YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1 (2015-2)

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: The role of neuroactive steroids in stress, alcohol craving and alcohol use in alcohol use disorders			
Principal Investigator: Rajita Sinha, PhD		**Yale Academic Appointment:** Professor	
Department: Psychiatry			
Campus Address: 2 Church Street South Suite 209, New Haven, CT 06519			
Campus Phone: 203-737-5805	**Fax:** 203-737-1272	**Pager:**	**E-mail:** rajita.sinha@yale.edu
Protocol Correspondent Name & Address (*if different than PI*): Julie Pinto, MS			
Campus Phone: 203-737-5489	**Fax:** 203-737-1272	**E-mail:** julie.pinto@yale.edu	
Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*):			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone :	**Fax :**	**E-mail**	
Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☒ **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Investigator Interests:

Does the principal investigator, or do any research personnel who are responsible for the design, conduct or reporting of this project or any of their family members (spouse or dependent child) have an incentive or interest, financial or otherwise, that may affect the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? Note: The Principal Investigator (Project Director), upon consideration of the individual's role and degree of independence in carrying out the work, will determine who is responsible for the design, conduct, or reporting of the research.

APPROVED BY THE YALE UNIVERSITY IRB 3/19/2021

See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI

☐ Yes x No

Do you or does anyone on the research team who is determined by you to be responsible for the design, conduct or reporting of this research have any patent (sole right to make, use or sell an invention) or copyright (exclusive rights to an original work) interests related to this research protocol?

☐ Yes x No

If yes to either question above, list names of the investigator or responsible person:

The Yale University Principal Investigator, all Yale University co-investigators, and all Yale University individuals who are responsible for the design, conduct or reporting of research must have a current financial disclosure form on file with the University's Conflict of Interest Office. Yale New Haven Hospital personnel who are listed as co-investigators on a protocol with a Yale University Principal Investigator must also have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form: http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

a. Internal Location[s] of the Study:

☐ Magnetic Resonance Research Center (MR-TAC)
☐ Yale Cancer Center/Clinical Trials Office (CTO)
☐ Yale Cancer Center/Smilow Cancer Data Repository/Tumor Registry
☐ YCCI/Church Street Research Unit (CSRU)
☐ YCCI/Hospital Research Unit (HRU)
☐ Yale-New Haven Hospital Yale-New Haven Hospital—Saint Raphael Campus
☐ University PET Center
☐ Laboratories
☒ Specify Other Yale Location: **Yale Stress Center, 2 Church Street South, Suite 209**

b. External Location[s]:

☐ APT Foundation, Inc. Haskins Laboratories ☐
☐ Connecticut Mental Health Center John B. ☐ Pierce Laboratory, Inc.
☐ Clinical Neuroscience Research Unit ☐ (CNRU) Veterans Affairs Hospital, West
☐ Haven Other Locations, Specify: ☐ International Research Site
(Specify location(s)):

c. Additional Required Documents *(check all that apply)*: ☐ N/A

☐ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC) Approval Date: *YCC Protocol
☐ Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date: *Radioactive Drug
☐ Research Committee (RDRC) Approval Date:
☐ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☐ Yale University RSC (YU-RSC) Approval Date: Magnetic Resonance Research
☐ Center PRC (MRRC-PRC) Approval Date: *Nursing Research Committee
Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at
http://radiology.yale.edu/research/ClinTrials.aspx)

Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.

   July 2018- July 2023

3. **Research Type/Phase: (Check all that apply)**
   a. **Study Type**
   ☒ Single Center Study
   ☐ Multi-Center Study
   Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☐
   ☐ Coordinating Center/Data Management Other:
   ☐

   b. **Study** ☐ **Phase N/A**
   ☒ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV
   ☐ Other (*Specify)*

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:

☐ Clinical Research: Patient-Oriented Clinical ☐ Research: Outcomes and
☒ Clinical Research: Epidemiologic and Behavioral Health Services
☐ Translational Research #1 ("Bench-to-Bedside") ☒ Interdisciplinary Research
☒ Translational Research #2 ("Bedside-to- ☐ Community") Community-Based Research

5. Is this study a clinical trial? Yes ☒ No ☐

NOTE the current ICMJE (International Committee of Medical Journal Editors) definition of a clinical trial: "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes." Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events" If yes, where is it registered?

Clinical Trials.gov registry ☐ Other (*Specify*)

The study is not yet registered on clinicaltrials.gov, but we will be registering it there shortly.

*Registration of clinical trials **at their initiation** is required by the FDA, NIH and by the ICMJE.*

If this study is registered on clinicaltrials.gov, there is new language in the consent form and compound authorization that should be used.

For more information on registering clinical trials, including whether your trial must be registered, see the YCCI webpage, http://ycci.yale.edu/researchers/ors/registerstudy.aspx or contact YCCI at 203.785.3482)

6. Does the Clinical Trials Agreement (CTA) require compliance with ICH GCP (E6)?
   Yes ☐ No ☐

7. Will this study have a billable service? *A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects*.

   Yes ☐ No ☒

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

8.. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ___ No _X__ *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform?

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

c. Will a novel approach using existing equipment be applied?

If you answered "no" to question 8a, or "yes" to question 8b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. ***By signing this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH****.*

SECTION III: FUNDING, RESEARCH TEAM AND TRAINING

1. **Funding Source:** Indicate all of the funding source(s) for this study. Check all boxes that apply. Provide information regarding the external funding source. This information should include identification of the agency/sponsor, the funding mechanism (grant or contract), and whether the award is pending or has been awarded. Provide the M/C# and Agency name (if grantfunded). If the funding source associated with a protocol is "pending" at the time of the protocol submission to the HIC (as is the case for most NIH submissions), the PI should note "Pending" in the appropriate section of the protocol application, provide the M/C# and Agency name (if grant-funded) and further note that University (departmental) funds support the research (until such time that an award is made). **Funding is listed in IRES IRB.**

2. **Research Team:** All members of the research team are listed in IRES IRB. **ALL members of the research team MUST complete Human Subject Protection Training (HSPT) and Health Insurance Portability and Accountability Act (HIPAA) Training before they may be listed on the protocol.**

NOTE: The HIC will remove from the protocol any personnel who have not completed required training. A personnel protocol amendment will need to be submitted when training is completed.

SECTION IV:
PRINCIPAL INVESTIGATOR/FACULTY ADVISOR/ DEPARTMENT CHAIR AGREEMENT

As the **principal investigator** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- Subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- All members of the research team will be kept apprised of research goals.
- I will obtain approval for this research study and any subsequent revisions prior to my initiating the study or any change and I will obtain continuing approval of this study prior to the expiration date of any approval period.
- I will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set by the University and qualify to serve as the principal investigator of this project or have acquired the appropriate approval from the Dean's Office or Office of the Provost, or the Human Subject Protection Administrator at Yale-New Haven Hospital, or have a faculty advisor.
- I will identify a qualified successor should I cease my role as principal investigator and facilitate a smooth transfer of investigator responsibilities.

_____ _____

PI Name (PRINT) and Signature Date

As the **faculty advisor** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- This project has scientific value and merit and that the student or trainee investigator has the necessary resources to complete the project and achieve the aims.
- I will train the student investigator in matters of appropriate research compliance, protection of human subjects and proper conduct of research.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- The student investigator will obtain approval for this research study and any subsequent revisions prior to initiating the study or revision and will obtain continuing approval prior to the expiration of any approval period.
- The student investigator will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set forth by the University and qualify to serve as the faculty advisor of this project.
- I assume all of the roles and responsibilities of a Principal Investigator even though the student may be called a PI.

_____ _____

Advisor Name (PRINT) and Signature Date

Department Chair's Assurance Statement

Do you know of any real or apparent institutional conflict of interest (e.g., Yale ownership of a sponsoring company, patents, licensure) associated with this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC)
☐ No

I assure the HIC that the principal investigator and all members of the research team are qualified by education, training, licensure and/or experience to assume participation in the conduct of this research trial. I also assure that the principal investigator has departmental support and sufficient resources to conduct this trial appropriately.

_____ _____ _____

Chair Name (PRINT) and Signature Date

_____

Department

YNHH Human Subjects Protection Administrator Assurance Statement

Required when the study is conducted solely at YNHH by YNHH health care providers.

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:

- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to notify the IRB if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and has the support of the hospital for this research project.

_____ _____ _____

YNHH HSPA Name (PRINT) and Signature Date

SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

To use pregnenolone (PREG; 300; 500mg) daily versus placebo (PLA) as a probe to assess the role of neuroactive steroids in individuals with alcohol use disorder (AUD). The study aims to examine the effects of PREG on a) alcohol craving, mood and neuroendocrine reactivity to brief, guided imagery exposure to stress, drug cues and neutral situations in the laboratory and b) daily alcohol intake, craving, cognition and mood in men and women with AUD; and c) sex differences in all of these outcomes. We hypothesize that PREG vs PLA will dose-specifically decrease

stress-induced and alcohol-cue induced alcohol craving, improve mood and cognitive performance, and normalize HPA axis response to stress and drug-cue imagery in the laboratory, and also reduce alcohol intake and craving in daily life in individuals with AUD.

The following aims will be addressed:

Specific Aim 1: To evaluate the safety/tolerability of 300mg and 500mg/daily of PREG in AUD individuals.
Specific Aim 2a: To evaluate PREG dose (PLA, 300 and 500 mg/day) effects on neuroactive steroids like ALLO and on experimentally provoked alcohol craving, HPA dysregulation, anxiety, mood and cognitive flexibility in AUD patients.
Specific Aim 2b: To assess whether PREG-stimulated ALLO levels mediate its effects on provoked craving, HPA dysregulation, anxiety, mood and cognitive flexibility in the laboratory component.
Specific Aim 3a: To assess the preliminary efficacy of 8-week PREG 300 and 500 doses vs. PLA treatment on primary alcohol use outcomes and secondary outcomes of alcohol craving, anxiety and mood.
Specific Aim3b: To assess whether PREG-stimulated ALLO levels mediate its effects on primary alcohol use outcomes and on secondary clinical outcomes during the 8-week treatment phase.
Exploratory Aim: To explore whether pre-treatment patient characteristics (gender, family history of alcoholism (FH), trauma history and co-morbid drug use) influence PREG-potentiated ALLO levels and primary and secondary alcohol use outcomes.

2. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

Evidence from clinical surveys indicates that stress frequently leads to continued drug use and relapse (Marlatt and Gordon 1985; Bradley, Phillips et al. 1989; Wallace, 1989; Hodgins, el Guebaly, & Armstrong, 1995; Miller & Tonigan, 1996). Animal studies have shown that acute behavioral stress facilitates drug self-administration and reinstatement to drug seeking behavior in drug-addicted animals that have been drug-free for extended time periods (Shaham et al., 1995). Previous work in our laboratory has shown that exposure to previous stressful experiences consistently increases drug craving and stress-related arousal in alcohol and alcohol dependent individuals (Sinha et al., 1999a; 2000; 2003a). Data also suggests that stress-induced alcohol craving and related HPA and autonomic arousal are predictive of alcohol relapse after inpatient treatment (Daughters et al., 2009; Fox et al., 2009; Sinha et al., 2003). These data suggest that attenuation of stress-induced alcohol craving, and their related arousal, may be a useful target in drug relapse prevention.

HPA axis is largely under GABAergic control (Herman et al., 2004). Chronic drug related adaptations and stress are each shown to down-regulate GABA-ergic transmission (Biggio et al., 2007) and increase neuroactive steroid levels in the brain and periphery (Purdy et al., 1991). Emerging evidence suggests that pregnenolone-derived neuroactive steroids may play a role in modulating negative reinforcement effects of drugs of abuse and also impact stress- and drug-cue associated relapse. Neuroactive steroids potentiate the GABA induced opening of the $GABA_A$ receptor chloride channel at nanomolar concentrations, and produce anxiolytic, anticonvulsant and hypnotic effects

similar to those induced by other $GABA_A$ receptor potentiating drugs (Lambert et al., 2001; Majewska et al., 1986). Most notably, fluctuations in peripheral and brain levels of the neuroactive steroid (3α,5α)-3-hydroxypregnan-20-one (a.k.a. allopregnanolone; ALLO) have been associated with motivational mood-related changes during pregnancy, menopause, and psychiatric disorders, including depression, premenstrual dysphoric disorder, schizophrenia and bipolar disorder (Barbaccia et al., 1996; Concas et al., 1998; Girdler et al., 2001; Marx et al., 2006; Uzunova et al., 2006). In addition, intraperitoneal (i.p.) injections of CRF and ACTH in rats increase brain and plasma levels of ALLO (Torres et al., 2001). These findings indicate that neuroactive steroids may play a crucial compensatory role in mediating homeostasis in response to stress and possibly chronic alcohol-related allostatic neuroadaptations (Biggio et al., 2007; Crowley and Girdler, 2014; Sarkar et al., 2011).

