

JHM IRB - eForm A – Protocol

Laboratory studies on oxytocin for treatment of alcohol use disorder

Protocol Version 3.3 Dated 06/08/16

Summary of Updates from Version 3.2 (dated 11/17/15) to Version 3.3 (dated 06/08/16)

- **Adding online screener information**
- **We are allowing participants to smoke ad lib while on the Bayview CRU. Nicotine patch will still be administered for subjects on the JHH CRU and it will be made available on session days.**
- **We will no longer exclude individuals who meet DSM-V criteria for mild cannabis use disorder; moderate and severe cannabis use disorder is still a rule out.**

1. Abstract

This study will lay the necessary groundwork for future comprehensive research to examine the utility of the neuropeptide oxytocin (OT) as a potential new medication for the treatment of Alcohol use disorder (AUD). OT modulates a number of key systems involved in addiction processes, including dopamine (DA) mesolimbic reward circuitry, and hypothalamic–pituitary–adrenal (HPA) axis and corticotrophin-releasing factor (CRF) stress systems, and has low abuse liability^[1, 2]. Our overarching hypothesis is that OT will attenuate several measures thought to drive compulsive alcohol drinking and relapse. Specifically, we will examine whether OT decreases acute stress responses, alleviates alcohol withdrawal symptoms, reduces craving and motivation to drink, and decreases alcohol self-administration. Since interactions with alcohol are an important focus of our study, we will enroll non-treatment seeking heavy drinkers with AUD in a double blind, placebo controlled inpatient protocol. Subjects will be randomized to receive intranasal OT (40 IU/dose) or placebo 3 times daily. Participants will complete alcohol detoxification; we will measure alcohol withdrawal symptoms, craving, and 24-hr urinary free CORT. Participants will then complete 3 laboratory procedures in fixed order. The Trier Social Stress Test (TSST) which includes public speaking and performance of mental arithmetic will be used to examine subjective and physiological stress responses. An alcohol motivated responding (AMR) procedure will be used to examine subjects' responding to earn either drinks or money. A cumulative alcohol-dosing (CAD) procedure will be used to examine physiological and subjective responses across several blood alcohol levels. CORT levels will also be assessed.

2. Objectives (include all primary and secondary objectives)

The primary goal of the study is to provide new information on OT efficacy across a range of different measures predictive of alcohol use and misuse, and, if OT shows efficacy, help clarify the mechanism of OT action. This research is needed to determine sample sizes for future research and will provide important preliminary evidence for the future of OT as a treatment for alcohol drinking problems. Towards this goal, we propose four specific aims:

Aim 1: Examine OT effects on severity of alcohol withdrawal symptoms, alcohol craving, and CORT during early alcohol abstinence. Hypothesis 1a: OT will reduce alcohol withdrawal symptom severity, and decrease alcohol craving, compared to placebo. Hypothesis 1b: OT will attenuate the elevation in urinary free CORT levels during early withdrawal compared to placebo.

Aim 2: Examine the effects of OT on response to social stress.

Hypothesis 2: OT will decrease CORT response and psychological responses to TSST, compared to placebo.

Aim 3: Examine the effects of OT on motivation to drink.

Hypothesis 3: OT will decrease alcohol-motivated responding and number of alcohol drinks earned, compared to placebo.

Aim 4: Examine the effects of OT on alcohol sensitivity.

Hypothesis 4a: OT will reduce the positive subjective effects of alcohol, when compared to placebo. Hypothesis 4b: OT will attenuate alcohol-induced increases in heart rate, when compared to placebo. Hypothesis 4c: OT will not produce significant side effects or adverse interactions with alcohol.

Since men and women will be enrolled, we will also explore possible sex differences in OT effects (secondary objective), which may have important treatment implications.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Stress and alcohol. Stress, generally defined as any stimulus that disrupts the body's internal balance, has long been suggested to be an important contributor to heavy alcohol drinking and relapse following a period of abstinence. Current theories suggest that changes in HPA-axis reactivity are involved in the behavioral and motivational processes associated with escalation of drinking, tolerance, dependence and withdrawal^[3]. In rats and monkeys, acute stress enhances alcohol preference and reward, and increased alcohol intake is correlated with stress-induced increases in cortisol/corticosterone (CORT)^[4-6]. In addition, with repeated social stress (e.g., defeat, low social rank, and maternal separation), rats and monkeys subsequently show greater alcohol intake when compared to non-stressed cohorts^[5-7]. CORT increases drug reward by increasing mesolimbic DA transmission^[8]. Rats self-administer CORT itself at levels similar to those elicited by stress, and intracerebroventricular infusions of CORT enhance the reinforcing effects of alcohol^[9]. During abstinence, rats previously exposed to chronic alcohol vapor escalate voluntary alcohol intake, and show persistent alcohol seeking and withdrawal symptoms^[10-12]. These effects are blocked by a glucocorticoid receptor antagonist^[12].

These findings may underlie the association between stress and alcohol consumption observed in humans. Large epidemiological studies report a variety of stressors including hazardous and demanding work environments, legal issues, family stress and low income are associated with increased alcohol consumption and binge drinking^[13-16]. People experiencing severe social stress following alcoholism treatment have higher rates of relapse compared with people not experiencing such stress^[17, 18]. Heavy alcohol drinking and AUD are associated with dysregulation of HPA-axis activity as shown by episodes of hypercortisolism between drinking bouts and a blunted cortisol (CORT) response to stress during early abstinence^[19-23]. Blunted CORT response has been

associated with increased anxiety and craving during acute abstinence and subsequent relapse to heavy drinking^[24-26]. The stress and AUD connection is well recognized by the NIAAA, as evidenced by a decade of funding of the Integrative Neuroscience Initiative on Alcoholism stress consortium and recent clinical trials of HPA-axis medications for AUD (e.g., arginine vasopressin receptor-1 antagonist). We selected OT because of its history of safety, lack of abuse liability and known effects on the HPA axis and mesolimbic reward systems.

Oxytocin and stress. In the human brain, OT receptors are primarily localized in thalamic, hypothalamic, basal ganglia and brain stem regions. OT neurons project throughout the CNS^[27]. Initially OT was thought to be primarily involved in sexual behaviors, female parturition and lactation. OT is important for a number of adaptive behavioral and physiological processes, such as the initiation and maintenance of social-attachment (mother-infant and pair bonding), memory, learning, feeding, pain, and stress responses^[28]. Studies in laboratory animals have shown that acute stress increases release of OT in blood and in hypothalamic and extra-hypothalamic brain regions^[29-32]. The increase in OT appears to be part of the homeostatic regulation of stress responses. Indeed, centrally administered OT decreases stress-induced increases in CORT^[30, 33, 34] and reduces stress-induced behaviors in rodent models of anxiety and depression^[33-36].

Investigations in human subjects are in line with the preclinical literature. When administered via the intranasal route, OT has an excellent safety profile and produces changes in autonomic arousal and mood^[37]. OT reduced CORT and psychological responses to social stress, increased positive communication during couples conflict discussions and improved recognition and processing of positive facial expressions^[38-46]. As indicated above, heavy drinking and AUD are associated with hypercortisolism during withdrawal, which may increase risk for relapse to heavy drinking^[24-26]. While the efficacy of OT in attenuating stress responses in normal healthy subjects have been shown, its effects on stress responses in persons with an AUD, in whom HPA-axis function is impaired, are unknown. The proposed study addresses this knowledge gap by evaluating OT effects in heavy drinkers with an AUD undergoing a well-established laboratory social stress procedure. If OT can reduce the negative impact of stress in this vulnerable population, this has important treatment implications particularly during alcohol withdrawal and early abstinence.

