PROJECT DESCRIPTION

Title: Effect of allopregnanolone on stress-induced craving

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2. Purpose:

The role of stress in the vulnerability, initiation, and maintenance of alcohol use disorders (AUDs) is supported by a rich literature and represents a clinically important area of study. The dysregulation of the stress response is seen as critical in the development and maintenance of AUD. Neurosteroids are considered central in the regulation of the stress response and the hypothalamic pituitary adrenal (HPA) axis, and have robust direct central nervous system (CNS) effects, as well as anxiolytic and neuroprotective effects. Neurosteroid modulation of the stress system has been identified as an important key to understanding the mechanisms of how stress-system dysregulation may lead to the development of AUD.

Neurosteroids act on a number of different receptors; most notably those that regulate CNS activity, gamma-amynobutyric acid (GABA) and glutamate, but also nicotinic receptors, glycine receptors and calcium and potassium channel receptors. However, their potent actions on GABAa receptors is of particular importance because it is through these most widely disseminated receptors in the brain that neurosteroids are believed to exert their powerful anxiolytic, analgesic, antiepileptic and neuroprotective properties. Of all neurosteroids, alloprognanolone (ALLO) is the most direct modulator of GABAa receptors and it is this action that has sparked the interest in ALLO's relevance for a number of stress-related psychiatric conditions. Considerable evidence suggests that ALLO mediates alcohol's subjective stimulant and sedative effects, tolerance, dependence and withdrawal. **The effects of ALLO administration have not been studied in humans in relationship to drinking outcomes and ALLO has not been administered to individuals with AUD.**

The goal of this Phase I study is to determine whether intravenous infusion of ALLO attenuates (1) stress-induced alcohol craving, stress-induced anxiety and (2) subjective stimulant/sedative effects of alcohol in individuals with AUD using a laboratory paradigm. The secondary objective of this project is to characterize the behavioral effects of ALLO in AUD. We propose a double-blind, randomized, between subject, placebo-controlled study in 60 individuals with AUD to compare *one dose (1.5 mg/ml solution) of continuous (200 min.) infusion of ALLO* (targeted plasma of 100 nM [IV bolus of 191 mcg/kg over 60 min. followed by continuous infusion of 57.4 mcg/kg/hr x 140 min.] n=30) to *placebo* (n=30). On a single test day, after 60 min of infusion - when ALLO levels stabilize - stress and neutral cues consisting of personalized 5 min. scripts will be presented in random order. The use of personalized scripts has been shown to produce robust and reliable craving and anxiety in subjects including heavy drinkers[1], alcohol dependent individuals (see the preliminary data section)[2] both with and without psychiatric comorbidity (including work by our group)[3-5] and, in those with other substance use disorders [6]. All participants will also receive alcohol administered intravenously using a clamp procedure, targeting a breath alcohol concentration (BrAc) of 40mg% (40 mg/dL). Alcohol will be administered following script presentation (20 min to target and clamped for

additional 30min). *The main outcomes are measures of stress-induced alcohol craving, stress-induced anxiety, and subjective alcohol effects;* other secondary outcomes include subjective mood effects, cognitive performance, and motor coordination.

Hypothesis 1 (Specific Aim 1): To determine if ALLO (targeted plasma of 100 nM), compared to placebo:
Aim #1a: attenuates stress-induced craving for alcohol, measured by the Alcohol Urge Questionnaire (AUQ).
Aim #1b: attenuates stress-induced anxiety, measured by the State Trait Anxiety Inventory (STAI-6).
Aim #1c: attenuates the subjective stimulant/sedative alcohol effects (target BrAC=40 mg%), measured by the Biphasic Alcohol Effects Scale (BAES).

Hypothesis 2 (Specific Aim 2): To characterize ALLO's behavioral effects:
Aim #2: to describe ALLO's subjective mood effects, we will use Differential Emotions Scale (DES-R), the Addiction Research Center Inventory (ARCI) and the Similarity to Drugs of Abuse Scale (SDAS).
To evaluate the effects of ALLO administration on cognition and motor coordination:
Aim #3a: to describe ALLO's effects on cognitive functioning, we will assess verbal memory using the Hopkins Verbal Learning Test-Revised (HVLT-R), and response inhibition using the Go No-Go task.
Aim #3b: to describe ALLO's effects on motor coordination, we will use the Grooved Pegboard Test.

Exploratory Aims:
Aim #4: For all the above aims, we will also examine if the effects of ALLO are gender specific.

3-4. Background and Significance

SIGNIFICANCE: Alcohol use disorders (AUDs) have been characterized as stress-related conditions because the dysregulation of the stress response is seen as critical in their development and maintenance. The stress response is a physiologic multi-level and multi-system cascade of events that arises each time a stressful event occurs. However, chronic stress and repeated challenges to the stress system result in the dysregulation of this highly integrated network. In the last few decades, a growing body of evidence suggests the importance of neurosteroid modulation of the stress system, and this has been identified as an important key to understanding the mechanisms of how stress-system dysregulation may lead to the development of AUD.

Neurosteroids: The hypothalamic pituitary adrenal (HPA) response to stress, and the dysregulation of this response in psychiatric disorders has been well characterized[7-10]. More recently, a growing literature has studied the regulation of the HPA axis response by stress-derived neurohormones and their neuroactive metabolites, also known as neurosteroids [11]. These are derived from cholesterol, are synthesized both centrally and peripherally, have direct action in the brain and spinal cord, are potent modulators of neurotransmitters including gamma-amynobutyric acid (GABA) and glutamate, and are involved in cognition, arousal, motivation and emotion[7]. While they act on a number of different receptors, most notably GABA and glutamate, they are also believed to act on nicotinic receptors, glycine receptors and calcium and potassium channel receptors. Nevertheless, their potent actions on GABAa receptors seem to be of particular clinical relevance and are thought to be key to their anxiolytic, analgesic, antiepileptic and neuroprotective properties. Neurosteroids include pregnanolone and alloprognanolone (ALLO), and their actions are of interest in the regulation of stress disorders and are relevant for a number of psychiatric disorders including anxiety, addictive, and mood disorders, and disorders of cognition including traumatic brain injury (TBI)[7-10, 12].

Compelling evidence indicates that neurosteroids, and ALLO in particular, have a significant therapeutic potential in psychiatric disorders.

Neurosteroids in alcohol drinking behavior and AUD: A substantial body of preclinical and human studies show that neuroactive steroids have a role in alcohol reinforcement, tolerance, dependence and withdrawal. Data also indicates that neurosteroids, and ALLO in particular, may have significant therapeutic potential in AUD because of their ability to normalize a dysregulated HPA axis response typically found in chronic alcohol abuse. **A2a.** *In preclinical studies:* Alcohol increases the levels of progesterone (PROG) and ALLO in alcohol-preferring and non-preferring rats [13-15]. Administration of ALLO increases alcohol self-administration in non-dependent rats and in non-dependent alcohol-preferring rats. However, chronic ethanol consumption in rats leads to decreases in plasma neurosteroid release [16-18], and decreased levels of ALLO after reintroduction of alcohol in alcohol-withdrawn animals[19]. Similarly, female monkeys who drink excessively tend to experience disruptions in their menstrual cycle, putatively due to neurosteroid depletion and low levels of ALLO [20]. *Rodents who consume high doses of alcohol for long periods of time show decreases in alcohol consumption after a single infusion of ALLO [19, 21] suggesting ALLO may play an important compensatory role in mediating alcohol effects*. **A2b.** *In healthy humans:* Similar to preclinical studies in healthy humans: a) alcohol intoxication increases plasma levels of ALLO [22, 23], b) ALLO levels are correlated with levels of intoxication [22, 23] and subjective effects ("liking" or "wanting" more alcohol)[24], c) pretreatment with finasteride (reduces the formation of ALLO and PROG) blocks subjective stimulant effects of alcohol[24] and reduces drinking in men[25], and a single dose of dutasteride attenuates subjective sedative effects in non AUD men [26], d) women are less sensitive to the effects of alcohol and drink more during the follicular phase of their cycle, when ALLO levels are at their lowest [27]. There are some negative studies that show no relationship between intoxication and ALLO levels at lower doses of alcohol [28], in women in the follicular phase[29] or the luteal phase [30], and find lower levels of PROG and ALLO after alcohol administration [31]. **A2c:** *In humans with AUD*: Mirroring preclinical studies, women who drink excessively tend to experience disruptions in their menstrual cycle presumably due to neurosteroid depletion and lower levels of ALLO[32]. Dysregulation in HPA axis function has been reported in individuals with AUD while they are actively drinking, during withdrawal, and during abstinence [33-35]. Also, suppression of neurosteroid release has been reported in individuals with AUD as well as their relatives after administration of naloxone [35, 36]. A recently published study examined the effect of progesterone stimulated ALLO in cocaine dependent men and women on stress-induced craving, mood, and cognitive functioning. High levels of ALLO were associated with decreased craving, increased mood ratings, and improved cognitive performance suggesting ALLO may have important therapeutic utility in addiction. *In conclusion, data from animals and humans show that ALLO may have a significant therapeutic potential although there are no studies to date that examine the role of ALLO administration in AUD.*

ALLO administration in humans: ALLO has been available in Europe for years and has been safely administered to healthy men and women and to women with premenstrual dysphoric disorder (PMDD) [37-40]. In healthy women, a total cumulative dose of 0.09 mg/kg increases sedation and decreases saccadic eye movement [40]. The same dose administered to both men and women (adjusted for weight) produces more sedation in men, although the saccadic eye velocity was significantly more decreased in women [39]. Bolus administration of ALLO (0.07 mg/kg) in healthy women produces a small deterioration in verbal memory but has no effect on semantic and working memory [37]. Finally, bolus administration of a low dose of ALLO (0.05 mg/kg) in healthy women and women with PMDD results in mild sedation and no effect on startle response [38].