Extensive research has examined the role that neuroactive steroids play in alcohol's effects (Morrow et al., 1999; Morrow et al., 2001). For example, acute alcohol increases levels of neuroactive steroids in plasma (VanDoren et al., 2000) and brain (Sanna et al., 2004), and blockade of neuroactive steroid production by the 5α-reductase (5α-R) inhibitor finasteride attenuated acquisition of alcohol preference in mice (Ford et al., 2008). In humans, the plasma concentration of ALLO was increased following severe intoxication (Torres and Ortega, 2003, 2004), but not moderate intoxication (Holdstock et al., 2006; Nyberg et al., 2005; Pierucci-Lagha et al., 2006). Importantly, chronic alcohol exposure reduces both plasma and brain levels of ALLO (Morrow et al., 2001). Clinical studies have also shown that variation in genes that encode neuroactive steroid synthesis enzymes was associated with alcohol dependence (Milivojevic et al., 2011) and subjective effects of alcohol (Milivojevic et al., 2014), which may modulate risk for the development of dependence. Moreover, nicotine has been found to increase neuroactive steroids (Porcu et al., 2003). Unlike alcohol dependence, their levels were found to be higher in smokers, in whom levels of ALLO positively correlated with nicotine dependence severity (Marx et al., 2006).

Recent studies using the neuroactive steroid precursor PREG up to 500 mg/day, found that it improved negative and cognitive symptoms, and functional capacity in men and women with schizophrenia, as compared to placebo (Marx et al., 2009; Marx et al., 2014)) and that PREGinduced increases in ALLO levels were associated with enhanced activation of the emotion regulation neurocircuitry in healthy controls (Sripada et al., 2013). A randomized placebocontrolled trial of PREG in men and women with bipolar depression improved depressive symptoms and showed PREG to be safe and well tolerated in all patients (Brown et al., 2014). Importantly, in all these studies PREG markedly increased ALLO levels, which significantly correlated with the outcome measures, representing a robust "precursor loading" strategy for ALLO enhancement (Marx et al., 2014). Although PREG use in psychiatric conditions is only emerging, preclinical evidence shows promise with recent reports of PREG reducing alcohol self-administration in alcohol-preferring rats (Besheer et al., 2010) and also reducing cannabis intoxication effects in mice and rats (Vallee et al., 2014). Recent evidence from our laboratory suggests that pregnenolone derived neuroactive steroidscould be important in a) mediating regulatory cognitive function in cocaine dependence (Milivojevic et al., 2014) and b) reducing stress and cue-induced ocaine craving, normalizing the HPA axis response to stress, and improving inhibitory function in cocaine dependent men and women (Milivojevic et al., 2016). On the basis of these data and previous preclinical research, we aim to use the neuroactive steroid precursor PREG as a mechanistic probe to assess the role of neuroactive steroids in a)

stress and cue-induced craving, b) physiological and endocrine arousal as provoked by stress and drug-cue imagery in the laboratory, and c) alcohol use and abstinence outcomes. Importantly, we will also examine the effects of gender in all these outcomes. **We hypothesize that PREG will decrease stress-induced and alcohol cue induced alcohol craving and intake in laboratory sessions, normalize HPA axis response to stress, and reduce daily alcohol intake, craving and mood symptoms**.

We have developed and validated a laboratory-based imagery induction method that reliably increases alcohol craving after imagery of stressful and drug cue events in individuals with alcohol use disorder (Sinha et al., 1999; 2000). However, whether a neuroactive steroid suppresses stress and alcohol-induced craving in individuals with alcohol use disorder has not been directly tested. In a sample of 90 treatment seeking men and women with alcohol use disorder, we will examine the effects of PREG on alcohol craving, alcohol motivation and intake, subjective mood states, and neurobiological responses in personalized stressful imagery and drug cues imagery. All subjects will be exposed to three imagery sessions (neutral, stressful and drug cues) and the order of imagery conditions will be counterbalanced across subjects.

We've selected PREG, because it is the precursor to potent neuroactive steroids, such as ALLO, and because it is easily available as a dietary supplement. We selected 300mg/day and 500 mg/day dosing based on prior clinical trials of PREG in psychiatric patients. In an 8-week clinical trial in schizophrenia patients, fixed escalating dosing from 100mg to 500mg of PREG (Marx et al., 2009) was well tolerated. The only side effects present in the PREG group to a greater degree than the PLA group were two reports of mild restlessness, one report of mild muscle pain/stiffness, and one report of mild cold extremities. A follow-up randomized clinical trial in men and women with schizophrenia used escalating doses of PREG, 100mg for 2 weeks, 300mg for 2 weeks, 500mg for 4 weeks, and found PREG to be safe and well tolerated (Marx et al., 2014). A 12-week randomized placebo-controlled trial of escalating doses of PREG to 400mg daily for 4 weeks in men and women with bipolar depression improved depressive symptoms and showed PREG to be safe and well tolerated in all patients (Brown et al., 2014). A recent study using an acute dose of PREG of 400mg to examine its effects on emotion regulation neurocircuitry (Sripada et al., 2013), also didn't find any side effects.

3. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

1. Study Design and Overview

Ninety treatment seeking men and women with AUD will be recruited to participate in and complete this preliminary study. Participants will be recruited through flyers and advertisements in local newspapers and from community substance abuse treatment facilities. Subjects will participate in an initial screening and intake session to obtain informed consent, followed by physical examination and blood work to determine eligibility. Eligible

participants will be randomly assigned to 2 doses of PREG (300/500 mg/day) vs placebo (PLA) treatment (N=30/group) over 8 weeks. In week 2, AUD patients will participate in a 3day human laboratory study where they will be exposed to three experimental sessions of stress, alcohol cue and neutral-relaxing imagery on separate days (Fox et al.,2007; 2009; Sinha et al., 2008; 2009; 2011). Throughout the study, participants will come twice-weekly to the Yale Stress Center to receive medication, counseling and provide urine samples for UTOX. Women will be enrolled during the mid-luteal phase of the menstrual cycle so that laboratory sessions will be scheduled during the early to mid-follicular phase of the cycle when endogenous sex hormone levels are low prior to ovulation. PREG doses and matching placebo will be obtained by Yale IDS for compounding and dosing to 300mg and 500mg of PREG or PLA. Participants will self-administer study medication on a twice-daily dosing schedule throughout entire study.

Subjects will begin with full respective dose of medication immediately without a titration period, as previous studies with similar doses of pregnenolone up to 500 mg/day at b.i.d. dosing to be well tolerated and with no side effects (Marx et. al., 2009; Marx et al., 2014; Brown et al., 2014; Sripada et al., 2013).

Participants will complete a variety of diagnostic, cognitive and psychological assessments, a comprehensive physical examination and blood work before enrollment into the study, and will be involved in development of imagery scripts from personal stress, drugrelated cue and neutral situations before the laboratory sessions. In week 2, subjects will participate in an imagery/relaxation training and habituation session p, followed by 3 laboratory sessions as close together as possible. In laboratory sessions, participants will be exposed to guided imagery of a personal stressful situation, a drug cue related situation and a neutral relaxing situation. Participants will receive one exposure per day, and order of imagery type will be randomly assigned across the three days.

2. Study Feasibility:

The Yale Stress Center currently screens approximately 100 subjects per month. On average, 10 patients meet criteria for the proposed study (individuals with alcohol use disorder not on other medications and otherwise healthy) and will be offered participation in this study. Thus we anticipate that 5 patients can be enrolled into the study monthly. The subjects will be recruited from those responding to advertisements and flyers for research.

3. General Procedures:

All participants will be screened and recruited into the study by the research assistant coordinator. Potential subjects will meet with the research assistant coordinator who will, after an initial screening over the telephone/in person, determine eligibility based on inclusion/exclusion criteria. The initial screening will collect basic demographic information and ask questions specific to the inclusion/exclusion criteria. The initial screening will take approximately 20-25 minutes. S/he will explain all study procedures and risk/benefits and obtain informed consent. Subjects will undergo breath alcohol testing/urine toxicology

screens at each in-person appointment to confirm self-report of alcohol use information. Subjects will also have the option to visit a Quest Diagnostics location to provide blood and urine specimens.

Laboratory Sessions:

Once a subject is deemed eligible, signs consent and completes all intake assessments, s/he will be randomized to 300mg PREG, 500mg PREG, or PLA and exposed to three laboratory sessions as close together as possible of guided imagery of stress, drug cues and neutral-relaxing cues in week 2 (identified as lab DAY 1, DAY 2, and DAY 3). In a session prior to laboratory sessions, subjects will participate in an imagery script development to provide descriptions of recent stressful, drug cue related and neutral relaxing situations to the research assistant. On the day before laboratory session 1 , subjects will participate in a relaxation and imagery training and IV habituation session, followed by three laboratory sessions that include IV blood drawing.

Daily surveys using a Smartphone platform (MetricWire) monitoring will prompt subjects to take study medication, and provide responses on their alcohol use, craving, stress and mood states for the day (see below for detailed description).

Genetic studies:

Ten mL of whole blood will be collected during physical examination to assess eligibility, and thirty-two mL of whole blood will be also collected from patients on the day of the first laboratory session (LAB DAY 1) for the purposes of understanding genetic factors that may affect craving.

Throughout the 8 week study, subjects will self-administer 300mg/day, 500mg/day or PLA/day and study staff will provide weekly medication packs to subjects.

Participants will be seen twice per week at the Yale Stress Center at 2 Church Street South, Suite 209 to receive once/week counseling, provide urine and receive contingency management for treatment attendance. Daily surveys using the smartphone platform (MetricWire) monitoring will prompt subjects to take study medication, and provide responses on their alcohol use, craving, stress and mood states for the day. Smartphone data will be collected daily. Subjects will be prompted at two random times in the morning and afternoon to complete a brief 1-minute survey of questions and a third 5-10 minute survey to be completed at night before bedtime. The surveys are set up with standard automated questions and prompts and subjects will use the smartphone key pad to answer the prompts (via MetricWire).
MetricWire, developed and owned by MetricWire Inc., has previously been utilized in clinical and academic research and is HIPPA compliant. Subjects will initially complete a training session which will last up to an hour and 30-minutes, where we will assist in installing the MetricWire app on their smartphone device. They will use the app to report their daily experiences throughout the study. They will be given instructions on how to operate the smartphone app and a guide that they may take home with them that will outline the information covered in the training session. They will also be given contact information should they have any problems or questions with the smartphone app over the course of the study. It will be explained

that all data on the app is encrypted and that the data is sent to a secure server where data is identified by subject id only. Subjects will also be informed that we will be monitoring the upload of data from the smartphone app to the secure server in order to verify that everything is working correctly. Subjects will be asked to use this app to report their mood, stress and alcohol use, once daily in the evening, for the course of the study. It will take approximately 10-15 minutes to complete these assessments each day.

Subjects will receive contingency management for each visit and 1 counseling session per week. As part of this process, participants will provide urine screens and breathalyzer samples twice per week. Blood draws of 15mL of whole blood each will also be done at weeks 2 or 5 and 7 of the study to measure pregnenolone levels. Subjects will also have the option to visit a Quest Diagnostics location to provide blood and urine specimens. These levels will allow staff to ensure subjects are taking medication as prescribed.

Medication compliance will also be monitored through the use of riboflavin as a marker. Riboflavin produces a bright yellow discoloration of the urine when the medication is taken 2 to 8 hours prior. During the study, staff will easily be able to visually inspect the subject's urine to determine whether the morning dose has been taken. 25mg of riboflavin will be added to all doses of pregnenolone and placebo by the pharmacist when capsules are made. Medication compliance will also be implemented through video Directly Observed Therapy (DOT) using the smartphone application eMocha. Subjects will use the eMocha app to video record themselves taking their medication. Research staff will log into a secure site to view the recorded videos to assess medication dose adherence.