Oxytocin and addiction. There is considerable interest in the role OT may play in neuropsychiatric disorders, including substance use disorders^[47]. Importantly, the mesolimbic DA and opioid systems, which are key substrates involved in drug reward and addiction, interact with the OT system during development of tolerance and dependence^[1]. Drug tolerance and compulsive drug seeking involve associative learning and memory processes, which are also regulated by OT systems. Of high significance, recent preclinical data show that OT administration disrupts biobehavioral adaptations associated with long-term alcohol exposure^[2]. In rats, OT reduces development of rapid tolerance to the hypothermic, hypnotic, and myorelaxant effects of alcohol, and produces a prolonged decrease in withdrawal symptoms^[2, 48-53]. OT decreases alcohol preference and produces long-term suppression of alcohol self-administration in rats^[54, 55]. The potential of OT as an AUD treatment is highlighted by a recent small clinical trial in alcohol dependent subjects^[56]. All subjects underwent alcohol detoxification with PRN administration of the benzodiazepine (BZ) lorazepam and concurrent, randomized intranasal OT or placebo. Compared with placebo, OT decreased withdrawal symptoms and amount of lorazepam needed to alleviate withdrawal during detoxification^[56]. It is unknown whether OT will reduce alcohol withdrawal symptoms when administered alone and whether OT alters subjective and reinforcing alcohol effects in human subjects.

There is tremendous need for new medications for treatment of AUD. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) estimates that 1 in 5 adults currently drinks at levels that increase risk for alcohol-related problems and 1 in 3 adults will experience lifetime problems with alcohol^[57]. Only three medications (naltrexone, acamprosate and disulfiram) are approved by the Food and Drug Administration for AUD treatment. These drugs have demonstrated clinical efficacy, but effect sizes during treatment are generally modest and the majority of patients relapse to heavy drinking after medication discontinuation. In addition, the current FDA-approved medications do not address the dysfunction of HPA-axis associated with heavy alcohol use and dependence. Although naltrexone acutely activates the HPA axis, its primary mechanism for AUD treatment is attenuation of alcohol reward and reduction of alcohol craving via blockade of opioid receptors^[58]. Disulfiram inhibits alcohol metabolism via aldehyde dehydrogenase to produce aversive effects if the patient drinks alcohol^[59]. Acamprosate decreases the negative reinforcing effects of alcohol by normalizing dysregulation of GABAergic and NMDA-mediated glutamatergic neurotransmission associated with chronic heavy alcohol use^[60]. In contrast, OT treatment may help to normalize the HPA-axis and reduce stress-related physiological and subjective responses (anxiety, craving) that increase drinking and trigger relapse. Additional benefits of OT may be promotion of social behavior and disruption of learned behaviors and neuroadaptive processes associated with heavy alcohol use and attenuation of the reinforcing effects of alcohol.

OT has no known abuse liability and an excellent safety profile in clinical research settings^[37]. The utility and feasibility of intranasal OT as a medication is supported by its success in clinical trials for the treatment of behavioral deficits associated with schizophrenia, anxiety, autism, and Prader-Willi syndrome^[61-64]. The rapid delivery and onset of intranasal OT effects allows the possibility of its administration as an acute medication under conditions of stress- or cue-induced craving. Examination of OT effects under controlled laboratory conditions is the first necessary step in developing OT for treatment of AUD, and will address important knowledge gaps. We propose to use multiple, well validated laboratory procedures and outcome measures to determine OT efficacy across a range of different measures associated with alcohol use and misuse. We will examine whether OT alleviates alcohol withdrawal symptoms and craving (Aim 1), alters subjective and physiological responses to social stress (Aim 2) and to alcohol intoxication (Aim 4), and reduces the motivation to drink after brief abstinence (Aim 3). Our findings will help clarify the mechanism of OT action (i.e., anti-stress effects vs. alleviating withdrawal vs. reward). By balancing treatment groups for gender, we can evaluate possible gender differences, which may have important treatment implications and influence future study designs and use of OT. Importantly, this study will establish effect sizes as the basis for future research. For example, if the current study demonstrates efficacy of OT on craving and motivation to drink, we would propose an outpatient clinical trial in which OT could be self-administered to reduce urges to drink. If OT is efficacious for alleviating withdrawal, we would propose an inpatient clinical trial to fully examine its utility in a broader sample.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Subjects: Healthy, non-treatment seeking, 21-50 years old, heavy drinking, male and female subjects will be recruited through the media. Minority subjects will be recruited in proportion to their representation in the Baltimore metropolitan area. Subjects will complete initial screening for eligibility by telephone or online. Subjects who are eligible following the phone screen or online

screen will provide informed consent and complete an in-person assessment using a standard battery of instruments (see **Table 1**). The Alcohol Use Disorders Identification Test (AUDIT) and the Mini International Neuropsychiatric Interview (M.I.N.I.)^[65], Version 7 for DSV-V will be used to assess the presence or absence of alcohol or other substance use disorders, and mood and anxiety disorders for study eligibility. The pattern and magnitude of drinking will be characterized using the 90-day Time Line Follow Back (TLFB) assessment^[66]. Subjects must meet DSM-V criteria for AUD, and be actively drinking >14 drinks/week for women and >21 drinks/week for men, with at least 2 heavy drinking days (>4 drinks/day for women, and >5 drinks/day for men) during a consecutive 30-day period on the 90-day TLFB (inclusion criteria). In addition to subject self-report of drinking on TLFB, levels of phosphatidylethanol (PEth) in blood will be used as a biomarker of recent drinking at assessment. Its formation in blood is specific to ethanol, is highly correlated with magnitude of alcohol consumption (g/day)^[67], and is detectable for 2 weeks or longer^[68]. We will use the USDTL (United States Drug Testing Laboratories) threshold of 8ng/mL, to verify alcohol drinking. The MINI for Tobacco Use Disorder (TUD) and the Fagerström Test for Nicotine Dependence (FTND)^[69] will be used to determine presence of TUD and severity of nicotine dependence symptoms, respectively. Subjects will undergo a medical history and physical examination, administration of the Clinical Institute Withdrawal Assessment Alcohol Revised (CIWA-Ar)^[70], and standard laboratory tests (complete blood count, comprehensive metabolic panel, and urinalysis).

Medical assessment results will be reviewed by Dr. Wand, the study physician, and persons in whom study participation is contraindicated will be excluded. Psychological and substance use interview results will be reviewed by Dr. McCaul, a licensed psychologist. We typically consent and screen 4 persons for each subject who meets study eligibility criteria. Inclusion and exclusion criteria are detailed in section 5. It is established that cycle phase can influence HPA-axis reactivity to social stress^[71, 72]. In females, cycle phase will be determined via menstrual diary. Ideally, women will be scheduled to complete procedures during their luteal phase. We recognize that heavy drinking can disrupt cycle regularity, and will therefor confirm cycle phase via progesterone level (gold standard), and will include this as a covariate in our analyses.

Subjects will be randomized to treatment groups (OT or placebo), matching for age, gender, and nicotine dependence status (as defined by FNDT score) by Johns Hopkins Investigational Drug Services (IDS). The FNDT score determined at assessment will be used to categorize participants according to nicotine dependence levels as defined by the instrument (Total Score of 0-2 = No Dependence; 3-5 = Moderate Dependence and 6-9 = Substantial Dependence) and provided to IDS for randomization.

Table 1: Assessment instruments:
The Alcohol Use Disorders Identification Test (AUDIT)
MINI for DSM-V
MINI Tobacco Use Disorder
Health Checklist
CIWA-Ar
90-day Time-line Follow-back (TLFB)
Shipley Institute of Living
REALM (if Shipley below score of 18)
Fagerström Nicotine Dependence Test
Spielberger State-Trait Anxiety Inventory (STAI)

Beck Depression Inventory (BDI- II)
Alcohol Dependence Scale (ADS)
Short Profile of Moods State (POMS)
Menstrual Cycle Questionnaire (Females only)

Phosphatidylethanol (PEth). A small blood sample will be collected via finger stick for the BloodSpot® collection test (US Drug Testing Laboratory, Des Plaines, IL). We selected dried blood spot vs. whole blood assays as it reduces subject risk associated with venipuncture and PEth is more stable in dried blood samples than in whole blood^[73, 74]. PEth provides a biomarker for heavy alcohol consumption due to its high specificity and slow degradation rate^[68, 75]. Participants must have a positive PEth (≥ 8 ng/mL) at assessment for study inclusion.