Laboratory stress paradigms can predict outcome and relapse in AUD: Clinical observations and laboratory studies – including work done by our group (see preliminary data) - show that the stress response is strongly linked to alcohol craving, relapse, and increased alcohol intake [6, 41]. Laboratory studies show that stress cues increase alcohol craving in active drinkers, abstinent alcoholics, and social drinkers[6, 41-44]. Laboratory studies using both stress inducing cues (fear, anxiety)[2, 45-49], and alcohol cues (smell of alcohol)[3, 50] show that stress cues are as powerful in eliciting self-reported craving for alcohol as is exposure to alcohol cues[48, 51, 52]. Also, stress-induced craving predicts drinking outcomes in alcoholics followed for 90 days after discharge from an inpatient treatment program[2]. Individuals with greater stress reactivity in the lab have a shorter time to relapse to their preferred substance than individuals with lesser stress reactivity[53]. This paradigm has been used to test medications (such as cloninide, guanfacine, prazosin) for their potential to attenuate the stress response in the laboratory prior to testing in clinical trials. *The laboratory stress paradigm we are testing has been successfully used by our group (preliminary data section) and others[42, 54], is a clinically relevant paradigm because of its potential to explore the link between reactivity to stress and alcohol use, and can be used to test underlying neurobiology and potential therapeutic agents.*

Proposed study: This project will investigate if pretreatment with the neurosteroid ALLO administered intravenously attenuates stress-induced craving, anxiety and subjective stimulant/sedative alcohol effects in those with AUD using a laboratory paradigm. Subjects (N=60 completers) will receive a continuous infusion of ALLO to achieve a targeted plasma level of 100nM or placebo over *200 minutes*. On a single test day, after 60 min of ALLO or placebo infusion all participants will undergo laboratory testing for stress-induced craving using personalized scripts based on an index stressful life event for each subject. This technique has been shown to produce robust and reliable craving and anxiety in subjects including heavy drinkers[1], alcohol dependent individuals[2] both with and without psychiatric comorbidity (including work by our group) and, in those with other substance use disorders[3-5]. All participants will also receive alcohol administered intravenously using a clamp procedure, targeting a breath alcohol concentration (BrAc) of 40mg% (40 mg/dL). This procedure will achieve steady-state blood alcohol levels without the individual variation from oral alcohol administration. IV alcohol administration will start after the script presentation; it will be infused over 20 min. and clamped for an additional 30 min. ALLO and placebo will be identical in color, clarity and viscosity and the blind will be maintained. The collaborator (Rogawski) in this study has extensive experience administering ALLO infusions in individuals with seizure disorders [55, 56], and TBI (NCT01673828). In doses proposed in this study, ALLO is very well tolerated with mild sedation being the main side effect.

INNOVATION: This study is among the first to evaluate administration of the neurosteroid ALLO for its effect on craving, anxiety, and subjective stimulant/sedative alcohol effects. Because of its CNS effects and its role in stress, it has a therapeutic potential for those with AUD and comorbidity, particularly PTSD and TBI. The investigators in this study represent a unique collaboration and together are ideally suited to conduct this research. Specifically, we have included experts on neurosteroids and the administration of ALLO (Rogawski), those who have expertise in alcohol dependence treatment (Ralevski, Petrakis), neurobiology and laboratory paradigms (Petrakis, Ralevski), and alcohol administration (Petrakis, Ralevski). Further, the investigators in this study have extensive expertise in the comorbidity of AUD with PTSD (Petrakis), and as such are well suited to further study this agent in this patient population. Finally, the study will carefully monitor the safety and effects of ALLO administration alone and in combination with alcohol in AUD, including its subjective effects, its effects on cognition, and on motor coordination.

APPROACH

Fig. 1 VAS craving

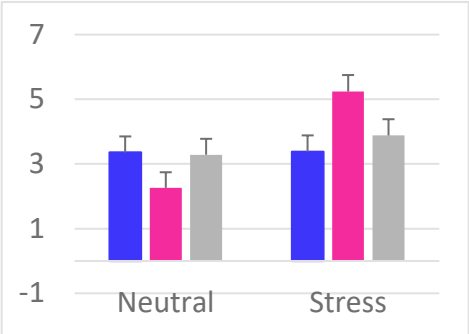
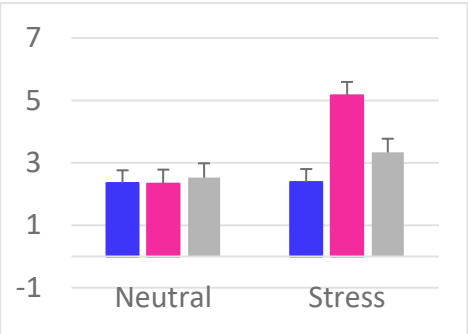
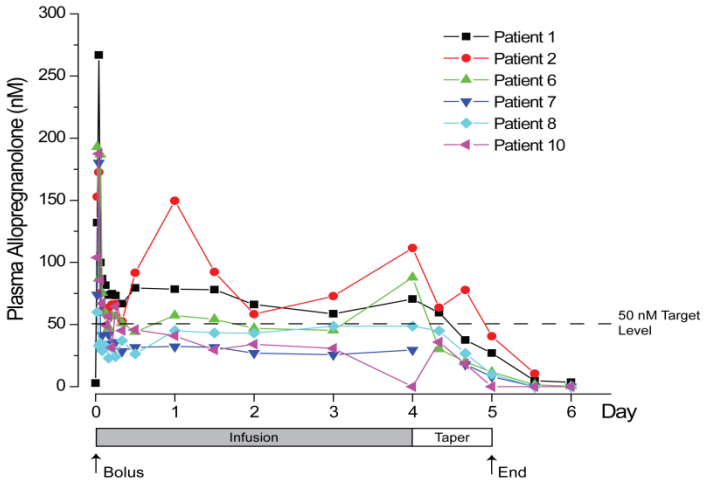


Fig. 2 VAS anxiety



A. Our experience with laboratory stress-induced paradigms: In a recent, randomized, within-subjects laboratory study, we compared the effects of neutral and stress cues (presented before [pre], and two times following the cues [post and recovery]) on craving and anxiety in 32 individuals diagnosed with DSM-IV alcohol dependence (AD). Fig. 1 and Fig 2 show the findings from the main outcome measures (Craving and anxiety measured by visual analog scales VAS) and indicate that stress when compared to neutral cues significantly increased alcohol craving and anxiety. The study shows that alcohol craving and anxiety can be reliably induced in the laboratory using this paradigm.

B. Experience with ALLO administration: We have established a collaborative relationship with Dr. Rogawski whose expertise include administration of ALLO to patients with TBI (see Fig. 3), Alzheimer's disease, and adults and children with seizure disorders [55, 56]. In this ongoing double-blind, placebo-controlled, randomized, dose-finding, two-stage adaptive clinical trial, two doses of ALLO are compared to placebo when administered intravenously for 5 days beginning within 8 hours after injury in patients with moderate to severe brain injury. Treatments are administered during a 4-day treatment period followed by a 1-day dose de-escalation period. Stage 1 of the study assesses safety and confirms the dosing plasma concentrations. Stage 2 allocates subjects to the 3 arms of treatment. Fig. 3 shows Stage 1 data for the low dose of ALLO in 6 participants. The primary outcome measure is The Glasgow Outcome Scale, administered 3 months after treatment to evaluate how the injury has affected functioning in major areas of patient life. Available data is currently not large enough for statistical analysis.



C. Our experience with alcohol clamp: In previous studies, we have used the clamp method to *safely* administer alcohol [57, 58] *to over 182 subjects*. The clamp method refers to a procedure where alcohol is infused intravenously over a period of about 20 minutes and after that the infusion is "clamped" or maintained at the same level for a set period of time. We have used the clamp to evaluate differences in subjective effects of alcohol in healthy subjects with and without a family history of alcoholism [59], to evaluate genetic bases for differences in subjective effects in healthy subjects [60], and to assess alcohol's effects on pain tolerance in healthy controls [57]. More recently, we demonstrated that IV nicotine reverses sedation and intoxication induced by IV alcohol in healthy volunteers [58]. This paradigm is ideally suited as a first step in testing a potential new medication for AUD as it allows the testing of subjective effects at a steady state of alcohol, without confounds of dose that are present when alcohol is orally administered.