Individual Counseling:

Subjects will participate in once weekly standard individual drug counseling as outlined in the Individual Drug Counseling Manual (IDC, Mercer & Woody, 1994) during the treatment phase in order to support treatment. Each subject will be seen by a Yale Stress Center staff counselor with expertise in IDC who will see the patient for the clinical component of the treatment research phase. The general purpose of the counseling sessions as described in the manual are to: (1) acquire information about important concepts and aspects of recovery from addiction; (2) increase self-awareness of specific problems and issues in relation to addiction and recovery, and (3) learn new coping skills to deal with problems contributing to or resulting from the addiction and to improve functioning. The focus of this psycho-educational approach is on providing patients with frequent supportive contact, introduce them to key concepts about the recovery approach, and develop a sense of personal responsibility for recovery.

**Participants will provide urines 2x/week to assess drug use, and they will receive contingency management for showing up on each of the twice/weekly appointments. Primary outcomes measures will be alcohol use, mood, stress and craving as assessed by MetricWire and urines.

After completion of the study, subjects will be referred to aftercare at the Substance Abuse Treatment Unit (SATU).

Contact and collateral information will have been obtained from subjects at intake. We currently use procedures that make it possible to locate and re-interview more than 90% of the patients recruited into our treatment and laboratory research studies. Of note, in our past completed stress studies with alcohol dependent individuals we obtained a 92% completion rate. Our procedures include informing the patient of the importance of follow-up evaluations at the time of initial assessment, emphasizing the patient's role as a research subject, guaranteeing complete confidentiality of all information, obtaining permission, addresses and telephone numbers of three collaterals to be contacted in order to help us locate the subject, arranging for home visits and telephone interviews when subjects fail to keep appointments, mailing of appointment reminders that are followed by telephone calls, and provision of incentive payments for successful completion of follow-up evaluations. All of these procedures will be incorporated into this study.

Laboratory Sessions:

Rationale for Use of Personalized Imagery Procedures. Emotional imagery paradigms have been widely used in behavioral research studies examining the pathophysiology of anxiety disorders, including panic disorder, obsessive compulsive disorder and PTSD (Cook, Melamed, Cuthbert, McNeil, & Lang, 1988; Foa & Kozak, 1986; McNeil, Vrana, Melamed, Cuthbert, & Lang, 1993; Pitman, Orr, Forgue, de Jong, & Claiborn, 1987; Shalev, Orr, & Pitman, 1993; Orr et al., 1998; Orr, Pitman, Lasko, & Herz, 1993). These procedures are known to activate the same physiological, subjective and behavioral components as the emotion trigger situations, thus being a useful clinical research procedure for inducing stress/emotional states. Similar imagery induction procedures have been successfully adapted by our group in previous studies to induce specific emotion states in normal volunteers and stress and drug cues in alcohol dependent individuals (Sinha, Lovallo, & Parsons, 1992; Sinha & Parsons, 1996; Sinha, Catapano, & O'Malley, 1999a;).

Imagery procedures have also been used in the drug abuse literature to induce drug cueinduced drug craving and stress/negative mood induced drug craving in the laboratory (CepedaBenito & Tiffany, 1996; Drobes & Tiffany, 1997; Maude-Griffin & Tiffany, 1996; Tiffany &
Drobes, 1990; Cooney, Litt, Morse, & Bauer, 1997; Tiffany & Hakenewerth, 1991; Payne, Rychtarik, Rappaport, & Smith, 1992; Rubonis et al., 1994; Sinha et al., 1999a). Tiffany and colleagues compared imagery induction vs. in-vivo exposure to cues, and found that while both methods were equally effective in eliciting high levels of self-reported craving, physiological reactivity was greater with the imagery manipulation. Furthermore, our previous work in alcohol dependent individuals showing activation of the HPA axis and increases in plasma catecholamines with stress and with drug cue imagery indicates that the method is effective in inducing drug craving and in activating brain stress circuits (Sinha et al., 1999a). Thus, the imagery procedure includes the following distinctive components based on prior experience:

(i) Personal stressful, drug cue and neutral situations rather than standard situations will be selected because personal events show greater physiological reactivity and generate more

intense emotional reactions than imagery of standardized non-personal emotions (Cook et al., 1988; McNeil et al., 1993; Miller et al., 1987).

(ii) Based on Lang's network theory on emotion processing and Tiffany's cognitive model of drug use and urges (Tiffany, 1990; Tiffany & Drobes, 1990) and our previous work, the addition of physiological, subjective and behavioral response descriptors are included in the imagery script so as to produce stronger activation of the experience.

(iii) To minimize subject variability in the imagery induction procedure, we include an imagery training session, in which each subject receives training on how to generate and maintain a mental image for 2-3 minutes. The imagery training procedures have been extensively used in previous studies by Lang and his colleagues and by the PI in her previous work on emotions and stress work with alcohol abusing samples.

Imagery Script Development Session: In a session prior to the laboratory sessions, scripts for the guided imagery induction will be developed. The ***stress imagery script*** will be based on subjects' description of a recent personal stressful event that they had experienced as "most stressful". "Most stressful" is determined by having the subjects rate the perceived stress experienced by them on a 10-point Likert scale where "1=not at all stressful" and "10=the most stress they felt recently in their life". Only situations rated as 8 or above on this scale are accepted as appropriate for script development. Stressful situations that involved drug-related stimuli, such as being arrested for possession of drugs or being caught in a police chase, are not allowed. Examples of acceptable stressful situations include breakup with significant other, a verbal argument with a significant other or family member or unemployment-related stress, such as being fired or laid off from work. As stressful situations often occur in the context of alcohol use, these situations are thought to be associated with drug use and therefore elicit alcohol craving.

The ***drug-related script*** will be developed by having subjects identify a recent situation that included drug-related stimuli and served as a trigger for subsequent alcohol use (e.g. buying crack, being at a bar and watching others use drugs/get high; getting together with drug using buddies). Drug-related situations that occurred in the context of negative affect or psychological distress will not be allowed, i.e., going to a bar after a marital conflict, or feeling depressed and calling a drug using buddy. A ***neutral-relaxing script*** will be developed from the subjects' commonly experienced neutral-relaxing situations. Neutral-relaxing events that involve drug cues will not be allowed. A 'script' or description of each situation will be developed using Scene Development Questionnaires which obtain specific stimulus and response details, including specific physical and interpersonal context details, verbal/cognitive attributions regarding the people involved, and physiological and bodily sensations experienced for the situation being described. The three scripts for each subject will then be recorded on an audio-tape for guided imagery in the laboratory sessions. The order of stress, neutral and drug cue scripts will be assigned randomly, and counterbalanced across subjects. Detailed procedures are outlined in the Imagery Development Procedures Manual (Sinha, 2001b).

Manipulation Check for Script Development: All three scripts will be rated on a Likert scale from 1 to 10 on a standard rating form (Independent Rating Scale) by two objective independent raters for stressful and emotional content. If a script scores below a rating of 8 for stressful content on a five-point rating scale the subject will be asked to develop a new script at

the next appointment prior to the laboratory sessions. These procedures ensure that the stress and drug cue scripts of all subjects are equated in intensity and content. It further ensures that differences in stress reactivity are not due to differences in intensity and emotional content of the stressor. The procedures for development of imagery scripts, rating of scripts for content and physiological activation are similar to those used by Miller et al. (Miller et al., 1987) and have been successfully used in our previous work on emotions (Sinha et al., 1992; Sinha & Parsons, 1996) and on stress reactivity with alcohol dependent individuals (Sinha et al., 1999a).

Imagery and Relaxation Training and IV Habituation Day. On LAB DAY 1, subjects will arrive at the lab at 11 AM on the day of the first laboratory session and an indwelling intravenous (IV) catheter will be inserted in the subject's arm so that subjects can habituate to the stress of an IV insertion. We have found that this period of adaptation is critical for stable baseline measurements on testing days. No blood will be drawn during this training period. After insertion of IV catheter, the subject will be provided with relaxation training followed by general imagery and physiological response training, as described in the imagery training procedures manual (Lang, Levin, Miller, & Kozak, 1983). The imagery training involves subjects' visualizing some commonplace scenes as they are presented to them. The scenes are neutral and non-emotional in content, such as reading a popular magazine. Following the imagery, the subject is asked questions about the visualization and given pointers regarding the process of imagining the scene. The subject also imagines scenes that are non-emotional but physically arousing in nature, such as doing sit-ups in gym class. With these scenes subjects are asked whether they notice any changes in their physiological response, such as change in heart rate or change in breathing. Once again, pointers with regard to imagining the situation "as if" they were really present in the situation are presented. This session takes approximately one hour and was developed to ensure that all subjects are trained on the method of generating an image and maintaining it for 2-3 minutes. At the end of training, the subject will be given lunch and prepared for the first laboratory session. .

Laboratory Sessions. In week 2 of the study PREG/PLA administration and hence DAY 1, 2 and 3 of the laboratory portion of the study, subjects will be brought into the research testing rooms of the Yale Stress Center (YSC) at 2:00 PM. The laboratory sessions will only be conducted if the subject has a breath alcohol level of 0, otherwise the session will be rescheduled. The subject will then be prepared for physiological assessments. An indwelling intravenous (IV) catheter will be inserted by the research nurse specialist in the antecubital region of the subject's non-preferred arm for blood sampling. Blood samples will be obtained periodically for assessment of neuroactive steroids, ACTH, cortisol, prolactin, NE and EPI levels. A blood pressure cuff will be placed on the subject's preferred arm to monitor blood pressure (BP) periodically. On-line assessment of physiological measures will be obtained at specified time periods during the laboratory sessions. Self-reports of emotional state, anxiety and alcohol craving will also be assessed periodically.

After preparation of assessments and a 40 minute adaptation period, two blood draws before imagery will be conducted (BASELINE). After the second blood draw, a 5-minute baseline period will follow to assess continuous pulse rate and BP assessments. Following the baseline period, instructions for conducting imagery will be provided. The order and content of the imagery condition will not be revealed to the subject or to the research staff conducting the

sessions. In the imagery task the subjects will be asked to imagine the situation being described vividly, 'as if' it were happening right now", until asked to stop. The imagery script will be played to the subject over headphones and the subject will be required to imagine the situation for 5 minutes. A tape recording for each subject's script will be developed prior to the testing situation and recorded by the same person for each subject. Immediately following imagery, a blood draw will be conducted (IMAGERY). Thereafter, four recovery blood draws will be conducted at 15 minute intervals (RECOVERY). Timeline for assessments is detailed in Table 1.

TABLE 1: Schedule for Laboratory Sessions

LAB DAY 1 11AM: Imagery and Relaxation Training and IV Habituation session (1 hour)

LAB DAY 1, 2 and 3: LABORATORY (IMAGERY) TESTING SESSIONS

2:00 PM	Set-up for plasma (IV insert) measures; physiological Setup - HR, BP, Finger Temp, SCL.
2:10 PM	Stabilization and Adaptation period, BP reading
2:40 PM (-20)	Initial Blood Draw (25 cc, includes 10 cc for RNA isolation), craving/DES ratings, physiological measurements
2:50 (-10)	BASELINE Period; on-line physiological recordings; baseline cognitive tasks
2:55 (-5)	Baseline blood draws; craving/DES ratings, BP assessments
3:00	IMAGE Period (Stress/Alcohol Cues/Neutral); physiological recordings
3:10 (0)	Post-Image blood draw (25 cc, includes 10 cc for RNA isolation); craving/DES ratings;
3:15 (+5)	RECOVERY Period; 5-min physiological recordings; craving/DES ratings; cognitive tasks;
3:25 (+15)	Recovery 1: Blood draws, craving/DES ratings; physiological assessments
3:45 (+25)	; Craving/DES and Physiological monitoring
3:55 (+45)	Recovery 2: Blood draws, craving/DES ratings; physiological assessments
4:10 (+60)	Recovery 3: Blood draws (25 cc, includes 10 cc for RNA isolation), craving/DES ratings; physiological assessments
4:25 (+75)	Recovery 4: Blood draws, craving/DES ratings; physiological assessments
4:40(+90)	Recovery 5: Blood draws, craving/DES ratings; physiological assessments
4:55 (+105)	Relaxation tape; assess physiological levels & subjective state for return to baseline
5:00 PM	BAC assessment continues until under .02. Once BAC reaches <.02, the subject is debriefed and leaves the session.

