Study Title: Neurosteroid Intervention for PTSD in Iraq/Afghanistan-era Veterans

NCT03799562

Document date: 4/10/2024

PROTOCOL TITLE: Neurosteroid Intervention for PTSD in Iraq/Afghanistan-Era Veterans

PRINCIPAL INVESTIGATOR: Jennifer C. Naylor, PhD

VERSION NUMBER AND DATE: Version #13, 04/10/2024

SPONSOR/FUNDING SOURCE: VA CSRD Merit Review

The PI is a VA clinician

Purpose

There is an urgent and immediate need to develop new and effective pharmacological treatments for posttraumatic stress disorder (PTSD) (Krystal et al., 2017). Currently only two medications for PTSD are approved by the Federal Drug Administration (FDA), both of which are from the same drug class, and resulted in only moderate effect sizes in FDA registration trials (Brady et al., 2000; Davidson et al., 2001; Marshall et al., 2001; Tucker et al., 2001). Since many Veterans with PTSD remain symptomatic despite treatment with these FDA-approved selective serotonin reuptake inhibitors (SSRI), prescribers often resort to off-label use of adjunctive or monotherapy medications that have not yet undergone extensive study for the treatment of PTSD. Needless to say, it is problematic that Veterans with PTSD are routinely treated with monotherapy medications or medication combinations that have little or no empirical evidence.
Moreover, extremely few investigations have focused specifically on optimal medication approaches for PTSD in Iraq/Afghanistan-era Veterans, a cohort that may be less treatment-refractory—particularly if treated early in the course of PTSD symptom development. Randomized clinical trials (RCT) utilizing new medication approaches for Iraq/Afghanistan-era Veterans are therefore acutely needed, and the investigation of promising pharmacological agents for the treatment of PTSD could thus not be more timely or urgent.

Background and Significance

Increasing preclinical and clinical evidence supports a potential role for neurosteroids in the neurobiology and treatment of PTSD. Pregnenolone and allopregnanolone (a downstream metabolite of pregnenolone) are neurosteroids that are synthesized endogenously in human brain, adrenals, and other tissues, and appear to have very favorable safety and tolerability profiles in clinical studies. Pregnenolone enhances learning and memory in rodents (Flood et al 1992), decreases apoptosis (Leskiewicz et al 2008), and increases myelination (Zhu and Glaser 2008). Allopregnanolone also has several properties that suggest potential therapeutic utility for PTSD and co-occurring conditions, including neuroprotective (Sayeed et al 2006; Djebaili et al 2005; Charalampopoulos et al 2004), neurotrophic (Ghoumari et al 2003), analgesic (Coronel et al 2016; Patte-Mensah et al 2014; Naylor et al 2016), and anti-inflammatory actions (Noorbakhsh et al 2014). Allopregnanolone levels are also significantly lower in individuals who have PTSD compared to control participants (Rasmusson et al., 2006; Marx et al, unpublished MIRECC; Marx et al, unpublished INTRUST; Stein PI), and rodent data demonstrate that reductions in allopregnanolone levels result in anxiety- and depression-like behaviors (Pinna & Rasmusson, 2012). Treatment with pregnenolone enhances downstream levels of allopregnanolone 5 to10-fold (Marx, 2009, 2014, 2016; Sripada et al., 2013), and may thus be a promising precursor loading strategy to restore deficient allopregnanolone levels in PTSD. The primary aim of this project is therefore to conduct an RCT in Iraq/Afghanistan-era Veterans with PTSD to evaluate the possible efficacy of pregnenolone compared to placebo (treatment for 8 weeks following a 2 week placebo lead-in period; total trial duration 10 weeks). We hypothesize that treatment with pregnenolone will be efficacious in Iraq/Afghanistan-era *Veterans with PTSD, and will* significantly reduce PTSD symptoms as assessed by

theCAPS-5 (primary endpoint) compared to placebo. Secondary endpoints will include the assessment of conditions that frequently co-occur with PTSD; specifically, we hypothesize that pregnenolone will also demonstrate efficacy for co-occurring chronic pain symptoms and depression symptoms.

Limited Pharmacological Treatments for PTSD: The estimated lifetime prevalence of posttraumatic stress disorder (PTSD) in the USA is approximately 7% (Kessler et al., 1995). Studies from Iraq/Afghanistan-era Veterans demonstrate combat-related PTSD prevalence is between 4 and 17 percent (Richardson et al., 2010), although the rates may be closer to 50% for those who have completed multiple deployments. PTSD is often chronic (Kessler et al., 1995), resulting in a substantial burden of disability and functional impairment (Amaya-Jackson et al., 1999; Thomas et al., 2010). PTSD is also associated with an increased risk for substance use disorders (Nock et al., 2010; Wilcox et al., 2009), suicidality (Davidson et al., 1991), and other psychiatric comorbidities (Dedert et al., 2009). In addition, patients with PTSD have increased rates of medical morbidities (Davidson et al., 1991), as well as mental and physical health service utilization rates (Calhoun et al., 2002). The current first-line pharmacological treatment for PTSD is [treatment with] SSRIs, but fewer than half of patients achieve full remission on these agents (Institute of Medicine, 2008; Berger et al., 2009), and the average remission rates may be lower in combat Veterans with chronic PTSD (Hertzberg et al., 2000; Davidson et al., 2003). In addition, more than 25% of patients prematurely discontinue SSRIs due to adverse side effects or treatment failure (Anderson, 2000; Nurnberg et al., 2003). Thus, despite demonstrated efficacy, SSRIs are often inadequate to manage PTSD symptoms, and other psychotropic medications that lack empirical support for the treatment of PTSD are frequently used for symptom management (Davidson et al., 2003). The 2009 update of the American Psychiatric Association guideline on PTSD states that “more effective pharmacological treatments must be identified, particularly for Veterans with combat related PTSD” (Benedek et al., 2009), and a Consensus Statement by the PTSD Psychopharmacology Working Group recommends that “effective pharmacological treatments for PTSD should be considered a national mental health priority” and that there is a “need for clinical trials conducted in Veterans that support the efficacy of pharmacological treatment of PTSD within the VA” (Krystal et al., 2017). New pharmacological intervention strategies thus are urgently needed for PTSD, especially in Veteran cohorts.

New Biomarker-Informed Therapeutics for PTSD: Neurosteroid Interventions: Compelling data from multiple preclinical and clinical research investigations suggest pregnenolone and downstream metabolites such as allopregnanolone which crosses the blood brain barrier (Wang et al., 1997; Marx et al., 2000; Naylor et al., 2008) and increases post-treatment with pregnenolone (Sripada et al 2013, Marx et al 2016, 2014) are promising biomarker candidates that can potentially be rapidly translated into safe pharmacological intervention strategies for the treatment of PTSD. Our laboratory has demonstrated that allopregnanolone and pregnenolone levels predict PTSD symptom improvement in an mTBI cohort of Veterans following 8 week treatment with pregnenolone. Increases in serum pregnenolone (r=-0.85;p=0.006) and allopregnanolone (r=-0.85; p=0.006) were significantly correlated with reductions in total CAPS scores in those receiving pregnenolone, and CAPS Cluster D symptoms were significantly correlated with

pregnenolone and allopregnanolone increases post- treatment (Marx et al., 2016). Furthermore, in a subsequent larger RCT of Veterans with mTBI, we found that Veterans randomized to pregnenolone reported a significant reduction in DSM-IV cluster D PTSD symptoms posttreatment, the primary behavioral endpoint (p=0.034; please see preliminary data section). Our laboratory also recently demonstrated that the improvement in pain ratings for Veterans with chronic low back pain is inversely associated with pregnenolone and allopregnanolone levels (Naylor, unpublished data), and that 4 week administration of pregnenolone significantly improved pain intensity ratings, the primary endpoint (Naylor, unpublished data).

Additionally, converging clinical evidence demonstrates that allopregnanolone and other endogenous neurosteroid levels are dysregulated in PTSD (Rasmusson et al. 2006, Marx et al. MIRECC unpublished data, Marx et al. unpublished INTRUST data-Stein PI; please see preliminary data section), as well as several other disorders that commonly occur with PTSD, such as chronic pain conditions (Kilts et al. 2010, Naylor et al., 2016a, 2016b) and depression (Uzunova et al., 1998). These disorders share several overlapping symptoms and are associated with the dysregulation of related pathophysiological processes (including neurohormonal/neuroendocrine, neurochemical, and inflammatory processes). It is therefore possible that PTSD and these other common psychiatric morbidities share common pathophysiological mechanisms associated with reductions in endogenous allopregnanolone levels.

Symptoms associated with these disorders may thus improve once allopregnanolone levels are restored via exogenous supplementation. The current project will thus determine if treatment with adjunctive pregnenolone as a precursor loading strategy to enhance endogenous allopregnanolone levels (quantified by mass spectrometry) has therapeutic utility for the treatment of PTSD and these commonly co-occurring disorders (pain and depression) in Iraq/Afghanistan-era Veterans. Supporting evidence and rationale for the use of neurosteroids for the treatment of PTSD and commonly comorbid conditions are provided below.

Allopregnanolone and PTSD: Allopregnanolone, a downstream metabolite of pregnenolone, is a potent, positive GABAA receptor modulator with several properties that strongly support its therapeutic potential for the treatment of PTSD, including neuroprotective (Sayeed et al., 2006; Djebaili et al., 2005; Charalampopoulos et al., 2004) and neurotrophic (Ghoumari et al., 2003) effects relevant to the underlying pathophysiology of PTSD. We have determined that allopregnanolone levels are lower in Iraq/Afghanistan-era Veterans with PTSD compared to those without PTSD in two separate and independent cohorts (N=480; N=212). Additional investigations have also demonstrated that allopregnanolone levels are significantly reduced in PTSD and negatively correlated with PTSD re-experiencing symptoms in both women (Rasmusson et al., 2006) and men (Rasmusson, 2016 poster presentation). In our recently completed RCT targeting Veterans with mTBI, Veterans randomized to the pregnenolone group, demonstrated a significant reduction in DSM-IV cluster D (hyperarousal; equivalent to DSM-5 Criterion E) PTSD symptoms compared to Veterans receiving placebo (p=0.034, please see preliminary data section). In healthy participants, allopregnanolone plays a role in the modulation of brain function associated with negative emotion and enhances

activity associated with regulatoryprocesses (Sripada et al., 2013; Priority Communication, *Biological Psychiatry*). Additionally, rodent models of PTSD demonstrate that reductions in endogenous allopregnanolone levels result in behaviors consistent with anxiety and depression, as well as increased aggression and enhanced fear conditioning (Pinna & Rasmusson, 2012). Using a predator stress paradigm, our lab has recently demonstrated that rats pre-treated with pregnenolone have significantly attenuated anxiety-like behaviors in the elevated plus maze task compared to vehicle-treated rats (p=0.04, manuscript in preparation, please see preliminary data section). There is thus substantial preclinical and clinical evidence to suggest that neurosteroid dysregulation is associated with PTSD symptomatology, and that restoration of low or depleted endogenous neurosteroid levels such as allopregnanolone may have therapeutic utility for the treatment of PTSD.

Neurosteroid Pain Analgesia: Increasing clinical and preclinical investigations demonstrate that neurosteroids including allopregnanolone have analgesic properties (Coronel et al., 2016; Patte-Mensah et al., 2014). Our laboratory has shown in multiple investigations that neurosteroid levels are inversely associated with pain symptoms (Naylor et al., 2016a, 2016b; Kilts et al., 2010). Additionally, we recently replicated these findings in an Iraq/Afghanistan-era cohort with a history of blast-related traumatic brain injury (TBI), in which allopregnanolone was significantly reduced in Veterans with co-occurring pain. Our lab also recently completed a VA-funded randomized, double- blind, placebo-controlled trial using pregnenolone for the treatment of chronic low back pain (PI Naylor; VA CDA). Veterans randomized to pregnenolone reported a significant reduction in pain intensity symptoms compared to placebo, p=0.006 post-treatment (see preliminary data section). In addition, increases in pregnenolone (r=-0.22; p=0.044) and allopregnanolone (r=-0.20; p=0.073, marginal) were associated with a reduction in low back pain intensity ratings. Several preclinical studies also provide strong evidence for the role of allopregnanolone, a GABAergic neurosteroid, in pain analgesia. For example, allopregnanolone increases response latencies to tail flick (Frye & Duncan, 1994) and protects against noxious mechanical stimuli in rats (Winfree et al., 1992), and increases response latencies to thermal stimuli in both rats (Pinna et al., 2003) and invertebrates (Kavaliers et al., 2000). Moreover, allopregnanolone has been implicated in the treatment of chemotherapy-induced neuropathies in cancer patients; specifically, allopregnanolone restored mechanical pain thresholds, sciatic nerve conduction, and neurochemical/cellular alterations to normal levels in rats treated with oxaliplatin (Meyer et al., 2010). Allopregnanolone may also be the active metabolite responsible for the analgesic actions of progesterone (Mok & Krieger, 1990; Korneyev & Costa, 1996). Collectively, these preclinical and clinical findings provide compelling evidence for the analgesic actions of neurosteroids such as allopregnanolone and pregnenolone. Additional clinical investigations of the analgesic effects of pregnenolone are warranted in larger samples that include common comorbidities like PTSD and depression.

