Does Oxytocin Alter Tolerance to Alcohol or Motivation for Alcohol in Heavy Drinking Human Subjects: Testing a Novel Anti-Addiction Mechanism

Martin Plawecki, M.D., Ph.D., 550 N University Blvd. UH5505
Indianapolis, IN 46202

Co-I: Ann Kosobud, Ph.D., Associate Professor, Dept. Neurology, IUSOM
Co-I: Sean O'Connor, M.D., Professor, Dept. Psychiatry, IUSOM

Support Provided by:
Hanlon Trust

Advisor (non-interacting): Cort A. Pedersen, MD
Advisor (non-interacting): Carolina Haas-Koffler, PharmD

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1.0 Background & Rationale

Alcohol use disorder (AUD; formerly termed alcohol dependence or addiction), is a chronic disorder that includes 1) compulsivity in and lack of control over alcohol seeking and consumption, 2) the development of tolerance, and eventually 3) the emergence of withdrawal (including physical and affective symptoms when alcohol is restricted). It is among the most common and costly mental health problems[1-4]. FDA-approved medications for alcohol dependence have had only modest efficacy and have been minimally used[5,6]. AUD is theorized to progress through three overlapping stages: binge-intoxication, preoccupation-anticipation, and withdrawal-negative affect[7]. **Binge-intoxication** is thought to consist of frequent ingestion of alcohol to achieve a pleasurable, inebriated state. Because of chemical adaptations in the brain with repeated alcohol exposure (neuroadaptations), achieving intoxication requires ingestion of steadily increasing amounts of alcohol, a process referred to as *tolerance formation*. While associated changes in activity of some brain neurotransmitter systems have been found, the precise mechanisms of alcohol tolerance formation remain poorly understood[8,9]. During the **preoccupation-anticipation** stage, drinking is increasingly driven by compulsion rather than pleasure. Sustained, heavy drinking leads to increasing tolerance, and eventually physiologic alcohol dependence. At this stage, cessation of drinking produces unpleasant, potentially fatal, withdrawal symptoms because the neurochemical balance in the alcohol-adapted brain is abruptly disrupted. In the **withdrawal-negative affect** stage, avoiding the discomfort of withdrawal, rather than seeking pleasurable inebriation, may become the primary motivation to continue heavy drinking[10,11].

Oxytocin (OT) is a small protein that is both a neurotransmitter in the brain and a hormone released into the blood from the pituitary gland. Because numerous studies in animals and, more recently, in humans have found a wide variety of prosocial effects of OT that are exerted in the brain, it has become known in the popular culture as the “love” hormone[12-14]. In humans, nasal OT administration has been shown in some studies to increase interpersonal trust and cooperation as well as accurate interpretation of social cues such as facial expressions[15-17].

However, animal research has also suggested some potentially clinically useful effects of OT on alcohol use and abuse. Numerous preclinical studies have shown that OT treatment: 1) prevents tolerance formation to repeated doses of alcohol non-dependent animals; 2) blocks withdrawal seizures in alcohol-dependent animals, and 3) in a recent study, results in decreased motivation for alcohol in alcohol-dependent animals[18-24]. For example, when non-dependent mice are given an impairing dose of alcohol on successive days, they adapt and steadily improve on daily tests of balance and coordination conducted after each alcohol dose. However, when OT is given before each alcohol dose, the development of tolerance is inhibited and balance and coordination improves more slowly on daily tests[18]. Treatment with the GABA-antagonist picrotoxin triggers withdrawal seizures in alcohol-dependent mice, an effect reduced by OT administration[23]. More recently, OT administration reduced motivation for alcohol and alcohol self-administration in alcohol dependent rats but not non-dependent animals[24].

In alcohol-dependent, heavy drinking individuals, studied on a research unit, Dr. Pedersen and colleagues recently discovered that twice-daily OT treatment (given in a nasal spray that results in significant brain delivery) potently and rapidly blocked alcohol withdrawal as judged by a decreased need for lorazepam intervention[25]. This, to the knowledge of the investigators, is the only journal publication on OT treatment of human AUD. Dr. Pedersen and colleagues have also recently completed a National Institute of Alcohol Abuse and Alcoholism (NIAAA) funded, randomized, placebo-controlled clinical trial; preliminary analysis found that 12 weeks of twice-daily intranasal administration of OT significantly decreased heavy-drinking days (5 standard drinks/day for men and 4 for women; a standard drink contains the same amount of alcohol as a 12 oz. beer) in heavy-drinking individuals. Other non-treatment examinations of the human response to OT in the context of an alcohol exposure or an AUD is limited, yet informative. While no effect of OT on the subjective response to alcohol was identified in light or social drinkers[26, 27], OT was reported to benefit baseline interoceptive accuracy

in heavy drinkers[27]. In contrast, when examined in veterans with comorbid post-traumatic stress disorder and AUD, OT was found to only marginally reduce stress reactivity (a core non-tolerance feature of the later stage of withdrawal-negative affect), and did not reduce self-reported craving[28]. Similarly, OT was not found to reduce emotional reactivity during a conflict task in couples with substance misuse[29]. These human studies, in conjunction with our own, suggest that: OT acts upon chronic tolerance, would be most effective in heavy drinkers, and its assessment should include subjective response to alcohol and craving, as well as objective measures of cognition and behavior.