Design Overview. This study will be completed as a 6-day inpatient protocol on the Johns Hopkins Hospital and/or Bayview Clinical Research Units (CRU). The sequence and timing of study procedures is shown below. Male (accrual n=12) and female (accrual n=12) participants will be randomized to receive intranasal OT (40 IU/dose) or placebo three times daily. During the CRU stay, participants will complete self-report assessments of alcohol craving, anxiety and depression; nursing staff will administer the CIWA-Ar to evaluate withdrawal symptom severity. Full descriptions for OT administration and assessment instruments are below. During the first 3 days, participants will undergo alcohol abstinence during which 24-hr urinary free cortisol (UFC) will be collected for the first 48 hours. Participants will then complete 3 laboratory procedures in fixed order. A urine toxicology screen, a breath alcohol test, and urine pregnancy test (women) are completed prior to all procedures. Subjects will be terminated from this protocol if they have positive drug toxicology tests (excluding THC) or a positive pregnancy test (females). On procedure days, subjects receive a calorie-controlled breakfast and lunch to control for dietary effects on alcohol absorption or stress response. On day 4, a Trier Social Stress Test (TSST), which includes performance of mental arithmetic and public speaking, will be used to examine subjective and physiological responses to stress. On day 5, an Alcohol Motivated Responding (AMR) procedure, in which subjects respond on the computer to earn either drinks or money and then have the opportunity to self-administer earned drinks, will be used to determine OT effects on motivation to drink and alcohol self-administration. On day 6, a Cumulative Alcohol Dosing (CAD) procedure will be used to examine physiological and subjective responses to fixed-dose alcohol administration. The TSST, AMR and CAD laboratory sessions will start at 1 pm, 30 min after the mid-day OT or placebo administration since OT reaches peak levels in plasma 30-40 min after intranasal administration^[76-78]. On day 7, subjects will have a brief intervention for their hazardous drinking before discharge.

Subjects who smoke cigarettes or other tobacco products will not be allowed to smoke at any time during the JHH CRU stay. Current smokers on the JHH CRU will be provided with a transdermal nicotine patch (21 mg) at admission and each morning to prevent the onset of nicotine withdrawal; smokers will be patched throughout their JHH CRU stay. Participants residing on the Bayview CRU will be allowed to smoke ad lib in the designated smoking room in the facility. Cigarettes will be held at the nursing station to prevent unauthorized smoking in the participant's bedroom. They will have the option to receive a transdermal patch (21 mg) on the days that they are transported to the JHH campus to complete study procedures.

All session procedures (TSST, ARM and CAD) will be completed in our research office (550 Building). Subjects staying at the JHH CRU will transfer to the Bayview CRU after the TSST for

the remainder of the study. Subjects will be transported via car service to the 550 Building for sessions, then return to the Bayview CRU. The sequence of conditions is:

- Day 1: Admission to Bayview CRU (or JHH CRU)*
** Subjects will be only be admitted to JHH Bayview if they meet defined parameters (see definition below)*
- Days 1-6: Intranasal self-administration of OT (40IU/dose) or placebo three times daily under nursing supervision.
Completion of daily self-report measures three times daily
- Days 1 - 2: Monitored alcohol abstinence
Staff administered CIWA-Ar every four hours while awake*
24-hr collection of urinary free cortisol
Practice AMR session procedures
** symptom triggered benzodiazepine treatment (see below)*
- Day 4: Session 1: Trier social stress test (TSST)
** JHH CRU participants transfer to the Bayview CRU (after TSST session) for the remainder of the study*
- Day 5: Session 2: Alcohol motivated responding (ARM) procedure
- Day 6: Session 3: Cumulative alcohol dosing (CAD) procedure
- Day 7: Brief Intervention for heavy drinking and CRU discharge

CRU admission criteria. Since persons at greatest risk for alcohol withdrawal complications will have been ruled out prior to study inclusion (e.g., those with prior history of withdrawal-related seizures, or serious alcohol withdrawal symptoms based on medical history or current CIWA score at the time of assessment see Section 5, Exclusion Criteria), we anticipate that most participants will be admitted to the Bayview CRU. Specifically, subjects will be admitted to Bayview CRU if they meet ALL of below criteria at assessment:

- 1) *No adult seizure history;*
- 2) *No serious alcohol withdrawal complications in prior alcohol withdrawal episodes;*
- 3) *Some non-drinking days on 90-day TLFB;*
- 4) *CIWA-Ar < 9 at time of assessment;*
- 5) *Study Physician and Investigator recommendations*

Since the JHH CRU and Bayview CRU offer different levels of care, subjects will be placed into the appropriate CRU, based on their withdrawal history and pattern of recent drinking. The Bayview CRU is primarily a domiciliary unit with nursing care. The JHH CRU is a licensed inpatient facility with full 24-hr medical staff coverage. Since subjects who do not meet the above criteria are at greater risk for complications associated with alcohol withdrawal, they will be admitted to the JHH CRU for clinical management of alcohol withdrawal.

Alcohol abstinence. For the first 24 hours, all subjects will receive an intravenous line with D5 NS 1000ml with MVI adult Inj 10ml, thiamine Inj 100mg, Folic acid Inj 1mg, Magnesium sulfate Inj max of 2g and infused at 84 ml/hour. Subjects will have PRN access to ibuprofen 400mg q 4hours, Maalox 30 ml po q 8hours. If systolic blood pressure is greater than 180 and/or diastolic blood

pressure greater than 105, atenolol 25 mg bid will be initiated. For all participants, the Clinical Institute Withdrawal Assessment Alcohol Revised (CIWA-Ar)^[70], which includes 10-items (nausea/vomiting, tremor, paroxysmal sweats, anxiety, agitation, tactile disturbances, auditory disturbances and visual disturbances) will be completed every 4 hours during waking hours. This well validated clinical tool is administered by a nurse, to assess severity of withdrawal symptoms during detoxification, and is the gold standard to direct use of benzodiazepine medication^[79, 80]. CIWA-Ar scores of 8 points or fewer correspond to mild withdrawal, scores of 9 to 15 points correspond to moderate withdrawal, and scores of greater than 15 points correspond to severe withdrawal symptoms. The benzodiazepine lorazepam will be administered as needed using the symptom-triggered method^[80-82]. Specifically, if CIWA-Ar score is ≥ 12 the subject is given a 2mg dose of lorazepam intravenously (IV), the study physician is notified, and CIWA-Ar scores are then repeated 1 hour later. If the score remains ≥ 10 then another 2 mg dose of lorazepam is given. Hourly CIWA-Ar scores and lorazepam treatment are repeated until CIWA-Ar score decreases to less than 9 or until a maximum of 4 doses of 2 mg lorazepam are administered; if CIWA is 9 or higher after 4 doses, subject will be removed from the study. Subjects will also be removed from the protocol for seizures, hallucinations, or disorientation. At that point in time, the physician on call will be contacted who will arrange for transfer to the medical service. The subject is now terminated from the study. Individuals who require benzodiazepine treatment and are successfully treated with lorazepam will remain in the study to complete the 3-day abstinence period, and then will be discontinued from further participation to avoid potential complications and confounds of treatment with benzodiazepines. Discontinued subjects would complete the brief intervention prior to early discharge.

Urinary free cortisol measurement. All subjects will provide urine samples for urinary free CORT (UFC), as a measure of withdrawal-related stress response. During the CRU stay, all urine voided per 24-hrs for the first 48 hours is collected in containers containing boric acid as a preservative as in our previous study^[19]; an aliquot is frozen (-70°C) for analysis of UFC via Liquid Chromatography, Tandem Mass Spectrometry (LC/MS/MS) by Quest Diagnostics Nichols Inst. San Juan Capistrano. The coefficient of variance is 5% and 7% for the intra-assay and the inter-assay, respectively.