Chronic pain is one of the most frequently co-occurring problems in those with PTSD (Asmundson & Katz, 2009), and depression is 3 to 5 times more likely in those with PTSD compared to those without PTSD (Kessler et al., 1995). Thus, the investigation of new agents with the potential to treat not just PTSD, but also commonly co-occurring conditions such as pain and depression, merits further study. *Neurosteroid interventions could target all 3 of these symptom domains.*

Neurosteroids for Depression: Both biomarker-informed data and clinical findings suggest neurosteroids may also have therapeutic potential for the treatment of depression symptoms. Neurosteroid levels are altered in individuals with depression (Girdler & Klatzkin, 2007; Schule et al., 2011) and reductions in pregnenolone (George et al., 1994) and its GABAergic metabolite allopregnanolone (Uzunova et al., 1998) are inversely associated with depressive symptoms. For example, allopregnanolone levels in cerebrospinal fluid are two times lower in patients with depression compared to controls (Uzunova et al., 1998). Restoration of depleted levels of allopregnanolone in depressed individuals has been shown as an effective treatment strategy. Compared to placebo, pregnenolone administration improves bipolar depression symptoms (Brown et al., 2014) and continuous intravenous administration of allopregnanolone (60 hours) significantly reduces severe depression symptoms postpartum—an effect that persisted for at least 30 days (Kanes et al., 2017). Administration of allopregnanolone also produces antidepressant-like effects in a rodent swim test (Khisti et al., 2000), and several serotonergic agents including fluoxetine, fenfluramine, and imipramine facilitate this antidepressant effect (Khisti and Chopde, 2000). Depressive symptoms associated with decreased plasma levels of allopregnanolone have additionally been shown to normalize following treatment with SSRIs and accompany clinical improvement in symptoms (Uzunova et al., 1998). These findings suggest that increases in allopregnanolone levels may be contributing to the therapeutic efficacy of SSRI antidepressant treatment. Moreover, the effects of SSRI medications on allopregnanolone upregulation is 10-50 times lower than what is required to inhibit serotonin activity (Pinna et al. 2009), suggesting that SSRIs may modulate GABA responses separately from serotonin re-uptake (i.e., a GABAergic mechanism). These findings suggest that pharmacological administration of pregnenolone might be beneficial in the treatment of depression and a variety of common psychiatric disorders with overlapping symptoms. Pregnenolone therefore could be used as a multifaceted approach for the treatment of PTSD, chronic pain and depression symptoms.

Neurosteroids and Inflammatory Markers in PTSD: PTSD is associated with elevated pro-inflammatory markers such as cytokines (interleukins IL-2, IL-4, IL-6, IL-8, IL-10; tumor necrosis factor-α, TNF-alpha) and C-reactive protein (CRP) (Michopoulos et al., 2017; Kibler et al., 2014; Guo et al., 2012; Oganesyan et al., 2009; Hoge et al., 2009). In addition, disorders commonly comorbid with PTSD, including depression (Maes et al., 1995; Bob et al., 2010; Dowlati et al., 2010; Hodes et al., 2014; Kohler-Forsberg et al., 2017) and chronic pain conditions (Hernandez et al., 2010; Della Vedova et al., 2010) are also associated with inflammatory dysregulation. *Neurosteroids such as allopregnanolone have anti-inflammatory properties (Noorbakhsh et al., 2014), and may thus reduce symptoms associated with PTSD, pain, and depression - in part via modulation of inflammatory processes.* Anti-inflammatory actions of neurosteroids include the ability of allopregnanolone to decrease IL-1β and TNF-α expression following brain injury (He et al., 2004) and to reduce microglia activation (Chen et al., 2011). Progesterone (precursor to allopregnanolone) has also been shown to reduce expression of TNF-α and IL-1β in cultured microglia (Jiang et al., 2012), and pregnenolone (precursor to allopregnanolone) reduces astrocyte activation and IL-1β

following experimentally induced spinal nerve ligation (Chen et al., 2016). Allopregnanolone has also been shown to reduce glial activation, pro-inflammatory cytokines, and pain symptoms following chronic constriction injury (Huang et al., 2016).

Our laboratory has found that allopregnanolone levels are inversely associated with several pro-inflammatory markers (potentially consistent with its anti-inflammatory actions). Specifically, in a cohort of 82 Iraq/Afghanistan Veterans, CRP (unpublished data, please see preliminary data section) was inversely correlated with allopregnanolone ($r=-0.23$; $p=0.047$) — a finding that we recently replicated in a larger, independent sample of 480 Veterans ($r=-0.26$; $p<0.0001$). Additionally, the inflammatory cytokine, IL-6 was also inversely correlated with allopregnanolone ($r=-0.22$; $p<0.0001$), pregnenolone ($r=-0.25$, $p<0.0001$), and androsterone levels ($r=-0.19$; $p<0.0001$) in this cohort of 480 Veterans. These findings are consistent with the anti- inflammatory actions that have been reported for several of these small molecules. In order to develop effective therapeutics for PTSD and common comorbidities, it will be critical to understand the pathophysiological underpinnings of these disorders, including possible alterations in neurosteroids that modulate inflammatory processes. Overall, both preclinical and clinical evidence suggests that neurosteroids may play a role in the modulation of inflammatory markers, and these effects could potentially contribute to their possible therapeutic efficacy in PTSD and other conditions. Neurosteroids may therefore have biomarker utility for the assessment of PTSD and commonly occurring disorders, as well as therapeutic utility for the mitigation of symptoms via their anti-inflammatory effects (among other possible mechanisms of action).

Design

The proposed design is a randomized, placebo-controlled, double-blind trial of treatment with pregnenolone or placebo in Iraq/Afghanistan-era Veterans with PTSD. The total study duration is 10 weeks. Following a 2-week placebo lead-in period, 90 subjects will be enrolled and randomly assigned to receive pregnenolone (n=45) or placebo (n=45) for 8 weeks (fixed escalating dose strategy). There will be one follow up phone call after the final study visit.

Specific objectives of the proposed project include:
1) To conduct an RCT in Iraq/Afghanistan-era Veterans that is supported by compelling preliminary data and investigates the possible efficacy of pregnenolone compared to placebo in PTSD (n=90 randomized; 45 per group). We hypothesize that treatment with pregnenolone for 8 weeks will significantly reduce PTSD symptoms in Iraq/Afghanistan-era Veterans compared to placebo, as assessed by the CAPS-5 (primary endpoint).

To determine if treatment with pregnenolone also improves co-occurring pain and depression symptoms in Iraq/Afghanistan-era Veterans with PTSD. Our preliminary data show that treatment with pregnenolone significantly decreases low back pain in Iraq/Afghanistan-era Veterans, and pregnenolone has also shown efficacy in bipoladepression. We thus hypothesize that treatment with pregnenolone will reduce pain and depression symptoms compared to treatment with placebo (secondary endpoints).

2) To quantify serum neurosteroid levels at baseline and post-treatment by highly sensitive and specific mass spectrometry-based techniques, and to determine if pregnenolone and downstream neurosteroid metabolites such as allopregnanolone (and/or other biomarker candidates such as inflammatory markers) are predictors of therapeutic response. We hypothesize that increases in pregnenolone and other neurosteroids (and decreases in inflammatory markers) will predict improvements in PTSD, depression, and chronic pain symptoms. We will also characterize genetic predictors of response. Specifically, we will: a.) characterize identify potential neurosteroid predictors of therapeutic response, and b.) examine single nucleotide polymorphisms (SNPs) of genes coding for enzymes involved in neurosteroid synthesis and metabolism. We also hypothesize that neurosteroids are dysregulated in PTSD, and that specific SNPs of genes coding for neurosteroidogenic enzymes will be associated with therapeutic response.

Primary Outcome Measure: The primary outcome for this RCT will be changes in total CAPS-5 score at Visit 6 (from baseline, Visit 2) for this modified intent-to-treat sample (mITT). The mITT population will consist of randomized subjects dispensed study medication and having at least one post-baseline (Visit 3) data point. PhenX toolkit measures, such as the CAPS-5 will be utilized as recommended.

Secondary Outcome Measures: Secondary clinical outcomes for this RCT include changes in pain intensity and functional interference, as measured by the Brief Pain Inventory, Short Form (BPI-SF) and depression symptoms by the Hamilton-Depression Rating Scale (HAM-D). Blood samples will be collected for serum analysis at all study visits and frozen in a -80 degree freezer. Upon completion of the study, samples will be thawed and analyzed using Gas Chromatography/Mass Spectrometry for neurosteroid analyses and inflammatory markers will be quantified with QuickPlex instrumentation. Genetic analyses to determine therapeutic response will be conducted at Duke University.

Risk/Benefit Assessment

Potential Risks:

a) *Physical Risk:* Physical risks associated with participation in this study include the risks of drawing blood (for serum and clinical lab tests). Possible side effects from drawing blood include bruising, bleeding, or pain at the injection site, and (rarely) fainting and infection. Blood draws are minimal risk. In addition, the electrodes from the ECG may cause mild irritation, slight redness, and itching of the skin.

Emotional Distress: Participants may experience emotional distress as a result of participating in this study. The study procedures could potentially lead to emotional distress from discussing medical histories or deployment experiences. Each participant will be closely monitored for emotional distress and emergent suicidal ideation and homicidal ideation at screening and each study visit (it is anticipated that

this will be rare). If a participant becomes significantly distressed or if intervention is deemed appropriate, they will be escorted to the Durham VA Emergency Room for additional assessment. Please see COVID-19 addendum for safety-related procedures associated with visits conducted by telephone.

b) *Unauthorized Access to Data:* There is a possibility of an unauthorized party gaining access to secure PHI contained in the database. Several precautions will be implemented to reduce this risk (please see "Risk Management and Emergency Response" below).

Protection Against Risk

Risk Management and Emergency Response:

a) *Physical Risk and Participant Safety*: Possible side effects from drawing blood (as mentioned above) will be attended to as usual by a trained phlebotomist or study personnel with phlebotomy competency. All serious adverse effects will be documented and reported as required by the IRB and Data Monitoring Committee (DMC).

b) *Emotional Distress and Participant Safety*: All participants will be carefully assessed before the study and will be made aware of psychiatric emergency services. During the informed consent process, participants will be advised that the study procedures could potentially lead to distress and that they may withdraw from the study at any time without adversely affecting their medical care or any benefits they may be receiving. They will also be assured that they will receive full compensation for the visit, even if it is not completed. If a participant withdraws from the study and they are not currently in medical treatment, they will be referred to the appropriate primary care provider or medical specialist. If a participant is significantly distressed during the study visit and an intervention is deemed appropriate, they will be escorted to the VA emergency care clinic. Dr. Naylor and her medical team (first line physician will be Dr. Chris Marx, MD) will be immediately available by cell phone at all times for any concerns regarding potential worsening medical symptoms or the emergence of suicidal or homicidal ideation (if Dr. Naylor and Dr. Marx, MD are traveling or on leave, another team study physician- Dr. Michael Hertzberg, MD will be contacted). Should any emergent issues arise that require immediate medical and/or psychiatric attention, the Durham VA has fully staffed Emergency Department and Psychiatric Emergency Care (PEC) services – which provide acute medical and psychiatric care 24 hours per day/7 days per week.

Should a participant report an intention to harm self or others during a phone visit, the study member on the phone will immediately contact the study PI (VA Clinical Psychologist) and/or the primary study physician (VA Psychiatrist) by cell phone while the participant remains on the phone. If clinically indicated, the study team member will also call 911 and provide emergency services with the participant's identification and location and/or provide the name of the individual the participant has threatened to harm. Study team personnel likely to be contacting participants by phone consist of several mental health professionals (MD, LCSW, RN) who have substantial psychiatric training.

In terms of risks to confidentiality, pertinent information regarding potential harm, including suicidal and homicidal intent will be shared as necessary and required by law with clinicians and/or the appropriate authorities. In such circumstances, records may be made available to authorities, even without the participant's consent. Upon completion of the study any participants not currently in psychiatric treatment will be referred for treatment as deemed appropriate.

c) *Unauthorized Access to Data:* There is a possibility of an unauthorized party gaining access to secure PHI contained in the database. This is also a low risk, and several precautions have been implemented to minimize unauthorized access. First, reports from participants' clinical records concerning research observations will not be made available to outside medical facilities without the written consent of the participant. All clinical and biological data obtained from research interviews and the laboratory will be coded. The data will be kept in locked file cabinets and accessible only to authorized research personnel. Only study numbers will appear on specimens, data and documents used for evaluation or statistical analysis. In addition, any publications resulting from this research will not identify individual participants.

d) *Risks for Study Personnel:* There is minimal risk involved to study personnel. A laboratory phlebotomist or study personnel with phlebotomy competency will draw participant blood for laboratory analysis and serum/blood storage. There is a slight risk of potential needle stick, however the VA Chemical Laboratories provides extensive risk prevention training for all phlebotomists, thus the likelihood of an accidental needle stick is low. The blood samples will be transported to -80º freezer for storage by study personnel. In order to reduce the risk of direct exposure, personnel will wear personal protective equipment while transporting participant serum/blood.