Based on OT effects in rodents, and the available human literature, we hypothesize that OT may exert therapeutic effects by rapidly reversing alcohol-induced neuroadaptations in the brain. This putative reduction of tolerance would represent a unique approach to treatment that could revolutionize clinical AUD care. If clinicians could reduce or eliminate tolerance, it would mitigate one of the key drivers of relapse. Furthermore, the availability of a treatment that reduces acute withdrawal and risk of relapse would provide a continuum of medical care that is now lacking. The proposed project will directly test whether OT reverses tolerance and associated alcohol seeking in humans by employing state-of-the-art computer-assisted intravenous alcohol administration. Two separate experiments will be run, one of which combines a standardized breath and therefore brain alcohol exposure with sensitive tests of subjective response and cognitive function. The second is an alcohol self-administration paradigm, assessing objective change in motivation for alcohol. Demonstrating that OT (compared to placebo, PL) worsens test performances in alcohol-dependent individuals and/or reduces the compulsive drive to self-administer alcohol would be strong evidence for its potential AUD treatment utility.

2.0 Objective(s)

The goal of this project is to provide evidence supporting the hypothesis that intranasal OT treatment blocks or reduces tolerance to alcohol and motivation for alcohol in heavy drinking individuals. Evidence supporting either outcome would indicate that OT has a potentially novel therapeutic effect and would then be used in a subsequent, larger study of the effect. Such an outcome would open new fields of investigation into a potential role for OT in AUD treatment, notably the prevention of relapse and support of abstinence. This could have profound effect on treatment approaches for AUDs. The project will examine whether OT reverses tolerance and associated alcohol seeking in humans. Tolerance to and motivation for alcohol will be assessed with sensitive tests of subjective response and cognitive function tested prior to, at the beginning, and at the end of an intravenous alcohol BrAC clamp (a standardized brain alcohol exposure). Alcohol seeking will be tested using a progressive ratio alcohol self-administration paradigm, assessing objective change in motivation for alcohol.

2.1 Primary Objective:

Aim 1: Demonstrate that OT alters alcohol tolerance. This will be assessed using a within-subjects design comparing OT and PL infusion sessions. Cognitive, motor and subjective responses will be tested prior to, at the beginning, and at the end of a steady-state alcohol clamp.

2.2 Secondary Objective:

Aim 2: Demonstrate that OT decreases motivation for alcohol. Motivation for alcohol will be measured directly when participants will be able to earn standardized alcohol exposures by performing a button pressing task on a progressive ratio scale in the active versus control condition. Motivation will also be assessed by measures given during the clamp (and its respective baseline and control assessments) including an alcohol purchase task (APT) that assesses the value of alcohol from a behavioral economics perspective and measures of subjective perception and craving for alcohol.

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures:

Aim 1: OT will alter alcohol tolerance. Compared to their performance under PL and respective baseline assessment, participants given intranasal OT will show, in one or more of the following, greater alcohol-related perturbation of the subjective response to alcohol, the Stroop test, and Stop Signal task performance.

3.2 Primary Outcome Measures:

Aim 2: OT will reduce motivation for alcohol. Participants will display lower motivation for alcohol under OT compared to PL, reflected by one of more of the following: During alcohol self-administration, less cumulative work for alcohol on the progressive ratio task, and reduced overall alcohol exposure as reflected by the breath alcohol concentration (BrAC) curve during alcohol self-administration; During the BrAC clamp, altered APT derived behavioral economic indices and/or reduced subjective craving for alcohol.

4.0 Eligibility Criteria

4.1 Inclusion Criteria

- Overtly healthy men and women aged 21 – 60.
- Heavy alcohol drinkers [greater than 14 (men) or 7 (women) drinks per week, and at least 5 binges per month, with a binge defined as 5 (men) or 4 (women) drinks on the same occasion[30]], unless determined by PI that drinking history meets study objectives.
- Able to understand/complete questionnaires and procedures in English.
- Have venous access sufficient to allow blood sampling.

4.2 Exclusion Criteria

- Latex allergy (due to oxytocin/latex cross-reactivity).
- Nasal condition that compromises delivery and/or absorption of intra-nasal oxytocin
- Pregnant or breast-feeding women.
- Desire to be treated for any substance use disorder or court ordered to not drink alcohol
- Medical disorders or other conditions such as alcohol withdrawal seizures or delirium tremens that may influence study outcome or participant safety.
- Positive urine drug screen for amphetamines/ methamphetamines, barbiturates, benzodiazapines, cocaine, opiates, or phencyclidine if determined by the PI to adversely affect participant safety or data integrity.
- Medications (past 30 days) that could influence participant safety or data integrity (e.g. antidepressants, antipsychotics, benzodiazepines, etc.) as determined by the PI.
- DSM 5 Disorders (non-AUD) or current/history of neurological disease of cerebral origin, or head injury with > 20 min loss of consciousness, if determined by the PI to affect participant safety or data integrity.
- Positive BrAC reading at beginning of the experimental session.
- Actively suicidal (for example, any current suicidal intent, including a plan) or are at serious suicidal risk, by clinical judgment of the PI.
- Any condition for which the PI and investigative team determine it is unsafe or not prudent to enroll a participant.

5.0 Study Design

Overview: This project employs a double-blind, within-subject design. Each participant will be randomly assigned to either the clamp or self-administration condition, and be studied twice, once with intranasal administration of OT and once with intranasal PL. At least 3 weeks will elapse between each participant's tests to ensure that residual effects of OT administration have dissipated.