5. Research Plan:

Overview: This is a double-blind, placebo-controlled, between-subjects study with 1 test day. Subjects will receive intravenous ALLO infusion to achieve a targeted plasma (100 nM; N=30, n=15 men, 15 women) or placebo (PLA; N=30, n=15 men, n=15 women) in a randomized fashion. We recognize that the double-blind in the study may be compromised by the lack of an active placebo. However, ALLO side effects or their intensity have not been extensively studied at this dose. The dose of alcohol is also so low that possible side effects of alcohol are also very slim. In addition, our group has extensive experience conducting similar studies where, for example, high dose of alcohol (significantly higher than in this study) is compared to a placebo. In order to protect the blind for the participants we have combined the possible side effects for ALLO, alcohol and placebo. For each subject, the rate of infusion will be calculated based on their weight. After 60 min. of infusion, participants will be exposed to 2 conditions in random order: stress cues and neutral cues. The cues will consist of a 5 min. presentation of the stimulus (stress or neutral) followed by immediate evaluation of craving and anxiety. There will be a relaxation procedure between each condition (see Table in Human Subjects section), and the presentation of the second cue will be initiated after stress levels have decreased to baseline. During the first hour of infusion and before the start of the stress paradigm, all participants will complete a number of measures to characterize ALLO's effects on mood, cognitive performance and motor coordination. Following the script presentations all participants will receive IV alcohol (40 mg%) that will be infused over 20 min and clamped at a steady level for an additional 30 min (Study procedures are also presented in tabular form in the Human Subjects section). All subjects will make 2 visits, one to develop the scripts and one for testing. In order to control for baseline differences in ALLO we will study women in their follicular phase of the menstrual cycle when levels of ALLO are most stable. In the follicular phase, women have low ALLO levels that are comparable to those in men, < 1 nmol/L [61]. Women have higher ALLO levels than men in the luteal phase (> 4 nmol/L), and especially high during pregnancy (100 to 160 nmol/L)[62]. For females, all sessions will be scheduled within the first 4-7 days of a woman's menstrual cycle.

Please note: Since this is the first study that combines ALLO with alcohol we propose to run at least one subject under open-label condition at a targeted plasma of 50nM (To target 50 nM: 95.5 mcg/kg/hr x 1 hour loading dose, then 28.7 mcg/kg/hr maintenance infusion, and at least one subject under open-label condition at a targeted plasma of 100Nm (To target 100 nM: 191 mcg/kg/hr x 1 hour loading dose, then 57.4 mcg/kg/hr maintenance infusion).

Justification for use of ALLO and dose: ALLO was chosen because it can be administered directly, and for its robust action on the GABAa receptors; this is believed to be responsible for the anxiolytic, analgesic, antiepileptic and neuroprotective properties of neurosteroids. We considered other neurosteroids, such as PROG. PROG is not of interest in this study because it does not bind directly to GABAa receptors and it has been widely accepted that the non-reproductive actions of PROG are mediated by ALLO. ALLO is currently not widely available in the US, but it has been studied previously in Europe. Dr. Rogawski's laboratory has extensive experience with ALLO, and is the only laboratory that holds an IND for the clinical use of ALLO in the US. He will be providing ALLO for this study. Dr. Ralevski will hold the IND (140598) for this study and will reference Dr. Rogawski's IND for ALLO (IND111085) and Dr. Petrakis' IND for Alcohol (IND121915). However, the sterile injectable vials of ALLO will be compounded at an FDA registered 503B outsourcing facility. His lab is now investigating the efficacy of ALLO in TBI (NCT01673828) and Alzheimer's disease (NCT02221622). We selected the dose in consultation with Dr. Rogawski, and is designed to maximize behavioral effects while limiting side effects such as sedation, alcohol-like intoxication, mild nausea, and flushing.

Justification for study design: 1. We decided to use continuous infusion of ALLO because bolus (i.e., rapid IV push) infusion leads to rapid and dramatic increases in ALLO followed by a precipitous

drop (in about 20-40 min.). The approach of delivering the dose continuously with an infusion pump over the 200 min period will still result in fluctuating plasma levels but the fluctuations will not be as precipitous as those using bolus infusion. **2.** The high cost of ALLO ($4175 per subject) combined with limited funding restricted our design to a single dose of ALLO, although a dose-ranging strategy would have generated important results regarding ALLO's dose-ranging efficacy. **3.** Personalized scripts were selected for stress induction rather than other stressors (Trier Social Stress test, or Cold Pressor Test) because they most reliably invoke stress in the lab [63], and they have been used to induce drug and alcohol craving. **4.** We decided to administer both scripts in one laboratory session based on the review of the evidence from laboratory studies in addition to practical considerations. The proposed design has been successfully used by our group and others in numerous imaging studies with healthy subjects and individuals with Axis I disorders. The current design will also limit subject drop outs by significantly reducing the number of lab sessions. **5.** Because the same stress condition cannot be administered on more than one occasion, a within subject design is not feasible for this study.

Justification for inclusion/exclusion criteria: 1. We plan to enroll both men and women since ALLO has been safely given to both genders. We believe that gender limitations will make recruitment very difficult, and including both genders will make the study more generalizable. Also, since there are some indications that healthy men may be more sensitive to the effects of ALLO infusion than healthy women, it seems imperative to further elucidate possible gender differences by including both genders in the study design. **2.** We are including smokers because of very high rates of smoking among heavy drinkers. In order to control for possible withdrawal during the lab session, all smokers will be allowed to have a cigarette before the beginning of the laboratory session.

Justification for measures of specific aims: We selected the primary measures of craving and anxiety based on work using stress induction in the lab with AUD subjects. The selection of measures for subjective stimulant /sedative effects of alcohol was based on numerous studies using IV clamp procedures. The selection of measures to characterize the subjective/ behavioral effects of ALLO in this population was guided by documented effects of ALLO in healthy subjects and on well-characterized effects of GABAa agonists (such as ethanol or benzodiazepines) in various populations.

A. Human subjects: Subjects will be recruited from the New Haven area by newspaper advertisements, Craigslist, online postings through Trialfacts/Rewards, and flyers**.** Based on previous human laboratory studies, approximately 10 percent of the subjects are expected to either drop out or be terminated because of non-compliance with the study procedures. To meet the target sample of 60 completers, about n=200 will be consented. After the initial phone screening, potential subjects will undergo a comprehensive evaluation that is summarized in Table 1. The administration of alcohol to human subjects as proposed in this study is in compliance with NIAAA guidelines for alcohol administration.

TABLE 1. Assessments

Study Procedures	Screening/ Baseline	Test Day	Follow up (1 Day and 4 Weeks after Test Day)
SCREENING & EVALUATION			
Screening Form	X		
CIWA-Ar	X	X	

Structured Clinical Interview for DSM-5 (SCID)	X		
EKG	X		
Physical and Laboratory Examination	X		
Vital Signs, Blood/Urine Pregnancy, Toxicology, BrAC	X	X	
Timeline Follow-Back (TLFB)	X	X	X
Script development	X		
LABORATORY ASSESSMENTS			
Alcohol Urge Questionnaire (AUQ)		X	
State Trait Anxiety Inventory (STAI-6)		X	
Visual Analogue Scale (VAS)		X	
Number of Drinks Scale (NDS)		X	
Biphasic Alcohol Affects Scale (BAES)		X	
Differential Emotions Scale (DES-R)		X	
Addiction Research Center Inventory (ARCI)		X	
Similarity to Drugs of Abuse Scale (SDAS)		X	
Hopkins Verbal Learning Test-Revised (HVLT-R)		X	
Go No-Go task		X	
Grooved Pegboard Test		X	
Plasma ALLO		X	
Follow-up Survey			X

Inclusion criteria:
1) Males and females, between the ages of 21 and 65; 2) Non-treatment seeking individuals with current DSM-5 AUD (please note we will exclude those with physiologic dependence on alcohol). This will be determined by the study physician conducting the physical examination, and by the Clinical Institute Withdrawal Assessment for Alcohol Scale (CIWA-Ar)[64] with those with score of ≥8 excluded; 3) No current substance use disorder (except tobacco, alcohol, and marijuana); 4) No current medical problems and normal ECG; 5) For women, not pregnant as determined by pregnancy screening, not breast feeding.

Exclusion criteria:
1) Current major psychiatric illnesses including mood, psychotic, or anxiety disorders; 2) History of major medical illnesses; including liver diseases, heart disease, chronic pain or other medical conditions that the physician investigator deems contraindicated for the subject to be in the study; 3) Liver function tests (ALT or AST) greater than 3 times normal; 4) weight >120kg; 5) renal impairment; and 6) patients on the following medications: a) medications for alcoholism (e.g. naltrexone, disulfiram, topiramate, acamprosate); b) psychotropic medications that promote sedation (please note patients on psychotropic medications for current psychiatric conditions will also be excluded); and c) patients currently taking antibiotics or antifungals.