Potential Benefits of Research

Benefits for Participants: Participants may experience an acute or perhaps short term reduction of symptoms associated with PTSD conditions. Alternatively, study participants may not receive benefits from the proposed research other than monetary compensation; their participation may lead to a better understanding of PTSD symptomatology. For those not currently treated, study participation may lead to referral for treatment upon completion (or withdrawal) of the study. In terms of benefit to others, knowledge gained from the study may help the evaluation and treatment of Veterans with PTSD.

Selection of Subjects

We will randomize 90 Iraq/Afghanistan-era Veterans. Approximately 45 will be randomized to receive pregnenolone, and approximately 45 will be randomized to receive placebo (randomization code held by the VA research pharmacist). Based on enrollment and recruitment from our other RCTs in Iraq/Afghanistan-era Veterans, we project that approximately 120 Veterans will need to be randomized to reach this enrollment benchmark. Based on our pre- screening strategies, we anticipant approximately 270 Veterans will be consented (randomization to consent ratio is anticipated to be approximately 1:2).

Inclusion/Exclusion Criteria:
Inclusion Criteria:

1. 18-65 years of age, any ethnic group, either sex.
2. DSM-5 diagnosis of PTSD as determined by CAPS-5 (At least 1 Criterion B and C symptoms and at least 2 Criterion D and E symptoms).
3. Women will be required to use a medically and study approved contraceptive or otherwise not be of child-bearing potential. Birth control methods must be non-hormonal.
4. No anticipated need to alter psychiatric medications for duration of study involvement.
5. Ability to participate fully in the informed consent process.

Exclusion Criteria:
1. History of allergy to pregnenolone.
2. Medical disorders that may preclude safe administration of pregnenolone or exacerbate PTSD symptoms.
3. Current suicidal or homicidal ideation necessitating clinical intervention or representing an imminent concern. Prior suicide attempt history or suicidal ideation that does not require clinical intervention or represent an imminent concern is permitted.
4. Serious unstable medical illness, such as history of cerebrovascular accident, prostate, uterine or breast cancer, others (at the discretion of the PI and medical oversight team).
5. Medical conditions not well controlled will be excluded, at the discretion of the PI and Medical Team.
6. Standard pharmacological interventions for PTSD will not be exclusionary, including, but not limited to: antidepressant medications such as SSRIs, SNRIs, tricyclics, bupropion, mirtazapine, venlafaxine, and nefazodone; mood stabilizers such as carbamazepine, divalproex, lamotrigine, topiramate; atypical antipsychotics, and other agents including prazosin. However, there may be no changes in psychotropic medications for PTSD 4 weeks prior to study randomization.
7. Benzodiazepine use
8. Current diagnosis of bipolar disorder, schizophrenia or other psychotic disorder, or cognitive disorder due to a general medical condition other than mild TBI (assessed at screening).
9. Initiation or change in psychotherapy within 3 months of randomization (i.e., psychotherapy must be stable for 3 months prior to study start.
10. Participants on hormonal therapies such as finasteride or hormonal birth control.
11. Female participants who are pregnant or breast-feeding.
12. As indicated by the DSM-5, moderate or severe Substance Use Disorders (excluding caffeine and tobacco) within 1 month of study entry. Mild Alcohol Use Disorder is not exclusionary, at the judgment of the PI and her medical team.

Screening Procedures: The following evaluations are required to determine eligibility/suitability for study participation. Informed consent will be obtained prior to initiation of any procedures used to determine eligibility.

i. Informed Consent
ii. Inclusion/Exclusion Criteria
iii. Current Medications
iv. Medical History
v. Vital Signs, Weight
vi. Physical Examination by study physician or Physical Assessment by RN
vii. Urine Drug Screen; Urinalysis; pregnancy test (females only)
viii. Clinical Labs: serum electrolytes, creatinine, blood urea nitrogen, and glucose; liver function tests; complete blood cell count.
ix. ECG

Subject Recruitment

Description of Recruitment Process: Research participants will be recruited by study staff (i.e., clinical coordinators) from Durham VA IRB-approved local advertising (flyers), Durham VA IRB-approved letters that will include an IRB-approved tri-fold brochure with information on the study (and follow-up phone calls / electronic mail messages utilizing a Durham VA IRB-approved phone / email scripts), and by referral from their Durham VA medical and behavioral health providers. Recruitment emails will be encrypted using AZURE RMS. Participants will also be recruited from a variety of sources including community-based PCPs, VA clinics, Vet Centers, the Durham VA Medical Center and VISN-6 VA Mid-Atlantic MIRECC Post-Deployment Mental Health Data Repository in Iraq/Afghanistan-era Veterans (over 94% of these Registry participants have already provided permission to be re-contacted for future research studies; there are over 3,600 Iraq/Afghanistan-era Veterans enrolled in this Repository). We plan to recruit OEF/OIF/OND-era Veterans from the MIRECC Repository using a recruitment letter (see attached recruitment letter and/or follow up email, and a follow up phone call). To recruit these Veterans, the recruitment letter will only be sent to subjects who have agreed to be re- contacted for future research studies as part of the VISN 6 MIRECC Post-Deployment Mental Health Data Repository (IRB # 01706). In order to target Veterans who are likely to meet our inclusion/exclusion criteria we will request that the VISN 6 MIRECC provide the name and mailing address of Veterans who meet the study inclusion/exclusion criteria (diagnosis of PTSD, between the ages of 18-65).

Additionally, we will use a Data Access Request Tracker (DART) research request to obtain Corporate Data Warehouse (CDW) Repository data to recruit veterans who meet this study's inclusion/exclusion criteria. Respondents to recruitment letters will be screened over the phone using an approved telephone script (see attached telephone script) and eligible participants will be scheduled for a study visit.

Information about the study may be disseminated through a variety of conduits and outreach efforts including brochures, flyers, MIRECC website, VA mailings and newsletters, radio, TV, university/college campuses, newspaper ads, and educational

presentations to health care professionals. All advertisements will be approved by the Durham VA IRB prior to use.

A select number of participants who have completed study participation will be re-contacted by study personnel (telephone) and invited to complete a short questionnaire about how study procedures could be modified during the COVID-19 pandemic. The study team is interested in learning which, if any modifications to face-to-face procedures, such as blood draws and ECG assessments would provide participants with a greater sense of safety and increase willingness to engage in study participation.

Inclusion of Women and Minorities

We will make special efforts to recruit women Veterans from the Durham VA Women's Health Clinic, and by patient referral from primary care providers at the Durham VA. Based on enrollment demographics of participants who have enrolled in adjunctive neurosteroid (pregnenolone) pilot studies previously at the Durham VA, approximately half of the participant cohort will likely be African American and approximately half or less will be Caucasian. Approximately 5% will likely be Hispanic, based upon the demographic characteristics of participants enrolled in our prior studies.

For women of childbearing age: Since this research may have harmful effects on an unborn child and should not be done during pregnancy, it is necessary that a pregnancy test be done before dispensing any study drug. Female participants must not be pregnant during the study and must also agree to avoid becoming pregnant during study participation. Acceptable forms of birth control include non-hormonal contraception (as per CDC Effectiveness of Family Planning Methods) include:

A. Non-hormonal intrauterine devices (fewer than 1 pregnancy per 100 women in a year)
B. Permanent sterilization (abdominal, laparoscopic, heteroscopic) (fewer than 1 pregnancy per 100 women in a year)
C. Diaphragm (12 pregnancies per 100 women in a year)
D. Male condom use (18 pregnancies per 100 women in a year)
E. Female condom use (21 pregnancies per 100 women in a year)

2 methods of non-hormonal birth control must be used if neither A. nor B. (above) are applicable. Participants in this research study cannot currently be taking oral contraceptives (birth control pills or any other type of birth control that affects hormone levels).

Consent Process

Description of Informed Consent Process: An Informed Consent Waiver along with a HIPAA Waiver will be utilized for screening/recruiting purposes. All potential participants will be mailed (email or US mail) an IRB-approved recruitment letter (which will include an IRB-approved tri-fold brochure with information on the study). Letters sent through US mail will befollowed up by an encrypted email and/or a phone call and letters sent through email only (not through US mail) will be followed up via phone call. An IRB-

approved phone screen will be administered, and participants judged likely to meet all of the inclusion criteria and none of the exclusion criteria will meet with a member of the research team (i.e., PI, clinical coordinator, study team) to discuss the research protocol, and to determine if the participant is capable of providing informed consent. This study utilizes a written informed consent form. Informed consent will be obtained in a face-to-face interaction between the participant and a member of the research team. The participant will be provided with a description (verbal and written) of the informed consent form, which includes the risks along with procedures to minimize these risks, and the participants' rights and responsibilities. Participants will be given the opportunity to read the consent form and ask questions during the screening process at the first study visit. Participants may discuss potential enrollment in the study if desired with others prior to signing the consent form. Participants will be assured that participation in this research study is voluntary and that they may withdraw from the study at any time without adversely affecting their medical care or any benefits they might be receiving. They may also refuse to answer any research questions during interviews at any time during study participation. Participants who are eligible for the study and choose to participate will sign the informed consent form in the presence of a research team member. The study team will not enroll any participant with a compromised mental capacity (due to substances, brain injury, etc). If any participant's mental capacity is compromised, the study team will alert the PI who will facilitate appropriate medical or psychiatric care. Populations who cannot provide informed consent will not be included in the research study. Individuals who may not consent themselves will not be enrolled into the study, thus there no Legally Authorized Representatives will be involved in the consenting process.

Text Message Reminders:
Participants will receive text message reminders about upcoming study visit appointments at the Durham VAHCS using the VA-approved software ANNIE app. Participants will provide study staff with their preferred phone number used for recruitment/research communication.

Digital Audio-recording:
Participants will be explicitly asked for permission to digitally audio-tape CAPS-5 interviews in the IRB-approved written Informed Consent Form. Participants will be informed that audiotaped sessions may be reviewed by an expert for the purposes of interviewer fidelity. We anticipate that the expert will review CAPS-5 interviews of the first 2 participants seen by each assessor, and 1-2 randomly selected interviews every 3-6 months. Digital audio recordings and corresponding CAPS-5 assessments will be identified only by subject ID and stored on the S-drive, behind the VA firewall at S:\MIRECC\Collaborators. Audio recordings will be captured using a Philips Pocket Memo Voice Recorder DPM8000, Voice Recorder Application on a VA-issued computer, or other approved audio recorder. Only approved study staff and the expert reviewer will have access to this file path/folder. No other portion of study visits will be recorded. Procedures for audio recording by telephone are in the COVID-19 addendum.

Study Interventions

Research Design and Methods

Overview: This study will be a 10-week randomized, placebo-controlled, double-blind, fixed escalating dose, clinical trial of pregnenolone or matching placebo in Veterans with PTSD. This fixed, escalating dosing strategy in divided doses has been well-tolerated with minimal side effects in our multiple other RCTs in Veterans. The trial will include a 2-week single-blind placebo lead-in phase followed by 8 weeks of study medication (placebo or pregnenolone). Forty-five subjects meeting DSM-5 criteria for PTSD will be randomized to receive pregnenolone, and 45 subjects meeting DSM-5 criteria for PTSD will be randomized to receive placebo. There will be no changes in current clinical treatment (i.e., pregnenolone or placebo will be adjunctive to other clinical pharmacological treatments), although eligible participants will be required to be on stable doses of medication for 4 weeks prior to randomization with no intended or actual changes of clinical psychopharmacological treatment throughout study participation.

Study Treatment: During the screening visit (Visit 1), participants will receive a physical examination/assessment from a qualified study medical team member (MD, PA, RN, etc.), complete medical and psychiatric questionnaires, and vital sign measurements will be collected. Physical examination may be conducted at Visit 2, prior to randomization, subject to physician and/or RN availability. Participants will have a venous puncture for clinical laboratory and neurosteroid/genetic tests. Clinical laboratory tests will be conducted including measurement of serum electrolytes, creatinine, blood urea nitrogen, and glucose; liver function tests; complete blood cell count; urinalysis; urine drug screen and urine pregnancy tests for females of childbearing age will be collected at each visit throughout study duration. Electrocardiograms (ECG) will be conducted by clinical staff in the heart lab at screening and at study exit and will be reviewed by a cardiologist at the Durham VAMC.

Eligible participants will receive a 2-week, single-blind placebo lead-in. Study medication will be dispensed at the time of the participant's in-person visit or mailed to their residence through expedited shipping. Participants will be randomly assigned to pregnenolone or placebo in a 1:1 ratio by the VA Study Pharmacist, who will create the randomization schedule (using a permuted block randomization strategy) and will be the only person with the randomization code. Participants will return to the Durham VA (i.e., study site) for study visits at weeks 2, 4, 6, 8, and 10 and will receive phone call check-ins during the weeks they do not have on- site study visits. These phone calls will include questions about safety, adverse events, and query medication changes. Additionally, a follow up phone call will occur one week following study completion (Study Procedures Table). Please see COVID-19 addendum which reflects safety procedures to be implemented due to the COVID-19 pandemic.