6.0 Enrollment/Randomization

Participants will be randomized to study session order (OT-PL or PL-OT) at time of scheduling, using a procedure that ensures an equal number of participants in each group. Stratification of other potentially key risk or analytical variables, such as sex and age distribution, will be periodically monitored and individual subject order assignment may be adjusted to achieve balance. Neither subjects nor technicians will know which session is PL, and which is OT.

7.0 Study Procedures

Recruitment and Screening

We expect to recruit most of the participants for this study from our database of subjects who have completed a past experiment and have consented to be contacted for additional studies. Some participants may learn about the study from print, electronic advertising, or word of mouth, and then contact our phone line or visit the study website. Participants who meet basic criteria will be invited for an interview. Participants who have not previously completed an interview in our lab will complete a full interview. Participants who have interview data on record may be asked to update some interview information as needed for participant safety or data integrity. Updates may be done by phone, online, or in person. Interview measures may include some or all of the following measures, at the discretion of the principal investigator:

Self-Report Measures:
- The Alcohol Use Disorders Identification Test (AUDIT)
- The Urgent Premeditation, Perseverance, Sensation Seeking and Positive Urgency (UPPS-P) Impulsive Behaviors Scale measures impulsivity traits, including urgency.
- Self-Rating of Effect of Alcohol assessing retrospective sensitivity to the subjective response to alcohol (SRE).
- Short Inventory of Problems – Revised (SIP-r)
- Medical History (including menstrual phase for women)
- Nicotine History Measure
- Penn Alcohol Craving Scale (PACS)

Interviews:
- Brief medical history (including menstrual phase for women)
- The Semi-Structured Assessment for the Genetics of Alcoholism – IV (SSAGA-IV), Alcohol
- The Semi-Structured Assessment for the Genetics of Alcoholism – IV (SSAGA-IV), Family History of Alcoholism

Diary Measures:
- Timeline Follow-back (TLFB) modified to assess for one-month recall of alcohol use

Screening tools:
- Clinical Institute Withdrawal Assessment for Alcohol Scale (CIWA)
- Generalized Anxiety Disorder Assessment (GAD-2, GAD-7). Anxiety symptoms over the last 2 weeks are queried in a 2 item scale which expands to 5 additional items if the first 2 are endorsed.
- Patient Health Questionnaire (PHQ-2 and PHQ-9). Depressive symptoms over the last 2 weeks are queried in a 2 item scale which expands to 7 additional items if the first 2 are endorsed.
- Urine pregnancy and drug screen (UPS, UDS, the latter including EtG urine screen at discretion of PI)
- Breath Alcohol Concentration (BrAC) Measurement
- Liver function assessment (standard liver panel)

Participants: We plan to recruit up to 30 subjects in order to complete study of 10-15 heavy drinking participants (minimum 5 each in the clamp and self-administration arms). Approximately equal numbers of men and women will be recruited.

Payment: Participation fees are paid in cash at the end of each study visit, by email using an Amazon gift card, or by mail using a visa card or check. For the interview, participants will receive $15 if they complete the online portion only, and $25 if they complete both the online and in person portions, or complete the full interview in person. Subjects who completed and were paid for the interview for another study, and who provide only minimal updates to their information, will not be paid an additional interview fee. For the IV alcohol infusion sessions, the experimental day lasts about 11 hours; session 1 pays $130, and session 2 pays $200. In addition, subjects will be reimbursed for parking, bus pass or up to $10 for taxi/ride sharing. Thus a subject can receive up to $365 for participation. Payments are calculated to reimburse subjects at a rate of about $15/hour if they complete all sessions; session 2 pays more than session 1 because of the increased value of the subject's participation. If an infusion session is terminated early, participants will be paid $15 per hour, calculated in 20 min increments, for time they are at the Clinical Research Center, up to the total promised for the study day.

Method: We will assess the effects of OT on alcohol tolerance in heavy drinking participants using the computer-assisted alcohol infusion system (CAIS; Figure 1) developed[30-32] and maintained at Indiana University with the support of the Indiana Alcohol Research Center. Individual characteristics (height, weight, age and sex) are used to compute physiologically-based pharmacokinetic models of the distribution and elimination of alcohol[41-42], which in combination with model-based control techniques, allows for the precise control of breath, and therefore arterial and brain, alcohol concentrations[43]. CAIS IV alcohol techniques span a