Screening Labs:
EKG, CBC w/differential, Chem7, LFTs, Calcium Profile, Amylase, Lipase, Free T4, TSH, GGT, Cholesterol (including LDH), PT/PTT/INR, urine toxicology, and urinalysis (routine and microscopic). **Females only:** beta HCG (blood, quantitative).

B. Assessments: The assessments that will be used in this study are summarized in Table 1.

Main outcome measures: Craving for alcohol will be assessed using the Alcohol Urge Questionnaire (AUQ), 8 items measured on a 7-point Likert scale. Anxiety will be assessed using the State Trait Anxiety Inventory **(STAI-6**) – 6 item measure designed to assess trait and state aspects of anxiety. Sedation during the stress paradigm will be measured using a Visual Analogue Scale **(VAS)** (0=not sleepy at all to 100=falling asleep). **Biphasic Alcohol Effects Scale (BAES)** [65] is a 14-item self-report adjective rating scale that will be used to measure the stimulant and sedative effects of alcohol.
Cognitive functioning: Little is known about the effects of ALLO on cognitive functioning. However, GABA receptor agonists have been studied extensively and are known to negatively affect cognition. The effects of ALLO on various aspects of memory have been studied in animals and the data show that ALLO impairs spatial, working, and long-term memory [66, 67]. Only one study examined the effects of ALLO administration in healthy women and found that 0.07 mg/kg of ALLO had a statistically significant but relatively minor effect on verbal memory but no effect on semantic or working memory [37]. Verbal memory will be evaluated using *The* ***Hopkins Verbal Learning Test-Revised*** **(HVLT-R**). The HVLT-R is a word list learning test of verbal memory. The main outcomes for the HVLT-R are percent corrected for immediate and delayed recalls. GABAa agonists have also been shown to affect response inhibition or the ability to suppress a response in rare and irregular situations[68, 69]. We will use the **Go No-Go task** to evaluate response inhibition [70]. A series of rectangles are presented every 1150 ms and participants are instructed to press a spacebar when they see a green rectangle (=go) but refrain from pressing the spacebar when they see a blue rectangle (=no go), and to give equal importance to speed and accuracy. The primary outcome is the number of commission errors. *Motor Coordination*: The negative effects of GABAa agonists on motor coordination have been well documented and studied for decades[71]. Motor coordination will be assessed using the **Grooved Pegboard Test** (Lafayette Instrument Company). This test is a manipulative dexterity test, consisting of a board with randomly positioned slots in which subjects insert pegs. It is an eye-to-hand coordination test that has been used in research studies, including pharmacotherapy studies [72].
Plasma ALLO: Deidentified plasma levels of ALLO will be analyzed by Dr. Rogawski's lab at UCDavis. Plasma samples will be obtained on multiple time points during the lab session (see Table in Human Subjects section) to examine how levels of ALLO relate to: a) mood, cognitive performance and motor coordination, and b) stress-induced craving and anxiety.

Clinician Administered Assessments*:*

Structured Clinical Interview for DSM-IV (SCID) [73]: Assessment used to determine psychiatric diagnoses. This interview assesses DSM-IV current and lifetime psychiatric diagnoses for anxiety, mood, psychotic, alcohol and substance use, somatoform, and eating disorders.

Time-Line Follow-Back Assessment Method (TLFB)**):** [74] Interview technique that will be used to obtain quantity/frequency of alcohol consumption data for each day during the 90-day period prior to the study, throughout the period of study participation (measuring drinking on days outside of test session day) and the follow-up. Participants are given a blank calendar covering the time interval to be re-constructed and are asked to reconstruct retrospectively their drinking behavior over that interval. The process is facilitated by establishing anchor points (e.g., holidays, anniversaries, major national events, etc.). It can be scored to provide the number of days on which various levels of consumption occurred. The time-line method has good test-retest reliability and good validity for verifiable events. It has been used in numerous studies to compare pre- to post-treatment drinking.

Self-Rated Craving Measures:

Alcohol Urge Questionnaire (AUQ) *[75]:* The AUQ is an 8 item questionnaire, derived from a larger 49 item "Questionnaire of Alcohol Urges," that assesses *desire for a drink, expectation of positive effect from drinking,* and *inability to avoid drinking if alcohol was available.* The AUQ is a reliable and valid scale for the measurement of self-reported alcohol urges, and scores have been shown to be strongly related to alcohol dependence severity (as measured by ADS scores) and to cognitive preoccupation with alcohol. Its brevity and time frame for ratings (i.e., right now) makes it suitable for administration during the alcohol infusion period.

Biphasic Alcohol Effects Scale (BAES) *[65]:* The BAES is a 14-item self-report adjective rating scale that will be used to measure the stimulant and sedative effects of alcohol during the test sessions. This instrument has been found to be a sensitive and reliable measure to study medication influences on alcohol effects [76-78].

Visual Analog Scales (VAS): In addition to sedation, the VAS has the following items that will also be assessed: craving for alcohol, high, anxious, drowsy, irritable, and nauseous. We recognize that single item scales may have greater variability than multiple item scales. However, they present a significant time saving advantage during busy test days. Many of these subjective measures have been utilized in several previous studies at our Center where they show very good sensitivity to drug effects and convergent validity with other measures of these mood states and symptoms.

State Trait Anxiety Inventory **(STAI-6**): The STAI-6 is a 6 item measure designed to assess trait and state aspects of anxiety.

C. Study Procedures: All participants will be asked to arrive alcohol and drug free (except for marijuana) for the test session. Urine tests and a breathalyzer will be performed for verification. Those with positive urines or breathalyzer will be rescheduled one time. Additional non-compliance with this or any other procedure will result in discharge from the study. Since women will be recruited during the follicular phase of their menstrual cycle, they will be asked to call within the few days following the start of their menstrual bleeding to be scheduled for the laboratory session.

Screening: Potential participants responding to advertisements will be given a brief description of the study over the phone and will then be asked to complete a phone interview to determine their preliminary eligibility to participate in the study. If the caller appears to meet the eligibility criteria, he/she will be invited in for a 3 hour (approximate) screening session.* During the screening visit, a detailed assessment will be completed to assess current health. Participants will be asked specifically about alcohol and drug use, and any traumatic experiences that they may have had. In addition, we will gather information about medical history, do an electrocardiogram (EKG), blood work (including pregnancy test for women), and urine will be tested for drugs of abuse (cocaine, marijuana, benzodiazepines, etc.). This information will become part of the participant's VA medical record. The screening will last approximately 2-3 hours.

***In order to reduce face-to-face interactions during COVID-19 potential participants will be given the option to complete a portion of the screening procedures remotely using a VA approved video platform (or by telephone when video is not feasible). If the psychological screening is conducted remotely, the participant will be mailed/emailed a blank consent form and an informed consent process with study staff will then take place either over the phone or via VA-approved video platform. We will go over the consent form in detail with the participants. Consents that are mailed will be sent using US Mail (USPS/UPS/FedEx). Consents that are e-mailed will be encrypted using a VA approved encryption platform (such as Azure).**

A fully signed consent and HIPAA will be obtained prior to starting any screening procedures (remote or in-person) should they still be interested and eligible to continue in the study. We will accept the following forms of signed consent and HIPAA: a) signed in person; b) signed at home and mailed back to the research team using a pre-addressed envelope; c) signed at home and the patient will take a photo of the consent and HIPAA pages and email the images to a member of the research team; or d) signed at home during a video visit where we will ask the patient to hold each consent and HIPAA page up to the camera and we will take a screenshot of the signature pages (ensuring the patient's face is not in view) and print and file the screenshots in the patient's research record.

- The following assessments will NOT be conducted remotely: physical exam, blood pressure/pulse, blood work, urine toxicology, pregnancy test, and EKG.

Randomization: Stratified block randomization will be used to balance treatment assignments within each gender. Our group has used this procedure with repeated success in previous and ongoing studies. The Research Pharmacy will create the randomization tables.

Script Development: Two scripts will be developed based on a procedure developed by Lang et al [79-81]and using Scene Construction Questionnaires [81-83]. The questionnaires elicit details on the event and individuals involved, including physical sensations, thoughts, emotions, and cues related to the event described. The stress related scripts will be based on the "most stressful" recent experience. Only stressful experiences rated 8 and above will be used for script development. Stressful experiences related to substance use will not be considered. The neutral script will be based on previously developed neutral scripts that will consist of a relaxed beach scene commonly experienced by most individuals but personalized for each participant. The script development can also be done virtually.

Alcohol Clamp Procedure: In this study, we will be using a modified alcohol-IV clamp procedure developed and standardized by Subramanian and colleagues [84], that has been used previously and in ongoing studies by the Co-PI [59]. We will use the latest state-of-the art infusion method CAIS that uses a computerized control of the alcohol infusion that includes real-time pharmacokinetic modeling to optimize the reliability and standardization of the procedure [85]. The infusion will be performed using a 6% ethanol solution in 0.9% saline. The computer assisted administration program automatically calculates and corrects the infusion rate based on real-time BrAc data entry by staff, based on the pharmacokinetic profile of each subject.