Dosing: Pregnenolone and placebo doses will be fixed escalating up to 500mg/day, tapered up as follows:
Visit 1 (week 0) screening visit and placebo lead-in (all participants); Visit 2 (week 2): 50mg PO, BID x 2 weeks (100mg daily, or placebo)
Visit 3 (week 4): 150mg PO, BID x 2 weeks (300mg daily or placebo),
Visit 4 (week 6): 250mg PO, BID for 2 weeks (500mg daily or placebo),

Visit 5 (week 8): 250mg PO, BID for 2 weeks (500 mg daily or placebo),
Visit 6 (week 10): No study medication will be dispensed.

The dosing strategy for the proposed study is the same as several prior RCTs in which all doses were well tolerated by Iraq/Afghanistan-era Veterans. Additionally, we have extensive experience conducting RCTs with neurosteroids and currently hold several FDA IND numbers. We currently hold an FDA IND number for the use of pregnenolone in PTSD specifically; this study will thus be conducted under FDA IND #142320 (Marx) for the administration of pregnenolone in participants with PTSD.

Safety Evaluations: Any adverse events will be recorded by administration of the Hillside Adverse Event Form and reviewed by a study physician (please see Medical Monitoring and Physician On-Call Schedule/Cascade). Adverse events will be assessed at all study visits and phone visits after the screening visit (i.e. Visits 2-6; phone visits). In addition, adverse events will be assessed by a follow-up telephone one week after study medication has been stopped (Week 11). Clinical lab values will also be monitored throughout the study, and any aberrant values will be reported to the IRB, any relevant Data Monitoring Boards, and FDA, as required. When determined appropriate by the medical monitoring team, participants and/or providers will be notified of aberrant lab values and appropriate recommendations and/or referrals will be made. In some instances, participants may be asked to attend a non-scheduled study visit to ensure appropriate medical follow-up (such as repeat labs, etc), which will documented as a protocol deviation; participants will be compensated for any unscheduled visits. In addition to receiving regular adverse event assessments and ongoing medical/psychiatric monitoring, participants will be provided a toll-free study number they can call if they have questions regarding the dose escalation schedule or medication side effects. They will also be provided with contact information of the PI, Dr. Naylor, as well as her medical team leader, Dr. Marx, MD.Neurosteroid Quantification by Gas Chromatography Tandem Mass Spectrometry (GC/MS/MS): 1 mL serum samples will be initially subjected to triplicate liquid-liquid extractions using ethyl acetate. Combined extracts will further purified by collecting fractions of interest from an Agilent 1100 HPLC system equipped with a 25cm x 4.6mm x 5um Lichrosorb Diol-5 HPLC column under normal phase conditions. Purified samples will be subsequently derivatized with heptafluoroacetic anhydride and injected onto an Agilent 7013A GC/MS/MS gas chromatography - triple quadrupole mass spectrometer with chromatographic separations performed on the interfaced Agilent 7890B GC. A splitless injection of 2 uL will be used on a 30.0 m × 0.25 mm × 0.25 um installed HP- 5MS GC column. Injections will be performed in duplicate. Multiple reaction monitoring transition states will be optimized for precursor and product ions using the two most intense precursor-to-product ion transitions for each analyte. The more intense transition will be used for target ion quantitation and the second one as the reference ion for structural confirmation (qualifier ion) and added specificity.

SNP Analyses of Genes Coding for Neurosteroidogenic Enzymes as Potential Predictors of Therapeutic Response: SNP analyses of genes coding for neurosteroidogenic enzymes will be performed by the Center for Human Genetics at Duke. Additional genetic analyses may also be conducted. Blood will also be collected

for mRNA analyses. This component of the study will focus on determining possible relationships between these SNPs, serum neurosteroid levels, symptoms, and the prediction of therapeutic response. DNA will be extracted from blood using standard methods. Functional variants will be genotyped directly to assess allozyme functional status using TaqMan allelic discrimination or direct sequencing. In addition, common variation in the gene and its flanking genomic region will be assessed by tagging methods. Genotyping of tagging SNPs will be carried out using TaqMan allelic discrimination assays. Any unused material will be returned to the Durham VA Medical Center or destroyed.

Medical Monitoring and Physician On-Call Schedule / Cascade: Dr. Chris Marx, MD will be the primary study physician for the proposed randomized clinical trial and will be the first call physician for any medical issues that should arise. If Dr. Marx is not immediately accessible within 5 minutes on her cell phone, then Dr. Hertzberg, MD will be contacted. Dr. Hertzberg has extensive experience with PTSD and in the conduction of randomized clinical trials. Dr. Hertzberg and Dr. Marx frequently cover for each other's clinical trials if one of them is traveling or on annual leave. There will be a master schedule available that adapts the above call-schedule to the annual leave and travel schedules of the individual MDs listed in this cascade. This on-call schedule willbe circulated weekly, so that the order of the call cascade is always up-to-date for any particular week (and incorporates any changes that may be necessary secondary to individual MD schedules).

Study Procedure Table:	Visit 1	Phone	Visit 2	Phone	Visit 3	Phone	Visit 4	Phone	Visit 5	Phone	Visit 6	Follow up Phone
	Screening										FINAL	
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11
Procedures	PLACEBO Lead-In		BASELINE								FINAL	
Informed Consent (15 min)	X											
Inclusion/Exclusion (5 min)	X											
Current Medications (5 min)	X	X	X	X	X	X	X	X	X	X	X	X
Medical History (10 min)	X											
COVID-19 Questionnaire	x											
Psychiatric Diagnosis SCID-5-CT Mini International Neuropsychiatric Interview 7.0.2 (15 min)	X											
Vital Signs, Weight (5 min)	X		X		X		X		X		X	
Physical Exam (10 min)	X										X	
Urine Collection (tox screen/urinalysis) (5 min)	X		X				X				X	
Urine Pregnancy Test (females only)	X		X		X		X		X		X	
Clinical Labs (Visit 1-6) [all blood draw labs = 15 min)	X		X		X		X		X		X	
Serum for Neurosteroids, small molecules, and Inflammatory Markers (3 red top tubes)	X		X		X		X		X		X	
Blood draw for genetic analysis (purple tops. paxgene)	X											
ECG (10 min)	X										X	
PhenX History of Head Trauma (5 min)	X										X	
*PhenX Clinician Admin. PTSD Scale (CAPS-5) (Visit #1 = 60 min, Visits #2,4,6= 30 min)	X		X				X				X	
PhenX PTSD Checklist - DSM-5 (5 min)	X		X		X		X		X		X	
Davidson Trauma Scale (DTS) (7 min)	X		X		X		X		X		X	
Hamilton Depression Rating Scale (HAM-D) (5 min)	X		X		X		X		X		X	
***PhenX Brief Pain Inventory. Short Form (BPI-SF) (5 min)	X		X		X		X		X		X	
Hamilton Anxiety Rating Scale (HAM-A) (5 min)	X		X		X		X		X		X	
PROMIS Sleep Disturbance Short Form (5 min)	X		X		X		X		X		X	
Connor-Davidson Resilience Scale (CDRSC) (5 min)	X		X		X		X		X		X	
PhenX Cigarette Smoking Status (2 min)	X		X		X		X		X		X	
Columbia Suicide Severity Rating Scale (5 min)	X		X		X		X		X		X	
Clinical Global Impressions Scale (CGI) (2 min)	X		X		X		X		X		X	
Side Effect Scale (10 min)		X	X	X	X	X	X	X	X	X	X	X
Dispense Study Medication (5 min)	X		X		X		X		X			
Breaks (2 x 10 min)	X		X		X		X		X		X	
Pregnenolone (mg) total daily dose:	0		50 mg BID		150 mg BID		250 BID mg		250 BID mg		0	
	PLACEBO (all patients)		or PLACEBO		or PLACEBO		or PLACEBO		or PLACEBO			
Duration of Visit (minutes)	226	15	141	15	141	15	141	15	141	15	176	15
Total Time (minutes)												1056
*Primary PTSD Outcome												
**Secondary Depression Outcome												
***Secondary Pain Outcome												

Note: Sleep disturbance is not a primary or secondary outcome for this project. After the protocol was funded, we noted that the proposed sleep assessments did not measure the outcome we sought to measure (prior proposed measures assessed for sleep disorder diagnosis, and we are interested in sleep disturbance). In addition, we recognized the potential time burden to complete these sleep assessments were substantial, consisting of an additional 30-45 minutes of interview time at each study visit. Therefore, after consultation with a local VA sleep expert, we have chosen to replace those assessments with a PROMIS sleep measure that is consistent what we would like to measure, and will significantly reduce participant burden.

All study physicians are fully credentialed active practicing clinicians at the Durham VA, with extensive experience treating Veterans with PTSD and other psychiatric disorders. The designated on-call physician will have the following responsibilities: conduct physical exams at baseline and study completion, medical chart review, determination of participant eligibility for study entry, review of all clinical laboratory results and ECG tracings within 24 hours (with ECG confirmation by VA cardiologist), assessments of potential side effects to study medication by reviewing a structured rating assessment administered to each research participant at each study visit and by interviewing and examining the participant as clinically indicated, and will attend to any other medical issues that arise during the course of this study. Should any emergent issues arise that require immediate medical and/or psychiatric attention, the Durham VA has fully staffed Emergency Department and Psychiatric Emergency Care (PEC) services – which provides acute medical and psychiatric care 24 hours per day/7 days per week. The PEC is staffed by a psychiatry resident, psychiatric social worker, and a VA psychiatry attending. In addition, the Durham VA has a 28 bed, onsite inpatient psychiatric unit, staffed by 3 VA Psychiatry attending physicians, 3 Duke Medical Center psychiatry residents, and other mental health care professionals.

Adverse Events

There are no known severe risks that have been associated with taking pregnenolone. In prior studies, pregnenolone was very well-tolerated with few side effects. There have been rare reports of stomach upset, headache, difficulty sleeping, irregular heartbeat, hair loss (in women), acne, and rash. Adverse events will be reported to all appropriate regulatory agencies (FDA, IRB, DMC, etc) within mandated timeframes. All adverse events will be reported per Durham VAMC requirements. All Serious, Unanticipated and Related adverse events will be reported to IRB within 5 business days of hearing of the event. All other adverse events will be reported at continuing review.

Similar to our prior RCTs, the CSR&D Centralized Data Monitoring Committee is anticipated to provide specific guidance on the structure of reporting for recruitment/retention, safety plan, monitoring of adverse events, data accrual, and analysis plan.

Protocol Deviations

Participants often need to reschedule appointments. Any rescheduled appointments (study visits or phone calls) will not be considered protocol deviations unless the time lapse is long enough to require study medication to be sent to the participant by FedEx.

Data Monitoring Committee
The current study will be monitored by the Clinical Science Research and Development Central Data Monitoring Committee (DMC). This committee will provide independent monitoring of project safety, data gathering and analyses, as well as overall

performance of the clinical trial. The DMC will provide an initial review of the project and ongoing oversight from initiation through completion of data gathering, including any changes to the protocol and/or amendments.

Costs and/or Payments to Subjects

There are no research-related costs to participants. Participants will be compensated for their time, participation, and travel. Participants will receive $125 for each study visit (6 visits per subject) attended plus travel costs per on-site subject visit. If a participant attends all 6 study visits, they will receive $750 in compensation, plus travel for any on-site. There will be no compensation for telephone visits (which are anticipated to last 10-15 minutes). Participants may receive payment after each study visit in the form of a check at the Durham VA's Cashier's Office or by electronic fund transfer (EFT). The travel stipend is determined by distance traveled (approximately $0.41/ mile; table below, See COVID-19 addendum for compensation associated with telephone visits):

One Way Distance (miles)	Total Allowance	Typical towns and cities
0-25	$10	Raleigh, Durham, Cary, Chapel Hill
25-50	$20	Henderson, Wake Forest, Burlington
50-100	$40	Greensboro, Fayetteville, Goldsboro
100-150	$60	Salisbury, Greenville, Rocky Mount
150-200	$80	Wilmington, Charlotte, Hickory

We estimate that approximately 25 percent of the study participants in the study will travel from areas outside the local catchment area and will be traveling at least an hour and a half (one way) to complete study visits. Reimbursement of up to $150, matching the government rate for overnight accommodation at a local hotel, meals, and parking will be offered to Veterans traveling to the Durham VA from a distance greater than 70 miles and with a research appointment scheduled at 10am or earlier. This reimbursement will be provided with approval by the PI. Qualifying Veterans will be informed of this option and will be asked to provide a receipt for lodging prior to reimbursement.

Data and Safety Monitoring

We will collect only the minimum amount of information required to report serious adverse events (SAE) to reporting agencies; SAE date, date of study visit, and medical information related to the SAE. The SAE information will be collected during the study visit and by telephone calls with study participants and by data collection from study files and Electronic Medical Records. Safety data collection will begin upon study enrollment. The principal investigator is responsible for monitoring the data, assuring

protocol compliance, and conducting the safety reviews at intervals appropriate to the study. During the review process the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Specifically, the principal investigator will assess whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol (at Risks to Subjects) or consent form (at Risks and Inconveniences) are required.

Identifiers: Each participant will be assigned a study number and all data and specimens will be identified by that study number. The data and specimens will thus be coded data. The list linking this number to participant identity will be kept in a file on a secure VA network drive (S:\MIRECC\PI Folders\Naylor), accessed through a password-protected computer to which only the Principal Investigator and study staff have access.