15 ml of whole blood will be collected at each time point (total of 360 mls over 3 days).

Relaxation instructions will be provided to ameliorate any residual effects of imagining personal stressful and drug cue situations. Subjects will then be free to leave the testing room. Relaxation instructions have been found to be effective in reducing drug cue-induced craving (Margolin, Avants, & Kosten, 1994; Sinha et al., 1999a) in the laboratory and we will use

these instructions to reduce any residual alcohol craving. At the end of the third laboratory session, subject will meet with the PI or her designee for a motivational interviewing session to consider the harmful effects of alcohol on their body and to increase their motivation to initiate recovery. To facilitate and support subjects' commitment to treatment, all subjects will meet with a clinician once/weekly for treatment counseling for the 4-week study period.

Laboratory Assessments.

a. Self-Reports (a) Differential Emotion Scale (DES; 246). Subjects will be asked to rate their current emotional state for the following: anger, fear, sadness, anxiety, joy, neutral-relaxed feelings. These ratings will be conducted for each assessment period during the imagery session (as indicated in Table 1). An abbreviated version of the DES (Izard, 1972) includes five adjectives (a total of 30 items) are used to describe each affect state and subjects are required to rate on a 5-point scale the extent to which each word describes the way she feels at the present time. The DES has been used in human laboratory studies to assess subjective states after induction of moods either via imagery or other standard tasks such as mental arithmetic, Stroop test or in a confederate situation (Schwartz & Weinberger, 1980; Schwartz, Weinberger, & Singer, 1981). It has provided useful emotion state data for alcohol dependent individuals during stress exposure and in drug craving (Sinha et al., 1999a; Sinha, Fuse, Aubin, & O'Malley, 2000; Sinha et al., 2002a. (b) Cocaine Craving. Cocaine craving will be measured using an adapted brief 10-item version of the Cocaine Craving Questionnaire-NOW (CCQ- Tiffany, Singleton et al., 1993,). The brief 10-item version of the CCQ-NOW scale has been adapted for use in the laboratory studies and has a reliability of .92 (Tiffany, personal communication). (c) Alcohol Craving: All subjects will be asked to rate alcohol craving during the laboratory sessions at timepoints specified in Table 3. Alcohol craving will be measured using the Alcohol Urge Questionnaire (AUQ) (Bohn, Krahn et al., 1995), which is a brief 8-item self-report craving scale, and is designed so that it can be used in laboratory studies that require repeated assessments of alcohol craving such as in cue-reactivity studies. The AUQ has high test-retest reliability (0.84) and was found to be a valid measure of alcohol urges that was significantly associated with drinking measures and dependence severity. We have previously used the AUQ in our laboratory study assessing the effects of naltrexone on craving and ad-lib drinking in alcoholics (O'Malley, Krishnan-Sarin et al, 2002).

b. Physiological Measures. A pulse sensor will be attached to the subject's forefinger to obtain a measure of pulse rate. Blood pressure will be measured using the IBS dynamap.

c. HPA and Catecholamine Measures. To assess plasma levels of ACTH, prolactin, cortisol, norepinephrine (NE) and epinephrine (EPI), 5ml of plasma (15 ml of whole blood) will be collected at each time point (total of 360 mls over 3 days). The total amount of blood to be drawn is within HHS guidelines of 450 ml within 8 research weeks. The samples will be split into five heparinized tubes (.15 ul of heparin) and the tubes will be placed on ice immediately after blood drawing. Within 30 minutes of collection, the blood will be centrifuged at 4C and the

serum removed into separate test tubes, 1 ml of plasma each for ACTH, cortisol, prolactin, NE and EPI. Similar blood drawing procedures have been established successfully at the Yale Stress Center in our previous studies with alcohol dependent individuals. Analysis of HPA measures will be conducted at the YCCI Core Labs. This Laboratory has well-developed procedures for handling and shipping of blood samples, and the Stress Center has an excellent collaboration with the YCCI Core Labs.. All tubes will be stored at -70C until they are ready for transport to the YCCI Core Labs. ACTH, prolactin and cortisol will be measured using standard radioimmunoassay procedures.

In addition, saliva samples will be collected at the same time points as the plasma samples. Saliva samples will be processed at the YCCI Core Laboratories. This will be done to compare salivary cortisol measurements with the plasma cortisol assessments.
Catecholamine samples will be analyzed by Dr. Anderson's laboratory at the Yale University Medical School. These samples will be labeled with a serial number and will not have any identifying information. These samples will be used to determine adreno-medullary and sympathetic response to the challenges employed. Although both plasma norepinephrine (NE) and epinephrine (EPI) levels increase rapidly with a range of stressors, differential response is often seen (Goldstein, 1998). The plasma concentrations of NE and EPI will be determined in serial blood samples using high performance liquid chromatography (HPLC). Catecholamines will be alumina-extracted from 1.0 ml plasma samples, separated by reverse phase ion pair chromatography and detected using coulometry (Coulochem II, ESA, Inc.). Concentration detection limits of 1-2 pg/ml are observed; typical baseline plasma NE and EPI levels are 150 pg/ml and 20 pg/ml, respectively. Within-day and day-to-day coefficients of variation are less than 10%.

For both HPA and catecholamine assays, data will be analyzed for the time-points outlined in Table 1. In addition, as each measure may show different response profiles over time, we will also assess maximal or peak response and area under the curve (AUC) for each measure. Peak and AUC assessments of these measures are commonly used methods to reduce variability in responses and have been used in several studies (Kirschbaum, Wust, Faig, & Hellhammer, 1992; O'Malley, Krishnan-Sarin et al. 2002).

d. Neuroactive Steroid Measures. To assess plasma levels of pregnenolone, allopregnanolone and androstanediol, a 1mL of plasma will be collected at each timepoint, and processed as per above. Analysis of these neuroactive steroids will be conducted at The Chemical Instrumentation Center at Yale, which has a state of the art gas chromatography/ mass spectrometry (GC/MS) instrumentation (Agilent 5973 GC-MS w/autosampler, HP 5971A GC-MS) following procedures outlined in Milivojevic et al., 2016).

Assessments: A trained research assistant will meet with the client at various time-points to assess the client on selected substance use and psychological functioning measures using structured interviews, self-report assessments and to obtain urine samples (at the Yale Stress Center) for toxicology screens. A description of the assessment battery follows.

Screening Assessments:

Assessment of IQ and Executive Functioning. The Shipley Institute of Living Scale (Shipley 1940) is a paper and pencil test taking about 15-20 minutes to complete. It consists of two parts (vocabulary and abstraction) which when combined provides a global measure of cognitive functioning and IQ. The Shipley will be administered to ensure that subjects will be able to successfully participate in the self-report components and comprehend all aspects of the protocol.

Sociodemographic/general patient information. Demographic data, medical history, and family psychiatric history will be assessed with interviews and self-report forms that provide data on age, race, socioeconomic status, marital status, psychiatric family history, educational and occupational levels, and significant medical history.

The *Structured Clinical Interview for DSM-V (SCID, First et al., 1995)* will be used to obtain DSM-V alcohol and other substance use disorder diagnoses. The SCID's reliability and validity among substance-using populations has been established in large-scale surveys (Kranzler et al., 1996). The number of dependence syndrome elements endorsed from the DSM-V substance abuse/dependence criteria will assess severity of alcohol and other drug use disorder. The SCID will also be used to obtain previous psychiatric diagnoses.

Medical History and Physical Examination: All patients will be required to participate in a physical examination including a medical history, electrocardiogram (EKG), blood, and urine laboratory assessments prior to initiation of PREG/PLA to ensure eligibility for pregnenolone study protocol. Female patients will also have a pregnancy test. These tests will be conducted at the Yale Stress Center under Dr. Gretchen Hermes' supervision. Subjects will also have the option to visit a Quest Diagnostics location to provide blood and urine specimens.

The California Psychological Inventory (CPI-So) (Gough, 1951) is the socialization scale of the CPI personality test used to assess the degree of social maturity, integrity, and rectitude that the individual has attained.

The Patient-Reported Outcomes Measurement Information System (PROMIS) 29 Profile is a NIH developed patient reported outcome measurement tool that measures physical functioning, anxiety, depression, fatigue, sleep disturbance, social role participation and pain, that have a major impact on quality-of-life across a variety of chronic diseases.

Substance Use: Alcohol and Drug Use Measures:

The Alcohol Urge Questionnaire (AUQ) is a brief 8-item self-report craving scale used to measure alcohol craving. The AUQ will be given weekly and at follow-up interviews.

The *Addiction Severity Index (ASI: McLellan et al., 1992)* is a structured interview that assesses the severity of substance use and substance-related problems in the areas of medical, employment, legal, family/social and psychiatric functioning. The ASI is the most widely used instrument for assessment of substance use and related problems and its psychometric properties are well-established (Cacciola et al., 1997). The ASI will be used at pre-treatment and week 8. Follow-up ASI interviews will be conducted at 30 days post-treatment.

The Substance Use Calendar was developed by Miller and DelBoca (1994) and is based on the Time-Line Follow-Back Method (TLFB) (Sobell, Maisto et al. 1980). The SUC uses a calendar prompt and a number of other memory aids (e.g., holiday, payday, and other personally relevant dates) to facilitate accurate recall of daily drug use during the targeted period. It has demonstrated adequate levels of reliability and validity when administered as an in-person interview, over the telephone, and when administered via computer (e.g. Carey, 1997; Sobell, Brown, Leo, & Sobell, 1996; Sobell, Sobell, Leo & Cancilla, 1988). It's reliability and validity in drug abusing samples has also been established (Fals-Stewart, et al., 2000). In the current study we will use to obtain self-report of day to day use of alcohol, and other drugs, for the 90 days prior to treatment and weekly during treatment.

The Smoking History Questionnaire will be given to collect information on current and past smoking habits. The questionnaire also questions the subject's number of past quit attempts, their perception of health risks, and perceived barriers to quitting.
*Fagerstrom Test for Nicotine Dependence (*FTND: Fagerstrom, 1978*)* is a 6-item questionnaire for assessing tobacco dependence, which evaluates, among other factors, depth of inhalation, time from awakening to day's first cigarette, and difficulty in smoking cessation.

The Alcohol Use Disorders Identification Test (AUDIT) (Bohn et al., 1995) is a 10-item screening tool developed by the World Health Organization (WHO) to assess alcohol consumption, drinking behaviors, and alcohol-related problems.

Alcohol Dependence Scale (ADS) is a 25 item to measure severity of alcohol dependence.

Abstinence Measures:

Clinical Institute Withdrawal Assessment (CIWA) is a 13 item scale that measures alcohol withdrawal signs and symptoms. The assessment will be administered weekly and at followup interviews.

Cocaine Selective Severity Assessment (CSSA) is an 18-item scale that measures the cocaine withdrawal signs and symptoms within the past 24 hours. The CSSA will be used weekly and at the follow-up interview.

Urine Toxicology and Breathalyzer Screening: A urine toxicology screen will be conducted at intake to confirm alcohol, and nicotine use. During treatment, urines will be collected 2 times weekly to confirm sobriety. In addition, urine samples will be taken weekly during treatment and at follow-up appointment using on-site TESTCUP5 Drug Screen (Roche Diagnostics Inc., Totowa, NJ) to monitor opiate and other drug use. The TestCup5 kit provides results for opioids, cocaine, THC, PCP and barbiturates. Continine levels in the urine will also be measured. Breath alcohol levels will also be assessed with the Alcosensor III Intoximeter, and carbon monoxide levels with a carbon monoxide breathalyzer, at each face-to-face contact, 2 times weekly.

Alcohol Urge Questionnaire (AUQ) is an 8-item self-report questionnaire that measures current craving state. This self-report will be completed weekly throughout the study.

Cocaine Craving Questionnaire (CCQ) is a 10-item self-report questionnaire that measures current craving state. This self-report will be completed weekly throughout the study.

The Treatment Review Scale (TRS), an adapted version of the Treatment Services Review (TSR) (McLellan, Kushner et al. 1992) will be administered weekly during treatment to assess participation in self-help. *The Questionnaire on Smoking Urges (QSU* (brief) is a 10item self-report measure designed to assess urges and cravings to smoke, with higher scores indicating stronger urges. Subjects will complete the questionnaire weekly and at follow-up interviews.

Thought About Abstinence Scale (TAAS) (Hall et al, 1990) is a four-item scale used to assess motivation to quit and abstinence self-efficacy.

