated and if so, to what extent. If the non-informative missingness assumption is deemed to be severely violated, we will report treatment effects using the methods that account for informative missingness.

9.4.7 Planned Interim Analysis

Not applicable

9.4.8 Safety Review

See above re safety monitoring, reporting and DSMB including individual participant and study halting guidelines.

9.4.9 Efficacy Review

Interim analyses will not be conducted. All analyses will be conducted after breaking the blind at the end of the study unless a blinded review is requested by the DSMB.

9.4.10 Multiple Comparison/Multiplicity

As noted above, the study has baseline, post-treatment, and two follow-up assessments, creating clustered data due to repeated measures. We describe the plan of analysis and adjustments in section 10.4.6.

9.5 Sample Size

Pre to post treatment assessment using our experimental approach has not been previously conducted, and therefore it is challenging to calculate power to estimate potential sample or effect size comparing the pre to post time-points. The following sample size calculations pertain to the expected changes related the psychophysiological and neurobiological indices to be obtained. The sample size selected for this proposal is based on the detection of between-group significant differences at an alpha of .05 using data collected in our previous study of fear conditioning and extinction recall in controls and patients (PTSD) and using our preliminary fMRI data in women with high (HE) vs. low estradiol (LE) (Zeidan et al, 2011). We have calculated our power to detect between-group differences with a sample size of 25 subjects per group. Based on our previous experience with this population and this paradigm, we expect that (at each of the two time points) it is possible that we will have unusable data in approximately 20% of the subjects due to data of insufficient quality for at least one phase of the experiment. Reasons for this include poor SCR responses, head motion during scanning, or technical difficulties with SCR or fMRI. In addition, we anticipate dropout of ~20 % of patients after the initiation of the study. Thus out of the total of 80 patients, we expect to obtain a full data set (from all time points) from 50 patients. By using the effect size obtained from our previous study (see above), an alpha level of 0.05 and 50 patients, we will have 85% power to detect a significant between-group difference. Regarding the clinical measures, we do not have data to calculate power because of the novelty, but since it is a preliminary proof-of-concept aim, the results to be obtained will help inform future power analyses and calculations for this line of research.

9.6 Measures to Minimize Bias

9.6.1 Enrollment/Randomization/Masking Procedures

The study randomization table is created by the study statistician and provided to the UTHealth Houston and U Penn site pharmacies to be used during the study. A research assistant will be responsible for randomizing the participant by entering the CAPS Severity in REDCap. A randomization number is generated, which is then reported to the Investigational Pharmacy which has the key indicating which randomizations numbers correlate to estradiol or placebo pills. The research team and the subject will be blinded and will not know the investigational product contained in the prescription bottle. The pharmacy has the names of participants in their log. The blind may be broken in the case of an emergency. To request unblinding, after permission to do so is granted by the PI or the study clinical lead, a member of the study team will contact the Investigational Pharmacy to receive the condition of the subject.

10 Source Documents and Access to Source Data/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.

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 blue or black ink or recorded in any direct entry forms. 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.

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

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

11 Quality Assurance and Quality Control

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the study team will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The investigative team has extensive experience conducting clinical psychotherapy and medication studies for PTSD (Dr. Foa) and fMRI methodologies (Dr. Milad). They will train and oversee the overall conduct of the staff in clinical trials methodology. They will ensure that on-going supervision is provided, study assessments are completed, and there is good compliance of staff with all research procedures. They will assume responsibility to see that project staff is sufficiently trained to administer project interviews, review assessments with participants, carry out laboratory/scanning procedures, and maintain participants in the study throughout follow-ups. Every quarter, participants’ casebooks will be checked by Dr. Milad for accuracy with regard to inclusion/exclusion criteria; staff adherence to study procedures; completeness; and documentation of participant attrition, adverse events, and sources of potential protocol violations. Discrepancies will be noted, brought to the attention of the PI and project staff, investigated and resolved. IEs and RAs will be given specific training on follow-up methods similar to those of our numerous prior studies, and a set of MINI and CAPS recordings will be reviewed to assess inter-rater reliability for each independent evaluator prior to the start of independent evaluations. The criterion of each tape in the set will be 80% agreement, with agreement on the CAPS defined as the scores of the rater within 10% of the total scale range.

Before beginning any experimental procedures, Dr. Milad provide extensive training to all team members regarding data acquisition and maintenance as well as safety of participants. Dr. Milad will also run data quality checks every 3 months to ensure adequate quality and consistency of fMRI and SCR data.

12 Ethics/Protection of Human Subjects

12.1 Ethical Standard

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 The UTHealth Houston Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the 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 NIH before commencement of this study.

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 A for a copy of the Subject Informed Consent Forms. These consent forms will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the 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.

12.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

12.3 Informed Consent Process

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention. The following consent materials are submitted with this protocol:

1. Consent for adult participants
2. Video consent for adult participants
3. Audio consent for adult participants
4. Consent for adult participants for training study
5. Video consent for adult participants for training study

12.3.1 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants (and their families, if requested). Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate.