Confidentiality: Reports from participants' clinical records concerning research observations will not be made available to outside medical facilities without the written consent of the participant. All clinical and biological data obtained from research interviews and the laboratory will be coded with a unique subject ID. Hard copy data will be kept in locked file cabinets, in locked offices within locked (card accessible only) buildings and will be accessible only to qualified research personnel and kept in accordance with VA Records Control Schedule. Only study numbers will appear on data, documents and biological specimens used for evaluation or statistical analysis (i.e. coded). The key-code linking participants to study data identified by study number will be kept on a secure VA network drive. In addition, any publications resulting from this research will not identify individual participants. Access to research data will be removed for staff that are no longer part of the study team. Participants will be informed during the consent process about the limits of confidentiality.

As part of the study, participant information and medical and/or research records may be disclosed to the Office for Human Research Protections (OHRP), the VA Office of the Inspector General (OIG), the Office of Research Oversight (ORO), the Department of Defense (DoD) or other government agencies, the Durham VAMC Institutional Review Board (IRB), and/or local Research Compliance Officers, and/or local Research Compliance Officers, and Food and Drug Administration (FDA). In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. Any sensitive information requested from regulatory bodies will be reported with the minimal information required and in compliance with HIPAA regulations.

Data Capture, Verification, and Disposition
Data Capture: Participant data will be collected by paper and/or computer kiosk. All data collected will be de-identified/coded. Any PHI collected on paper which needs to be maintained (i.e. Informed Consent Form, HIPAA Authorization, Participant Payment Forms, etc.) will be stored in a folder separate from de-identified/coded paper forms (i.e.

study assessments). These two folders will be filed in two different locked file cabinets inside a locked room within the Durham VAMC. Only approved VA researchers and study personnel will have access to this information. No PHI will be removed from the Durham VAMC. De-identified/coded information collected using paper forms (i.e. study assessments) will be stored in a locked filing cabinet inside VAMC Building 15, VA Main Hospital Building, or MIRECC (3022 Croasdaile Rd). These paper forms will be entered into an approved database stored on a separate, secure, password protected database within the Durham VAMC (so that information cannot be linked to participant PHI) by study personnel and will be stored indefinitely according to VA guidelines. Research records will be maintained and destroyed according to the National Archives and Records Administration, Records Schedule Number: DAA-0015-2015-0004. Records destruction, when authorized, will be accomplished using the then current requirements for the secure disposal of paper and electronic records. Currently, destruction of research records (see DAA-0015-2015-0004, section 7.6 “Research Investigator Files” for materials included in research records) is scheduled for 6 years after the cut-off (the cut-off is the completion of the research project) and may be retained longer if required by other federal agencies. Records will not be destroyed without pre-notification to the facility records manager.Electronic records are in compliance with FDA 21 CFR 11.

Participant blood and serum samples will be collected during the study visit(s) and coded with a corresponding unique, linked numerical code (i.e. the study ID number). Blood and serum samples will be stored at the Durham VAMC in a locked freezer room located in a secure research wing (requires special permission and a security activated badge to gain access).

Any incident regarding theft/loss of data, authorized access of sensitive data or storage devices or non-compliance with security controls will be addressed according to VA policy (VHA Handbook 1200.05, 10j; 1058.01 11.a; 6500, Appendix D and 6500.2) and will be reported to Durham VAMC PO and ISO immediately.

Study members who are initially part of the study team, but leave the study team for whatever reason will no longer have access to participant study data.

Sharing Study Results: Any medical concerns (i.e., clinically relevant abnormal lab values, medical findings during routine physical examination, or ECG) will be followed up by the clinical coordinator, study PI, and the medical team (Drs. Marx, Szabo and Hertzberg). If necessary, appropriate referrals will be made (including notification of current providers).

Monitoring: The database manager will review completed forms with the study coordinator and generate queries, which the study coordinator will resolve before data entry.

Data Monitoring/Quality Assurance: The study coordinators will assure that all assessments have been completed and check all forms for accuracy and completeness prior to the termination of each session. The database will provide information on which

assessments are to be completed at each visit. In addition, the data entry procedures will notify those entering the data when information has been omitted or is required. These procedures should greatly increase overall quality of the data. The data entry system itself forces data entry personnel to enter complete within range data or produce a reason, which is stored in the database.

Protocol Fidelity Monitoring: The data manager will continuously track adherence to the protocol, and will report any problems to the PI and the statistician. Monitoring will include adherence to inclusion/exclusion criteria, completion of measures at each assessment point, following procedures for termination of participants from the protocol, and training and certification for all research personnel. The monitor and data manager will verify that valid informed consent has been obtained from all participants. Research personnel must log the date of informed consent, the staff member obtaining the informed consent, acknowledge that the signature has been witnessed, and that the participant has received a copy of the informed consent document. In addition, all participants must verify that they have provided informed consent and received a copy of the informed consent document. All these steps must be completed prior to entering any information about the participant into the database.

Data Storage: All data will be stored at the Durham VAMC. Data will be coded and collected either electronically or through pen/paper and stored on a secure VA computer behind the VA firewall. When data are ready for analysis, the data will be provided to the statisticians at Duke using the AZURE Rights Management Service (RMS) platform. Alternatively, coded data, without identifiers, will be emailed to Duke University statisticians for analyses. All data handling and storage will be compliant with VA policies on information security and data storage.

Laboratory Evaluation:
Specimens Collected, Scheduled, and Amount: Blood samples will be collected at the screening visit (approximately 3.5 tablespoons), and at each subsequent (approximately 2.5 tablespoons). Laboratory testing will be conducted (2 red tops, 1 lavender tube) and serum samples collected (3 red tops) on all visits (screening and Visits 2-6). One paxgene tube and one purple top tube will be collected at visit 1 only (genetic analyses).

Clinical Laboratory Tests: Results of clinical lab tests will be used to screen for inclusion into the study and throughout participation (each study visit). Participants with clinically relevant abnormal levels will be excluded at the discretion of the PI, in consultation with her medical team. Active medical issues requiring attention take precedence over voluntary research participation.

Storage: Participant blood and serum samples will be collected during the study visit(s) and coded with a corresponding unique, linked numerical code (i.e. the study ID number). Blood and serum samples to be tested for inflammatory markers and analytes will be stored at the Durham VAMC in a locked freezer room in a secure research wing (requires special permission and a security activated badge to gain access). At different points, a small portion of this de-identified/coded blood samples will be hand-carried by

study personnel to Duke University for the purposes of genetic analysis. Any remaining samples will be returned to the Durham VA or destroyed after analyses have been completed. All individuals who will be transporting or shipping biological specimens will be adequately trained and monitored annually by the Durham VAMC Biosafety Committees.

Laboratory Performing Evaluations and Special Precautions: Laboratory Performing Evaluations and Special Precautions: Dr. Chris Marx's laboratory will conduct neurosteroid analyses by mass spectrometry. The Durham VAMC will complete all clinical labs. Point-of-care (POC) tests (urine drug screen and serum pregnancy test) will be conducted by trained research team members and/or the Durham VA chemistry laboratory. Dr. Naylor and Dr. Doug Williamson's laboratories will conduct inflammatory marker analyses. All individuals who will be transporting or shipping biological specimens will be adequately trained and monitored annually by the Durham VA Biosafety Committee.

Withdrawal of Participants

Patients will be withdrawn from the study for the following reasons: Medical hospitalization for more than 24 hours, seizure, cerebral vascular event (e.g. TIA), rash consistent with a drug reaction on the trunk, pregnancy, non-compliance with study medication for 3 days (6 doses) or longer in between study visits, physician request, or if it is not judged to be in the subject's best interest to continue (i.e. evaluation of a medical issue takes precedence over study participation, among other possibilities).

Participants will be assured that participation in this research study is voluntary and that they may withdraw from the study at any time without adversely affecting their medical care or any benefits they might be receiving. If a participant withdraws or is withdrawn from the study and they are not currently in medical treatment, they will be referred to the appropriate primary care provider or medical specialist

Data Analysis and Statistical Considerations

Power Analysis:

The study is powered to detect a minimum 5.5 unit decrease in the primary outcome comparison of change from baseline in PTSD symptoms as measured by the CAPS-5 total symptom severity score. Treatment of at least 89 subjects will provide 90% power to detect a treatment difference in the change from baseline in the mean CAPS-5 severity score, assuming a reduction in the CAPS-5 score of at least 5.5 in the PREG arm compared to a reduction of 2 in the placebo arm by the treatment week 8 visit. A linear decline in the CAPS-5 severity score from baseline through assessment at treatment weeks 2, 4, 6, and 8 was assumed. Further assumptions include a standard deviation of the difference of 2.582 (derived assuming a standard deviation in both treatment arms of 10 exactly as had been assumed since the outset, and with this value confirmed by examining baseline study date collected to date at the time of protocol amendment), alpha of 0.05, and an attrition rate of 35% by study end (based on experience in a recent study with 8 weeks of treatment, unpublished data). We assumed a correlation structure between time points as 0.7 between visits 2 weeks

apart, 0.6 between visits 4 weeks apart, and 0.5 between visits 6 weeks apart. The sample size calculation aligns with a mixed model repeated measures analysis and was developed using the SAS procedure GLMPOWER (SAS Institute, Cary, NC).

A second set of estimates was estimated using SAS PROC POWER under a simplified set of assumptions to calculate power over a range of hypothetical mean difference scores; mean residual variance, derived from the above models, was adjusted for sample size per Brown and Prescott (2006). Under an assumed level of power 1 – β = 80%, estimates yielded an MDD = -3.28 units under a total sample size n = 90, and MDD = -3.60 units with a total post-attrition sample size of n = 76 subjects. In all instances the sensitivity of MDDs exceeded our anticipated -8 units.]

Analysis of the Primary & Secondary Hypotheses: The analysis plan is organized around the study's hypotheses using a variety of statistical techniques. Univariate summary statistics, including means, medians, rates, proportions, and variances, will be calculated as appropriate for all variables. In addition to statistics, outcome measures will be examined graphically to assist in determining distributional form as a precursor to model selection. Preliminary bivariate analyses by treatment condition will use standard Chi-square procedures for categorical outcomes; continuous outcomes meeting distributional requirements will be tested using Student's t-test, whereas data not meeting distributional requirements will be analyzed by nonparametric procedures (e.g., Kruskal-Wallis). Differences between covariates will be tested at baseline to assess randomization. Putative covariates with significant asymmetry between groups will be considered for inclusion in subsequent modeling and testing (however, see Miller & Chapman, 2001). Inference for all analyses will be evaluated relative to p=0.05 within a given hypothesis. As interest in general is in demonstrating superior outcomes among subjects randomized to the intervention condition (pregnenolone), hypotheses are often structured as one-directional.

Tests of the primary hypotheses will proceed through three stages: The initial stage will assess the distributional characteristics of the tested outcome to determine requisite regression form and whether transformations are necessary. As a second step, a series of models will be estimated to assess linearity; in instances of non-linearity, measures will be transformed or quadratics will be added to models as necessary. Subsequent final models testing significance of the treatment condition will be as follows (assuming linearity): For hypotheses related to Specific Aim 1, hypotheses will be tested using LMM regression procedures using an ANOVA paradigm. The tested outcome will be regressed on a dichotomous treatment proxy (Preg=1; Placebo=0) variable, a second proxy variable denoting time (Weeks 2, 6 and 10), and an interaction term crossing treatment with time; models will also include the subject-level intercept as a random effect. Tests of the outcome measure will be based on a post-hoc least squares contrast of the difference between randomization levels at the end-of-study (Week10) time point. Hypotheses are structured to be unidimensional. Formally, support for the hypothesis will require rejection of the null hypothesis positing no difference between treatment groups in favor of the alternative (H0: βpregXseek10 = 0; H1: β pregXseek10 <> 0; < or > depending on the structure of the hypothesis). Hypotheses outlined in Specific Aim 2 will be estimated using similar procedures but modified as follows: Tests

of hypotheses positing associations between baseline levels of symptoms and the identified neurosteroid will regress symptoms – PTSD in tests of the primary hypothesis and pain and depression symptoms as tests of secondary hypotheses – on baseline levels of the identified neurosteroid (ignoring randomization status); tests of association will be based on the significance level of the estimated coefficient associated with the tested neurosteroid. Models testing the association between symptom change and change in neurosteroid levels between baseline and end-of-study will be restricted to the pregnenolone-treated cohort. As above, levels of symptom change will be regressed on levels of steroid change. Because distribution of neurosteroid levels post- treatment are typically highly right-skewed, steroid levels will be logged prior to analysis; all models will include subject intercept as a random effect.

Missing Data: Current approaches to missing data favor one of two approaches. The first requires the use of full-information maximum likelihood (FIML) procedures for analyses where analytical results are derived using all available data. The second approach is based on data augmentation procedures as described by Schafer (1997) and as implemented in SAS (Version 9.4, Cary, NC) PROC MI (Multiple Imputation). Per the SAS approach, imputation, using one of a number of available options, creates multiple complete data sets (five are suggested as sufficient). The latter are subsequently separately analyzed using standard complete-data methods, and the resulting estimates from each model are pooled to yield a final estimate. Both of the latter procedures assume the missing data are at a minimum missing at random. That is, the missingness should not be associated with the outcome measure. The missing at random (MAR) assumption is difficult—if not impossible to assess. Verbeke & Molenberghs (2000) strongly advocate sensitivity analysis as a part of the imputation process, i.e., comparing models estimated under differing assumptions to assess stability and consistency and as a tool to gain insight and understanding of the missing data mechanism as well as an assessment of how robustness of the results to missingness. A sensitivity analysis will thus be conducted in the current project for missing data. Instances where the MAR assumption is not tenable requires that missingness itself be modeled, perhaps utilizing prior scores in serial data.