Oxytocin (OT) administration. OT (syntocinon, Novartis) 40 IU/ml, Pharmaworld, Zurich, Switzerland) and placebo (generic saline nasal spray, various vendors) will be transferred by JHH IDS in identical sterile metered-dose nasal spray bottles (Lukas-Bottles, 6-ml) to deliver 0.1 mL/spray. Pharmaworld has provided oxytocin for numerous clinical trials in the US, including prior studies by Dr. Lee (Co-I). Syntocinon previously was an FDA-approved product (NDA 012285) used medically for labor induction, but was discontinued. It is no longer marketed by Novartis. Oxytocin will be used in these studies under FDA-approval via IND 110,562, which held by our collaborator Mary Lee.

Johns Hopkins Hospital Investigational Drug Services will maintain and dispense spray bottles per study randomization schedule. Subjects will self-administer 5 sprays per nostril, 30s apart, of placebo or OT (4 IU/spray x 10 sprays = 40IU total dose) 3-times/day under nurse supervision (e.g., 7 am, 12:30 pm, 7 pm).

Daily self-report measures. The CRU nurses and research staff will insure completion of self-report instruments for alcohol craving (visual analog scale, VAS^[83]; Alcohol Urges Questionnaire^[84]), POMS short ^[85, 86] and medication side effects using a modified version of the SAFTEE^[87]. Alcohol craving, a new diagnostic criterion for AUD in the DSM-V, is a primary trigger for relapse.

Reductions in craving are strongly associated with better treatment outcomes for behavioral interventions and AUD medications^[88-90].

Trier Social Stress Test (TSST). On day 4, subjects will complete the TSST. We selected the TSST as it is a well-validated procedure for induction of stress responses in the laboratory setting in a safe manner without inducing serious mental or physical distress^[91]. It stimulates a more robust CORT response when compared to other validated stress tests (e.g. guided imagery^[92, 93] or Paced Auditory Serial Addition Test^[94]). The TSST consists of a 5-min public speaking component and a 5-min oral mental arithmetic component completed in front of a panel of 2 people (confederates). Subjects are told the session will be videotaped and their performance will be rated for speech content, speed and accuracy of their mathematical performance when compared to other TSST completers. Mood, craving and anxiety self-report measures are completed before, during and after the session.

For the public speaking component, the subject is seated in a chair facing a table. Two chairs to seat the test panel are placed at the opposite end of the table. The subject is asked to sit quietly, relax, and await instructions for the test protocol. During this 20-minute waiting period, saliva samples are collected for baseline cortisol measurement. Following baseline sampling, the subject is told to listen carefully to the taped instructions for the first task, the job interview. He/she is told that they are interviewing for the position of a hospital administrator and that in a 5-minute speech he/she should convince the panel that he/she is the best candidate for the job. They are told that they must maintain eye contact with the panel throughout the interview. He/she is given 10 minutes to mentally prepare for the interview. The test period begins (Time 0) when a two-member panel (confederates) is seated across the table from the subject. During the session, one of the confederates pretends to be filming the subject with a video camera. In actuality, the camera has no tape. Following the speech, subjects are given instructions for the mental arithmetic test.

For the mental arithmetic test, the subject is told to repeat a four-digit number after the tester, subtract 13 from it, and call out the answer. The subject continues subtracting and calling out answers for 1 min. A new number is introduced every minute for a total of 5 minutes. Throughout this challenge, the tester distracts the subject by commenting on the speed and accuracy of his/her responses and urges the subject to look at the tester at all times. Following all study procedures, subjects are debriefed about the speech task. They are informed that the video camera did not contain film, that their performance was not actually being rated.

Salivary CORT will be collected at three times before the TSST (-30, -15 and 0 minutes for baseline), immediately after the TSST and every 10 minutes for 120 minutes. Salivary CORT was selected to avoid stress of IV placement and blood draws. Unlike serum CORT, salivary CORT is unbound to protein and reflects the physiologically relevant fraction. For each sample, subjects chew a pad for 1 min, which is then placed into a salivette. Saliva is harvested by centrifugation, and stored in a cryotube at -70 until assayed in duplicate (Diagnostic Systems Laboratories; Webster, Texas). The intra- and inter-assay coefficients of variation are <5%. The POMS Tension and Anxiety (POMS-TA) subscale will be administered before and after the TSST to capture perceived stress. We have extensive experience with the TSST and have used it to examine differences in HPA-axis response associated with gender, AUD, family history, and several gene variants^[19, 71, 95-98].

Alcohol Motivated Responding (AMR). On day 5, subjects will complete the AMR procedure. The AMR utilizes a progressive ratio schedule of reinforcement in which the number of required responses increases for each successive reinforcer, until the person fails to complete the requirement

(breaking point) or the session ends. The maximum response ratio completed that resulted in reinforcement provides a quantifiable measure of motivation to drink. A second reinforcer (\$1 per unit) is included to permit examination of relative rates and distribution of responses between the two reinforcers. Using the NIAAA definition of a standard drink unit (SDU, 14 g alcohol), each alcohol reinforcer equals 0.5 SDU (7 g alcohol). Alcohol content was standardized to 0.5 SDU to ensure equivalency across subjects for amount of alcohol. A maximum of 10 reinforcers (money and alcohol) is available. The response ratio requirement begins at 400 and increases by a factor of 1.2 (400, 480, 576, 692, 830, 995, 1194, 1433, 1720, 2064). We will administer a priming drink (0.5 standard drink of alcohol (liquor type is based on subject preference) immediately before the AMR. This drink volume is sufficient to increase craving or desire to drink in heavy drinkers without producing a significant increase in blood alcohol levels or interfering with responding under the PR procedure. Participants will complete self-report measures of alcohol craving and alcohol effects following drink completion, and then begin the AMR session.

The AMR session will terminate after 60 min, or after no response occurs for 10 min, whichever occurs first. Alcohol is not available for consumption until 60 min elapses. After 60 min, participants receive money vouchers and self-administer earned drinks of their preferred liquor (vodka or whiskey). Subjects will be allowed up to 90 minutes to finish drinking; Staff will provide drinks paced to ensure that subjects cannot exceed 0.5 SDU every 5 min or all earned drinks (5 SDU maximum) in a 50-minute period. A preferred mixer and snacks is provided. Using this procedure, we have established a baseline in which high levels of responding were maintained. Subjects distribute responses to earn about half of available alcohol drinks on average. We targeted this distribution to 1) avoid a floor effect in which response cost is too high, and subjects stop responding, 2) prevent a ceiling effect in which all 10 drinks are obtained, and 3) allow detection of either increases or decreases in drinks during OT treatment.

Cumulative Alcohol Dosing (CAD). On day 6, subjects will complete a CAD procedure in which 1 placebo and 3 active alcohol drinks are administered at timed intervals to increase BAL progressively (0.03-0.1%) Subject doses will be determined using a Computerized Blood Alcohol Calculator^[99] which adjusts for age, height and gender differences in body water and time spent drinking, to target similar BAL in males and females. IDS will prepare each 120 mL drink by mixing the appropriate mL of 95% ethanol in a sweet beverage using a w/v metric. The placebo drink is blinded by floating 1 mL ethanol on top of the drink and by placing an ethanol-soaked wristband around the glass to deliver a strong alcohol odor. Active alcohol drinks are prepared the same. Staff monitor subjects and ensure consumption of each drink is paced over 10 min. Subjects will complete a computerized subjective report battery at baseline (-30 minutes) and every 30-min. The 10-min computerized battery is shown in **Table 3**. The placebo drink will be administered at time 0, followed by active alcohol drinks at 30 min intervals. Breath alcohol levels (BAL) will be determined at baseline and 15 min after each drink. Heart rate, skin conductance, skin temperature and blood pressure are recorded via a noninvasive monitor. Subjects return to the CRU for monitoring, and complete the battery and an Alcohol Hangover Scale^[100] every hour for 4 hours post session and once the next morning. We have safely administered alcohol in social and heavy drinkers^[83, 100-102], and demonstrated reliable alcohol dose-related increases in heart rate and subjective effects; these effects are sensitive to AUD medications (naltrexone, acamprosate)^[103-106]. The CAD was chosen to reduce subject research burden and dropout rates.