Written or electronic (via REDCap) informed consent will be obtained by one of the IRB approved UTHealth Houston study investigators prior to participation in the study. Subjects must be competent to provide informed consent. Study procedures and potential risks and benefits will be described to subjects at the time participation in the study is sought. Ample opportunity will be provided for potential subjects to carefully read the consent form and to ask any questions they may have. The PI will retain copies of the signed consent

form patients will be given a copy of the consent form. A subject may end their study participation at any time if they chose to do so.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

12.4 Participant and Data 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.

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

Facial images in the video files will not be obscured because the emotional expression of the participants' faces during the training PE sessions are an essential component for the training. Thus, blurring the images may hinder some aspects of the training and therefore will not be done. To ensure confidentiality, all files containing video recordings will be password protected and stored on UTHealth Houston computers that are encrypted and password protected. Those computers will be located in locked offices and only viewed by IRB-approved personnel for clinician training purposes. Videos that need to be viewed by personnel located at UPenn will remain within the UTHealth Houston firewall and accessed only after approvals for the UPenn personnel to access the UTHealth Houston system through creating an ID and gaining access to a shared network server.

12.4.1 Research Use of Stored Human Samples, Specimens, or Data

- Intended Use: Samples and data collected under this protocol may be used to study PTSD and its treatment. No blood samples will be stored and no genetic testing will be performed.
- Storage: Data will be kept in password-protected computers within the UTHealth Houston firewall. Only investigators will have access to the study data.

13 Data Handling and Record Keeping

13.1 Data Collection and Management Responsibilities

Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Prior to access to any study-related information, personnel will be required to sign statements agreeing to protect the security and confidentiality of identifiable information. Wherever

feasible, identifiers will be removed from study-related information. A Certificate of Confidentiality will be obtained, because the research could place the subject at risk of criminal or civil liability or cause damage to the subject's financial standing, employability, or liability. Precautions are in place to ensure the data is secure by using passwords and encryption, because the research involves web-based surveys. We will use Research Electronic Data Capture (UTHealth Houston's REDCap) to support direct data entry by patients and study staff. RedCap will be used for data collection and management. RedCap is a secure web platform for delivery of surveys and management of databases. For this study we will take additional steps outlined by UTHealth to ensure that it is 21 CFR Part 11 compliant for data collection and management. These requirements/steps are outlined in the Attachment labeled UTHealth Redcap 21CRFPart11. Lastly, any electronic data collected will be stored on the UTHealth Houston's secured and managed network servers and devices. Only cleared study personnel will have access to the server and devices.

Data and image recordings will be accessed by IRB-approved Penn personnel only through the UTHealth Houston firewall. This will ensure data and image recordings are not being transferred outside of the UTHealth Houston firewall to maintain subject confidentiality. For neuroimaging and psychophysiological data, UPenn personnel will de-identify the data and then transfer data via secure servers into UTHealth Houston computers. This will be done once a month. Once deposited into UTHealth Houston secure servers, the PI and the team will maintain the security of the data storage as described. For clinical data to be input into RedCap, UPenn will use UTHealth Houston's RedCap, located within UTHealth Houston's secure firewall.

13.2 Study Records Retention

Study essential documents and data for this project will be retained for at least 5 years after the publication of the last findings from this study. These documents may be retained for a longer period if required for additional research activities.

13.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations. Protocol deviations must be reported to the IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

13.4 Publication and Data Sharing Policy

The PI (Mohammed Milad) holds the primary responsibility for publication of the results of the study and oversight of the data storage and utilization.

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

14 Study Finances

14.1 Funding Source

This study is financed through a grant from the National Institute of Mental Health.

14.2 Costs to the Participant

Participants will not incur any costs associated with their participation in the study. Participants will benefit from receiving well-delivered treatment for PTSD. Each subject will be compensated for completing the study procedures and will be reimbursed for their parking expenses. In the event of premature termination, subjects will receive partial reimbursement for the procedures they have completed.

14.3 Participant Reimbursements or Payments

Participants will receive $60 for visit 1, $20 for visits 2, 5 and 10, $15 for visits 3, 4, 6-9 and 11, to help defray costs of travel and time. Participants will then receive $235 upon completion of visit 12. Participants will receive $50 at each remaining follow-up visit (visits 13 and 14). The total compensation for all completed study visits will be $560. Participants who drop out of the study will be paid for the visits they attended. Participants who are deemed ineligible at /after the baseline assessment visit will be paid $60 for completing the initial assessment.

15 Study Administration

The study will be overseen and managed by the PI Dr. Milad with the clinical trial additionally overseen by Dr. Mira Milad, Dr. Jair C. Soares and Dr. Foa.

16 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities 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.) must have the conflict reviewed by the UTHealth Houston Conflict of Interest Management Unit (CIMU) with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All UTHealth Houston investigators will follow the applicable conflict of interest policies.