The data will be analyzed by Dr. Ryan Wagner and Dr. Cynthia Coffman (DUKE University) following completion of the entire study and once the study has been unblended. Study data will be sent to the statisticians via email using AZURE RMS.

Privacy, Confidentiality, and Information Security

1. Lists of Data Reviewed and/or Collected for Screening/Recruitment and Conduction of Study:

The Personal Health Information that will be obtained, used, and/or shared for this study includes:

Identifier(s)	Source(s) of Health Information
☒ Names	☒ Medical history & physical exam information
☒ All geographic subdivisions smaller than a State, including street address, city, county, precinct, and zip code. Describe: street address, city, state, zip code	☒ Photographs, videotapes, audiotapes, or digital or other images CAPS-5 interviews will be audio-recorded
☒ All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, visit or treatment dates, etc.; and all ages over 89, Describe: Date of birth, date of death, visit dates, treatment dates, admission and/or discharge dates	☒ Biologic specimens (e.g., blood, tissue, urine, saliva). Describe: blood, urine
☒ Telephone numbers	☒ Progress notes
Fax numbers	☒ Diagnostic / Laboratory test results
☒ Electronic mail addresses	☒ Operative reports
☒ Social Security Numbers	☒ Imaging (x-ray, CT, MRI, etc.)
☒ Medical record numbers	☒ Discharge summaries
Health plan beneficiary numbers	☒ Survey / Questionnaire responses
☒ Account numbers	Billing records
Certificate and/or license numbers	HIV testing or infection records
Vehicle identifiers and serial numbers, including license plate numbers	Sickle cell anemia information
Device identifiers and serial numbers	☒ Alcoholism or alcohol use information
Web Universal Resource Locators (URLs)	☒ Drug abuse information
Internet Protocol (IP) address numbers	☒ Mental health (not psychotherapy) notes

Identifier(s)	Source(s) of Health Information
☐ Biometric identifiers, including finger & voice prints	☒ Psychological test results
☐ Full-face photographic images and any comparable images	☒ Genetic testing
☒ Any other unique identifying number, linked study ID, characteristic, or code, describe: Unique study ID and unique study blood sample barcode	☒ Other, describe: Close relative's name, close relative's phone number

2. Data and/or Specimen Acquisition:

Data for this study will be collected through (*check all that apply*):

☒ Prospective data and/or specimen collection obtained from participants. Provide description of processes: Prospective data will be collected via self-report, questionnaires, and interviews. Blood specimen collection will be via venipuncture. Urine will also be provided by voided sample (free catch).

☒ Retrospective data collection and/or specimens obtained from medical chart review/data access. Describe how data will be obtained (e.g., fileman, CDW, etc.): Electronic Medical Chart reviewed by study staff.

☐ Retrospective data collection and/or specimens obtained from an IRB-approved data and/or specimen repository. Indicate the repository source including name, VA location, and IRB number

Note: for data and/or specimens obtained from a VA approved data repository, a Data Use Agreement (DUA) must be executed prior to obtaining data and/or specimens. See VHA Handbook 1200.12 for further information.

3. Level of Data:

The following level(s) of data will be acquired/maintained for this study (*check all that apply*):

☒ Identified (e.g., names, addresses or other identifiers included)

☒ Coded (direct and/or all identifiers removed, but study code/ID included)

☐ De-Identified (all HIPAA 18 and study ID/code removed):

- ☐ Verified Statistically

  OR

- ☐ Verified by Absence or Removal of HIPAA 18 and study ID

☒ Limited Data Set

☐ Other: Describe:

4. Location of Data and/or Specimens, and Data Retention Plan:

A. Data and/or Specimen Location:

Data will be stored electronically behind the VA firewall in *S:\MIRECC\PI FOLDER\Naylor*. Data that will be stored electronically include PHI and study assessment responses. PHI will be stored in a database separate from any study assessment responses. Paper records of data include study assessments, informed consent form, HIPAA authorization, subject payment forms, and will be stored in Building 15, 4th floor; VA Building 1, room D3009, and/or the MIRECC Croasdaile Building, 3rd floor. Specimens include coded subject blood and serum samples, and wil

be frozen at -80 degrees Celsius and stored in the freezer rooms in MIRECC Croasdaile Building, 3rd Floor; Building 6 and/or Building 15 at the Durham VA Health Care System. Audio recordings and CAPS-5 assessments will be coded with a subject ID and stored on the VA S-drive at: S:\MIRECC\Collaborators. When the CAPS-5 assessment is administered via video platform, it will only be audio recorded. This folder will be accessible to approved study staff and to the expert PTSD assessor, Dr. Kleiman. Dr. Kleiman will not have access to the key code and will not be able to identify participants from the audio tape, video platform meeting information, or written CAPS-5 assessment.

☒ Data will be also be placed at the VA Informatics and Computing Interface (VINCI; http://vaww.vinci.med.va.gov/vincicentral/VINCIWorkspace.aspx). The VA Informatics and Computing Infrastructure is a partnership between the VA Office of Information Technology and the Veterans' Health Administration Office of Research and Development. Researchers and operations staff can use VINCI to access data and statistical analysis tools in a virtual working environment through a certified VHA network computer using the VA Intranet or Virtual Private Network (VPN).

B. Data Retention Plan

☒ Research records will be maintained and destroyed according to the National Archives and Records Administration, Records Schedule Number: DAA-0015-2015-0004. Records destruction, when authorized, will be accomplished using the then current requirements for the secure disposal of paper and electronic records. Currently, destruction of research records (see DAA-0015-2015-0004, section 7.6 "Research Investigator Files" for materials included in research records) is scheduled for 6 years after the cut-off (the cut-off is the completion of the research project) and may be retained longer if required by other federal agencies. Records will not be destroyed without pre-notification to the facility records manager.

☐ Other data retention plan, describe:

5. Data Access and Data Recipients:

Only members of our DVAHS research team will have access to identifiers, whether they reside behind the VA firewall or in paper form or coded data. For the purpose of analysis, coded data will be shared, via the AZURE Rights Management Service (RMS) platform, with the study statisticians, R. Henry Wagner, PhD at Duke University and Cynthia Coffman, PhD. Data will be returned to us via the AZURE RMS platform. Alternative methods to send data to the statisticians includes sending coded data by email that do not contain any personal identifiers.

All VA research personnel who have access to VHA records are instructed, in accordance with VA policy, on the requirements of Federal privacy and information laws and regulations, VA regulations and policies, and VHA policy. All study personnel who are VA employees working within the VA system have fulfilled all required HIPAA and other VA security and privacy policy training requirements and have agreed to follow guidelines pertaining to the protection of patient data. All research staff sign VA Rules of Behavior, and all study staff are up-to-date with VHA Privacy Policy Training and the VA Office of Cyber and Information Security Awareness Training Course. The data security and privacy procedures summarized in that course include logging off or locking the computer when walking away from it; no sharing of access codes, verify codes or passwords; not allowing anyone else to use the computer under one's password; and disposing of sensitive information using VA-approved methods (e.g., shredder bins).

Access to study data will be removed for all study personnel when they are no longer part of the research team.

6. Data and/or Specimen Transportation and/or Transmission for all data and/or specimens involved in the study:

I. ☐ Data and/or specimens will not be transported or transmitted outside of Durham VAMC environment.

II. ☒ Data and/or specimens will be transported BETWEEN sites that are under the auspices of the Durham VA Medical Center. Data and blood/urine specimens will be transported between the main DVAHS campus and 3022 Croasdaile Dr. for data entry and storage. Study staff (research coordinators, research assistants, study PI) will transport data and specimens. Specimens will be transported in properly packaged, leak-proof cooler labeled with as a biohazard. No additional stops will be made while transporting data and/or specimens.

III. ☐ Data and/or specimens will be transmitted to other VA sites using the following method(s):

A. Data

☐ Data are de-identified and thus will be sent via unencrypted e-mail or unencrypted disk (encryption is optional).

☐ Data are coded or contain identifiers and thus will be sent ☐ Other, describe:

B. Specimens

☐ Specimens are de-identified and thus will be sent via standard carrier (tracking is optional).

☐ Specimens are coded or contain identifiers and thus will be sent via VA-authorized carrier with tracking.

☐ Other, describe:

IV. ☒ Data and/or specimens will be transported to non-VA/VHA sites (e.g., academic affiliates, laboratories, etc.) using the following method(s):

A. Data

☐ Data are de-identified and thus will be sent via unencrypted e-mail or unencrypted CD.

☐ Data are 27 or contain identifiers and thus will be sent via using VA—approved carrier with tracking.

☒ Data are coded or identified and will be sent via the AZURE Rights Management Service (RMS) at https://safe.amrdec.army.mil/safe/. AZURE RMS is a secure method of exchanging files <2GB to and from individuals with a valid .gov, .mil, .com, or .edu email address. Recipients are to include statisticians at Duke University including Dr. Ryan Wagner, PhD and Cynthia Coffman, PhD.

☐ Data are coded or identified and will be uploaded to sponsor website using electronic case report form (eCRF)

☒ Other, describe: Coded data will be taken off site for data entry, evaluation, and statistical analysis. Local DVAMC memorandum

“Authorization to Use,

Process, Store, or Transmit VA Sensitive Information Outside VA Owned or Managed Facilities" has been pre-filled out for each study team member who may transport the data and/or specimens off-site. All data will be transported and stored in a locked briefcase and will be labeled in accordance with VHA Directive 6609:

B. Specimens

☐ Specimens are de-identified and thus will be sent via standard carrier (tracking is optional) or will be hand-delivered by research study personnel. Specify method of delivery:

☒ Specimens are coded and thus will be sent via VA-approved carrier with tracking or will be hand-delivered by research study personnel. Specify method of delivery: Coded blood samples will be hand carried and delivered to Duke University for the genetic analyses.

In accordance with the HIPAA and the Privacy Act, for any coded or identifiable data or specimens released from the Durham VAMC (with the exception of Limited Data Sets), an Accounting of Disclosure (AOD) will be maintained (e.g., in a database or spreadsheet) that includes the participant's name, date of the disclosure, description of the nature of the Individually Identifiable Information (III) disclosed, purpose of each disclosure, and the name and address of the person/agency to whom the disclosure was made.

☒ Local DVAMC memorandum "Authorization to Use, Process, Store, or Transmit VA Sensitive Information Outside VA Owned or Managed Facilities" has been pre-filled out for each study team member who may transport the data and/or specimens off-site. This (these) forms are included with the IRB materials.

☒ Containers (e.g., briefcase, bin) are labeled with the following notice (label placed on the outside of container) in accordance with VHA Directive 6609:

NOTICE!!!
Access to these records is limited to: AUTHORIZED PERSONS ONLY. Information may not be disclosed from this file unless permitted by all applicable legal authorities, which may include the Privacy Act; 38 U.S.C. §§ 5701, 5705, 7332; the Health Insurance Portability and Accountability Act; and regulations implementing those provisions, at 38 C.F.R. §§ 1.460 – 1.599 and 45 C.F.R. Parts 160 and 164. Anyone who discloses information in violation of the above provisions may subject to civil and criminal penalties.

V. ☐ We will communicate with veterans enrolled as participants in this research study through MyHealtheVet.

7. Risk Mitigation Strategies:
There is a possibility of an unauthorized party gaining access to secure PHI contained in the database. This is also a low risk, and several precautions have been implemented to minimize unauthorized access. First, reports from participants' clinical records concerning research observations will not be made available to outside medical facilities without the written consent of the participant. All clinical and biological data obtained from research interviews and the laboratory will be coded. The data will be kept in locked file cabinets and accessible only to authorized research personnel. Only study numbers will appear on specimens, data and documents used for evaluation or statistical analysis. In addition, any publications resulting from this research will not identify individual participants. In addition to the PI, study coordinators, laboratory staff, Co-Is, research assistants, statisticians, and the medical team bear responsibility for overseeing the privacy and security of the data.

☐ Data are fully de-identified (stripped of HIPAA 18 and study ID/code) before being shared outside of Durham VAMC.
☐ Specimens are fully de-identified (stripped of HIPAA 18 and study ID/code before being shared outside of Durham VAMC.
☒ Direct identifiers will be maintained separately from data and or specimens by using a code to "identify" subjects. In a separate database (i.e., a "linking" or "cross-walk" database) this code will be linked to identifying subject information.
☐ Other, specify:

8. Suspected Loss of VA Information:
Should any incident such as theft or loss of data, unauthorized access of sensitive data or non-compliance with security controls occur it will be immediately reported according to VA policy. All incidents regarding information security/privacy incidents will be reported to the ISO and PO within 1 hour of acknowledgement of issue and done so using the VHADUR Research Events Report e-mail group (VHADURResearchEventReport@va.gov).