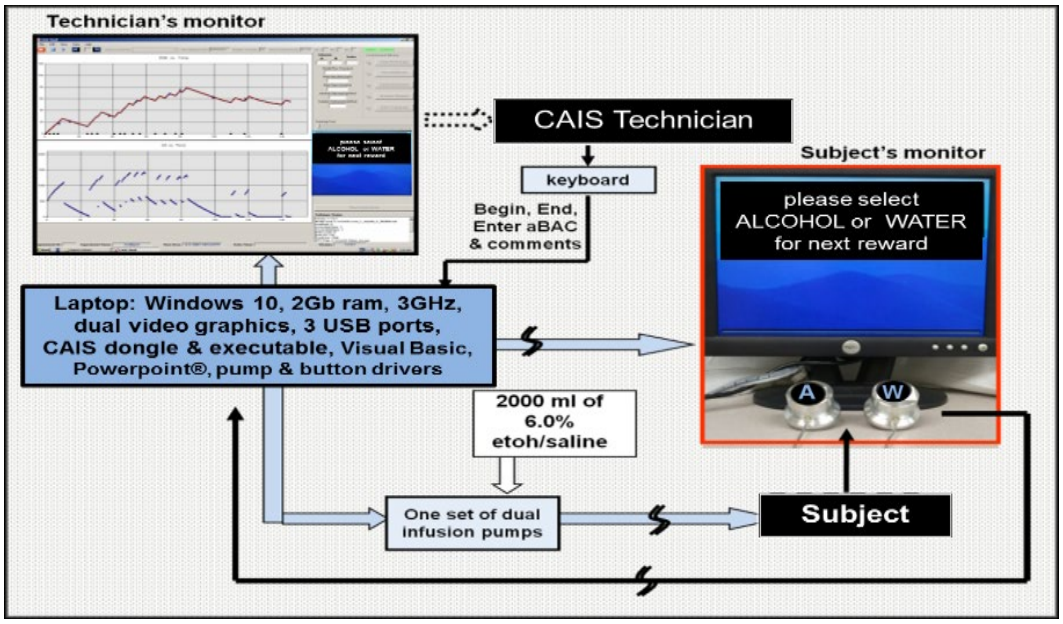

Figure 1. Schematic of CAIS system, including computer specifications, software, and accessories. The CAIS system consists of PC-based software interfacing with iv infusion pumps. Prior to infusion, the participant's age, height, weight, and gender are entered into the software, which transforms those measurements into parameters of a physiologically-based pharmacokinetic (PBPK) model of alcohol distribution and elimination. Infusate and breath alcohol concentration measurements provide feedback to adjust the PBPK model for each participant. Computation of the infusion rate profile is based on the updated model to achieve virtually identical clamp then incremental exposures to alcohol across participants. The subject and the infusion rate profile are monitored at all times by a technician.

broad range of applications, from the assessment of initial sensitivity and tolerance using the alcohol clamp[31-37] to the assessment of alcohol self-administration[39-40]. The need for controlled IV alcohol administration is illustrated in Figure 2 - Top, which shows the wide variation in alcohol exposure within and across individuals resulting from oral challenge paradigms. In contrast, (Figure 2 – Bottom) shows data from an alcohol clamp,

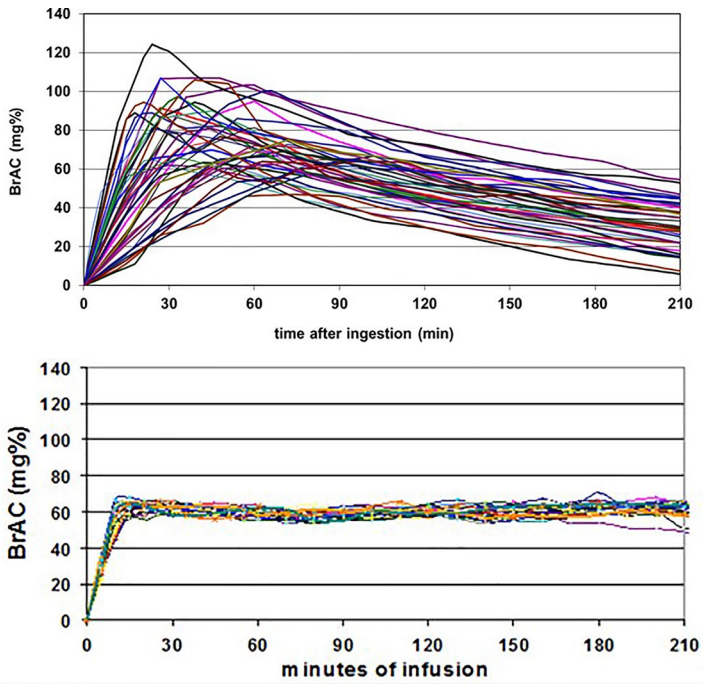

Figure 2.

Top. Individual BrAC trajectories in ingestion. Alcohol ingestion yields variable trajectories of BrAC. Exposure trajectories after ingestion of a dosage normalized by total body water to produce a peak BrAC of 80 mg/dL under standardized conditions for 44 participants is displayed[44,45].

Bottom. BrAC clamping trajectories. BrAC clamping experiment conducted in 50 young adults with the goal of raising BrAC to 60 mg/dl in 10 minutes, then maintaining it for 3 hours. The result minimizes the variability in BrAC trajectories across participants[44.45].

the first developed and most common IV alcohol paradigm. Breath alcohol concentration (BrAC) is raised to a target level, usually within 15-20 minutes, that can then be maintained ("clamped") at or near the target for whatever duration is necessary for a specific study. Recently, CAIS-based alcohol self-administration paradigms have been developed that provide the participants with control over individual alcohol increments that are virtually identical within and across participants. The overall alcohol exposure differs due to the participant's choice of timing and number of increments (see Figure 3, Alcohol Self-Administration phase). CAIS IV techniques allow investigators to study the higher BrAC typical of heavy drinkers safely, both due to the reduced variability of each incremental exposure and because BrAC immediately descends upon termination of the infusion; after oral intake, the BrAC continues to rise until absorption is complete. In addition, IV alcohol infusion reduces expectancy effects (arising from sight, odor, taste and the physical act of drinking), without affecting the subjective effects associated with intoxication[46]. In the present study, half of the subjects will be tested using a BrAC clamp to assess performance on a battery of perceptive, cognitive and behavioral tests, and half of the subjects will be tested in a self- administration session for a direct measure of motivation for alcohol and self-selected BrAC, consistent with our prior work.