Assessment of Psychological Symptoms:

A number of self- report measures of psychological functioning will be obtained weekly to examine pregnenolone's effects on psychological functioning. The following wellestablished and reliable instruments will be used:

*The Emotion Regulation Scale (ERS) (DERS) i*s a 36-item questionnaire to provide a comprehensive measure of the difficulties in emotion regulation.

The Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983) is a 7 item, brief measure of generalized symptoms of anxiety and fear. Respondents indicate how they currently feel and responses are rated on a 4-point Likert scale and range from 0 to 3.

*Obsessive Compulsive Disorder Scale (OCDS) i*s a 14 item test to measure obsessionality and compulsion.

The Barratt Impulsiveness Scale, Version 11 (BIS-11; Patton et al., 1995) is a 30 item selfreport questionnaire designed to assess general impulsiveness. The structure of the instrument allows for the assessment of six first-order factors (attention, motor, self-control, cognitive complexity, perseverance, cognitive instability) and three second-order factors (attentional impulsiveness [attention and cognitive instability], motor impulsiveness [motor and perseverance], nonplanning impulsiveness [self-control and cognitive complexity]).

The Life Orientation Test (LOT-R) (Scheier and Carver (1985) is a scale containing eight items that measures an individual's level of optimism.

The Brief Self-Control Survey (SCS) (Tangney et al., 2004) measures an individual's perceived ability to exercise his/her self-control in a variety of domains, including selfdiscipline, deliberate/nonimpulsive action, healthy habits, work ethic, and reliability.

Stress-Related Assessments:

The Chronic Stress Checklist is adapted from Turner's (1995) widely used Checklist Measures of Stressful Life Events. It will be used to assess for both chronic and recent life stress.

Perceived Stress Scale (PSS; Cohen et al., 1983) assesses the degree to which situations are appraised as threatening or otherwise demanding and that there are insufficient resources to cope with the situation. This global measure of perceived stress has been widely used in the behavioral medicine literature to examine the effects of stress on disease and treatment outcome. The PSS is a 14 item self-report measure with adequate reliability and both predictive and construct validity. It has been shown to predict smoking reduction in a smoking cessation treatment program (Cohen et al., 1983; Cohen & Lichtenstein, 1990). It will be used to assess stress levels weekly in study patients during treatment and at the follow-up appointment (copy included in Appendix B).

The COPE Questionnaire (Carver, Scheier et al. 1989) is a multidimensional coping inventory that assesses the different ways in which people respond to stress. It is a 53-item self-report measure that assesses 14 coping strategies categorized into three coping styles (Problem Focused Coping; Emotion Focused Coping; Less-Useful Coping). While most previously developed coping scales have lacked good empirical validation, the COPE questionnaire has been developed with good overall reliability and construct validity, and is judged as an adequate measure of coping strategies (Parker & Endler, 1992). In this study we will assess stress-related coping at baseline and once during treatment and at the follow-up to assess effects of study medication on stress-related coping.

Cognitive assessments

CANTAB (Cambridge Cognition, Inc) Battery will be administered at baseline (first week of study enrollment) and again at some point during pregnenolone treatment. All CANTAB tasks incorporate testing modes suitable for use across multiple time-points and are presented on a touch-screen computer.

Stroop Color Word Test (Golden, 1976). On the initial trial participants are given 45 seconds to read as quickly as possible a list of 100 color words (red, green, blue) randomly arranged and printed in black ink. On the second trial participants have 45 seconds to identify as quickly as possible the color of 100 "XXXX" printed in either red, green or blue ink. The final trial consists of the 100 words presented in the initial trial in the colors presented in the second trial. In all cases, the word (i.e. red) is different from the color it is printed in (i.e. blue. (e.g. "red"). Subjects are given 45 seconds to name the color of the ink as quickly as possible. The number of raw items read for each trial is recorded and converted to standardized T scores. (5 mins)

Safety and Side Effects Measures

The following safety measures will be assessed at each contact with the patient:

Sitting vital signs: including heart rate, systolic and diastolic blood pressure will be assessed every 15-30 minutes for one hour 2x weekly throughout study. Laboratory chemistry will be assessed at week 2, 5 and week 7 after initial assessment for abnormal values.

A Systematic Assessment of Treatment Emergent Events (SAFTEE) will be assessed at each visit to assess any adverse events of side effects that may have occurred in patients during the treatment study. The scale will assess physical or health problems since the last visit, including changes in physical appearance and changes in functioning due to physical condition . If the subject describes any changes a detailed assessed of the symptom or complaint will be conducted using the SAFTEE form pages 2-4. An adverse events form developed by our group will be completed for the event according to the criteria outlined in the Data Safety Monitoring Plan in the Human Subjects section. Thus, study staff and investigators will evaluate all adverse events, and an adverse event report will be completed and the Yale HIC and the Data Safety Monitor will be notified (see Data Safety Monitoring Plan for procedures for safety monitoring and reporting of adverse events). Adverse events/side effects will be assessed weekly throughout the study.

Other Relevant Measures:

QMI Vividness of Imagery Scale (Sheehan, 1967) will be used to assess the vividness of mental imagery. The 35 items of the QMI are statements regarding the imagery ability in seven different sensory modalities (visual, auditory, olfactory, cutaneous, kinaesthetic, gustatory, and organic). Subjects are asked to rate their imagery vividness on a 7-point scale (1 = I perceive it perfectly clearly, as if it were real; 7 = I think about it, but I cannot imagine it). A low score on the QMI indicates more vivid imagery.

The Menstrual Cycle Questionnaire (MCQ) is a 30-item standardized instrument including 23 questions about symptoms experienced during a typical menstrual cycle and questions regarding typical cycle and period length. Female subjects will complete the questionnaire at baseline.

4. Genetic Testing N/A ☐

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned

The samples will be used to examine specific genes, by group analysis, and also to examine changes in gene expression over time, time point analysis i.e. before and after medication. ii. the plan for the collection of material or the conditions under which material will be received The genetic testing materials will be extracted from whole blood, which will be collected in the study (during intake physical assessments, and during LAB DAY 1 of PREG/PLA

administration). iii. the types of information about the donor/individual contributors that will be entered into a database
The data base will only have group coding. Samples will be identified by study ID only, and no information about the individual donors will be entered. iv. the methods to uphold confidentiality
Samples will be marked only by study ID numbers and no other personal identifying information will be sent to the laboratory. Once genetic analysis is conducted the data will be sent back to our laboratory and the genetic data will be stored in computerized research records that will then be analyzed by group analysis. There is no plan to return specimens back. If the subject chooses to withdraw their sample, the subject will be informed that the genetic blood sample will be stripped of all identifiers (anonymized) including study id number.

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?

At this point in time, there are no plans for sharing of materials or distributing for future research projects. Should this occasion arise, all specimens will be stripped of all identifiers, including the study ID number.

C. Is widespread sharing of materials planned? See above
D. When and under what conditions will materials be stripped of all identifiers? See above
E. If the subject chooses to withdraw their sample, the subject will be informed that the genetic blood sample will be stripped of all identifiers (anonymized) including study id number. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?

Yes, the subject can withdraw the identifiers that connect them to their materials.

How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?
The genetic blood sample will be stripped of all identifiers (anonymized) including study id number.

F. Describe the provisions for protection of participant privacy

Samples will be marked only by study ID numbers and no other personal identifying information will be sent to the laboratory. Once genetic analysis is conducted the data will be sent back to our laboratory and the genetic data will be stored in computerized research records that will then be analyzed by group analysis.

G. Describe the methods for the security of storage and sharing of materials

Upon enrollment, all study subjects will be assigned a unique study number. The study number—and no personal identifiers—will be used as labels for study records, samples and any other related research documentation. All electronic and digital files will be stored on the secure Yale network, and the PC accessing the network will be password protected and encrypted. All paper files, such as consent forms, will be stored in a locked file cabinet in a locked office and access is limited to members of the study research team.

Genetic and Gene-Expression Measures.

During the physical examination at study entry, 10 ml of whole blood will also be drawn to analyze molecular adaptations.

Thirty-two mL of whole blood will be collected from patients on day 1 of the laboratory sessions, separately from the blood draws during the lab sessions, for the purposes of understanding genetic factors that may influence the way in which the subject behaves/feels.

Microarray profiling will be used to detect these molecular changes by comparing the molecular profile of the blood drawn during the physical to blood samples drawn on day 1 of the laboratory sessions. Total RNA will be isolated from whole blood collected at the indicated times using the PAXgene Blood RNA Isolation kit (QIAGEN, Valencia, CA) per the manufacturer's instructions. Total RNA will be depleted of globin mRNA message using GLOBINclear hybridization capture technology (Ambion, Austin, TX). Globin-reduced total RNA will then be used to carry out cDNA synthesis and overnight in vitro transcription utilizing the Illumina TotalPrep RNA Amplification Kit (Ambion). Biotinylated cRNA (1.5 μg) will be hybridized onto an Illumina Sentrix Beadchip (Human-6v2). This platform was chosen because of its lower cost in relation to Affymetrix GeneChips and demonstrated utility in detecting expression changes in RNA samples derived from whole blood (Barnes et al., 2005). Hybridizations and array scanning will be carried out at the Yale Neuroscience Microarray Center.

5. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.

   Ninety treatment seeking individuals with current alcohol use disorder (half men; half women) will be recruited to participate in this study. Subjects will be recruited from the community without regard to sex, racial, or ethnic background. We expect a representative sample will be drawn from the community.

6. **Subject classification:** Check off all classifications of subjects that will be <u>specifically recruited for enrollment</u> in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

Non- ☐ Children ☐ Healthy ☐ Fetal material, placenta, or ☐ dead fetus
☐ English Speaking ☐ Prisoners ☐ Economically ☐ disadvantaged persons
☐ Decisionally Impaired ☐ Employees ☐ Pregnant ☐ women and/or fetuses
☐ Yale Students ☐ Females of childbearing potential

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects? ☐ No ☒ Yes (If yes, see Instructions section VII #4 for further requirements)

7. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

A. Human Subjects Involvement, Characteristics and Design

Ninety participants who meet current DSM-V criteria for alcohol use disorder and who are treatment seeking will be recruited to participate in this study. Women will be included in subject sample, and will represent 50% of the study sample in order to explore gender effects. The AUD individuals recruited into our previous studies have tended to be of the following racial and ethnic background: 35% are Caucasian, 60% are African-American, 5% are Hispanic. As a result, we expect that the projected demographics of our sample will follow the above gender and race breakdown. The following inclusion and exclusion criteria will be followed: Inclusion Criteria:

i. Male or female individuals, ages 18 to 68.
ii. Subjects must meet current DSM-V criteria for alcohol use disorder; documented positive urine toxicology screen for alcohol at intake or collateral information from family members, significant others, room-mates etc., on recent use.
iii. Subject has voluntarily given informed consent and signed the informed consent document. iv. Able to read English and complete study evaluations.

Exclusion Criteria:

i. Women who are pregnant, or nursing or are of childbearing potential and not practicing an effective means of birth control.
ii.Meet current severe criteria for use disorder on another psychoactive substance, such as amphetamines, hallucinogens/PCP, excluding nicotine.
iii. Meet current criteria for an opiate use disorder, including heroin (assessed via urine toxicology and self report).
iv. Current use of any psychoactive drugs (urine toxicology), including naltrexone and antabuse, excluding stable use of SSRIs, SNRIs, and benzodiazepines.
v. Any psychotic disorder or current Axis I psychiatric symptoms requiring specific attention, excluding stable use of SSRIs, SNRIs, and benzodiazepines.
vi. Significant underlying medical conditions such as cerebral, renal, or cardiac pathology which in the opinion of study physician would preclude patient from fully cooperating or be of potential harm during the course of the study.
viii. Hypotensive individuals with sitting blood pressure below 90/50 mmHG.

8. How will **eligibility** be determined, and by whom?

Individuals with alcohol use disorder will be interviewed by a research assistant to determine interest in participating in this study. After obtaining written informed consent for participation in the study, one of the study physicians will interview the subject and review all medical and psychiatric data prior to admitting the subject and beginning medication.

9. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

I) Pregnenolone/Placebo Treatment:

We selected 300mg and 500 mg/day pregnenolone versus placebo dosing based on prior clinical trials of pregnenolone in psychiatric patients (men and women). In an 8-week clinical trial in schizophrenia patients, fixed escalating dosing from 100mg to 500mg of pregnenolone (Marx et al., 2009) was well tolerated. The only side effects present in the pregnenolone group to a greater degree than the placebo group were two reports of mild restlessness, one report of mild muscle pain/stiffness, and one report of mild cold extremities. A follow-up randomized clinical trial in men and women with schizophrenia used escalating doses of pregnenolone, 100mg for 2 weeks, 300mg for 2 weeks, 500mg for 4 weeks, and found pregnenolone to be safe and well tolerated (Marx et al., 2014). A 12-week randomized placebo-controlled trial of escalating doses of pregnenolone to 400mg daily for 4 weeks in men and women with bipolar depression improved depressive symptoms and showed pregnenolone to be safe and well tolerated in all patients (Brown et al., 2014). A recent study using an acute dose of pregnenolone of 400mg to examine the effects of ALLO its effects on emotion regulation neurocircuitry (Sripada et al., 2013), also didn't find any side effects. Pregnenolone and placebo will be obtained by Yale IDS, which will prepare fully matching pregnenolone and placebo pills for dosing each subject after randomization. IDS follows stringent procedures to ensure product quality, and will provide certificates of analysis for purity. Moreover, daily smartphone monitoring and twice-weekly face-to-face appointments will monitor medication safety and tolerability, and any adverse events will immediately be reported to a Data Safety monitor and the Yale IRB.

II) Drawing of Blood:

During each laboratory session, about 120mL of whole blood will be drawn to measure stress hormone levels that may change during the session, totaling 360 mL for all three laboratory sessions. The amount of blood drawn over three laboratory sessions is equal to a little over onehalf the blood obtained during a regular blood donation. In addition, 32 mL of whole blood will be drawn on the first day of laboratory session to look at genetic markers, and 10mL of whole blood will be drawn during the physical exam/admission procedure to assess eligibility and look at molecular adaptations during the study. Lastly, 15 mL of whole blood will also be drawn each at weeks 2, 5 and 7 to measure pregnenolone levels. These levels will allow staff to ensure subjects are taking medication as prescribed. Thus, a total amount of 432 mL of whole blood will be drawn throughout the entire study. People who are in good health are not usually affected by this kind of blood loss. However, to be safe, subjects will be warned against donating blood for at least eight weeks after completing this study and 5 weeks beforehand.

III) Laboratory Sessions:

(1). Intravenous (IV) Catheter: When an IV is started, there is some risk that subjects may develop a bruise or bleeding where the vein is punctured. If this occurs, appropriate treatment will be instituted immediately. On extremely rare occasions, fainting, blood clot or infection may occur.

(2). The Imagery Procedures: The imagery method involves reliving a personal stressful event and can be somewhat anxiety provoking at the time of the task. Our previous experience has shown that once the task is over, there is very little anxiety that carries over, thus posing minimal risk. The alcohol cue imagery involves reliving a personal experience involving alcohol-related stimuli associated with alcohol use. While subjects may feel aroused by the alcohol cue imagery, relaxation procedures have been shown to be successful in reducing the physiological arousal associated with cue exposure and stressful imagery. It is likely that moderate craving for alcohol may occur due to subjects' imagining and reliving stressful events and drug cue related events. It is also possible that craving for alcohol may linger even after the task. In order to ensure that subjects are not put at increased risk for alcohol consumption after the laboratory sessions, the following safeguards will be taken:

(a) Subjects who have difficulty reducing their craving levels will participate in relaxation procedures to return craving levels to baseline both after the imagery sessions and prior to being discharged from the unit. We have found that relaxation procedures are particularly effective in reducing craving levels after alcohol/drug cue exposure (Margolin, Avants et al., 1994; Sinha, Catapano et al., 1999a; Sinha, Fuse et al., 2000).

(b) To enhance their response to relaxation procedures, subjects will also be trained on relaxation procedures in a session prior to the laboratory sessions.

(c) Any subject that reports residual craving or emotional discomfort after completion of laboratory sessions will receive an individual counseling session by a licensed psychologist (the PI or her designee) who are experienced in therapy. The focus of this session will be coping with emotions and cravings.

(d) Transportation to and from all lab sessions to the subject’ residence will be provided, if they do not have a ride.

(e) Lab sessions will take place in a clinical research center, where subjects will participate in a relaxation exercise after each lab, which may reduce craving levels. In the event that a subjects’ craving level does not return to baseline at the conclusion of the lab sessions, a licensed clinical psychologist will be available to provide counseling targeting craving reduction. Finally, at the end of laboratory session 3, all subjects will meet with the PI or her designee for a motivational therapy session to consider the harmful effects of alcohol intake and initiate treatment and recovery.

IV) Breath, saliva, and urine collections

Breath screening, saliva collection, and urine collections are performed primarily as safeguards to contamination of data and should add no risks other than those normally associated with these procedures.

V) Rating Scales and Questionnaires

These are all noninvasive and should add no risk. The major disadvantages are the time taken to complete them, and possible breach of confidentiality. Our past experience with these measures indicates that they are acceptable to subjects. Careful efforts aimed at maintaining confidentiality have been effective in previous research, and only patients' code numbers will be recorded on the forms themselves to protect confidentiality.

VI) Nonspecific Risks. Other risks from the counseling, rating scales and urine collections are not beyond usual clinical procedures in a substance abuse treatment program. Confidentiality of these results are specifically protected by Federal laws, as a certificate of confidentiality will be sought for the study; all records will be identified by code number only, with the master file kept under lock by the Principal Investigator or Data Manager.

10. **Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

i. Pregnenolone treatment:

Inclusion criteria and the use of trained research assistants in the initial screening will help to avoid the acceptance of subjects that are inappropriate for the study. Moreover, subjects will participate in a comprehensive physical examination conducted at the Yale Stress Center, to rule out subjects who may be medically or psychiatrically unfit. These will incorporate stringent medical assessments that include electrocardiography and laboratory tests of renal, hepatic, pancreatic, haematopoietic and thyroid function. There are no known serious adverse effects of pregnenolone. The following precautions will be taken: 1. Vital signs (heart rate, blood pressure, body temperature) will be assessed 3 times weekly during the during the face-to-face study visits. 2. Any clinically significant physiological signs or other adverse symptoms will be monitored closely at each visit to the clinic.

ii. Laboratory Procedures:

The laboratory sessions will be conducted at the Yale Stress Center. For subjects who have difficulty reducing their craving levels, relaxation procedures will be conducted to return craving levels to baseline after the imagery sessions. We have found that relaxation procedures are particularly effective in reducing craving levels after drug cue exposure (Margolin et al., 1994; Sinha et al., 1999a).

To enhance their response to relaxation procedures, subjects will also be trained on relaxation procedures in a session prior to the laboratory sessions.

Any subject that reports residual alcohol/drug craving or emotional discomfort after completion of laboratory sessions will receive an individual counseling session by a licensed psychologist or her designee who is experienced in relapse prevention therapy. The focus of this session will be coping with high-risk situations and cravings.

11. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study?
    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://www.yale.edu/hrpp/forms-templates/biomedical.html for
        i. Minimal risk ii. Greater than minimal

    d. For multi-site studies for which the Yale PI serves as the lead investigator:
        i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
        ii. What provisions are in place for management of interim results?
        iii. What will the multi-site process be for protocol modifications?

This protocol has been deemed a moderate risk protocol.

As this is a pilot efficacy study it does not require a data safety monitoring board. However, as drug abusing patients who are considered to be a high-risk and vulnerable population are subjects in this study, a data safety monitoring board has been put in place.

The Data Safety Monitoring Board (DSMB) for this trial will be composed of the following three clinical investigators as listed below who are not affiliated with this study, and do not have an interest in the outcomes of this study.

1) Gerard Sanacora, M.D., Phone (203)-974-7535, Email: gerard.sanacora@yale.edu; Dr. Sanacora is a Professor at the Department of Psychiatry at Yale University. He is an experienced clinical researcher who is familiar with the population under study. He is PI on several pharmacological and behavioral treatment and laboratory studies and is extremely familiar with the NIH requirements for data safety monitoring.

2) Graeme Mason, Ph.D., Phone (203)737-1478, Email: graeme.mason@yale.edu; Dr. Mason is an Associate Professor jointly appointed at the Department of Diagnostic Radiology in the Division of Bioimaging Sciences and the Department of Psychiatry at Yale University.

He is the Director of Psychiatric MRS and the Director of Metabolic Modeling. He is PI on several imaging studies, including studies related to addiction.

3) Robert Beech, M.D., Ph.D., Phone (203)974-7550, Email: robert.beech@yale.edu; Dr. Robert Beech is an Assistant Professor at the Department of Psychiatry at Yale University. He has been PI and Co-I on several NIH funded studies examining neuropsychiatric disorders risk, treatment and the underlying etiology of the illnesses.

The DSMB members will evaluate all Adverse and Serious Adverse Events, and will assist the PI in preparing and sending the pertinent expedited reports to the Yale Human Investigation Committee (HIC)/IRB and the NIAAA PO as outlined below. They will monitor the studies quarterly and review all adverse event sheets completed during that period. Although the proposed clinical trial and laboratory study includes generally safe procedures and we do not foresee termination of the study as a direct consequence of study procedures, if there is a need, the DSMB will assist the PI in making critical decisions regarding a particular patient continuing in a study for safety reasons. The DSMB will also review the summary of all Adverse and Serious Adverse Events, which will be reported annually to the Yale Human Investigation Committee (HIC) and the appropriate NIH Project Officer.

Monitoring and Reporting of All Adverse and Serious Adverse Events (AEs and SAEs): Data on all unanticipated, serious-related Adverse Events occurring during the course of conducting this study will be collected, documented and reported to the Yale HIC. A summary of all AE's will be prepared annually, by the project PI and the Data Safety Monitor and submitted to the Yale HIC. The Yale HIC requires the re-approval of study protocols at least annually and will not re-approve the protocol without such reports.

Adverse Events (AE) will be defined on the basis of the NIDA Guidelines on Data and Safety Monitoring for Intervention Trials. These guidelines define an AE as any reaction, side effect or untoward event that occurs during the course of the clinical trial, whether or not the event is considered related to the study interventions. A new illness, symptom, unfavorable or unintended sign, or worsening of a pre-existing condition or abnormality will be considered an AE. Stable chronic conditions such as diabetes that are present prior to study entry and do not worsen will not be considered AEs. For all NIDA-MDC studies including this one, AEs will include events and symptoms reported by the subjects that are of clinical importance as noted by the study staff.

The following Yale HIC/FDA classification of AE "severity" and "attribution" will be used and reported on the Yale/HIC Adverse Reporting Form.

1. Mild adverse event
2. Moderate adverse event
3. Severe

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

Plan for Determining Seriousness of Adverse Events:

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

Each AE will be classified by the project PI as serious or non-serious and appropriate reporting procedures will be followed. Serious Adverse Events (SAEs) will be defined on the basis of the NIDA Guidelines on Data and Safety Monitoring for Intervention Trials. An SAE will be any fatal event, any immediately life-threatening event, any permanent or substantially disabling event, any event that requires or prolongs inpatient hospitalization, or any congenital anomaly. Any Unexpected Event that suggests a significant hazard, contraindication, side effect or precaution will also be reported.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether prompt reporting to the IRB is necessary.

Reporting UPIRSOs (including Adverse Events) to the IRB: The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND

2. Is related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND

3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – serious, unexpected, and related adverse events and unanticipated adverse device effects. Please note that adverse events are reportable to the IRB as UPIRSOs only if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the prompt reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

Timeframe for Reporting:

1. Events that may require a temporary or permanent interruption of study activities by the Principal Investigator or sponsor to avoid potential harm to subjects should be reported to the IRB immediately (if possible), followed by a written report to the IRB no more than 5 calendar days after the Yale Principal Investigator becomes aware of the event.
2. Internal Events (defined above) should be reported to the IRB within 5 calendar days of the Principal Investigator becoming aware of the event.

Reporting of Other Study-Safety Events: The project PI will inform the Data Safety Monitor, the Yale HIC, NIDA project officer and the sponsor promptly of any change in recruitment or other changes in this project that are relevant to safety, as well as any action taken by the Yale HIC as a result of continuing review of this project.