9. Reporting of Results:
☒ Reporting of results, such as in scientific papers and presentations, will never identify individual subjects. Data will be presented in aggregate and individual-level data will not be published.
☐ Other results reporting plan, describe:

10. Future Use of Data:
☐ Data will be retained for future use. This is described elsewhere in the protocol and is noted in the HIPAA authorization.
- ☐ Future Use of data is optional (i.e., not required by the research subject).
- ☐ Future Use of data is required for participation in the study.

☒ No future use of data is currently planned.

11. Use of Mail Merge Technology

☒ Mail merge programs will be used to generate letters and/or address labels for mailings to potential or already enrolled research subjects. The study team is aware that to reduce risk of mail merge related privacy incidents, use of mail merge programs requires a 25% accuracy check to verify that (potential) research subject name and mailing address are properly "matched". If discrepancies are found, a 100% accuracy check is required before letters may be mailed.

12. Use of Non-Standard Software

☒ I do NOT intend to use any new specialized software (i.e. Software that's not already approved OR installed) in this study.

☐ I intend to use specialized software that has not already been installed and it has been approved for use by the VA Technical Reference Model (TRM) Group.
(Note: All new software must be approved by TRM before it can be installed on VA systems.)

☐ I intend to use previously installed software on my VA computer.

13. Use of Cloud Computing Services

☒ Cloud computing services will NOT be used in this study.

☐ Cloud computing services WILL be used in this study as described below and have been approved nationally by the VA Chief Information Officer (CIO). (Note: ONLY cloud computing services that have been approved nationally may be used.)

References

1. Amaya-Jackson, L., J.R. Davidson, D.C. Hughes, M. Swartz, V. Reynolds, L.K.

George, and D.G. Blazer. Functional impairment and utilization of services associated with posttraumatic stress in the community. J Trauma Stress.(1999);12:4:709-24.
2. Asmundson, G.J. and J. Katz. Understanding the co-occurrence of anxiety disorders and chronic pain: state-of-the-art. Depress Anxiety.(2009);26:10:888-901.
3. Benedek, D., M. Friedman, D. Zatzick, and R. Ursano, Practice guideline for the treatment of patients with acute stress disorder and posttraumatic stress disorder, 2009, American Psychiatric Association: Washington, DC.
4. Berger, W., M.V. Mendlowicz, C. Marques-Portella, G. Kinrys, L.F. Fontenelle, C.R. Marmar, and I. Figueira. Pharmacologic alternatives to antidepressants in posttraumatic stress disorder: a systematic review. Prog Neuropsychopharmacol Biol Psychiatry.(2009);33:2:169-80.
5. Bob, P., J. Raboch, M. Maes, M. Susta, J. Pavlat, D. Jasova, J. Vevera, J. Uhrova, H. Benakova, and T. Zima. Depression, traumatic stress and interleukin-6. J Affect Disord.(2010);120:1-3:231-4.
6. Brady, K., T. Pearlstein, G.M. Asnis, D. Baker, B. Rothbaum, C.R. Sikes, and G.M. Farfel. Efficacy and safety of sertraline treatment of posttraumatic stress disorder: a randomized controlled trial. JAMA.(2000);283:14:1837-44.
7. Brancu, M., H.R. Wagner, R.A. Morey, J.C. Beckham, P.S. Calhoun, L.A. Tupler, C.E. Marx, K.H. Taber, R.A. Hurley, J. Rowland, S.D. McDonald, J.M. Hoerle, S.D. Moore, H.S. Kudler, R.D. Weiner, V.A.M.-A.M. Workgroup, and J.A.Fairbank. The Post-Deployment Mental Health (PDMH) study and repository: A multi-site study of US Afghanistan and Iraq era veterans. Int J Methods Psychiatr Res.(2017).
8. Brown, E.S., J. Park, C.E. Marx, L.S. Hynan, C. Gardner, D. Davila, A. Nakamura, P. Sunderajan, A. Lo, and T. Holmes. A randomized, double-blind, placebo-controlled trial of pregnenolone for bipolar depression. Neuropsychopharmacology.(2014);39:12:2867-73.
9. Calhoun, P.S., H.B. Bosworth, S.C. Grambow, T.K. Dudley, and J.C. Beckham. Medical service utilization by veterans seeking help for posttraumatic stress disorder. Am J Psychiatry.(2002);159:12:2081-6.
10. Charalampopoulos, I., C. Tsatsanis, E. Dermitzaki, V.I. Alexaki, E. Castanas, A.N. Margioris, and A. Gravanis. Dehydroepiandrosterone and allopregnanolone protect sympathoadrenal medulla cells against apoptosis via antiapoptotic Bcl-2 proteins. Proc Natl Acad Sci U S A.(2004);101:21:8209-14.
11. Chen, S., J.M. Wang, R.W. Irwin, J. Yao, L. Liu, and R.D. Brinton. Allopregnanolone promotes regeneration and reduces beta-amyloid burden in a preclinical model of Alzheimer's disease. PLoS One.(2011);6:8:e24293.
12. Chen, S.L., Y. Zang, W.H. Zheng, X.H. Wei, and X.G. Liu. Inhibition of Neuropathic Pain by a Single Intraperitoneal Injection of Diazepam in the Rat: Possible Role of Neurosteroids. Chin J Physiol.(2016);59:1:9-20.
13. Coronel, M.F., F. Labombarda, and S.L. Gonzalez. Neuroactive steroids, nociception and neuropathic pain: A flashback to go forward. Steroids.(2016);110:77-87.
14. Coronel, M.F., M.C. Raggio, N.S. Adler, A.F. De Nicola, F. Labombarda, and S.L. Gonzalez. Progesterone modulates pro-inflammatory cytokine expression profile

after spinal cord injury: Implications for neuropathic pain. J Neuroimmunol.(2016);292:85-92.
15. Davidson, J., T. Pearlstein, P. Londborg, K.T. Brady, B. Rothbaum, J. Bell, R. Maddock, M.T. Hegel, and G. Farfel. Efficacy of sertraline in preventing relapse of posttraumatic stress disorder: results of a 28-week double-blind, placebo-controlled study. Am J Psychiatry.(2001);158:12:1974-81.
16. Davidson, J.R. Treatment of posttraumatic stress disorder: the impact of paroxetine. Psychopharmacol Bull.(2003);37 Suppl 1:76-88.
17. Davidson, J.R., D. Hughes, D.G. Blazer, and L.K. George. Post-traumatic stress disorder in the community: an epidemiological study. Psychol Med.(1991);21:3:713-21.
18. Davidson, J.R., B.O. Rothbaum, B.A. van der Kolk, C.R. Sikes, and G.M. Farfel. Multicenter, double-blind comparison of sertraline and placebo in the treatment of posttraumatic stress disorder. Arch Gen Psychiatry.(2001);58:5:485-92.
19. Davidson, J.R., R.H. Weisler, M.I. Butterfield, C.D. Casat, K.M. Connor, S. Barnett, and S. van Meter. Mirtazapine vs. placebo in posttraumatic stress disorder: a pilot trial. Biol Psychiatry.(2003);53:2:188-91.
20. Dedert, E.A., K.T. Green, P.S. Calhoun, R. Yoash-Gantz, K.H. Taber, M.M. Mumford, L.A. Tupler, R.A. Morey, C.E. Marx, R.D. Weiner, and J.C. Beckham. Association of trauma exposure with psychiatric morbidity in military veterans who have served since September 11, 2001. J Psychiatr Res.(2009);43:9:830-6.Della Vedova, C., S. Cathcart, A. Dohnalek, V. Lee, M.R. Hutchinson, M.A. Immink, and J. Hayball. Peripheral interleukin-1ss levels are elevated in chronic tension-type headache patients. Pain Res Manag.(2013);18:6:301-6.
21. Djebaili, M., Q. Guo, E.H. Pettus, S.W. Hoffman, and D.G. Stein. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. J Neurotrauma.(2005);22:1:106-18.
22. Dowlati, Y., N. Herrmann, W. Swardfager, H. Liu, L. Sham, E.K. Reim, and K.L. Lanctot. A meta-analysis of cytokines in major depression. Biol Psychiatry.(2010);67:5:446-57.
23. Flood, J.F., J.E. Morley, and E. Roberts. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. Proc Natl Acad Sci U S A.(1992);89:5:1567-71.
24. Frye, C.A. and J.E. Duncan. Progesterone metabolites, effective at the GABAA receptor complex, attenuate pain sensitivity in rats. Brain Res.(1994);643:1-2:194-203.
25. George, M.S., A. Guidotti, D. Rubinow, B. Pan, K. Mikalauskas, and R.M. Post. CSF neuroactive steroids in affective disorders: pregnenolone, progesterone, and DBI. Biol Psychiatry.(1994);35:10:775-80.
26. Ghoumari, A.M., C. Ibanez, M. El-Etr, P. Leclerc, B. Eychenne, B.W. O'Malley, E.E. Baulieu, and M. Schumacher. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. J Neurochem.(2003);86:4:848-59.
27. Girdler, S.S. and R. Klatzkin. Neurosteroids in the context of stress: implications for depressive disorders. Pharmacol Ther.(2007);116:1:125-39.
28. Guo, M., T. Liu, J.C. Guo, X.L. Jiang, F. Chen, and Y.S. Gao. Study on serum

cytokine levels in posttraumatic stress disorder patients. Asian Pac J Trop Med.(2012);5:4:323-5.
29. He, J., C.O. Evans, S.W. Hoffman, N.M. Oyesiku, and D.G. Stein. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. Exp Neurol.(2004);189:2:404-12.
30. Hernandez, M.E., E. Becerril, M. Perez, P. Leff, B. Anton, S. Estrada, I. Estrada, M. Sarasa, E. Serrano, and L. Pavon. Proinflammatory cytokine levels in fibromyalgia patients are independent of body mass index. BMC Res Notes.(2010);3:1:156.
31. Hertzberg, M.A., M.E. Feldman, J.C. Beckham, H.S. Kudler, and J.R. Davidson. Lack of efficacy for fluoxetine in PTSD: a placebo controlled trial in combat veterans. Ann Clin Psychiatry.(2000);12:2:101-5.
32. Hodes, G.E., M.L. Pfau, M. Leboeuf, S.A. Golden, D.J. Christoffel, D. Bregman, N. Rebusi, M. Heshmati, H. Aleyasin, B.L. Warren, B. Lebonte, S. Horn, K.A. Lapidus, V. Stelzhammer, E.H. Wong, S. Bahn, V. Krishnan, C.A. Bolanos-Guzman, J.W. Murrough, M. Merad, and S.J. Russo. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc Natl Acad Sci U S A.(2014);111:45:16136-41.Hoge, E.A., K. Brandstetter, S. Moshier, M.H. Pollack, K.K. Wong, and N.M. Simon. Broad spectrum of cytokine abnormalities in panic disorder and posttraumatic stress disorder. Depress Anxiety.(2009);26:5:447-55.
33. Huang, Q., X.F. Mao, H.Y. Wu, T.F. Li, M.L. Sun, H. Liu, and Y.X. Wang. Bullatine A stimulates spinal microglial dynorphin A expression to produce anti-hypersensitivity in a variety of rat pain models. J Neuroinflammation.(2016);13:1:214.
34. Jiang, Z.Y., Y.Y. Guo, H.B. Ren, Y.F. Zou, M.S. Fan, Y. Lv, P. Han, W. De, and L.Z. Sun. Tumor necrosis factor (TNF)-alpha upregulates progesterone receptor-A by activating the NF-kappaB signaling pathway in human decidua after labor onset. Placenta.(2012);33:1:1-7.
35. Kanes, S., H. Colquhoun, H. Gunduz-Bruce, S. Raines, R. Arnold, A. Schacterle, J. Doherty, C.N. Epperson, K.M. Deligiannidis, R. Riesenberg, E. Hoffmann, D. Rubinow, J. Jonas, S. Paul, and S. Meltzer-Brody. Brexanolone (SAGE-547 injection) in post-partum depression: a randomised controlled trial. Lancet.(2017);390:10093:480-489.
36. Kavaliers, M., T.S. Perrot-Sinal, D.C. Desjardins, S.K. Cross-Mellor, and J.P. Wiebe. Antinociceptive effects of the neuroactive steroid, 3alpha-hydroxy-5alpha-pregnan-20-one and progesterone in the land snail, Cepaea nemoralis. Neuroscience.(2000);95:3:807-12.
37. Kessler, R.C., A. Sonnega, E. Bromet, M. Hughes, and C.B. Nelson. Posttraumatic stress disorder in the National Comorbidity Survey. Arch Gen Psychiatry.(1995);52:12:1048-60.
38. Khisti, R.T. and C.T. Chopde. Serotonergic agents modulate antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice. Brain Res.(2000);865:2:291-300.
39. Kibler, J.L., M. Tursich, M. Ma, L. Malcolm, and R. Greenbarg. Metabolic, autonomic and immune markers for cardiovascular disease in posttraumatic stress disorder. World J Cardiol.(2014);6:6:455-61.