Study day procedures: Upon arrival at the Indiana Clinical Research Center (ICRC), participants will be re-screened for drug use, pregnancy (if applicable), and BrAC. Participants are asked to abstain from alcohol after 8:00 pm the night before each session and must have a BrAC of zero at the beginning of an infusion. We will record the participant's recent drinking using a TLFB. Car keys will be held for safekeeping. Using sterile technique, an indwelling catheter will be placed in a vein of one arm. For the clamp, only a single pump is needed to deliver alcohol. For self-administration, the catheter will be connected to the two infusion pumps, one of which is used to deliver saline as the alternative reward; the second delivers ethanol infusate prepared by IU Health Investigational Drug Services (IDS) by mixing sterile half-normal saline with 95% sterile ethanol to create an approximately 6.0% (v/V) solution. Infusate concentration is checked using a refractometer prior to the start of the session and the result incorporated into the infusion profile determination. After the catheter is placed, a standardized 550 calorie breakfast is served. Testing will be performed in one of the lab's 5'x7' Industrial Acoustic Corporation® sound-dampened chambers or in a private room in the ICRC. Testing location will be identical across sessions for each subject. When testing is done in the lab chambers, a closed-loop intercom system enables the participant to talk to the technician at any time without manual effort. When testing is done in a private room in the CRC, technicians will be located behind a curtain and immediately available to the subject whenever needed.

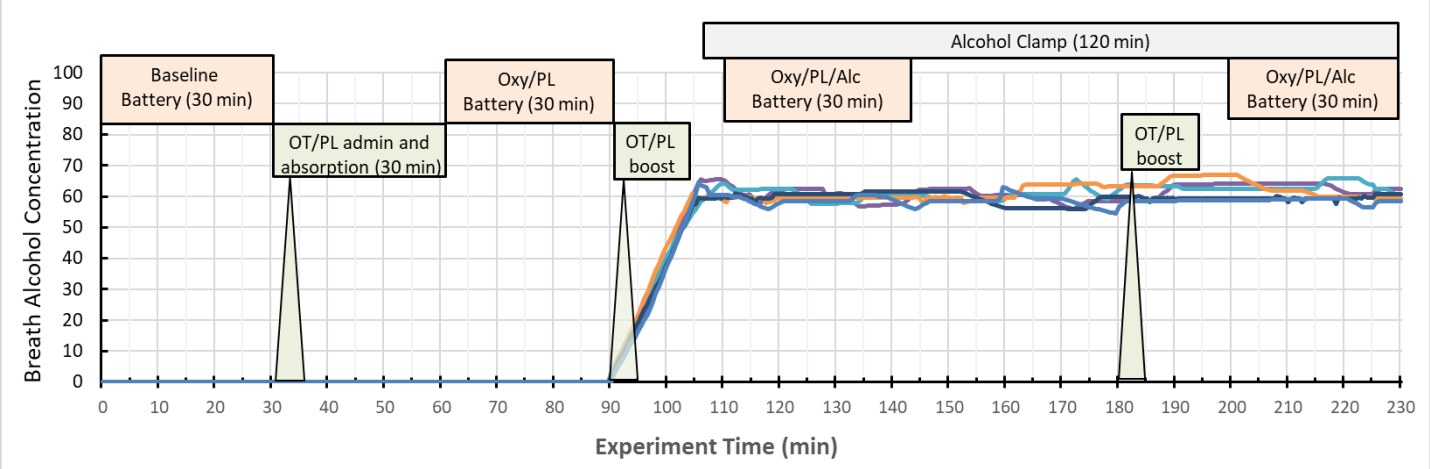

Figure 3: Alcohol Clamp. Diagram of the experimental session showing test batteries (orange boxes), OT and PL administrations (green boxes), alcohol clamp (grey box) and example subject breath alcohol concentrations (colored lines). **Experimental timeline:** Min 0-30: Baseline battery. Min 30-35: Intranasal OT or PL administered. Min 35-60: Pause for drug absorption. Min 60-90: OT/PL battery. Min 90-105: OT/PL booster dose at the beginning of a 15 min alcohol infusion ramp (4 mg/dL/min) to bring BrAC to 60 mg/dL. Min 110-140: Alcohol+OT or Alcohol+PL battery assessing initial response to alcohol during BrAC clamp at 60 mg/dL. Min 180-182.5: Booster dose of OT or PL. Min 200-230: Alcohol+OT or Alcohol+PL battery assessing adapted response to alcohol during BrAC clamp at 60 mg/dL.

Session procedures: Alcohol Clamp (Figure 3). Clamp sessions begin with a baseline battery lasting approximately 30 min and consisting of the Stop Signal Task (a motor impulsivity task that has demonstrated sensitivity to alcohol exposure); a subjective perceptions assessment, including craving; a side effects questionnaire; the Alcohol Purchase Task (a cognitive test providing measures of behavioral economics, revised for use during iv infusion); and the Stroop Test of mental concentration (accuracy and speed stating the color of a series of words printed in colors different from the colors they actually name). The battery is repeated after an initial dose of OT or PL, and at the beginning and end of a 2 h clamp, with booster doses of OT or PL 20 min prior to each of the clamp batteries. The dosing schedule of oxytocin relative to testing is derived from studies suggesting onset of effects in approximately 30 minutes and a duration of approximately 60 minutes[48-51]. The first dose of OT or PL will consist of 10 intranasal puffs [insufflations] alternating every 30 seconds between nostrils, delivering in total 40 IU of OT in 1 ml of solution or 1 ml of PL solution. A 20-30 min drug absorption period follows the first dose; booster doses of 24 IU of OT will be given 20 min prior to each of the remaining 2 test batteries. During the intervals between test batteries, the participant will be free to rest, read, or use an electronic device for entertainment.