12. **Statistical Considerations:** Describe the statistical analyses that support the study design. For this preliminary study data we will mostly use descriptive statistics for comparing the medication groups on primary outcome measures and safety measures. Repeated measures ANOVA and mixed model procedures to assess treatment group differences in alcohol craving and stress related arousal (heart rate, blood pressure, HPA and catecholamine responses) during laboratory exposure to stress and drug cues as compared to neutral imagery conditions. Non-parametric tests will be used to assess occurrence of side effects, alcohol use measures, CIWA scores and perceived stress scores during the treatment study phase. The number and type of AE's for the two medication groups will be conducted using t-tests, chisquare tests and ANOVAs as appropriate. Findings will be used to estimate effect sizes for the medication and guide the larger randomized clinical trial in the future.

    Primary Outcome Measures during 8 week treatment: The primary outcome measures will be:(1) alcohol use as measured by weekly urine drug screens and self-report of drug use on the Substance Use Calendar, (2) side effects and adverse events as measured by the SAFTEE, and (3) treatment adherence as measured by frequency of attendance for medication selfadministration and attendance for counseling appointments, including missed sessions.

    Secondary Outcome Measures: Secondary outcome measures will include alcohol craving scales, alcohol withdrawal assessment using the CIWA, perceived stress scale, the recent stressful life events scale (LES), and the COPE Questionnaire. In addition, other assessments will be used to ensure that medications groups are not different in various measures at baseline and also to assess their role in predicting treatment response. Examples of such

assessments would be demographic measures, depressive and anxiety symptoms, motivation to change and thoughts about abstinence, which are measures that have been found to predict substance abuse treatment outcomes (Hall et al., 1990; 1991; McMahon et al., 1986).

Data Analytic Procedures: Since patients will be randomly assigned to the pregnenolone doses and placebo treatment groups, we do not expect the groups to be different on demographic, prior drug use history or other predictor variables. Nevertheless, prior to undertaking the specific analyses, we will compare the treatment groups on demographic variables, substance use characteristics and previous psychiatric history/predictor variables using t-tests/chi-square analyses as appropriate. If any group differences are found, the specific variable will be entered as a covariate in all of the specific analyses.

The laboratory study component proposes a 3 X 2 X 3 mixed-factorial design with Treatment (300mg Pregnenolone vs. 500mg Pregnenolone vs. Placebo) and Sex (AUD women vs. men) as the Between-Subjects factors and Imagery Condition (Stress, Drug Cues and Neutral cues) X Time Period 7 (Baseline, Image, Recovery 1, recovery 2, recovery3, Recovery 4 and Recovery 5) as the Within-Subjects factors. Order of imagery conditions will be randomly assigned and counterbalanced across subjects. As measurements for imagery condition and time period will be repeated within subjects, these are not independent, leading to correlations between measurements. Mixed linear models provide a way not only to model the mean differences between levels of factors, but also allows for modeling of the variancecovariance matrices (Laird and Ware 1982) (Littell, Milliken et al. 1996). This is particularly advantageous for designs such as the one proposed here which require repeated laboratory measurements within subjects. Furthermore, these models also have the capacity to handle missing data, without excluding subjects with missing data points (Cnaan *et al.*, 1997; Littell *et al.*, 1996).

Thus, mixed linear models will be used to test the hypotheses for each of the specific aims. In each case, Subjects will be treated as a random effect and Sex, Imagery Condition and Timeperiod will be treated as fixed effects. PROC MIXED in the Statistical Analysis System (SAS, version 9.3) will be used to model the experimental effects. Each of the specific aims and hypotheses are listed below. For the treatment section of the study, Cox Proportional Hazards models will be used to ascertain differences in time to relapse between AUD individuals treated with pregnenolone compared with those treated with placebo. We anticipate that pregnenolone-treated individuals will demonstrate a survival function reflecting an increase in number of days to relapse compared to the placebo group.

Specific Aim #1: *To evaluate the safety/tolerability of 300mg and 500 mg/day of PREG vs PLA in AUD individuals. Hypothesis 1: PREG 300 and 500 mg/day will be found to be safe, well-tolerated and with fewer side effects/adverse events as compared to the PLA group. Statistics to test hypothesis 1 will compare safety and adverse events data using percentages of various side effects and number and type of AE's for the three treatment groups by each week in treatment using PROC MIXED procedures in SAS, but also frequencies of overall AEs compared across the 8-week period.*

Specific Aim #2a: To evaluate PREG dose (PLA, 300 and 500 mg/day) effects on ALLO and on provoked cue- and stress induced craving, HPA responses, anxiety/mood and cognitive flexibility in AUD patients.
Hypothesis 2a: Compared to PLA, PREG will dose dependently increase ALLO levels and also decrease stress and cue-induced alcohol craving, anxiety, normalize HPA axis basal and response levels of Cortisol, ACTH and adrenal sensitivity (Cortisol/ACTH ratio) and improve Stroop cognitive flexibility.
Specific Aim #2b: To assess whether PREG-stimulated ALLO levels mediate its effects on provoked craving, anxiety, mood, HPA axis response and cognitive flexibility in the laboratory component. Hypothesis 2b: Compared to PLA, higher ALLO levels in the PREG dose groups will mediate greater reductions in alcohol craving, anxiety, mood, cognitive flexibility and normalizing of HPA axis measures specified in Hypothesis 2a.
Specific Aim #3a: To determine the efficacy of 8-week PREG doses versus PLA treatment on primary alcohol use outcomes, and secondary outcomes including alcohol craving, anxiety and mood.
Hypothesis 3a: AUD patients on PREG doses versus those on PLA will dose dependently show (a) Primary: higher alcohol abstinence days, lower mean heavy drinking days and lower amounts of alcohol used in the 8-week treatment period, and (b) secondary: lower alcohol craving, anxiety and negative mood symptoms.
Specific Aim #3b: To assess whether PREG-stimulated ALLO levels mediate its effects on primary alcohol use outcomes and on secondary clinical outcomes during the 8-week treatment phase. Hypothesis 3b: Compared to PLA, higher ALLO levels in the PREG dose groups will dose dependently mediate primary and secondary alcohol use outcomes predicted in the above Hypothesis 3a with the PREG 500mg group showing better primary and secondary outcomes than the PREG 300mg group who will be better than outcomes in the PLA group.
Exploratory Aim: To explore whether pre-treatment patient characteristics (gender, family history of alcoholism (FH), trauma history and co-morbid drug use) influence PREGpotentiated ALLO levels and primary and secondary alcohol use outcomes. Exploratory analyses using mixed effects models and MRMs will be conducted to assess if pretreatment characteristics and laboratory responses (average or AUC) are predictive of primary alcohol use outcomes.

SECTION VI: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, state N/A and delete the rest of the section.

A. DRUGS, BIOLOGICS and RADIOTRACERS

1. **Identification of Drug, Biologic or Radiotracer:** What is (are) the **name(s)** of the drug(s) biologic(s) or radiotracer(s) being used? Identify whether FDA approval has been granted and for what indication(s).

Pregnenolone is a naturally occurring steroid, which is available as a dietary supplement in health stores. **Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), a dietary supplement is not considered a drug if the intended use is only to affect any function of the body. In this pilot study, we are looking at pregnenolone's effect on function in the body and we will be collecting preliminary alcohol and drug use outcome data.**

All protocols which utilize a drug, biologic or radiotracer **not** approved by, but regulated by, the FDA, or a radiotracer regulated by the RDRC, must provide the following information:

a. What is the Investigational New Drug (IND) **number** assigned by the FDA?
b. Who holds the IND?
c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: _____

Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step)
Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission.

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States. If there is no IND and an exemption is being sought, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1
The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☐ Yes ☐ No
ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. ☐ Yes ☐ No
iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☐ Yes ☐ No iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☐ Yes ☐ No

☐ ☐

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☐Yes ☐No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

   Recent studies using the neuroactive steroid precursor PREG found that it improved negative and cognitive symptoms, and functional capacity in men and women with schizophrenia, as compared to placebo (Marx et al., 2009; Marx et al., 2014)) and that PREG-induced increases in ALLO levels were associated with enhanced activation of the emotion regulation neurocircuitry in healthy controls (Sripada et al., 2013). A randomized placebo-controlled trial of PREG in men and women with bipolar depression improved depressive symptoms and showed PREG to be safe and well tolerated in all patients (Brown et al., 2014). Importantly, in all these studies PREG markedly increased ALLO levels, which significantly correlated with the outcome measures, representing a robust "precursor loading" strategy for ALLO enhancement (Marx et al., 2014). Although PREG use in psychiatric conditions is only emerging, preclinical evidence shows promise with recent reports of PREG reducing alcohol self-administration in alcohol-preferring rats (Besheer et al., 2010) and also reducing cannabis intoxication effects in mice and rats (Vallee et al., 2014). There is increasing interest in pregnenolone in psychiatric disorders, and current studies of pregnenolone on clinicaltrials.gov reveal that pregnenolone has been and/or is currently tested in a range of psychiatric disorders such as in veterans with mild TBI, in the treatment of schizophrenia, withdrawal symptoms and craving in male cigarette smokers, PTSD, bipolar depression and marijuana dependence, among others.

We selected 300mg and 500 mg/day versus placebo dosing based on prior clinical trials of pregnenolone in psychiatric patients (men and women). In an 8-week clinical trial in schizophrenia patients, fixed escalating dosing from 100mg to 500mg of pregnenolone (Marx et al., 2009) was well tolerated. The only side effects present in the pregnenolone group to a greater degree than the placebo group were two reports of mild restlessness, one report of mild muscle pain/stiffness, and one report of mild cold extremities. A follow-up randomized clinical trial in men and women with schizophrenia used escalating doses of pregnenolone, 100mg for 2 weeks, 300mg for 2 weeks, 500mg for 4 weeks, and found pregnenolone to be safe and well tolerated (Marx et al., 2014). A 12-week randomized placebo-controlled trial of escalating doses of pregnenolone to 400mg daily for 4 weeks in men and women with bipolar depression improved depressive symptoms and showed pregnenolone to be safe and well tolerated in all patients (Brown et al., 2014). A recent study using an acute dose of pregnenolone of 400mg to examine the effects of ALLO its effects on emotion regulation neurocircuitry (Sripada et al., 2013), also didn't find any side effects.

3. **Source:** a) Identify the source of the drug or biologic to be used.

   Medication Administration and Compliance: Pregnenolone and matching placebo pills will be provided by Yale IDS Pharmacy who will make up identical active and placebo capsules, packaged into weekly packs for medication self-administration. Subjects will be randomly assigned to twice daily dosing of 300mg and 500mg PREG/PLA. Subjects will selfadminister study medication twice daily. Study staff will monitor vital signs and side effects during bi-weekly face-to-face appointments. We expect all subjects to tolerate doses of 300mg and 500mg pregnenolone. Alcohol craving, abstinence symptoms, mood and perceived stress scale and side effects will be assessed twice weekly. Urines will be obtained twice weekly.

   Medication compliance will be monitored by blood draws done at weeks 2, 5, and 7, to measure PREG levels, and through the monitoring of riboflavin as a urine marker. Riboflavin produces a bright yellow discoloration of the urine when the medication is taken 2 to 8 hours prior. Subjects will be taking morning and bedtimes doses of the medication. After this time, staff will easily be able to visually inspect the subject's urine to determine whether the morning dose has been taken. 25mg of riboflavin will be added to all doses of PREG and PLA by the pharmacist when capsules are made.

   Subjects will self-administer study medication, and will be reminded via daily smartphone platform monitoring. Daily smartphone monitoring will be used to prompt patients to take study medication, and also to assess alcohol use, craving, craving resistance and mood states. Concordance between daily smartphone reports and alcohol positive urines will be evaluated for daily versus retrospective reporting.

   Medication compliance will also be implemented through video Directly Observed Therapy (DOT) using the smartphone application eMocha. Subjects will use the eMocha app to video record themselves taking their medication. Research staff will log into a secure site to view the recorded videos to assess medication dose adherence.

All participants and study staff will remain blind to medication condition.

b) Is the drug provided free of charge to subjects? ☒ Yes ☐ No If yes, by whom?

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

Commercially available pregnenolone and matching placebo will be obtained, formulated and packaged by Yale IDS Pharmacy in weekly bubble packs for self administration by subjects. Medication and placebo be stored at the Yale Stress Center in our designated medication room in a locked cabinet. Access will be provided to the research nurse, Ms. Mary Kurjanowicz, the study physician, Dr. Gretchen Hermes, and study PI and Co-I, Prof. Rajita Sinha and Dr. Verica Milivojevic. Self-administration and dispensing will be conducted by the research nurse or coordinator.