Table 3: Computerized battery of subjective report measures for CAD session

Drug Effect Visual Analog Scale (VAS) includes the following questions: "Do you feel ANY effect of the drink(s)?," "Do you feel GOOD effects from the drink(s)?," "Do you feel BAD effects of the drink(s)?," "Do you LIKE the effects of the drink(s)?," "Do you DISLIKE the effects of the drink(s)?," "Is this the WORST you have ever felt?," and "Is this the BEST you have ever felt?,". Subjects respond by positioning an arrow on a 100-point line anchored by "not at all" and "extremely."

Tiffany Brief Alcohol Craving Scale is based on a questionnaire developed by Cox, Tiffany and Cristen^[107] on which subjects rate their alcohol craving. Subjects rate each item on a scale from 0 (not at all) to 10 (extremely or very strong, depending on the question). Questions include items such as "How badly would you like an alcoholic drink right now?" and "Rate your desire to drink alcohol."

Biphasic Alcohol Effects Scale (BAES)^[108] is composed of fourteen items measuring stimulant (elated, energized, excited, stimulated, talkative, up, vigorous) and sedative (difficulty concentrating, down, heavy head, inactive, sedated, slow thoughts, sluggish) effects of alcohol. Items are presented one at a time in alphabetical order. Subjects rate each item on a scale from 1 (not at all) to 9 (extremely).

Subjective High Assessment Scale (SHAS)^[109] subjects rate alcohol effects by positioning an arrow on a 100-point line ranging from "normal" (0) to "extremely" (100) Subjects are told to assume their ratings were "normal" before they received a beverage. Items are uncomfortable, high, clumsy, muddled or confused, slurred speech, dizzy, nauseated, drunk or intoxicated, sleepy, distorted sense of time, effects of alcohol or drug, difficulty concentrating, feelings of floating, the worst I've ever felt, and the best I've ever felt.

Alcohol Hangover Scale^[100] is composed of ten symptoms (sweaty, loss of appetite, shaky, trouble concentrating, racing heart, anxious, alcohol craving, tired, restless and irritable). Each item is rated on an analog scale marked at opposite ends with "not at all" and "most ever."

Profile of Moods state tension and anxiety (POMS-TA) subscale^[85, 110] is composed of a list of feelings (tense, shaky, on edge, panicky, uneasy, restless, nervous, anxious, relaxed). Subjects rate each item for how they are feeling on a 4 point scale ranging from 0 (not at all) to 4 (extremely)

Brief Intervention: Prior to discharge on day 7, participants will receive a brief intervention that addresses their heavy drinking and any alcohol-related problems. The intervention will be delivered by one of the Master's-level research staff or a faculty member (Dr. McCaul, or Dr. Alvanzo). It will follow the NIAAA guidelines from Helping Patients Who Drink Too Much, including treatment referral as requested.

b. Study duration and number of study visits required of research participants.

Subjects have a maximum of 6 months to complete the entire study. We anticipate 2 visits to complete the protocol. This includes an in-person assessment (visit1) and a 6-day inpatient stay (visit 2). Additional 1 or 2 visits may be scheduled if labs at assessment need to be repeated or procedures are rescheduled due to study complications.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Alcohol and OT are administered under placebo controlled blind conditions. Blinding is done to control for expectancy effects.

Justification of why participants will not receive routine care or will have current therapy stopped.

N/A. The subjects are healthy volunteer participants.

c. Justification for inclusion of a placebo or non-treatment group.

Blinding is done to control for expectancy effects for the best test of medication efficacy.

d. Definition of treatment failure or participant removal criteria.

Subjects will be terminated from this protocol if they have positive drug toxicology test (excluding THC) or a positive pregnancy test (females) at any time during the study. Subjects may also be terminated from this protocol if they do not comply with CRU rules or study procedures; they will first be warned of possible dismissal and, if noncompliance persists, will be terminated from the protocol. Subject participation may also be terminated based on safety issues.

Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Subjects terminated prematurely from this protocol will be paid for procedures completed prior to termination. We will also attempt to complete the brief intervention interview for participants prior to CRU discharge. Subjects who complete the study will receive payment for all procedures completed plus the study bonus.

5. Inclusion/Exclusion Criteria

a. Inclusion Criteria:

- Healthy 21-50 years old male and female subjects
- Must meet DSM-V criteria for AUD and not be seeking treatment
- Actively drinking >14 drinks/week for women and >21 drinks/week for men, with at least 2 heavy drinking days (>4 drinks/day for women, and >5 drinks/day for men) for a consecutive 30-day period in the last 90 days from assessment (Time Line Follow Back, TLFB)
- Positive blood PEth (≥ 8 ng/mL)

b. Exclusion Criteria:

- Current DSM-V major current mood or anxiety disorder or drug use disorder (excluding alcohol and nicotine; excluding mild cannabis use disorder); in or in need of treatment
- Moderate to severe cannabis use disorder
- Drug use in last 30 days and/or positive urine toxicology screens (excluding marijuana/THC)
- History of seizure disorder or closed head trauma

- History of withdrawal-related seizures or serious alcohol withdrawal symptoms (CIWA ≥ 12)
- HIV positive
- Neuroendocrine disorder
- Any serious medical condition that would place subject at risk or interfere with study participation
- Liver function tests $> 3X$ times normal at screening
- Shipley vocabulary score < 18 and REALM score below 19, corresponding to 5th grade reading level
- Prescription medications in last 3 months that could affect CNS or HPA axis function
- Women who are pregnant, nursing or planning pregnancy cannot participate

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Oxytocin/syntonocin: We chose 40 IU to maximize the likelihood of observing OT effects based on evidence of increased cerebrospinal fluid levels after intranasal administration of OT^[111] and vasopressin (a similar peptide to OT), and prior studies showing efficacy of 40 IU OT^[38, 42, 112-114]. Doses as high as 320 IU/day OT have been safely administered, without adverse events^[115, 116]. OT has no known abuse liability and has an excellent safety profile in clinical research settings^[37]. The utility and feasibility of intranasal OT as a medication is supported by its success in clinical trials for the treatment of behavioral deficits associated HPA-axis dysfunction including schizophrenia, anxiety, autism, and Prader-Willi syndrome^[61-64].

Ethyl Alcohol/Alcohol: Alcoholic beverages are legally sold to adults. The dose of alcohol used in the CAD was selected to bring blood ethanol levels to approximately 0.1% was determined based on our previous studies and a comprehensive literature on administering alcohol to research subjects. This BAC corresponds to about 1 g/kg alcohol. The drink is prepared by IDS using 190 Proof ethanol (95%v/v, 0.775 g/ml Spectrum Chemical); the total alcohol dose is divided over three drinks, prepared by mixing the appropriate amount of pure ethanol with a sweet beverage. Using standardized drinking units, 1 (e.g. (One standard drink is equivalent to 12 ounces of beer, 5 ounces of wine, or 1.5 ounces of 80-proof spirits.), this amount is about 4-5 drinks for men and about 3-4 drinks for women when consumed in over 2-hour drinking episode. This amount is within the limits of the typical drinking episode of participants, and are not expected to produce significant adverse events.

After the AMR, subjects may consume up to up to 5 standard drinks (70g ethyl alcohol) of an alcoholic beverage of their choice (e.g., beer, wine or liquor) during the alcohol self-administration session, and drinking is paced. These amounts are within the limits of the typical drinking episode of participants and also are sufficiently large to promote alcohol-motivated responding.