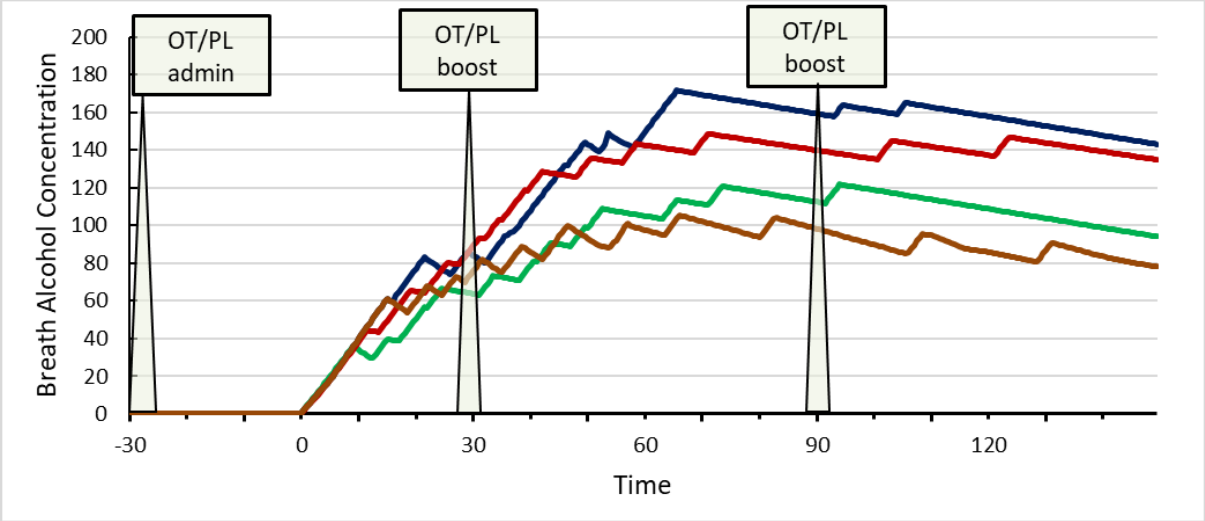

Figure 4: Alcohol Self-Administration session: Diagram of the experimental test session showing timing of OT/PL administration relative to the alcohol self-administration phase. The array of lines during the alcohol self-administration phase, derived from subject data from a past experiment, reflects a range of possible behavior, limited by the 180 mg/dL safety limit and the increasing demands of the progressive work schedule.

Session procedures: Self-administration (Figure 4). During the 150 min alcohol self-administration session, the participant initiates each work-set by choosing between 1 of 2 rewards, either alcohol or water. Work-sets consist of trials of the Constant Attention Task, or CAT (Figure 5), developed by our laboratory and with demonstrated sensitivity to pharmacogenomics and sex effects[38, 39]. Successive rewards require an increasing number of successful CAT trials. The work schedules required to obtain water or alcohol are identical, but progress is tracked separately and not communicated to the participants. Throughout any workset, participants are free to wait, work ad-lib, pause, or cease working. The available rewards are a standardized increment in BrAC (alcohol, labeled "A") or an infusion of saline (water, labeled "W"), each delivered over 2.5 min. Reward delivery begins immediately after the last correct CAT trial required for the work-set is performed. Alcohol reward increments raise the participant's BrAC by 10.0 mg/dL in 2.5 min before declining at a steady rate of -0.8 mg/dl/min, until the next alcohol reward is delivered or as long as pharmacokinetically possible. CAIS continuously estimates the future time course of BrAC and prohibits infusion that would yield a BrAC exceeding the predetermined, ecologically valid safety limit (180 mg/dL in this heavy alcohol drinking sample). The first dose of OT or PL will be administered 30 min before the start of the self-administration phase, and consist of 10 intranasal puffs [insufflations] alternating every 30 seconds between nostrils, delivering in total 40 IU of OT in 1 ml of solution or 1 ml of PL solution. Booster doses of 24 IU of OT will be given approximately hourly after the initial dose. Participants will complete the subjective perceptions assessment and side effects questionnaire prior to the first OT/PL administration, immediately before the start of alcohol self-administration, and at 20 min intervals throughout the session, timed with alcohol reward increments when possible. Except for ad-libitum bathroom breaks, participants remain in the chamber or room for the duration of the experiment; technician interaction is limited to occasional BrAC samples, data collection, and verbal checks to assure subject comfort at the beginning/end of each intervention.