Alternatively, the alcohol-IV clamp procedure may be completed by using a MATLAB [86] calculation package. The loading phase rate, determined by MATLAB, will use a formula that includes patient age, gender, height, and weight to generate a linear ascension to target BrAc in 30 minutes. The infusion will still be performed using a 6% ethanol solution in 0.9% saline. BrAc will be measured during the loading phase as well as during the target BrAc clamp. Once the target BrAc is achieved, the infusion pump rate will be adjusted so that the subjects are maintained within ±5 mg% of target BrAc for 30 minutes. In previous studies, this alcohol clamp method has been used safely and successfully to administer alcohol [57-60] *in over 182 subjects*.

Participants will be required to have a negative COVID test within 3 days of the Test Day. Participants who can show proof (completed vaccination card) that they are fully vaccinated will not be required to have a COVID test prior to any of the test days,

unless local guidelines (e.g., Yale University, VACT, the state of CT) require it at the time of study participation.

After the test day is completed, subjects will be asked to remain on the Biostudies Unit to make sure that there are no after effects of the alcohol or ALLO. A physician will examine them before they are permitted to go home. After the test day, subjects will be encouraged to make arrangements to be driven home. If they are driving themselves home, they will not be permitted to leave until their breathalyzer is at or below (≤) 0.02 and prior to discharge a physician will evaluate them to detect any signs of intoxication that would impair driving ability.

Blood Draw: In addition to collecting ALLO, we will also be collecting Ghrelin and Neuropeptide Y (NPY). Recent evidence suggests that peptides such as Ghrelin and NPY may play a role in stress, alcohol craving, and reward; however, this research is in its early stages. The role of these peptides in alcohol consumption and in alcohol use disorders is not well understood. Current evidence from both animal and human research demonstrates that the peptide Ghrelin is strongly and positively related to craving for alcohol. The role of NPY in relation to craving for alcohol is less understood. This project will attempt to understand the role of Ghrelin and NPY in drinking, stress, and craving in heavy drinkers. We will collect Ghrelin and NPY on a total of N=60 completers.

Follow-up: After the test day, participants will be contacted for 2 telephone follow-ups. The first telephone interview will be approximately 1 business day after the participant completes their lab session. They will be asked about how they have been doing since their last test day as well as about any alcohol, tobacco, or drugs they may have consumed. The questionnaire will take about 10 minutes to complete. The week 4 follow-up will also be over the phone and will last about 30-60 minutes, where they will be asked about how they have been doing since the test day and will complete a TLFB for the past 4 weeks as well as other surveys outlined in Table 1.

D. Data analysis and sample size considerations:

Study data will be collected and managed using REDCap electronic data capture tools hosted at VA CT Healthcare System. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources (Harris et al 2009).

Data Analysis: Descriptive statistics will be calculated and normality assumptions will be checked prior to analyses. Transformations or non-parametric methods will be used if normality assumptions are not satisfied. The primary analyses will use linear mixed models (LMMs). LMMs are flexible in modeling the variance-covariance structure of the repeated measures, use all available data on an individual, and provide unbiased and efficient results when data are missing at random. Based on Schwartz Bayesian information criterion (BIC) (smaller is better), we will select the appropriate correlation structure for each dependent variable. Medication will be entered as a categorical variable with two levels (placebo, ALLO 100 nM). Stress/Neutral cues will be entered as a within-subject variable with two levels: stress and neutral. Time will also be a within-subject factor (before cue presentation, and 2 times after each cue presentation). **Specific Primary Aims** will examine the effect of medication on craving (assessed with AUQ), and anxiety (assessed with STAI-6) under stress vs. neutral condition, and stimulant/sedative effects of 40 mg% alcohol. We will consider a significant interaction between medication and stress condition with smaller differences between the stress and neutral conditions on active drug (ALLO) supportive of our hypothesis. **Specific Secondary Aims** will examine the effect of medication, under stress vs. neutral, and in combination with alcohol on

subjective mood effects, cognitive functioning and motor coordination. As stated in the specific primary aims, a significant interaction between medication and stress condition with smaller differences between the stress and neutral conditions on active drug (ALLO) vs. placebo will be considered supportive of our hypothesis. **Exploratory Aims** will examine the gender-specific effects by adding gender as a between-subject factor in the models above and testing all possible interactions involving gender. All statistical testing for the primary hypotheses will be two-sided at 0.05 significance level. Post-hoc tests and analyses of secondary outcome measures will be adjusted for multiplicity using the Bonferroni correction.

Sample size considerations: With 30 subjects per treatment group, we have at least 80% power to detect large effects (f=0.5) for the interactions of interest for Aims 1a, 1b, and 1c assuming alpha=0.05. We can also detect large effects for the comparisons of ALLO dose to placebo (d= 0.74) under the same assumptions. The effects we are proposing are based on data from Timby et al. 2006 and van Broekhoven et al 2007 who reported that ALLO administration had a large effect (d>0.8) on saccadic eye movement and sedation among healthy subjects.

6. PROTECTION OF HUMAN SUBJECTS:

Human subjects: Non-treatment seeking individuals with AUD will be recruited from the New Haven area by newspaper advertisements, Craigslist, and flyers. Based on previous human laboratory studies, approximately 10 percent of the subjects who enroll in the study, meet all inclusion/exclusion criteria and are randomized are expected to either drop out or be terminated because of non-compliance with the study procedures. To meet the target sample of 60 completers, a total of 66 participants will be randomized. After the initial phone screening, potential subjects will undergo a comprehensive evaluation that is summarized in Table 1 in "Research Strategy".

Study Procedures: Table 1 delineates the timeline for study procedures and measures that will be administered at each time point during the laboratory session.

Table 2. Study Procedures (times are approximate)

Time	Measures and Events
Baseline	Urine and BAC check, HR/BP, CIWA, TLFB, AUQ, STAI-6, VAS; BAES, NDS, DES-R, ARCI, SDAS, HVLT-R, Go no-Go, Grooved Pegboard Test, Ghrelin, NPY and ALLO levels, breakfast
-62 min	Ghrelin and NPY levels
-60 min	ALLO infusion starts
-40 min	HR/BP, AUQ, STAI-6, VAS; BAES, NDS, DES-R, ARCI, SDAS, HVLT-R, Go no-Go, Grooved Pegboard Test, ALLO levels
-20 min	HR/BP, ALLO levels
-2 min	Ghrelin and NPY levels
Stress Reactivity Condition 1 (15 min break)	
0 min	Condition 1. (Neutral or Stress) Baseline/Relaxation; BP & HR, AUQ, STAI-6, VAS
+10 min	Image period, BP & HR
+15 min	BP&HR, AUQ, STAI-6, VAS, Ghrelin and NPY levels
+20 min	Recovery period. AUQ, STAI-6, VAS
+25 min	BP&HR, AUQ, STAI-6, VAS, Ghrelin, NPY and ALLO levels

Stress Reactivity Condition 2	
+30 min	Condition 2. (Neutral or Stress). Baseline/Relaxation as described above
+40 min	Image Period as described above
+45 min	BP&HR, AUQ, STAI-6, VAS, Ghrelin and NPY levels
+50 min	Recovery Period as described above
+55 min	BP&HR, AUQ, STAI-6, VAS, BAES, NDS, DES-R, ARCI, SDAS, Ghrelin, NPY, and ALLO levels
Alcohol Infusion	
+70 min	*start of IV alcohol infusion*
+90 min	Target reached STAI-6, VAS, BAES, NDS, ARCI, SDAS, HVLT-R, Go no-Go, Grooved Pegboard
+118 min	Ghrelin and NPY draw
+120 min	***IV alcohol infusion ends*, ALLO infusion ends**
+150 min	STAI-6, VAS, BAES, NDS, ARCI, SDAS, HVLT-R, Go no-Go, Grooved Pegboard, ALLO levels, Lunch

6.1 Risk to the subjects
Human subject's involvement and characteristics:

Sources of material: The subject will be the source of material. They will be informed that the research material collected will be for research purposes only. Blood, plasma, and urine samples will be obtained from study participants. The blood samples will be obtained with standard venipuncture techniques. In addition, physiological (heart rate and blood pressure), cognitive performance, and subjective measures will be obtained.

Potential risks: There are potential risks, discomforts and inconveniences associated with the participation in this study. These may be due to allopregnanolone (ALLO) administration, IV alcohol administration, combination of ALLO and alcohol, stress induction, blood draw and loss of privacy.

