Official Title: A Human Laboratory Study of n-Acetylcysteine for Alcohol Use Disorder
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1. BACKGROUND

Alcohol (ALC) use disorder (AUD) is an unrelenting public health concern. Approximately 17 million Americans meet criteria for AUD, with 85,000 deaths directly attributable to ALC each year, resulting in an annual economic burden of about $250 billion. Several medications have been approved for treating AUD (i.e., disulfiram, naltrexone, acamprosate), but these pharmacotherapies are not widely used, nor are they universally effective or appropriate to use in all AUD patients due to contraindications. To address the continued need to improve AUD treatment, identifying novel AUD pharmacotherapies is an NIAAA priority.

A wealth of data indicates that ALC use produces profound changes in the brain glutamate system. Preclinical studies show that chronic or binge-like ALC administration decreases extracellular glutamate levels, which are then increased during ALC withdrawal. These elevated glutamate levels observed during ALC withdrawal can be reduced with further ALC administration, driving a cycle of heavy ALC use and withdrawal periods that characterize AUD. The changes in glutamate homeostasis that occur during the cycle of chronic ALC intoxication and withdrawal have been attributed to altered glutamate transport (e.g., changes in cystine-glutamate exchanger [xCT] and glial glutamate transporter [GLT-1] expression and function). Normalization of extracellular glutamate levels through administration of compounds like ceftriaxone or n-acetylcysteine (NAC), which increase expression of the xCT and GLT-1, attenuates maladaptive brain changes produced by ALC, as well as ALC self-administration and behavioral sensitization in preclinical models.

These preclinical data provide a strong scientific premise for the hypothesis that normalization of extracellular glutamate levels (i.e., restoration of glutamate homeostasis) is a promising pharmacotherapeutic approach for AUD, yet this research area remains unexamined in prospective clinical studies. Because ceftriaxone must be administered intravenously and can result in serious side effects (e.g., *C. Difficile* infection), as well as antibiotic resistance, it is not feasible for testing as an AUD treatment in clinical research. NAC can be administered orally and has a low incidence of side effects making it an ideal lead compound to be evaluated as a potential medication for treating AUD. Although we are not aware of any clinical studies that have prospectively tested NAC for AUD, there is clinical evidence, including data from our laboratory, supporting its utility in treating other substance use disorders linked to disrupted glutamate homeostasis.

The overarching goal of this application is to demonstrate that restoration of glutamate homeostasis with NAC is a viable strategy for treating AUD. This goal will be achieved through the conduct of a rigorous human laboratory study designed to accomplish three specific aims.

The first aim is to demonstrate that maintenance on NAC reduces the pharmacodynamic effects of ALC that contribute to its abuse. A within-subjects study will be conducted in which non-treatment-seeking subjects with AUD (N=25) will be maintained on placebo and two doses of NAC (1.2 and 2.4 g/day). Two active doses will be tested in order to identify which dose is most effective and should be advanced to a clinical trial. Order of maintenance conditions will be randomly determined for each subject. After being maintained on each dose condition for 4 days, the reinforcing and subjective effects of ALC, including ALC craving, will be determined. Self-reported ALC use in the natural environment during maintenance will also be assessed. This reverse-engineered human laboratory approach has good predictive validity for clinical pharmacotherapy efficacy. After completing a session, subjects will undergo a 1-week washout and begin maintenance on the next condition, repeating this cycle until all conditions have been completed.

The second aim is to demonstrate the safety and tolerability of NAC when combined with ALC. Physiological responses (e.g., heart rate) and breath alcohol levels will be measured throughout each experimental session. Adverse events will also be assessed across each maintenance period in order to demonstrate that NAC can safely be administered to subjects with AUD.

A third, exploratory, aim is to evaluate potential mechanisms contributing to the influence of NAC on the pharmacodynamic effects of ALC. To this end, a number of validated trait measures (e.g., the Zuckerman-Kuhlman Personality Questionnaire, the Barratt Impulsivity Scale) and cognitive-behavioral tasks (e.g., Go/Stop Task, Alcohol Delay Discounting Task) will be completed. Outcomes from these measures will be correlated with pharmacodynamic and safety/tolerability outcomes.