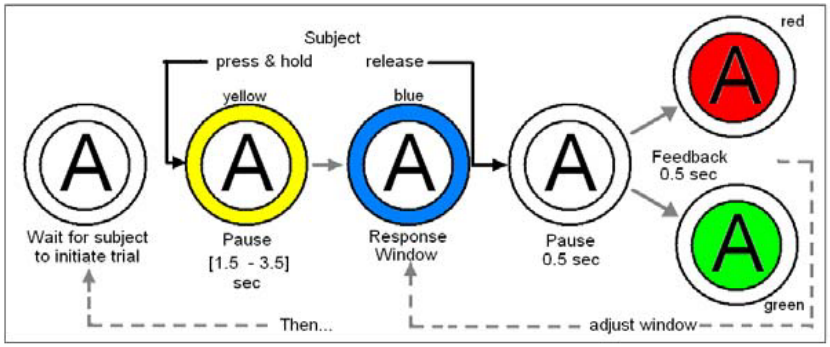

Figure 5: The CAT task. Subjects initiate the task by pressing down on one of two buttons, labeled "A" (alcohol) or "W" (Water). Holding the button down results in a screen graphic showing the choice button ("A" in this example) surrounded by a yellow circle. When the yellow changes to blue, the subject must release as quickly as possible. On release, the circle returns to white and the subject must indicate whether they thought they were successful. The screen graphic then shows green for successful release, and red for an unsuccessful (too slow) release. The task adapts by increasing the release time window required after errors, and decreasing it after successes. Thus, the percent of successful trials remains constant across subjects and is not affected by basal reaction time, practice, fatigue, or intoxication.

Recovery/Discharge procedures: Alcohol clamp sessions. Upon completion of the alcohol clamp, participants will stay in an inpatient room until they can be safely discharged with a BrAC <35mg/dL. They are served lunch from the ICRC kitchen. Subjects will be told that they should expect to stay at the CRC until 7:00 pm, although they will be dismissed earlier if their BrAC is below 35 mg/dL. Participants with a BrAC > 35 mg/dL at 7:00 pm will be compensated at the rate of $15/hour in 20-minute increments for the time stayed past 7:00 pm. Participants staying past 5:00 pm will be offered dinner.

Recovery/Discharge procedures, Alcohol self-administration sessions. Subjects participating in the self-administration sessions will be required to stay until at least 7:00 pm and until BrAC < 35 mg/dL, whichever is later. Participants are served lunch from the ICRC kitchen; they will also be given dinner at about 5:30 pm. Participants with a BrAC of 35 mg/dl or below but who show signs of intoxication and/or self-report any concerns regarding their safe passage home at the time of discharge will be provided transport home via car service. Participants with a BrAC > 35 mg/dL at 7:00 pm will be compensated at the rate of $15/hour in 20-minute increments for the time stayed past 7:00 pm in a payment made at the second session. Any participants who must stay past 7:00 pm for the first session will be informed that they have to stay until the same time for the second session (in order to reduce discharge-motivated drinking behavior during the second session compared to the first). However, for the second session, participants will only actually be required to stay past 7:00 pm if their BrAC remains at or above 35 mg/dL or if a concern regarding their ability to be safely discharged exists. At the discretion of the PI, subjects may be discharged before 7:00 pm if their BrAC is at or below 35 mg/dL and an early discharge would not jeopardize subject safety or study objectives.

8.0 Study Calendar

	Screening		Study Day				
Interventions	**Phone**	**Interview**	**Before session**	**Baseline Block**	**OT/PL Block**	**Alcohol Clamp**	**Alcohol Self-administration**
Phone Screen	X						
Liver Function panel		X					
Medical History		X					
UPPS-P Impulsive Behaviors Scale		X					
Generalized Anxiety Disorder Assessment (GAD-7)		X					
Patient Health Questionnaire for depressive symptoms (PHQ-9)		X					

Alcohol Use Disorders Identification Test (AUDIT)		X					
Semi-structured Assessment for the Genetics of Alcoholism (SSAGA); Alcohol and Family History modules		X					
Self-Rating of Effects of Alcohol (SRE).		X					
Short Inventory of Problems – Revised (SIP-r)		X					
Clinical Institute Withdrawal Assessment for Alcohol Scale (CIWA)		X					
Nicotine History (NHF)		X					
Penn Alcohol Craving Scale (PACS)		X					
Timeline Followback (TLFB) recall of alcohol and drug use		X	X				
Urine Pregnancy Screen (UPS)		X	X				
Urine Drug Screen (UDS)		X	X				
Breath Alcohol Concentration (BrAC)		X	X			X	X
Alcohol Purchase Task (APT)		X		X	X	X	
Stop Signal Response Task				X	X	X	
Stroop Test				X	X	X	
Subjective Perceptions Assessment				X	X	X	X
Side-Effects Questionnaire				X	X	X	X

9.0 Reportable Events

Serious adverse events resulting in any physical harm associated with testing will be reported to the IRB as required by applicable policies and guidance. Minor, and expected, adverse events (e.g. nausea, infusate infiltration, discomfort associated with infusion, nasal irritation) will be reported to the Alcohol Studies DSMB meeting at least annually. Side effects attributable to the interventions will be reported in any manuscripts or scientific reports based upon the collected data and entered into any mandated database.

10.0 Data Safety Monitoring

The Data Safety and Monitoring Board (DSMB) that monitors virtually all the alcohol studies at IUSM will review this study at least annually. This board is comprised of all IU faculty engaged in IV alcohol research, and chaired by Dr. Laura Tormoehlen, who is independent of the sponsor and investigators. The following will be monitored as part of the Data Safety Monitoring Plan (DSMP): data quality, recruitment, accrual, retention, outcome and adverse event data, assessment of scientific reports or therapeutic development, results of related studies that may impact participant safety, and procedures designed to protect the privacy of participants.