Check applicable Investigational Drug Service utilized:

☒ **YNHH IDS** ☐ **Yale Cancer Center**

☐ **CMHC Pharmacy** ☐ **West Haven VA**

☐ **PET Center** **None** ☐

☐ **Other:**

Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

5. **Use of Placebo:** ☐ **Not applicable to this research project**

If use of a placebo is planned, provide a justification which addresses the following:

1. Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.

b. State the maximum total length of time a participant may receive placebo while on the study.

c. Address the greatest potential harm that may come to a participant as a result of receiving placebo.

d. Describe the procedures that are in place to safeguard participants receiving placebo.

All randomized AUD patients in the study will be provided standard behavioral counseling (12Step counseling and contingency management for treatment attendance) that is efficacious for alcohol treatment. While there are FDA approved medications for AUDs, the effect size is modest and no better than behavioral counseling. Thus, by providing behavioral counseling to all patient, we are testing whether pregnenolone improves AUD clinical outcomes over and above standard behavioral treatment for AUDs. Subjects will receive placebo for no more than 8 weeks. All subjects will receive psychosocial treatment that is currently used in adjunct to any pharmacological treatment available. All participants will receive weekly 12-step alcohol/drug recovery counseling with an addiction expert.

6. **Use of Controlled Substances:**
Will this research project involve the use of controlled substances in human subjects?
☐ Yes ☒ No *See HIC Application Instructions to view controlled substance listings*.

7. **Continuation of Drug Therapy After Study Closure** ☒ **Not applicable to this project** Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?
☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

☐ No If no, explain why this is acceptable.

B. DEVICES- NA

SECTION VII: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects: 90

a. targeted for enrollment at Yale for this protocol_90__
b. If this is a multi-site study, give the total number of subjects targeted across all sites___

2. Indicate recruitment methods below. Attach copies of any recruitment materials that will be used.

☒ Flyers ☐ Internet/Web Postings ☐ Radio
☐ Posters ☐ Mass E-mail Solicitation ☒ Telephone
☐ Letter ☐ Departmental/Center ☐ Website ☐ Television
☐ Medical Record Review ☐ Departmental/Center Research Boards ☒
☐ Newspaper
☒ Departmental/Center Newsletters ☒ Web-Based Clinical Trial Registries
☐ YCCI Recruitment database ☐ Clinicaltrials.gov Registry (do not send materials to HIC) ☐ Other (describe):

3. Recruitment Procedures:

a. Describe how potential subjects will be identified.
b. Describe how potential subjects are contacted.
c. Who is recruiting potential subjects?

Subjects will be recruited through flyers and advertisements in local newspapers, internet/web postings, through YCCI's recruitment database Yale's clinical research management system (CRMS)-OnCore, and from community substance abuse treatment facilities. Subjects will participate in an initial phone screening and intake session to obtain informed consent with the research coordinator or research assistant, followed by physical examination and blood work to determine eligibility.

4. Screening Procedures

a. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No

b. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:

Subjects will be asked if they have any current or past medical, e.g. neurological, cardiac, endocrine, cancer etc., or psychiatric conditions, e.g. depression, suicidal attempts. They will be asked if they received any past medications for psychiatric conditions. Also, they will be asked if they are currently on any medications, and if so, to list them. Subjects will also be asked about past and current drug/alcohol use.

HIPAA identifiers:

☒ Names

☒ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.

☒ Telephone numbers

☐ Fax numbers

☒ E-mail addresses

☐ Social Security numbers

☐ Medical record numbers

☐ Health plan beneficiary numbers

☐ Account numbers

☐ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older

☐ Certificate/license numbers

☐ Vehicle identifiers and serial numbers, including license plate numbers

☐ Device identifiers and serial numbers

☐ Web Universal Resource Locators (URLs)

☐ Internet Protocol (IP) address numbers

☐ Biometric identifiers, including finger and voice prints

☐ Full face photographic images and any comparable images

☐ Any other unique identifying numbers, characteristics, or codes

5. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐ Yes, all subjects

☐ Yes, some of the subjects

☒ No

If yes, describe the nature of this relationship.

6. **Request for waiver of HIPAA authorization:** (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

   Choose one:

   ☐ For entire study

   ☒ For recruitment purposes only

   ☐ For inclusion of non-English speaking subject if short form is being used

   i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data;
   ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data;

   The recruitment will begin with a screening questionnaire over the phone, for which subjects will be asked to give a verbal consent, but a signature cannot be obtained. Once potential subjects have completed the phone screening and appear to be eligible, they will be invited for an intake appointment, during which a signed authorization and consent will be obtained. Subjects will also have the option to complete an eConsent using the eConsent module in Epic.

   By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:
   ☒ Compound Consent and Authorization form
   ☐ HIPAA Research Authorization Form

8. **Consent Personnel:** See IRES IRB Study Team.

9. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

   Potential study participants will be interviewed by a research assistant to determine interest in participating in this research study. After obtaining written informed consent for participation in the study, one of the study physicians will interview the subject and review all medical and psychiatric data prior to admitting the subject and beginning medication.

10. E**valuation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

    The Investigator will not enroll subjects in the study who are determined to have limited decision-making capacity. Prior to consent procedures, breathalyzers will be conducted. Subjects whose breathalyzer registers above the legal limit for alcohol will not be consented for the study. Subjects will be assessed throughout the consent process to ensure their understanding of consent procedures.

11. **Documentation of Consent/Assent:** Specify the documents that will be used during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.

    1 Document:
    Research study consent

12. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.

    Non-English speaking subjects will not be enrolled into this study

    12(a) As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment?

    YES ☐ NO ☐

    Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

    Several translated short form templates are found on our website at:

http://www.yale.edu/hrpp/forms-templates/biomedical.html. If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via amendment prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

13. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐ **Not Requesting a consent waiver**
☒ **Requesting a waiver of signed consent**
☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)

☒ **Requesting a waiver of signed consent for Recruitment/Screening only** If requesting a waiver of signed consent, please address the following:

a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☒ No

b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☒ No

OR

c. Does the research activity pose greater than minimal risk?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity ☒ No

AND

d. Does the research include any activities that would require signed consent in a nonresearch context? ☐ Yes ☒ No

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

Results of the physical examination, psychological assessments, self-reports, and data collected during the lab session will be collected and used for research. The proposed study will be conducted by specialized and trained research staff using standardized biophysiological and psychosocial assessments. Data collected and analyzed in the study will be derived from three main sources (1) semi-structured clinical interviews and self-rating scales: psychiatric history, medical history, levels of alcohol and alcohol use and demographic self-reports of age, race, socioeconomic status, marital status, educational and occupational levels. (2) Biophysiological data: includes heart rate, blood pressure, saliva, pregnenolone levels, and plasma HPA axis markers (cortisol and ACTH). Additionally, health checks prior to enrollment will comprise a physical examination and blood work. (3) Urine and breathalyzer data: will be used to confirm alcohol, opioids, cocaine, THC, PCP and barbiturates. It will be collected at intake, 3x weekly during treatment appointments, and at follow-up interviews.

The screening of subjects using the inclusion and exclusion criteria, and the comprehensive medical and psychiatric evaluation will minimize the risk of including subjects who are otherwise inappropriate. Confidentiality in regard to collected materials will be maintained via a numbered reference system maintained by the Project Coordinator. Subjects' names will appear only on a consent form and "key" form kept by the PI and the Project Coordinator in a separate location.

b. How will the research data be collected, recorded and stored?

c. How will the digital data be stored? ☐ CD ☐ DVD ☐ Flash Drive ☐ Portable Hard Drive ☒ Secured Server ☐ Laptop Computer ☐ Desktop Computer ☐ Other

d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?

Upon enrollment, all study subjects will be assigned a unique study number. The study number—and no personal identifiers—will be used as labels for study records, samples and any other related research documentation. All electronic and digital files will be stored on the secure Yale network, and the PC accessing the network will be password protected and encrypted. All paper files, such as consent forms, will be stored in a locked file cabinet in a locked office and access is limited to members of the study research team.

Do all portable devices contain encryption software? ☐ Yes ☐ No NA as per above

If no, see http://hipaa.yale.edu/guidance/policy.html

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

Upon completion of study and data analysis, a professional information protection, storage, and disposal company will be retained to dispose of research files and informed consent documentation.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data)

All de-identified data will be available to the research investigator PI, Co-Investigator, statistician, DSM, research staff responsible for entering data, NIH/NIAAA, Yale HRRP, and Yale's HIC. Protected Health Information will be available to the research physician, and research staff only when deemed necessary.

g. If appropriate, has a Certificate of Confidentiality been obtained?

A Certificate of Confidentiality has been obtained from the National Institutes of Health.

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g. HIV testing – reporting of communicable diseases; parent interview -incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported.

Mandatory reporting of incidents of abuse or neglect of a child or elderly person as required by Connecticut State law will be complied with. Additionally, certain reportable infectious diseases such as HIV and Hepatitis B and C will be reported to the appropriate state agency, although these will not be tested by the study. We may also need to intervene and report information regarding threat of injury to self or others to the proper authorities. All of these requirements will be complied with.

SECTION IX: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

The proposed study has the potential to reveal novel mechanisms that may play a role in alcohol addiction. A better understanding of alcohol use disorder is of utmost importance in order to develop improved treatments for alcohol use disorders. Understanding the potential role on neuroactive steroids in alcohol addiction could also aid in our knowledge of the role of sex hormones and their precursors and derivatives in this disorder, and thereby allow us to potentially address gender differences that exist in the development, progression and relapse of alcohol use disorder.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?

   Subjects need not participate in this study to receive treatment for alcohol use disorder, such as approved pharmacotherapies and counseling, group therapy, and psychotherapy.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

   Subjects completing the Study will receive $20 for a remote intake interview session or $25 for an in-person intake interview session, $25 for the imagery and relaxation training session, and $100 for each of the 3 laboratory sessions. Subjects will also be paid a $50 bonus for completing the laboratory sessions. Therefore, for the completion of all procedures leading up to and including the laboratory sessions, subjects will receive up to a total of $400.

   If a subjects continue on to complete the treatment phase, they will earn chances to win dollar prizes for kept appointments to encourage appointment attendance. For every twice/weekly appointments they attend, the chance to draw a prize from the fishbowl will increase by one. The fishbowl prizes are in increments of $1, $5, $20, and $100. Subjects will participate in this at every twice/weekly appointment they attend. If they miss any appointment, or fail to take study medication, the chances will be reset back to one. For example, if no appointments are missed the subject will have a total of 8 chances at the last appointment. Each prize increment that is won from the 8 attempts is then added together into a lump sum. There is no limit on the amounts that can be won. A standard payment rate of $20 per week can be used in lieu of drawing from the fish bowl in order to encourage completion of appointments.

   Subjects participating in smartphone monitoring will be compensated for their participation at $2 a day, with a $6 bonus for completing all 7 days, hence a possible total of $20 a week, or $160 for all 8 weeks of the study. Subjects will receive $25 per week for taking study medication using eMocha for a total of $200 for completing all 8 weeks. In addition, subjects will receive $25 for week 3, $25 for week 5, $25 for week 7 , and a $25 bonus for completing the treatment phase. The total that subjects may be reimbursed if they complete all aspects of the study will be $860 plus the various amounts won for keeping appointments during the treatment phase.
   If a subject decides to not continue with the treatment phase, twice-weekly follow-up appointments for one week will be scheduled after the laboratory sessions are complete. They will receive $25 for each visit. Therefore, if subjects only complete the laboratory phase with the follow up visits and smartphone monitoring, the maximum total payment will be $490.

3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

There will be no costs charged to subjects who participate in this study. All evaluations including interviews, physical exams, diagnostic tests will be provided at no cost to the subjects.

4. **In Case of Injury:** This section is required for any research involving more than minimal risk.
   a. Will medical treatment be available if research-related injury occurs?
   b. Where and from whom may treatment be obtained?
   c. Are there any limits to the treatment being provided?
   d. Who will pay for this treatment?
   e. How will the medical treatment be accessed by subjects?

   While medical therapy will be offered for any physical injuries sustained as a consequence of participation in this research, the subject and their insurance carrier will be responsible for the cost of such treatment. Financial compensation for injury is not available.

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