Use of intranasal OT and alcohol will be under FDA-approval via IND 110,562 (holder Mary Lee, MD, Co-I, *amendment submitted to FDA 8/4/14*). Amendment application is uploaded to eIRB protocol.

Nicotine Patch: Active nicotine patch (21 mg) is the standard of care for management of nicotine withdrawal.

- b. **Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.**

n/a

- c. **Justification and safety information if non-FDA approved drugs without an IND will be administered.**

n/a/

7. Study Statistics

a. Primary Outcome variables:

- Peak scores for CIWA-Ar and Craving Scales for abstinence days 1-3.
- Total and subscale scores on POMs Short at assessment and during the CRU stay
- UFC for abstinence days 1-3
- Peak salivary CORT in the TSST
- Number of drinks earned in the AMR
- Peak drug effect VAS scores and peak increase in heart beats per minute (bpm) in the CAD

b. Secondary Outcome variables:

- Stimulation and sedation subscales of the BAES in the CAD
- Total score of the SHAS in the CAD
- Total scores for the tension and anxiety subscale of the POMS at assessment, during the TSST, and during withdrawal
- Side effects reported on the SAFTEE during the CRU stay

c. Statistical plan including sample size justification and interim data analysis:

In AUD treatment research, a moderate effect of 30-40% improvement is typical. The primary objective of this protocol is to generate estimates of effect size and variance between the OT and placebo treatment groups that could be used to calculate sample size for a larger scale, Phase III study. We have effect sizes for some of our variables using data drawn from the literature and our prior studies. For our power analyses we set type I error (α) = 0.05 where power ($1-\beta$) = 0.8 for the specified effect size for each outcome measure between the OT and placebo treatment groups (n=12 per group, 24 total). Data from prior small trial^[56] showed the OT group (n=7) had 3-fold lower CIWA-Ar scores and a 40% decrease in craving vs. the placebo group (n=4) on day 1. In our prior study in AUD subjects (n=25)^[117], the mean (SD) peak alcohol craving on day 1 of abstinence was 23.4 (7.9). We have power (0.89) to detect a 40% difference in CIWA-Ar and craving between treatment groups (Aim 1). In a previous study, OT reduced stress-induced salivary CORT by 30%^[38]. Based on our TSST data (n=414), with mean (SD) peak salivary CORT of 0.48 (0.46), we will have power (0.8) to detect a 30% difference in CORT between treatment groups (Aim 2). In our pilot study (n=9), subjects drank an average of 3.1 (1.9 SD) drinks, although the 3 drinkers most similar to those in the proposed study drank a

mean of 5 drinks (2.7 SD). We have power (>0.8) to detect a 30% difference in drinks between groups (Aim 3). In our preliminary data ($n=101$), mean (SD) was 118 (62.8) for VAS scores and +13(6.6) for bpm. We have power >0.8 to detect a 40% reduction in peak VAS and bpm (Aim 4). Thus, our sample size of 24 subjects should be sufficient to detect differences between treatment groups, assuming a moderate effect of OT.

The outcome variables for each Aim are listed above. They are all continuous measures. We will first exam the normality by plotting histograms and Shapiro-Wilks tests. The need for transformations and/or use of non-parametric analysis will be determined. In each aim, we compare the outcomes between two groups (OT vs. Placebo), so the statistical analyses will be similar across aims. We will run two-sample t-tests or a non-parametric equivalent method to test if the outcomes are different between the OT-treated and placebo-treated groups. Randomized treatment groups were matched for age, gender and FNDT score; we will evaluate for possible unbalance between the two groups for other baseline measures (e.g., AUD, drinking severity). For each outcome variable, we will construct an ANCOVA model. Potential confounding factors (e.g., gender, mean drinks/week) will be added as covariates to the models, based on sensitivity analysis. We will also complete exploratory analyses of the effects other assessment instruments and alcohol measures (secondary outcome measures) on treatment outcomes.

d. Early stopping rules.

Participants with significant alcohol withdrawal symptoms as indicated by a CIWA-Ar score ≥ 12 , will be treated with benzodiazepines. Individuals who require 4 lorazepam doses (2mg) in and do not show improvement in symptoms (CIWA= 9 or higher) will be removed from the study. Subjects will also be removed from the protocol for seizures, hallucinations, or disorientation. Other individuals in need of benzodiazepine treatment or upon physician recommendation will end participation after completion of the 3-day withdrawal phase; subjects will be discharged pending approval of a physician on day 4 and will not complete the laboratory sessions. Subjects also may be terminated from this protocol if they do not comply with CRU rules or study procedures. They will first be warned of possible dismissal and, if noncompliance persists, will be terminated from the protocol.

Subjects will also be discontinued from the protocol for positive pregnancy test (females) or positive drug toxicology tests (excluding THC).

8. Risks

- a. ***Medical risks, listing all procedures, their major and minor risks and expected frequency.***
Subjects will be carefully screened and fully informed of study procedures and risks prior to participation in the study. This study involves several separate procedures, each of which entails some risk of discomfort or side-effects. These risks are discussed by procedure. Participants will receive a thorough description of all potential medical risks in the consent form.
- i. ***Assessment procedures:*** The major disadvantage is the time taken to complete study instruments and questionnaires. Our experience with these evaluations indicates they are acceptable to patients. This study involves questions about dangerous or illegal behavior, psychiatric history, a medical history, and a physical exam. There is a small risk that

participants will become upset during the assessment interview. There also is a risk of breach of confidentiality if the responses were to be disclosed.

- ii. *Oxytocin Administration:* The use of oxytocin in these studies will be under FDA-approval via IND 110,562; this IND is held by Dr. Mary R. Lee, who is a collaborator on the study. While many studies have used 24 IU OT^[37], we chose the 40 IU dose to maximize the likelihood of observing OT effects based on evidence of increases in cerebrospinal fluid levels for vasopressin, a similar peptide to oxytocin^[112], and use of the 40IU dose in prior cognition and stress studies^[38, 42, 113], including a recent study by Dr. Lee^[114]. It has been used in many clinical trials for other medical purposes and only a few people experienced any side effects (nasal irritation, runny nose, sleepiness, light-headedness, euphoria, stomach pain, anxiousness, or headache). Based on both Dr. Lee's experience and as cited in the literature, we expect the side effects for OT to be minimal. For example, a recent meta-analysis of the safety and side effects of intranasal OT drawn from 38 randomized trials determined OT produces no reliable side effects, and is not associated with adverse outcomes when delivered in 18-40 IU/dose for short-term use in controlled research^[37]. In addition, doses as high as 320 IU/day OT have been safely administered to human subjects, without report of adverse events^[115, 116].
- iii. *Blood collection:* Blood draw procedures involve minimal risks, such as a slight risk of discomfort at the intravenous site. A small amount of bleeding under the skin will produce a bruise in about 5% of cases. The risk of temporary clotting of the vein is about 1%. The risk of infection or significant blood loss is less than 1 in 1000. In rare cases, fainting could occur. For PEth, a small blood sample will be collected via finger stick for the BloodSpot® collection test (US Drug Testing Laboratory, Des Plaines, IL). We selected dried blood spot vs. whole blood assays as it reduces subject risk associated with venipuncture and PEth is more stable in dried blood samples than in whole blood^[73, 74].
- iv. *Urine Collection:* During the CRU stay, all urine voided during the first 48 hours of the CRU stay is collected. There is no risk associated with urine collection, but subjects may find this inconvenient.
- v. *Medically supervised alcohol withdrawal and monitored abstinence:* Eligible participants will undergo monitored alcohol abstinence and alcohol withdrawal on the CRU. There is a risk of complications (e.g., elevated BP, seizures) during alcohol detoxification; treatment with atenolol and/or lorazepam will be provided if needed, and as detailed below. To reduce risk, applicants with a history of withdrawal-related seizures or other serious alcohol withdrawal symptoms will be excluded from participation. We have been safely using this procedure under IRB approval for over 15 years.
 - a. *Atenolol.* Participants that have elevated blood pressure that are above safe limits (systolic blood pressure >180 and/or diastolic blood pressure >105) will be given atenolol (25 mg BID), which is approved by the Food and Drug Administration for treatment of hypertension. The most common symptoms of atenolol include dizziness, lightheadedness, tiredness, drowsiness, depression, nausea and diarrhea. Less common but more serious side-effects include: shortness of breath, and swelling of the hands, feet, ankles or lower legs.
 - b. *Lorazepam.* Participants that experience serious withdrawal symptoms as determined by CIWA scores ≥ 12 or physician recommendations, will receive 2 mg lorazepam