17 References

1. Arnberg FK, Bergh Johannesson K, Michel P-O. Prevalence and duration of PTSD in survivors 6 years after a natural disaster. J Anxiety Disord. 2013;27:347–52.
2. Bouton ME. Context and behavioral processes in extinction. Learn Mem. 2004;11:485–94.
3. Foa EB. Psychosocial treatment of posttraumatic stress disorder. J Clin Psychiatry. 2000;61:43–51.
4. Foa EB, Gillihan SJ, Bryant RA. Challenges and successes in dissemination of evidence-based treatments for posttraumatic stress: Lessons learned from prolonged exposure therapy for PTSD. Psychol Sci Public Interest. 2013;14:65–111.
5. Foa EB, Hembree E, Rothbaum BO. Prolonged Exposure Therapy for PTSD: Emotional Processing of Traumatic Experiences Therapist Guide (Treatments That Work). New York, NY: Oxford University Press; 2007.
6. Gillihan SJ, Foa EB. Fear extinction and emotional processing theory: A critical review. (2011). In Conditioning and animal learning: Human and non-human animal applications. Edited by Schactman TR & Reily SS. Oxford, UK, Oxford University Press.
7. Glover EM, Jovanovic T, Mercer KB, Kerley K, Bradley B, Ressler KJ, et al. Estrogen levels are associated with extinction deficits in women with posttraumatic stress disorder. Biol Psychiatry. 2012;72:19–24.
8. Graham BM, Milad MR. The study of fear extinction: implications for anxiety disorders. Am J Psychiatry. 2011;168:1255–65
9. Graham BM, Milad MR. Blockade of estrogen by hormonal contraceptives impairs fear extinction in female rats and women. Biol Psychiatry. 2013;73:371–8.
10. Weathers FW, Blake DD, Schnurr PP, Kaloupek DG, Marx BP, & Keane TM. The Life Events Checklist for DSM-5 (LEC-5) – Extended. [Measurement instrument]. 2013. Available from https://www.ptsd.va.govHergueta, T., Baker, R., & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IVand ICD-10. J Clin Psychiatry, 59(Suppl 20), 2233.
11. Inslicht SS, Metzler TJ, Garcia NM, Pineles SL, Milad MR, Orr SP, et al. Sex differences in fear conditioning in posttraumatic stress disorder. J Psychiatr Res. 2013;47:64–71.
12. Kalisch R, Korenfeld E, Stephan KE, Weiskopf N, Seymour B, Dolan RJ. Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. J Neurosci. 2006;26:9503–11.
13. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen H-U. Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. Int J Methods Psychiatr Res. 2012;21:169–84.
14. Marin MF, Milad MR, et al. Association of resting metabolism in the fear neural network with extinction recall activations and clinical measures in trauma-exposed individuals. Am J Psychiatry. 2016; 173(9):930-8.
15. McLean CP, Foa EB. Dissemination and implementation of prolonged exposure therapy for posttraumatic stress disorder. J Anxiety Disord. 2013;27:788–92.
16. McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res. 2011;45:1027–35.
17. Milad MR, Igoe SA, Lebron-Milad K, Novales JE. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience. 2009b;164:887–95.
18. Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, et al. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol Psychiatry. 2009a;66:1075–82.
19. Milad MR, Quirk GJ. Fear extinction as a model for translational neuroscience: ten years of progress. Annu Rev Psychol. 2012;63:129–51
20. Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol Psychiatry. 2007;62:446–54.
21. Milad MR, Zeidan MA, Contero A, Pitman RK, Klibanski A, Rauch SL, et al. The influence of gonadal hormones on conditioned fear extinction in healthy humans. Neuroscience. 2010;168:652–8.
22. Phelps EA, Delgado MR, Nearing KI, LeDoux JE. Extinction learning in humans: role of the amygdala and vmPFC. Neuron. 2004;43:897–905.

23. Pitman RK, Rasmusson A., Koenen KC, Shin L., Orr S., Gilbertson MW, et al. Biological studies of post-traumatic stress disorder. Nat Rev Neurosci. 2012;13:769–87.
24. Powers MB, Halpern JM, Ferenschak MP, Gillihan SJ, Foa EB. A meta-analytic review of prolonged exposure for posttraumatic stress disorder. Clin Psychol Rev. 2010;30:635–41.
25. Steenkamp MM, Dickstein BD, Salters-Pedneault K, Hofmann SG, Litz BT. Trajectories of PTSD symptoms following sexual assault: is resilience the modal outcome? J Trauma Stress. 2012;25:469–74.
26. Zeidan MA, Igoe SA, Linnman C, Vitalo A, Levine JB, Klibanski A, et al. Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biol Psychiatry. 2011;70:920–7.

18 Attachments

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

- Consent Form
- Consent Form for Training Study
- Video Consent Form for Training Study
- Audio consent for Assessments Primary Study
- Questionnaire assessments
- Recruitment materials