The research proposed here will directly translate findings from preclinical research and provide the initial prospective clinical evidence that restoration of glutamate homeostasis with NAC has promise for treating AUD. This study will also provide basic science information about the glutamatergic mechanisms

underlying the pharmacodynamic effects of ALC in humans. Overall, the proposed research seeks to expand the scope of current AUD treatment research by focusing on glutamate homeostasis, which has strong preclinical evidence supporting its critical role in AUD but remains unstudied in clinical research.

2. OBJECTIVES

A within-subject, double-blind, NAC placebo-controlled human laboratory experiment will be conducted to demonstrate that maintenance on NAC attenuates the pharmacodynamic effects of ALC that contribute to its use (Drobes et al., 2003; Hendershot et al., 2016; O'Malley et al., 2002). ALC use in the natural environment, as well as the safety and tolerability of combining NAC with ALC, will also be evaluated.

3. STUDY DESIGN

This study will use a rigorous within-subject, double-blind, NAC placebo-controlled human laboratory design with subjects receiving all NAC doses in a randomized order (i.e., NAC is a within subject factor).

4. STUDY POPULATION

Up to 150 individuals will be screened to participate in this study. We intend to enroll 25 (17 male and 8 female) completers into the study. Screening procedures will be conducted under our lab's screening protocol (03-0509). Inclusion and exclusion criteria are outlined below.

Inclusion/Exclusion Criteria

The following criteria must be met for an individual to participate in the study: 1) able to speak/read English; 2) not seeking treatment at the time of the study; 3) female or male between the ages of 21 and 55 years; 4) one binge drinking episode (5+/4+ standard alcoholic drinks per drinking session for men and women, respectively) in the past 30 days; 5) recent ALC use verified by ethyl glucuronide positive urine, as well as fulfillment of DSM-5 diagnostic criteria for AUD; 6) other than the diagnosis for AUD at the time of the interview, subjects must be otherwise healthy; 7) ECG, read by cardiologist, within normal limits; 8) body mass index of 19-35; 9) females using an effective form of birth control and not pregnant or breast feeding; 10) judged by the medical staff to be psychiatrically and physically healthy; 11) able to abstain from ALC for 12 hours prior to session; and 12) no contraindications/allergies to NAC or ALC. Potential research subjects who are seeking treatment for substance abuse/dependence will be excluded from research participation, and will be referred to an appropriate treatment program.

Other than the diagnoses for AUD at the time of the interview, subjects must be healthy. Screening procedures for all subjects will include a medical-history questionnaire, drug-use questionnaire, physical examination, laboratory chemistries (e.g., blood chemistry screen including liver and kidney function tests, hemogram with differential, urinalysis), electrocardiogram (ECG) and a mini-mental status examination. All subjects must have a Body Mass Index (BMI) of 19-35. Dr. Rayapati must consider blood-chemistry, complete blood count and urinalysis values that are not within the normal range clinically insignificant. Dr. John Gurley, a cardiologist, or an authorized colleague, will review the results of the ECG. The ECG must be considered to be within normal limits in the opinion of Dr. Gurley for a potential subject to be enrolled. Any potential subject with a history of serious physical disease, current physical disease (e.g., impaired cardiovascular functioning, histories of seizure, head trauma, or CNS tumors) or current or past histories of psychiatric disorder, other than AUD or tobacco use disorder, will be excluded from research participation. Female subjects must be using an effective form of birth control (e.g., birth control pills, surgical sterilization, IUD, cervical cap with a spermicide or abstinence) in order to participate and must not be pregnant. A urine pregnancy test will be conducted prior to each session to ensure female subjects do not continue in the study if pregnant. All study subjects will be judged by the medical staff to be psychiatrically and physically healthy and without contraindications/allergies to NAC or ALC.