1) Allopregnanolone: In this study we will use a targeted plasma of 100 nM of ALLO. This dose of ALLO is similar to the levels found in women in their third trimester of pregnancy. Similar doses have been safely given to healthy participants with minimal and transient side effects. Common adverse effects associated with single administration of ALLO are sedation, alcohol-like intoxication, headaches, mild nausea and flushing. Less common side effects associated with neurosteroids, such as progesterone, and reported after long-term use include depression, anxiety, blockage of blood vessels, and increased risk for heart attack or stroke.
2) IV alcohol administration: Adverse effects resulting from ethanol consumption include blurred vision, nausea, vomiting, flushing, headache, and lightheadedness. These side effects should reach their peak within 1.5 hours following consumption of alcohol and decline thereafter.
3) Combined administration of ALLO and alcohol: We anticipate that ALLO will attenuate the sedative and stimulant effects of alcohol. However, since this will be the first instance the two compounds are given together all participants will be continuously monitored, a physician and/or a nurse will be present during the laboratory session at all times.
4) Stress induction: The interview, questionnaires, and imagery sessions will require subjects to discuss stressful experiences. These procedures are expected to cause a moderate degree of anxiety, psychological discomfort, and alcohol craving that will return to baseline levels before the subjects are sent home. If the subject continues to experience unpleasant symptoms a few hours after the end of the procedures, clinically-trained staff will guide the subject through relaxation techniques. If urges to drink or emotional distress persist after an hour of relaxation training, the subject will receive an individual counseling session with a psychologist who is

experienced in psychotherapy. If urges to drink or emotional distress persist after the psychotherapy session, the subject will be escorted to the Emergency Department and treated by the on-call psychiatrist.

5) Blood Drawing: Subjects will have approximately 140 ml of blood drawn as a result of their participation in the study. Blood drawing can cause some pain and may result in bruising.
6) Loss of confidentiality: Participation in the study may lead to loss of confidentiality.

6.2 Adequacy of protection against risks.

General procedures to protect against risks: Before initiating any research activity, each subject must give informed consent that will detail the risks of study participation. Eligibility will be determined by the medical and psychiatric history, drug use history, and the physical examination done prior to beginning this research protocol. Subjects will be provided a number to call to reach an on-call psychiatrist (24 hours/day) should unpleasant effects occur after subjects have left the testing facility. We ask subjects to contact us on the day following tests on a routine basis and if we are not contacted, we will call the subjects to monitor their status.

1) Protection against adverse events from ALLO: To minimize risks associated with ALLO, subjects will be closely monitored throughout the laboratory session. The targeted plasma level of ALLO (100 nM) used in this study has been used safely in studies with healthy human subjects. The dose is higher than circulating levels of ALLO in men and women but comparable to circulating levels recorded in women during pregnancy.

2) a) The administration of alcohol to human subjects proposed in this study is in compliance with NIAAA guidelines for alcohol administration. Subjects are carefully screened so only those who have experience with the dose of alcohol will be enrolled and those that are treatment seeking will be excluded. The clamping procedure is safe and has been used safely with blood alcohol levels up to 0.15 g/dL. The ethanol dose employed in this study is equivalent to doses used in previous studies in healthy human subjects and falls within ranges that alcohol dependent individuals self-administer. No fetus will be exposed to alcohol: pregnancy tests will be administered on the day of testing to every female subject with positive results excluding participation. Women who are breastfeeding will not be included in this study.
   b) We have instituted several levels of medical monitoring including: i) the testing facility is covered at all times by the VA Connecticut Healthcare System (VACHS) medical emergency ("Medical Code") team, ii) the nurses in the testing facility are all certified in advanced cardiac life support, iii) there is continuous medical and nursing presence throughout the test day, iv) subjects will be administered alcohol only if the systolic blood pressure is <150 mmHg and heart rate is <90 beats/minute. Subjects will be terminated from the study if the blood pressure at any time is >170/110 mm Hg or if the heart rate is >130 beats/min.
   c) At the end of each test day, the research staff will review the experiences of the day with the research subject. Subjects will not be released from the Laboratory until the BrAc is less than or equal to 0.02 g/dL (0.02 g/dL is below the threshold of BrAc associated with any impairment in locomotor coordination or judgment in humans) and research personnel have documented that participants exhibit an alert and oriented demeanor.
   d) At the end of the test session, we will provide participants with a brief motivational interview based on the principles of Miller's Motivational Enhancement Therapy. This interview will be conducted by a member of our research group, an M.D. or Ph.D. previously trained in motivational interviewing. During this 30minute interview, participants' drinking and smoking behavior (for the subgroup of smokers), and experience of alcohol and smoking-related problems will be reviewed. Participants will be presented with normative drinking information, as well as guidelines for non-hazardous alcohol consumption. If interested,

participants will be given treatment referrals to address their drinking and smoking behavior (for smokers). It has been found that similar brief advice is associated with decreases in alcohol drinking behavior and increased motivation to quit drinking.
e) All participants will be contacted approximately 1 business day and 1 month after the end of their participation to ensure no residual effects from ALLO or alcohol administrations.

3) Stress induction: Similarly to above we have instituted several levels of medical monitoring including: i) the testing facility is covered at all times by the VA Connecticut Healthcare System (VACHS) medical emergency ("Medical Code") team, ii) the nurses in the testing facility are all certified in advanced cardiac life support and one of the four nurses have extensive medical ICU experience, iii) there is continuous medical and nursing presence throughout the test day, iv) at the end of the test day, the research staff will review the experiences of the day with the research subject. The subject will not be released from the unit unless his/her anxiety, psychological discomfort, and alcohol craving have returned to baseline levels. At the end of the lab session, we will provide participants with a brief motivational interview based on the principles of Miller's Motivational Enhancement Therapy (details outlined above).

4) Blood drawing: The medical staff who perform blood drawing are trained individuals and follow Standard Precautions.

5) Confidentiality will be protected by having records identified by code number only with the master list including names kept in a sealed envelope in a locked file in the Principal Investigator's office and by the pharmacy, or on the VA secure server. Subjects will be given telephone numbers to call in case of emergency, 24 hours a day.

Stopping rules: Subjects will be terminated from the study if the blood pressure at any time is >170/110 mm Hg or if the heart rate is >130 beats/min. Subjects will remain in the laboratory until the breathalyzer reaches ≤ 0.02 g/dL. These procedures have been developed as part of our routine procedures for IV alcohol administration. Blood pressure and heart rate will be monitored frequently during the lab session. If blood pressure and/or heart rate exceeds these parameters, the infusion will be terminated. If blood pressure does not improve after the infusion is terminated, an MD will evaluate the subject and make a determination regarding rescue medications. If symptoms do not improve the patient will be escorted to the ER if deemed necessary.

6.3 Potential benefits of the proposed research to the subjects and others. Study participation may help the participants to seek help for their alcohol use. At the end of the study, if interested, participants will be referred to clinical programs.

6.4 Importance of the knowledge to be gained. This proposed study may help to develop new and more effective treatments for AUD. We believe that the risk/benefit ratio for this study is acceptable, and that the benefits of the proposed studies outweigh the potential risks to subjects.

6.5 Data safety and monitoring plan. The risk associated with participating in this study is moderate, because ALLO administration may be associated with mild side effects and the effects of the stress-induction procedure are expected to normalize quickly. Serious side effects associated with this study are not expected. This project will be monitored by a Data and Safety Monitoring Board (DSMB), because the study involves double-blind administration of ALLO <u>in combination with alcohol in AUD participants.</u> This board is composed of persons not otherwise affiliated with the study who are

experienced in various aspects of the conduct of clinical trials, laboratory studies and treatment of addictive disorders. We propose three investigators located here in Connecticut who are not directly involved in this study – Declan Barry, Ph.D., Sherry McKee, Ph.D., and David Fiellin, M.D. as the members of the DSMB. The members of the DSMB and all study Investigators will complete Conflict of Interest forms created by Yale's IRB in accordance with NIH guidelines.

We will report recruitment, follow-up, and adverse events to this panel on a quarterly fashion. Prior to study initiation, critical parameters for collection of side effects and for study discontinuation will be recommended to the DSMB who may use these or other measures to monitor safety of the ongoing study. The DSMB will be available to convene outside of scheduled meetings, if necessary, due to concerns regarding a particular subject or due to any troublesome developments in subjects' experiences during the study. The DSMB will make appropriate recommendations for changes in the study protocol, if needed.

This monitoring will be consistent with NIH policy regarding the protection of human subjects in research, and FDA guidance on statistical practices for clinical trials (ICH E9) and good clinical practices (ICH E6). In general, the data to be reviewed will include screening data, baseline data, laboratory data, and safety data.
The Principal Investigator will conduct a review of all adverse events and determine the attribution and grade of severity of the adverse event by using the following scales:
Attribution of Risk Categories:
Definite: Adverse event(s) will clearly be related to investigational agent(s) or other intervention
Probable: Adverse event(s) will likely be related to investigational agent(s)
Possible: Adverse event(s) may be related to investigational agent(s)
Unlikely: Adverse event(s) will doubtfully be related to investigational agent(s)
Unrelated: Adverse event(s) will clearly not be related to the investigational agents(s)
Grades of Risk:

0: No adverse event or within normal limits
1: Mild adverse event
2: Moderate adverse event
3: Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect
4: Life-threatening or disabling adverse event
5: Fatal adverse event

Serious adverse events (SAEs) include any untoward medical occurrence that results in death or the immediate risk of death, hospitalization or the prolonging of an existing hospitalization, persistent or significant disability/incapacity or a congenital anomaly/birth defect. Subjects will be terminated from participation if the investigator feels that subjects' health or well-being may be threatened by continuation in the study. Serious unanticipated and anticipated adverse events will be reported within 48 hours to the VA Hospital and Yale IRBs, and NIAAA. We will directly report to the FDA, whenever their magnitude or frequency exceeds expectations.