using the system triggered method, and additional monitoring of symptoms. A maximum of 4 doses (2 mg IV) will be administered. Lorazepam has been approved by the Food and Drug Administration for treating alcohol withdrawal in this manner. The most common side effects of lorazepam are drowsiness, fatigue and ataxia (decreased muscle control). Other less common side effects include: confusion, depression, headache, hypoactivity, slurred speech, feeling light-headed or dizzy, constipation, nausea, incontinence, changes in libido, urinary retention, slower pulse, cardiovascular problems, low blood pressure, blurred vision or other visual disturbances, blood clots and skin rash. Participants who receive lorazepam will stay in the study to complete alcohol abstinence and withdrawal monitoring only if symptoms are controlled ($CIWA < 9$), but will not complete the laboratory sessions; participation will end on day 4 of the CRU stay.

- vi. *Nicotine Patch*: Nicotine dependent subjects will be administered a nicotine patch at the time of JHH CRU admission and every morning for the duration of the study. The nicotine patch dose selected for study in this protocol is FDA-approved and has been shown to have a low incidence rate of serious side-effects or adverse events in clinical trials with nicotine-dependent patients. Less than 5% of smokers have to stop using a nicotine replacement product because of side effects. Side effects of nicotine patches may include: skin rash at the location of the patch; sleep problems when using a 24-hour patch, such as having trouble sleeping or having especially vivid dreams. On rare occasions, there have been reports of severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); fast or irregular heartbeat; mouth, teeth, or jaw problems; pounding in the chest; severe diarrhea, dizziness, nausea, vomiting, or weakness.
- vii. *Trier Social Stress Test (TSST)*: Subjects will participate in the Trier Social Stress Test as part of the experimental protocol. This test includes public speaking and mental arithmetic components, which have been shown to elevate a variety of physiological measures, including neuroendocrine and autonomic variables. There is a small risk that clinically significant symptoms of anxiety or grossly elevated autonomic reactivity may occur. We have completed the Trier Stress Test (TSST) in over 400 subjects without inducing serious mental or physical distress. This includes completing the TSST in 27 alcohol dependent subjects. Participants are monitored throughout the procedure.
- viii. *Alcohol procedures (AMR and CAD)*: Alcohol may produce mood and behavioral effects that are dysphoric and that can result in impairment in performance and judgment and, in cases of overdose, ethanol can produce medically serious toxic effects. In the AMR and alcohol self-administration session, the maximum dose of alcohol that subjects can self-administer is five standard drinks over 50 minutes; this dose will produce a blood ethanol level of approximately 120mg/dL. In the CAD session, the maximal proposed dose is one that produces a blood ethanol level of approximately 100mg/dL. Subjects invited for study participation are those who regularly drink doses of ethanol greater than the doses selected in this study, reducing the likelihood of adverse events. These are medically safe but behaviorally intoxicating levels, associated with impairment of psychomotor and cognitive performance and with emotional and behavioral effects that may range from sedation and drowsiness to agitation, irritability, depression, and emotional lability. These effects dissipate as blood ethanol levels decline.

- ix. *IV placement and fluid delivery.* IV placement can cause some pain. There is a slight risk of bleeding, bruising, or irritation whenever blood is drawn or IV lines are started. In addition, IV fluid can occasionally escape under the skin, causing pain and swelling for a few days. When an intravenous product is given, there is always a chance that the needle placed in the vein may infiltrate causing temporary swelling, bruising, bleeding and/or discomfort. The risk of temporary clotting of the vein is about 1%. In rare cases, fainting or infection may occur.

b. Steps to Minimize Risks.

- i. *Recruitment and Informed Consent.* Participants will be identified through the media. To date, we have used newspaper, radio and social media advertisements. All ads and screening materials are reviewed and approved by the IRB prior to use. At the initial contact, the research assistant will discuss the study purpose and requirements with the participants. This information will also be displayed to participants who complete the online screener.
- ii. *Assessment.* Prior to the start of the assessment, subjects provide written informed consent using a document approved by the Johns Hopkins IRB. The staff member will discuss the consent form with the volunteer and answer any questions before they are asked to sign it. Volunteers will receive a copy of the signed consent form to keep. The consent form describes the experimental procedures and their associated risks. It provides an assurance that volunteers may ask and will receive answers to questions, assures volunteers that their participation is voluntary and may be terminated by them at any point if they wish, gives the conditions for investigator termination of research participation, and provides names and numbers to contact in the event of questions or concerns.

Prior to completing any study assessment materials, subjects are breathalyzed and must provide a 0mg% reading to participate in the interview. Subjects also must provide a urine sample and test negative for illicit drug use (excluding THC). Female participants must also test negative in a urine pregnancy test. Subjects with any contraindications are excluded from participation. Subjects are permitted to discontinue their participation at any time. Subjects are carefully and continually monitored throughout their participation. In case of an adverse event, a physician or nurse practitioner is available by beeper for assistance.

- iii. *Insuring protocol comprehension:* We exclude potential subjects with a Shipley or REALM score below the 5th grade reading level because of concerns about their ability to adequately participate in the study procedures. Many of our behavioral/subjective measures are self-administered and require basic literacy and language skills (e.g., English is not primary language). If subjects are not at a 5th grade reading level, they have difficulty responding accurately to the study questionnaires.
- iv. *Psychosocial assessments:* The risk of distress or personal discomfort elicited during assessment is minimized by the use of standardized assessment procedures widely and successfully used in research settings. Also, all study staff are trained in nonjudgmental interview techniques and crisis intervention procedures. Employees are carefully trained to monitor subjects for any adverse effect and to contact an Investigator immediately if such an event occurs. If serious psychological concerns (e.g., suicide risk) arise, staff has been trained to immediately refer these to study investigators who are licensed psychologists (Dr. McCaul) and Psychiatrists (Dr. Alvanzo). In the unlikely event neither of them is available, staff will escort the participant to the emergency room.

Medically supervised alcohol withdrawal and monitored abstinence: For the first 24 hours, all subjects will receive an intravenous line with D5NS with MVI adult Inj 10ml, thiamine Inj 100mg, Folic acid Inj 1mg, Magnesium sulfate Inj max of 2g infused at 84 ml/hour. Subjects will have PRN access to ibuprofen 400mg q 4hours, Maalox 30 ml po q 8hours. Subjects will be closely monitored by nursing and physician staff. Severity of withdrawal will be determined using the Clinical Institute Withdrawal Assessment -- Alcohol Revised (CIWA-Ar), a standardized withdrawal assessment instrument and determination of vital signs every 4 hrs during waking hours. Participants that have elevated blood pressure that are above safe limits, will be given atenolol .We will initiate system-triggered lorazepam treatment for alcohol withdrawal symptoms if the withdrawal score exceeds the clinical cutoff of CIWA-Ar ≥ 12 or on the advice of the study physician. If the CIWA-Ar score is ≥ 12 the subject is given a 2mg dose of lorazepam, and CIWA-Ar scores are then repeated 1 hour later. If the score remains ≥ 10 then another 2 mg dose of lorazepam is given. Hourly CIWA-Ar scores and lorazepam treatment are repeated until CIWA-Ar score decreases to less than 9 or until a maximum of 4 doses of 2 mg lorazepam are administered; if CIWA is 9 or higher after 4 doses, subject will be removed from the study. Subjects will also be removed from the protocol for seizures, hallucinations, or disorientation. At that point in time, the physician on call will be contacted who will arrange for transfer to the medical service. Individuals who require benzodiazepine treatment and are successfully treated with lorazepam will remain in the study to complete the 3-day withdrawal period, but will not complete laboratory session in order to avoid potential complications and confounds of treatment with benzodiazepines.