5. SUBJECT RECRUITMENT METHODS AND PRIVACY

Subjects are recruited primarily through formal advertisement (i.e., regular newspaper advertisements placed generally in free newspapers), local flyers posted in public areas (e.g., bars, restaurants, stores) and by word-of-mouth. These advertisements are approved under our screening protocol (IRB # 03-0509). Subjects make initial contact by phone with one of our recruiters who have completed the research training and HIPAA compliance web-based modules. If the subject self-discloses information that would make him/her potentially eligible for the study, they will be invited to a screening appointment. Screening is completed by one of our research assistants at the UK Laboratory of Human Behavioral Pharmacology (LHBP). Study investigators may interact with subjects in this setting and appropriate cautions are in place to ensure privacy during the intake process.

6. INFORMED CONSENT PROCESS

All potential subjects that are identified using the subject recruitment methods noted above will provide informed consent prior to participating in the protocol. Subjects that meet the eligibility criteria noted above will come to the LHBP and will undergo a field sobriety test and provide an expired air sample that will be tested for the presence of alcohol. If the subject passes the field sobriety test (walk and turn, one-leg balance [timed], finger-to-nose and backwards-counting tasks) and the expired air sample is negative, he or she will then be given a copy of the approved informed consent document to read and sign. After reading the consent document, the PI or one of the Co-Is on this protocol will address any questions the subject may have in order to assess the subject's understanding of the protocol. After this, the subject will receive a copy of the informed consent document and will sign a form indicating that they have received a copy of the form they read and signed.

7. RESEARCH PROCEDURES

Subjects that meet the inclusion criteria will participate as outpatients at the LHBP and as inpatients at the Clinical Services Core (CSC) unit of the University of Kentucky Medical Center.

Twenty-five (≈8 women) non-treatment-seeking subjects with AUD will complete the study. Subjects, recruited from the community, will earn $40/session and a $40/session completion bonus if they finish the protocol. To maximize medication adherence, a contingency management approach will be used wherein subjects will receive a monetary bonus each day that medications are administered per protocol as verified by Wisepill®. Payments will start at $5 and increase by $2 each consecutive day of dosing adherence.

The study protocol will use previously developed methods that have predictive validity for AUD treatment efficacy (Drobes et al., 2003; Hendershot et al., 2016; O'Malley et al., 2002). More specifically, the sensitivity of this clinical laboratory approach was established with a medication already known to be effective for AUD (i.e., naltrexone; see above). After screening, subjects will complete the protocol as outlined in Table 1. After completing the protocol, all subjects will be offered referral to a treatment program per the NIAAA National Advisory Council guidelines (National Council on Alcohol, 1988; Sinha et al., 1999).

Day	Table 1: Protocol Schedule
0	Practice Session at the Laboratory of Human Behavioral Pharmacology (LHBP). Matches Experimental Session, except no ALC administered, nor is there an overnight stay at the CSC. Subjects will receive NAC doses for take-home administration.
1-5	NAC (0, 0.6 or 1.2 g/dose; dose order randomly assigned) administered at 0700 and 1900h.
5	Experimental Session 1 at Inpatient Clinical Services Core (CSC) unit.
6-12	Washout.
13-17	NAC (0, 0.6 or 1.2 g/dose; dose order randomly assigned) administered at 0700 and 1900h.
17	Experimental Session 2 at Inpatient CSC unit.
18-24	Washout.
25-29	NAC (0, 0.6 or 1.2 g/dose; dose order randomly assigned) administered at 0700 and 1900h.
29	Experimental Session 3 at Inpatient CSC unit.
30	Subject discharged from study.

Subjects will be instructed to maintain their standard diet, ALC and cigarette intake throughout participation, with the exception that they will be asked to abstain from ALC for at least 12h prior to a session. All subjects will provide urine and expired-breath samples prior to experimental sessions. The presence of drugs of abuse in the urine or positive BALs upon arrival for experimental sessions will result in rescheduling of the session. Repeated occurrences will result in study dismissal. Subjects will not be allowed to smoke tobacco cigarettes during experimental sessions, consistent with previous work (O'Malley et al., 2002).