40. Kilts, J.D., L.A. Tupler, F.J. Keefe, V.M. Payne, R.M. Hamer, J.C. Naylor, R.P. Calnaido, R.A. Morey, J.L. Strauss, G. Parke, M.W. Massing, N.A. Youssef, L.J. Shampine, R.E. tVeterans Affairs Mid-Atlantic Mental Illness, W. Clinical Center, and C.E. Marx. Neurosteroids and self-reported pain in veterans who served in the U.S. Military after September 11, 2001. Pain Med.(2010);11:10:1469-76.
41. Kohler-Forsberg, O., H.N. Buttenschon, K.E. Tansey, W. Maier, J. Hauser, M.Z. Dernovsek, N. Henigsberg, D. Souery, A. Farmer, M. Rietschel, P. McGuffin, K.J. Aitchison, R. Uher, and O. Mors. Association between C-reactive protein (CRP) with depression symptom severity and specific depressive symptoms in major depression. Brain Behav Immun.(2017);62:344-350.
42. Korneyev, A. and E. Costa. Allopregnanolone (THP) mediates anesthetic effects of progesterone in rat brain. Horm Behav.(1996);30:1:37-43.
43. Krystal, J.H., L.L. Davis, T.C. Neylan, A.R. M, P.P. Schnurr, M.B. Stein, J. Vessicchio, B. Shiner, T.D. Gleason, and G.D. Huang. It Is Time to Address the Crisis in the Pharmacotherapy of Posttraumatic Stress Disorder: A Consensus Statement of the PTSD Psychopharmacology Working Group. Biol Psychiatry.(2017);82:7:e51-e59.
44. Leskiewicz, M., D. Jantas, B. Budziszewska, and W. Lason. Excitatory neurosteroids attenuate apoptotic and excitotoxic cell death in primary cortical neurons. J Physiol Pharmacol.(2008);59:3:457-75.
45. Maes, M., E. Bosmans, and H.Y. Meltzer. Immunoendocrine aspects of major depression. Relationships between plasma interleukin-6 and soluble interleukin-2 receptor, prolactin and cortisol. Eur Arch Psychiatry Clin Neurosci.(1995);245:3:172-8.
46. Marshall, R.D., K.L. Beebe, M. Oldham, and R. Zaninelli. Efficacy and safety of paroxetine treatment for chronic PTSD: a fixed-dose, placebo-controlled study. Am J Psychiatry.(2001);158:12:1982-8.
47. Marx, C.E., D.W. Bradford, R.M. Hamer, J.C. Naylor, T.B. Allen, J.A. Lieberman, J.L. Strauss, and J.D. Kilts. Pregnenolone as a novel therapeutic candidate in schizophrenia: emerging preclinical and clinical evidence. Neuroscience.(2011);191:78-90.
48. Medicine, I.o.(year)Treatment of Posttraumatic Stress Disorder: An Assessment of the Evidence.Washington, DC: The National Academies Press.224.
49. Michopoulos, V., S.D. Norrholm, J.S. Stevens, E.M. Glover, B.O. Rothbaum, C.F. Gillespie, A.C. Schwartz, K.J. Ressler, and T. Jovanovic. Dexamethasone facilitates fear extinction and safety discrimination in PTSD: A placebo-controlled, double-blind study. Psychoneuroendocrinology.(2017);83:65-71.
50. Michopoulos, V., A. Powers, C.F. Gillespie, K.J. Ressler, and T. Jovanovic. Inflammation in Fear- and Anxiety-Based Disorders: PTSD, GAD, and Beyond. Neuropsychopharmacology.(2017);42:1:254-270.
51. Mok, W.M. and N.R. Krieger. Evidence that 5 alpha-pregnan-3 alpha-ol-20-one is the metabolite responsible for progesterone anesthesia. Brain Res.(1990);533:1:42-5.
52. Naylor, J.C., C.M. Hulette, D.C. Steffens, L.J. Shampine, J.F. Ervin, V.M. Payne, M.W. Massing, J.D. Kilts, J.L. Strauss, P.S. Calhoun, R.P. Calnaido, D.G. Blazer, J.A. Lieberman, R.D. Madison, and C.E. Marx. Cerebrospinal fluid

dehydroepiandrosterone levels are correlated with brain dehydroepiandrosterone levels, elevated in Alzheimer's disease, and related to neuropathological disease stage. J Clin Endocrinol Metab.(2008);93:8:3173-8.
53. Naylor, J.C., J.D. Kilts, C.M. Hulette, D.C. Steffens, D.G. Blazer, J.F. Ervin, J.L. Strauss, T.B. Allen, M.W. Massing, V.M. Payne, N.A. Youssef, L.J. Shampine, and C.E. Marx. Allopregnanolone levels are reduced in temporal cortex in patients with Alzheimer's disease compared to cognitively intact control subjects. Biochim Biophys Acta.(2010);1801:8:951-9.
54. Naylor, J.C., J.D. Kilts, J.L. Strauss, S.T. Szabo, C.E. Dunn, H.R. Wagner, R.M. Hamer, L.J. Shampine, J.R. Zanga, C.E. Marx, E. Department of Veterans Affairs Mid-Atlantic Mental Illness Research, and W. Clinical Center. An exploratory pilot investigation of neurosteroids and self-reported pain in female Iraq/Afghanistan-era Veterans. J Rehabil Res Dev.(2016);53:4:499-510.
55. Naylor, J.C., J.D. Kilts, S.T. Szabo, C.E. Dunn, F.J. Keefe, L.A. Tupler, L.J. Shampine, R.A. Morey, J.L. Strauss, R.M. Hamer, H.R. Wagner, M. Workgroup, and C.E. Marx. Allopregnanolone Levels Are Inversely Associated with Self-Reported Pain Symptoms in U.S. Iraq and Afghanistan-Era Veterans: Implications for Biomarkers and Therapeutics. Pain Med.(2016);17:1:25-32.
56. Nock, M.K., I. Hwang, N.A. Sampson, and R.C. Kessler. Mental disorders, comorbidity and suicidal behavior: results from the National Comorbidity Survey Replication. Mol Psychiatry.(2010);15:8:868-76.
57. Noorbakhsh, F., G.B. Baker, and C. Power. Allopregnanolone and neuroinflammation: a focus on multiple sclerosis. Front Cell Neurosci.(2014);8:134.
58. Nurnberg, H.G., P.L. Hensley, A.J. Gelenberg, M. Fava, J. Lauriello, and S. Paine. Treatment of antidepressant-associated sexual dysfunction with sildenafil: a randomized controlled trial. JAMA.(2003);289:1:56-64.
59. Oganesyan, L.P., G.M. Mkrtchyan, S.H. Sukiasyan, and A.S. Boyajyan. Classic and alternative complement cascades in post-traumatic stress disorder. Bull Exp Biol Med.(2009);148:6:859-61.
60. Patte-Mensah, C., L. Meyer, O. Taleb, and A.G. Mensah-Nyagan. Potential role of allopregnanolone for a safe and effective therapy of neuropathic pain. Prog Neurobiol.(2014);113:70-8.
61. Pinna, G., E. Costa, and A. Guidotti. SSRIs act as selective brain steroidogenic stimulants (SBSSs) at low doses that are inactive on 5-HT reuptake. Curr Opin Pharmacol.(2009);9:1:24-30.
62. Pinna, G., E. Dong, K. Matsumoto, E. Costa, and A. Guidotti. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc Natl Acad Sci U S A.(2003);100:4:2035-40.
63. Pinna, G., E. Dong, K. Matsumoto, E. Costa, and A. Guidotti. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc Natl Acad Sci U S A.(2003);100:4:2035-40.
64. Pinna, G. and A.M. Rasmusson. Up-regulation of neurosteroid biosynthesis as a pharmacological strategy to improve behavioural deficits in a putative mouse model of post-traumatic stress disorder. J Neuroendocrinol.(2012);24:1:102-16.
65. Rasmusson, A.M., C.E. Marx, S. Jain, G.M. Farfel, J. Tsai, X. Sun, T.D.

Geracioti, M.B. Hamner, J. Lohr, R. Rosse, L. Summerall, J.C. Naylor, C. Cusin, A.J. Lang, R. Raman, and M.B. Stein. A randomized controlled trial of ganaxolone in posttraumatic stress disorder. Psychopharmacology (Berl).(2017);234:15:2245-2257.

66. Rasmusson, A.M., G. Pinna, P. Paliwal, D. Weisman, C. Gottschalk, D. Charney, J. Krystal, and A. Guidotti. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. Biol Psychiatry.(2006);60:7:704-13.
67. Richardson, L.K., B.C. Frueh, and R. Acierno. Prevalence estimates of combat-related post-traumatic stress disorder: critical review. Aust N Z J Psychiatry.(2010);44:1:4-19.
68. Sayeed, I., Q. Guo, S.W. Hoffman, and D.G. Stein. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. Ann Emerg Med.(2006);47:4:381-9.Schule, C., D. Eser, T.C. Baghai, C. Nothdurfter, J.S. Kessler, and R. Rupprecht. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? Neuroscience.(2011);191:55-77.
69. Sripada, R.K., C.E. Marx, A.P. King, J.C. Rampton, S.S. Ho, and I. Liberzon. Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits. Biol Psychiatry.(2013);73:11:1045-53.
70. Thomas, J.L., J.E. Wilk, L.A. Riviere, D. McGurk, C.A. Castro, and C.W. Hoge. Prevalence of mental health problems and functional impairment among active component and National Guard soldiers 3 and 12 months following combat in Iraq. Arch Gen Psychiatry.(2010);67:6:614-23.
71. Tucker, P., R. Zaninelli, R. Yehuda, L. Ruggiero, K. Dillingham, and C.D. Pitts. Paroxetine in the treatment of chronic posttraumatic stress disorder: results of a placebo-controlled, flexible-dosage trial. J Clin Psychiatry.(2001);62:11:860-8.
72. Uzunova, V., Y. Sheline, J.M. Davis, A. Rasmusson, D.P. Uzunov, E. Costa, and A. Guidotti. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci U S A.(1998);95:6:3239-44.
73. Wilcox, H.C., C.L. Storr, and N. Breslau. Posttraumatic stress disorder and suicide attempts in a community sample of urban american young adults. Arch Gen Psychiatry.(2009);66:3:305-11.
74. Winfree, C.J., D.W. Coombs, J.A. DeLeo, and R.W. Colburn. Analgesic effects of intrathecally-administered 3 alpha-hydroxy-5 alpha-pregnan-20-one in a rat mechanical visceral pain model. Life Sci.(1992);50:14:1007-12.
75. Zhu, T.S. and M. Glaser. Regulatory role of cytochrome P450scc and pregnenolone in myelination by rat Schwann cells. Mol Cell Biochem.(2008);313:1-2:79-89.

Neurosteroid Intervention for PTSD in Iraq/Afghanistan-Era Veterans
Protocol Addendum

This document is an addendum to the protocol describing changes in response to the COVID-19 pandemic to safely resume research study activity for this randomized clinical trial, per Durham VAHCS clinical guidelines.

Study Interventions:

During the COVID-19 pandemic, and to minimize possible exposure to COVID-19 for both participants and study staff, we will conduct several study visits by telephone, rather than in-person. There will be no changes in the assessments to be administered other than the addition of the COVID -19 questionnaire. During the three study visits (visits 1, 4, 6) in which participants must come to the Durham VAHCS for safety evaluations (including clinical labs and EKGs on visits 1 and 6), participants will have the choice to complete study assessments by telephone at the Durham VAHCS or at home. Telephone assessments may be conducted with the participant at the Durham VAHCS (alone in a room with study staff talking with them by phone in a separate room), or outside of the Durham VAMHCS; presumably at their home.

Specific COVID-19 Procedural Changes

1. Clinical interviews and assessments will be conducted by phone (either on-site with the study coordinator in another room, separate from the study participant, or the study participant may be at home). This will remain an option for participants even will the threat of COVID-19 has subsided.
2. Visits 2, 3, and 5 will be conducted remotely by telephone (either on-site with the study coordinator in another room, separate from the study participant, or the study participant may be at home). This will remain an option for participants even will the threat of COVID-19 has subsided.
3. Audio recording by telephone: CAPS-5 will be administered by phone and will be recorded using the recording device already approved for this study, then uploaded to the S-Drive for review by consultant per protocol.
4. Video platform use: CAPS-5 will be administered via Microsoft Teams or other VA approved platforms. Video assessments will only be audio recorded using the recording device already approved for this study, then uploaded to the S-Drive for review by consultant per protocol.
5. Participant reimbursement and travel: Participants will not receive travel reimbursement if they do not travel to the VA for their study visit. Participants will receive compensation for their participation at every visit regardless of whether it is in person or by phone.

Telephone Contact Safety Measures:

Study staff conducting telephone visits will confirm with the study participant their location and contact information, should a medical or psychiatric emergency arise. Study staff will alert the PI (Clinical Psychologist) or the study team Durham VA Attending Psychiatrist (Dr. Marx) should any participant express suicidal or homicidal ideation. If any study participant experiences a medical emergency or expresses suicidal or homicidal ideation that the study team determines requires immediate intervention, 911 will be dispatched by the study team and the participant will be maintained on the phone until emergency personnel arrive.

We anticipate that once the COVID-19 pandemic ends, some participants will prefer to participate by telephone as often as possible, thus we intend to continue to offer participants the option to engage in the aforementioned manner even after the COVID-19 pandemic ends. This study will follow all ORD and Durham VAHCS Research Guidelines and Medical Center policies.