6.6 Clinicaltrials.gov requirements. This study will be registered at the clinicaltrials.gov website.

7. Payment:
Participants will be paid $30 for the screening session, $30 for the script development session, $150 for attending the test session, and $0 for the 1 month follow-up. Thus, the total amount that could be earned during the study is $210. This payment is for the time and effort associated with study assessments and procedures. This payment will be given in the form of cash. Participants will not be paid if a test day is cancelled because they test positive for alcohol or illicit drugs in their system. If a

patient participates in a virtual screening and is not eligible to come for the in-person visits they can choose to come in to pick up their cash payment or be mailed a gift card or check. If they choose to receive payment by gift card or check they may need to complete a tax form.

We will provide participants with 2 meals during the lab visit (breakfast and lunch).

8. References:

1. Ray, L.A., et al., *A human laboratory study of the effects of quetiapine on subjective intoxication and alcohol craving*. Psychopharmacology (Berl), 2011. 217(3): p. 341-51.
2. Breese, G.R., et al., *Stress enhancement of craving during sobriety: a risk for relapse*. Alcoholism: Clinical & Experimental Research, 2005. 29(2): p. 185-95.
3. Coffey, S.F., et al., *Trauma and substance cue reactivity in individuals with comorbid posttraumatic stress disorder and cocaine or alcohol dependence*. Drug and Alcohol Dependence, 2002. 65(2): p. 115-127.
4. Coffey, S.F., et al., *Trauma-focused imaginal exposure for individuals with comorbid posttraumatic stress disorder and alcohol dependence: revealing mechanisms of alcohol craving in a cue reactivity paradigm*. Psychology of Addictive Behaviors, 2006. 20(4): p. 425-35.
5. Chaplin, T.M., et al., *Behavioral arousal in response to stress and drug cue in alcohol and cocained addicted individuals versus healthy controls*. Hum Psychopharmacol Clin Exp, 2010. 25: p. 368 - 376.
6. Sinha, R., et al., *Effects of lofexidine on stress-induced and cue-induced opioid craving and opioid abstinence rates: preliminary findings*. Psychopharmacology, 2007. 190(4): p. 569-74.
7. Zorumski, C.F., et al., *Neurosteroids, stress and depression: potential therapeutic opportunities*. Neurosci Biobehav Rev, 2013. 37(1): p. 109-22.
8. Irwin, K.C., et al., *PTSD symptoms and pain in Canadian military veterans: the mediating roles of anxiety, depression, and alcohol use*. J Trauma Stress, 2014. 27(2): p. 175-81.
9. Magnaghi, V. and G. Puia, *Editorial on "New perspectives in neurosteroids action: a special player allopregnanolone"*. Front Cell Neurosci, 2015. 9: p. 133.
10. Strous, R.D., R. Maayan, and A. Weizman, *The relevance of neurosteroids to clinical psychiatry: from the laboratory to the bedside*. Eur Neuropsychopharmacol, 2006. 16(3): p. 155-69.
11. Crowley, S.K. and S.S. Girdler, *Neurosteroid, GABAergic and hypothalamic pituitary adrenal (HPA) axis regulation: what is the current state of knowledge in humans?* Psychopharmacology (Berl), 2014. 231(17): p. 3619-34.
12. Schumacher, M., et al., *Revisiting the roles of progesterone and allopregnanolone in the nervous system: resurgence of the progesterone receptors*. Prog Neurobiol, 2014. 113: p. 6-39.
13. O'Dell, L.E., et al., *Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats*. Eur J Pharmacol, 2004. 484(2-3): p. 241-7.
14. Korneyev, A. and E. Costa, *Allopregnanolone (THP) mediates anesthetic effects of progesterone in rat brain*. Horm Behav, 1996. 30(1): p. 37-43.
15. Barbaccia, M.L., et al., *Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats*. Eur J Pharmacol, 1999. 384(2-3): p. R1-2.
16. Cagetti, E., et al., *Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function*. Neuropharmacology, 2004. 46(4): p. 570-9.
17. Devaud, L.L., et al., *Sensitization of gamma-aminobutyric acidA receptors to neuroactive steroids in rats during ethanol withdrawal*. J Pharmacol Exp Ther, 1996. 278(2): p. 510-7.
18. Devaud, L.L., J.M. Fritschy, and A.L. Morrow, *Influence of gender on chronic ethanol-induced alterations in GABAA receptors in rats*. Brain Res, 1998. 796(1-2): p. 222-30.
19. Morrow, A.L., et al., *The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence*. Brain Research Brain Research Reviews, 2001. 37(1-3): p. 98-109.

20. Mello, N.K., et al., *Alcohol self-administration by female macaque monkeys: a model for study of alcohol dependence, hyperprolactinemia and amenorrhea*. J Stud Alcohol, 1988. 49(6): p. 551-60.
21. Martin-Garcia, E., S. Darbra, and M. Pallares, *Intrahippocampal allopregnanolone decreases voluntary chronic alcohol consumption in non-selected rats*. Prog Neuropsychopharmacol Biol Psychiatry, 2007. 31(4): p. 823-31.
22. Torres, J.M. and E. Ortega, *Alcohol intoxication increases allopregnanolone levels in male adolescent humans*. Psychopharmacology (Berl), 2004. 172(3): p. 352-5.
23. Torres, J.M. and E. Ortega, *Alcohol intoxication increases allopregnanolone levels in female adolescent humans*. Neuropsychopharmacology, 2003. 28(6): p. 1207-9.
24. Pierucci-Lagha, A., et al., *GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride*. Neuropsychopharmacology, 2005. 30(6): p. 1193-203.
25. Irwig, M.S., *Decreased alcohol consumption among former male users of finasteride with persistent sexual side effects: a preliminary report*. Alcohol Clin Exp Res, 2013. 37(11): p. 1823-6.
26. Covault, J., et al., *Dutasteride reduces alcohol's sedative effects in men in a human laboratory setting and reduces drinking in the natural environment*. Psychopharmacology (Berl), 2014. 231(17): p. 3609-18.
27. Mello, N.K., J.H. Mendelson, and B.W. Lex, *Alcohol use and premenstrual symptoms in social drinkers*. Psychopharmacology (Berl), 1990. 101(4): p. 448-55.
28. Holdstock, L., et al., *Moderate doses of ethanol fail to increase plasma levels of neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one-like immunoreactivity in healthy men and women*. Psychopharmacology (Berl), 2006. 186(3): p. 442-50.
29. Nyberg, S., et al., *The effect of a low dose of alcohol on allopregnanolone serum concentrations across the menstrual cycle in women with severe premenstrual syndrome and controls*. Psychoneuroendocrinology, 2005. 30(9): p. 892-901.
30. Nyberg, S., et al., *Altered sensitivity to alcohol in the late luteal phase among patients with premenstrual dysphoric disorder*. Psychoneuroendocrinology, 2004. 29(6): p. 767-77.
31. Pierucci-Lagha, A., et al., *Subjective effects and changes in steroid hormone concentrations in humans following acute consumption of alcohol*. Psychopharmacology (Berl), 2006. 186(3): p. 451-61.
32. Mello, N.K., J.H. Mendelson, and S.K. Teoh, *Neuroendocrine consequences of alcohol abuse in women*. Ann N Y Acad Sci, 1989. 562: p. 211-40.
33. Romeo, E., et al., *Marked decrease of plasma neuroactive steroids during alcohol withdrawal*. Clinical Neuropharmacology, 1996. 19(4): p. 366-9.
34. Hill, M., et al., *Altered profiles of serum neuroactive steroids in premenopausal women treated for alcohol addiction*. Steroids, 2005. 70(8): p. 515-24.
35. Adinoff, B., et al., *Suppression of the HPA axis stress-response: implications for relapse*. Alcohol Clin Exp Res, 2005. 29(7): p. 1351-5.
36. Hernandez-Avila, C.A., et al., *Adrenocorticotropin and cortisol responses to a naloxone challenge and risk of alcoholism*. Biol Psychiatry, 2002. 51(8): p. 652-8.
37. Kask, K., et al., *Allopregnanolone impairs episodic memory in healthy women*. Psychopharmacology (Berl), 2008. 199(2): p. 161-8.
38. Kask, K., et al., *Allopregnanolone has no effect on startle response and prepulse inhibition of startle response in patients with premenstrual dysphoric disorder or healthy controls*. Pharmacol Biochem Behav, 2009. 92(4): p. 608-13.
39. van Broekhoven, F., et al., *Effects of allopregnanolone on sedation in men, and in women on oral contraceptives*. Psychoneuroendocrinology, 2007. 32(5): p. 555-64.
40. Timby, E., et al., *Pharmacokinetic and behavioral effects of allopregnanolone in healthy women*. Psychopharmacology (Berl), 2006. 186(3): p. 414-24.