11.0 Study Withdrawal/Discontinuation

The participant may withdraw from the study at any time for any reason. The PI may choose to terminate any subject's participation at any time if it is deemed that s/he cannot participate or cooperate in testing procedures safely or if a subject's behavior jeopardizes integrity of the collected data. Partial days will be compensated at a rate of $15/hour, including time to recover to a BrAC < 35 mg/dL. Payment will be calculated in 20 min increments.

12.0 Statistical Considerations

The primary goal of the study is to generate data that would be supportive of a large, properly powered study of the effects of OT on tolerance and/or motivation for alcohol. For this exploratory study, we anticipate examining within-subject effects of OT compared to PL using paired t-tests, accommodating baseline assessments as appropriate for the specific measures.

Aim 1 Hypothesis Testing Plan
Hypothesis 1: Tolerance to alcohol will be altered by OT, such that participants exhibit greater effects of alcohol when tested with OT compared to PL and each with respect to its own baseline assessment. Specifically, we predict the following differences in one or more of the following measures:

Stop Signal Response Task:
Participants will have more rapid reaction times under PL compared to OT.
Subjective Perceptions of Alcohol and Craving
Participants will report weaker subjective effects of alcohol and higher craving under PL compared to OT.
Stroop Test
Participants will have shorter response times and fewer errors under PL compared to OT.

Aim 2 Hypothesis Testing Plan
Hypothesis 2: OT will reduce motivation for alcohol as demonstrated by changes in one or more of the following measures:

Alcohol Purchase Task
Participants will display alteration in behavioral economic indices derived from the Alcohol Purchase task under OT compared to PL (for example, a reduction in intensity, greater elasticity, lower breakpoint, lower total outcome, etc.).
Subjective Perceptions of Alcohol and Craving
Participants will report weaker subjective effects of alcohol and higher craving under PL compared to OT.
Alcohol Self-Administration
Participants will demonstrate reduced cumulative work for alcohol rewards and less overall alcohol exposure under OT compared to PL.

Missing Data

Missing data may occur intermittently or due to attrition. Due to the within-subjects design, participants completing only one of two experimental sessions will be excluded from final analyses.

Power Analyses

While OT is a commonly used agent in human studies, this is a novel investigation of the effect of OT on laboratory measures of alcohol tolerance and motivation for alcohol. Consequently, no directly relevant preliminary data exists upon which to base proper power analyses – collection of such data is the primary objective of this investigation. However, our previous alcohol self-administration work showed effect sizes from 0.49 to 1.31 according to the phenotype of interest. Effect size estimates attributable to an alcohol clamp vary considerably in the literature depending on the participant population, exposure, and outcome of interest. For example, we and our colleagues have identified effect size for the differences in alcohol elimination rate in participants stratified by sex is moderate ($f2=0.07$). We recently identified an alcohol effect on P300 amplitude in response to the stop signal using the clamp ($f2 = 0.57$). At baseline, we have also documented both large and small sex differences in subjective responses in both general (large effect of $f2 = 0.35$) and specifically as a function of abstinence (small effect of $f2 = 0.034$).

13.0 Statistical Data Management

Primary data will be collected via paper, electronic forms, and computer software output and stored electronically on a department server. The storage location is backed up automatically daily. Other data sources include pathology lab data that will be stored in separate electronic files and merged with the primary data as needed. The following data quality control methods will be used: single entry with random checks of

accuracy, range checks, testing of database by study team prior to moving to data analysis, and regular, periodic extraction and cleaning of data.

14.0 Privacy/Confidentiality Issues

Procedures for protecting privacy interest in the study include advising the participant to ensure his/her privacy during the phone screen and interview if performed electronically, and conducting all further recruiting and testing from or on the Indiana CTSI Clinical Research Center, in a private room during regular hours of operation. Research data is stored either in a lockable file cabinet in a locked and secured area, or on password protected network file servers.

15.0 Follow-up and Record Retention

We expect project completion no later than December 2022. The identifiable project participant files will be destroyed at the conclusion of data analysis. The records will be kept for 7 years after completion of the study. The databases will be kept on a secure server in password-protected files. The database files will be over-written with null data at the completion of the retention interval.

16.0 Appendix

The following materials are included:

1. The Urgent Premeditation, Perseverance, Sensation Seeking and Positive Urgency Impulsive Behaviors Scale (UPPS-P)
2. Generalized Anxiety Disorder Assessment (GAD-2 and GAD-7)
3. Patient Health Questionnaire for depressive symptoms (PHQ-2 and PHQ-9)
4. Alcohol Use Disorders Identification Test (AUDIT)
5. Semi-structured Assessment for the Genetics of Alcoholism -IV (SSAGA-IV) – Alcohol module
6. Semi-structured Assessment for the Genetics of Alcoholism -IV (SSAGA-IV) – Family History module
7. Self-Rating of Effect of Alcohol (SRE).
8. Short Inventory of Problems – Revised (SIP-r)
9. Clinical Institute Withdrawal Assessment for Alcohol Scale (CIWA)
10. Nicotine History Form (NHF)
11. Penn Alcohol Craving Scale (PACS)
12. Timeline Followback (TLFB) recall of alcohol and drug use
13. Alcohol Purchase Task (APT)
14. Subjective Perceptions Assessment (Squizzer) – Hanlon
15. Side-Effects Questionnaire
16. Adverse Events Reporting Form
17. Intranasal Administration Training Guidelines

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