NAC Maintenance Conditions. After completing screening, a subject will be randomized to a NAC dose order condition using a random number generator. Completing subjects will experience all dose conditions. A pharmacist in the Investigational Drug Service will maintain the randomization key to preserve the double blind. NAC doses will be prepared with commercially available drug placed into opaque capsules. Cornstarch will be used as filler. Placebo capsules will contain only cornstarch, but will be visually identical to capsules that contain NAC. Placebo capsules will also be washed in a NAC slurry to control for the

distinct smell of NAC as has been done in previous research (Kevin M. Gray, M.D., Personal Communication). Subjects will receive practice taking the study capsules during the practice session. Subjects will be provided two placebo capsules during this session and instructed on how to correctly administer those capsules. Subjects will be told that these capsules are placebos, but that placebo and NAC capsules will look visually identical.

Total daily doses are 0, 1.2 and 2.4 g/day (i.e., 0, 0.6g and 1.2 g administered two times daily at 0700 and 1900h; Table 1). One of the doses reduced ALC use in one study (i.e., 1.2 g/day; Squeglia et al., 2016) and the other dose reduced elevated brain glutamate levels in another (i.e., 2.4 g/day; Schmaal et al., 2012). Testing two doses in this study will inform dose selection when NAC is advanced to a clinical trial. The dosing regimen and maintenance period were chosen based on previous studies (Amen et al., 2011; LaRowe et al., 2013; Squeglia et al., 2016).

Medication adherence will be assessed with the Wisepill® digital monitoring system. This device uses mobile phone and Internet technologies to provide real-time and comprehensive medication management. When the subject opens the device, it sends a signal to the server with a time-stamped confirmation. The Wisepill® digital monitoring system is currently being used successfully in other studies conducted in our laboratory. Subjects will receive the Wisepill® device packed with the appropriate doses in the practice session and in the afternoon of Days 13 and 25 of participation (see Table 1). They will receive $10 for coming to pick up doses.

Apparatus. After 4 full days of maintenance (i.e., on the 5th day of maintenance in each condition), subjects will be admitted to the CSC for an overnight stay while they participate in each of the three experimental sessions (see Table 2). Subjects will be tested in individual rooms on the CSC in order to eliminate the effects of social factors on the behavioral and physiological effects of ALC (e.g., Kirkpatrick and de Wit, 2013). Subjects will complete the subjective-effects questionnaires on a computer, which automates the collection of behavioral data, increasing the efficiency and accuracy of data collection and management. The TLFB and trait questionnaires (see below) will be administered using pen and paper and double verified by study staff.

Time	Table 2: Experimental Session Activities
1400-1500h	Arrival at LHBP. If the subject drove to the LHBP, the subject's car keys will be collected. Urinalysis (including pregnancy test for females), Breath alcohol level (BAL), field sobriety test, vital signs assessment, self-reported times of maintenance dosing, TLFB completed.
1500-1530h	Admission and acclimation to the CSC. Subject provided a light standardized snack.
1530-1615h	Baseline subjective, physiological and cognitive-behavioral measures.
1615-1730h	ALC Sampling. Subjective and physiological measures completed at 10, 20 and 30 minutes after dosing. Cognitive-behavioral measures completed 30 minutes after dosing.
1730-1830h	Choice Block 1. Subjects can consume ALC (up to 4 drinks total) or receive an alternative reinforcer. Subjective and physiological measures completed every 30 minutes.
1830-1930h	Choice Block 2. Details the same as noted for the 1640-1740h period.
1930-1975h	Udvalg for Kliniske UndersØgelser (UKU) Side Effects Rating Scale. Subject provided a standardized dinner.
1975-2300h	Recreational activities (i.e., movies, magazines, and books).
2300h	Lights out.
0700h	Subject provided a standardized breakfast.
0730-0800h	BAL sample and field sobriety test.
0800h	Discharged from CSC and car keys returned.