To date, we have successfully withdrawn over 98% of our alcohol-dependent subjects without medications requiring protocol termination in studies using a similar population. Exclusion of subjects with a history of alcohol withdrawal complications, and/or high CIWA-Ar scores is a successful strategy to help ensure subject safety in studies of new medications^[118-120].

- v. *Oxytocin administration:* Oxytocin (40 IU/ml, Syntocinon spray, Novartis) and placebo (containing the carrier without the neuropeptide) in identical metered-dose nasal spray bottles (Lukas-Bottles, 5-mL, 0.1 mL/actuation) will be obtained from Pharmaworld, Zurich, Switzerland. Oxytocin will be used in these studies under FDA-approval via IND 110,562. The IND 110,562 is held by our collaborator Mary Lee. This supplier and formulation has been used by Dr. Lee in her prior studies. Full information sheets of the nasal spray formulation are uploaded. Oxytocin and placebo will be dispensed by The Johns Hopkins Investigational Drug Services. Throughout the study, subjects will remain on the inpatient CRU, and will complete medication side-effects questionnaires. Forms are reviewed by nurses and study staff.
- vi. *Blood collection:* Experienced medical personnel using sterile equipment will be performing the blood draws, minimizing the risk associated with venipuncture.
- vii. *Trier Social Stress Test:* Participants will complete the TSST in our research offices. Subjects will be monitored throughout the procedure. If a subject shows clinically significant symptoms of anxiety the study physician or nurse practitioner is available by beeper for assistance. Subjects are debriefed about the procedure at the end of the study participation and prior to CRU discharge. Specifically, they are informed that the video camera actually did not contain film, that their performance was not rated, and that the interviewers were actually persons associated with the study.

- viii. *Alcohol Administration Sessions:* In conducting alcohol administration procedures in heavy drinkers, we will take several important steps to ensure the safety and well-being of subjects participating in this protocol. First, we will follow the guidelines of the National Institute on Alcohol Abuse and Alcoholism for alcohol administration to persons with an alcohol use disorder. Second, we recruit only persons who are not seeking treatment and who are volunteering for remuneration. Third, we house subjects on the CRU under observation to reduce potential harm during alcohol withdrawal and alcohol intoxication. Fourth, the dose of alcohol that will be available during the alcohol motivated responding (AMR) and self-administration procedure (i.e., 5 standard drinks) and the cumulative alcohol dosing (CAD) procedures sessions is well below doses routinely self-administered by the participants and drinking will be paced to prevent very rapid ingestion of the available alcohol drinks. Fifth, all subjects will participate in a brief intervention at the conclusion of their study participation; the intervention provides feedback on their hazardous drinking levels and encourages acceptance of treatment referral, as appropriate.

Our laboratory has substantial experience administering ethanol to human subjects under a variety of experimental and dosing conditions. All ethanol doses are administered under doctor's orders and prepared by the Johns Hopkins Investigational Drug Service using the participant's preferred alcoholic beverage (e.g., beer, wine or liquor for AMR and self-administration) or pharmacy-grade ethanol (for CAD). Participants are monitored carefully throughout the laboratory sessions; should a subject evidence a medical or behavioral adverse effect, a physician will be called immediately and the subject will receive appropriate evaluation and treatment. Thus, the careful choice of doses, the medical observation and monitoring, and the use of human volunteers who regularly drink doses of ethanol comparable to and greater than the ones selected for this study render the likelihood of serious adverse ethanol-induced effects unlikely.

- ix. *Confidentiality:* Our staff is well trained in the matters of confidentiality. Subject numbers will be used to code all data forms for computer entry and storage. Study findings are reported using group data only. No information about subjects will be provided to anyone outside of the study including family members, third persons or organizations. Experimental sessions will take place in the Johns Hopkins 550 Building and the Johns Hopkins Clinical Research Unit. We will obtain a Certificate of Confidentiality for this study from the funding agency.
- x. *IV placement and fluid delivery.* To minimize risks, only experienced medical personnel using sterile equipment will be performing IV placements and fluid delivery, and procedures are completed on the CRU.

c. Plan for reporting unanticipated problems or study deviations

An adverse event (AE), is defined as any untoward medical occurrence in a subject, not necessarily having a causal relationship with the study. A serious adverse event (SAE) is any untoward medical occurrence that a) results in death, b) is life-threatening, c) requires inpatient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability/incapacity, or e) is a congenital anomaly/birth defect.

AE's may be graded as: Mild (no limitation of usual activities), Moderate (some limitation) or Severe (inability to carry out usual activities). The relationship of the AE to the study procedures is classified as: Not related, Unlikely, Possible, Probable, or Definite.

Dr. Wand has responsibility for communication with the IRB and will be responsible for distinguishing between an AE and SAE. SAEs that are deemed to be severe and have a high probable or definite relationship to study procedures will be reported to the Johns Hopkins Medical Institute IRB and NIAAA project officers within 48 hours.

Annual reports will be submitted to the NIAAA project officer and the IRB summarizing protocol deviations, as well as AEs and how they were addressed by the study team. Dr. Lee (holder of the IND) will be responsible for all reports to the FDA per their guidelines and regulations.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Subject numbers will be used to code all data forms for computer entry and storage. Study findings are reported using group data only. No information about subjects will be provided to family members, third persons or organizations. Experimental sessions will take place in the Johns Hopkins 550 Building, the Johns Hopkins CRU and the JH Bayview CRU. Our study personnel are trained in the protection of human subject confidentiality and are HIPAA trained and certified.

e. Financial risks to the participants.

All costs for the CRU stay and study related procedures will be covered by NIH grants.

9. Benefits

a. Description of the probable benefits for the participant and for society.

This study is conducted for the advancement of science. It is clinically and scientifically important to determine the effects of OT on alcohol withdrawal symptoms, stress response, alcohol motivation, consumption and sensitivity in heavy drinkers during acute alcohol abstinence. These studies will provide new information that is relevant for the development of OT as a potential pharmacotherapy for AUD.

At the end of study participation, subjects receive a brief psychosocial intervention for their heavy drinking. Brief psychosocial intervention has been shown to be efficacious in reducing alcohol consumption and alcohol-related problems.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Since participants receive payment depending on which parts of the protocol they participate we have divided payments into payment for procedures for study eligibility and procedures for study completion.

	Compensation (\$)
Eligibility Assessment procedures	
In-person Assessment	\$75

extra visit assessment (if needed)	\$25
Assessment compensation	\$100

Study Protocol Elements for eligible subjects	Compensation (\$)
3 Session days (\$75 ea)	\$225
6 overnight CRU (\$25 + \$15 ea)	\$240
Bonus for study completion	\$150
max \$ bonus AMR (\$1 per drink)	\$10
Inpatient procedures compensation	\$625
Total max study compensation	\$725

Subjects who are terminated for noncompliance or who drop out of the study will be paid only the amount earned to date for completed procedures, CRU stay or outpatient visits. They will forfeit future possible earnings and bonus payments. To eliminate any financial incentive for early termination, subjects who are noncompliant or drop out will not receive payment until the day when they were scheduled to complete their study procedures. If subjects are terminated by the Investigators for safety reasons, they will be paid the amount earned to date for completed procedures, CRU stay or outpatient visits. Additionally, they may be eligible for bonus payments prorated for length of study participation.

11. Costs

- a. **Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.**

n/a

12. References

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