41. Sinha, R., J. Robinson, and S. O'Malley, *Stress response dampening: effects of gender and family history of alcoholism and anxiety disorders*. Psychopharmacology (Berl), 1998. 137(4): p. 311-20.
42. Sinha, R., et al., *Effects of adrenal sensitivity, stress- and cue-induced craving, and anxiety on subsequent alcohol relapse and treatment outcomes*. Arch Gen Psychiatry, 2011. 68(9): p. 942-52.
43. Breese, G.R., R. Sinha, and M. Heilig, *Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse*. Pharmacol Ther, 2011. 129(2): p. 149-71.
44. Spanagel, R., H.R. Noori, and M. Heilig, *Stress and alcohol interactions: animal studies and clinical significance*. Trends Neurosci, 2014. 37(4): p. 219-27.
45. Childress, A.R., et al., *Can induced moods trigger drug-related responses in opiate abuse patients?* Journal of Substance Abuse Treatment, 1994. 11(1): p. 17-23.
46. Cooney, N.L., et al., *Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men*. Journal of Abnormal Psychology, 1997. 106(2): p. 243-50.
47. Stasiewicz, P.R., et al., *Exposure to negative emotional cues and alcohol cue reactivity with alcoholics: a preliminary investigation*. Behaviour Research & Therapy, 1997. 35(12): p. 1143-9.
48. Sinha, S., et al., *Enhanced negative emotion and alcohol craving, and altered physiological responses following stress and cue exposure in alcohol dependent individuals*. Neuropsychopharmacology, 2009. 34(5): p. 1198 - 1208.
49. Sinha, R., *Modeling stress and drug craving in the laboratory: implications for addiction treatment development*. Addict Biol, 2009. 14(1): p. 84 - 98.
50. Smith-Hoerter, K., P.R. Stasiewicz, and C.M. Bradizza, *Subjective reactions to alcohol cue exposure: a qualitative analysis of patients' self-reports*. Psychology of Addictive Behaviors, 2004. 18(4): p. 402-6.
51. Meisler, A.W., *PTSD and substance abuse in Vietnam*, in *International Society of Traumatic Stress Studies*. 1996: San Francisco, CA.
52. Sinha, R., et al., *Alcohol craving and subjective emotional state during stress and alcohol cue exposure in alcoholics and social drinkers*. Alcoholism: Clinical & Experimental Research, 2005. 29(5): p. 150A.
53. Sinha, R., et al., *Stress-Induced Cocaine Craving and Hypothalamic-Pituitary-Adrenal Responses are Predictive of Cocaine Relapse Outcomes*. Archives of General Psychiatry, 2006. 63: p. 324-331.
54. Fox, H.C., et al., *Prazosin effects on stress- and cue-induced craving and stress response in alcohol-dependent individuals: preliminary findings*. Alcohol Clin Exp Res, 2012. 36(2): p. 351-60.
55. Broomall, E., et al., *Pediatric super-refractory status epilepticus treated with allopregnanolone*. Ann Neurol, 2014. 76(6): p. 911-5.
56. Rogawski, M.A., et al., *Neuroactive steroids for the treatment of status epilepticus*. Epilepsia, 2013. 54 Suppl 6: p. 93-8.
57. Perrino, A.C., Jr., et al., *Ethanol and pain sensitivity: effects in healthy subjects using an acute pain paradigm*. Alcohol Clin Exp Res, 2008. 32(6): p. 952-8.
58. Ralevski, E., et al., *Preliminary findings on the interactive effects of IV ethanol and IV nicotine on human behavior and cognition: a laboratory study*. Nicotine Tob Res, 2012. 14(5): p. 596-606.
59. Kerfoot, K., et al., *Effects of family history of alcohol dependence on the subjective response to alcohol using the intravenous alcohol clamp*. Alcohol Clin Exp Res, 2013. 37(12): p. 2011-8.
60. Petrakis, I.L., et al., *Role of Gaba-Related Genes in Mediating Subjective Response to Iv Ethanol in Healthy Subjects*. Alcoholism: Clinical and Experimental Research, 2011. 35(6): p. 131a-131a.

61. Genazzani, A.R., et al., *Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences*. J Clin Endocrinol Metab, 1998. 83(6): p. 2099-103.
62. Chabbert Buffet, N., et al., *Regulation of the human menstrual cycle.* Front Neuroendocrinol, 1998. 19(3): p. 151-86.
63. Thomas, S., et al., *Clinical laboratory stressors used to study alcohol-stress relationships.* Alcohol Res, 2012. 34(4): p. 459-67.
64. Sullivan, J., et al., *Assessment of alcohol withdrawal: the revised Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar).* British Journal of Addiction, 1989. 84: p. 1353-1357.
65. Martin, C.S., et al., *Development and validation of the Biphasic Alcohol Effects Scale.* Alcoholism: Clinical & Experimental Research, 1993. 93: p. 8 - 15.
66. Frye, C.A. and J.D. Sturgis, *Neurosteroids affect spatial/reference, working, and long-term memory of female rats*. Neurobiol Learn Mem, 1995. 64(1): p. 83-96.
67. Johansson, I.M., et al., *Allopregnanolone inhibits learning in the Morris water maze.* Brain Res, 2002. 934(2): p. 125-31.
68. Bari, A. and T.W. Robbins, *Inhibition and impulsivity: behavioral and neural basis of response control.* Prog Neurobiol, 2013. 108: p. 44-79.
69. Oddy, B.W. and R.J. Barry, *The relationship of N2 and P3 to inhibitory processing of social drinkers in a Go/NoGo task*. Int J Psychophysiol, 2009. 72(3): p. 323-30.
70. Stock, A.K., et al., *High-dose alcohol intoxication differentially modulates cognitive subprocesses involved in response inhibition*. Addict Biol, 2016. 21(1): p. 136-45.
71. Marczinski, C.A., et al., *Effects of energy drinks mixed with alcohol on information processing, motor coordination and subjective reports of intoxication.* Exp Clin Psychopharmacol, 2012. 20(2): p. 129-38.
72. Rosen, M., et al., *Neuropsychological correlates of suboptimal adherence to metformin.* Journal of Behavioral Medicine, 2003. 26(4): p. 349-360.
73. First, M.B., et al., *Structured Clinical Interview for DSM-IV Axis I Disorders (Patient Edition) (SCID-P)*. 1996, Biometric Research, New York State Psychiatric Institute: New York, N.Y.
74. Sobell, L.C. and M.B. Sobell, *Timeline Follow-Back: A technique for assessing self-reported alcohol consumption*, in *Measuring Alcohol Consumption: Psychosoical and biological methods*, R.Z. Litten and J. Allen, Editors. 1992, Humana Press: Totowa, NJ. p. 41-72.
75. Bohn, M.J., D.D. Krahn, and B.A. Staehler, *Development and initial validation of a measure of drinking urges in abstinent alcoholics.* Alcoholism, Clinical & Experimental Research, 1995. 19(3): p. 600-6.
76. Kranzler, H., V. Modesto-Lowe, and J. Van Kirk, *Naltrexone vs. Nefazodone for Treatment of Alcohol Dependence. A Placebo-controlled Trial.* Neuropsychopharmacology, 2000. 22(5): p. 493-503.
77. Reynolds, B. and R. Schiffbauer, *Measuring state changes in human delay discounting: an experiential discounting task*. Behavioural Processes, 2004. 67(3): p. 343-56.
78. Swift, R.M., et al., *Naltrexone-induced alterations in human ethanol intoxication*. American Journal of Psychiatry, 1994. 151(10): p. 1463-7.
79. Lang, P.J., et al., *Emotional imagery: conceptual structure and pattern of somato-visceral response.* Psychophysiology, 1980. 17(2): p. 179-92.
80. Lang, P.J., et al., *Fear behavior, fear imagery, and the psychophysiology of emotion: the problem of affective response integration*. Journal of Abnormal Psychology, 1983. 92(3): p. 276-306.
81. Miller, G.A., et al., *Individual differences in imagery and the psychophysiology of emotion.* Cognition & Emotion, 1987. 1: p. 367-390.
82. Sinha, R., W.R. Lovallo, and O.A. Parsons, *Cardiovascular differentiation of emotions.* Psychosomatic Medicine, 1992. 54(4): p. 422-35.

83. Sinha, R., et al., *Enhanced subjective, behavioral and physiological sensitivity to stress and alcohol craving during abstinence from chronic alcohol abuse*. Neuropsychopharmacology, in press.
84. Subramanian, M., et al., *A three-stage alcohol clamp procedure in human subjects*. Alcoholism: Clinical and Experimental Research, 2002. 26(10): p. 1479-83.
85. Zimmermann, U.S., S. O'Connor, and V.A. Ramchandani, *Modeling alcohol self-administration in the human laboratory*. Curr Top Behav Neurosci, 2013. 13: p. 315-53.
86. MATLAB, *Mac II-MATLAB*. 1.2c ed. 1987, South Natick, MA: The Mathworks, Inc.