Session Activities. On experimental session days, subjects will arrive at the LHBP at the University of Kentucky at 1400h. Upon arrival to the LHBP, the LHBP staff will take the subject's car keys and will return them when the subject is released from the hospital the following morning. Should the subject leave the hospital prior to that time, the LHBP staff or subject will arrange transportation (e.g., arrange a taxi). The car keys will be returned the morning following the experimental session upon successful completion of a field sobriety test.

Urine and expired breath samples will be obtained for drug and ALC testing. A field sobriety test will be completed. Time of maintenance dosing will be confirmed by self-report and Wisepill® log. If subjects miss more than 3 doses or take doses more than 2 hours outside their scheduled window on 3 occasions within a single dose condition, they will be coached on adherence and that dose condition will be repeated. If this non-adherence occurs again during any other dose condition (or during the repeat condition), the subject will be dropped from the study. Subjects will report their ALC consumption during each 4-day maintenance block using the TLFB (Sobell et al., 1992). Subjects will be then admitted to the CSC at 1500h for their overnight stay and provided a light snack upon admission.

ALC Self-Administration Procedures. The reinforcing effects of ALC, alone and during maintenance on NAC will be assessed using a sophisticated clinical laboratory approach that has predictive validity for AUD pharmacotherapy efficacy (Drobes et al., 2003; Hendershot et al., 2016; O'Malley et al., 2002). The primary outcome measure for this procedure is Number of Drinks Chosen. Testing under this procedure involves two phases: 1) Sampling and 2) Self-Administration.

Sampling Phase. Subjects will complete a Sampling Phase at 1600h during experimental sessions to acquaint them with ALC effects. During each Sampling Phase, subjects will receive a single administration of 95% ALC mixed with non-alcoholic mixer (e.g., lemon lime soda, tonic water) (designed to raise BALs to 0.03 g/dl), to be consumed over 5 minutes.

Self-Administration Phase. After sampling the ALC dose, subjects will complete the Self-Administration Phase. During each Self-Administration Phase, subjects will complete 2 1-h choice blocks in which they will be provided with a tray containing 4 drinks, with each drink prepared with 95% ALC mixed with non-alcoholic mixer (e.g., lemon lime soda, tonic water) (designed to raise BALs 0.015 g/dl/drink) and a "tab" detailing the cost of each drink, based on the cost of a drink purchased in a local bar (i.e., $3.00). Subjects will be instructed to consume as many drinks on the tray as they want during the 1-h choice block. Subjects may instead choose to receive the corresponding dollar amount for the drinks that they do not consume. At the end of the first choice block, the drink tray will be replaced with a second tray of 4 drinks for the second choice block. Subjects can thus consume a maximum of 8 drinks or earn maximum of $24, but may choose freely between these two options. Including money as an alternative reinforcer provides an incentive for not drinking ALC, which models the natural ecology. Any money earned will be added to that subject's $40 payment for attending the session.

Subjective-Effects Questionnaires. Five standardized subjective-effects questionnaires that measure various aspects of mood and ALC effects (Ray et al., 2009; Roche et al., 2016) will be completed as outlined in Table 2: 1) ALC Urge Questionnaire to measure craving (Bohn et al., 1995); 2) Subjective High Assessment Scale (Schuckit, 1984); 3) Biphasic ALC Effects Scale (Martin et al., 1993); 4) Profile of Mood States (Schacham, 1983) and 5) Drug Effects Questionnaire (Johanson and Uhlenhuth, 1980).

Safety and Tolerability Measures. Physiological measures (i.e., BAL measurements, heart rate and blood pressure) will be recorded upon arrival at the LHBP, before ALC administration and throughout the session at the intervals noted in Table 2 using digital monitors. ALC will not be administered if a subject's heart rate is ≥100 bpm, systolic pressure is ≥150 mmHg or diastolic pressure is ≥100 mmHg. A subject will be excluded from further participation if he/she exhibits hypersensitivity (i.e., heart rate > 130 bpm, systolic pressure > 180 mmHg, diastolic pressure > 120 mmHg) during maintenance on NAC and/or to the effects of ALC. The UKU Side Effects Rating Scale will be completed at the end of sessions (Lingjaerde et al., 1987).

Trait Questionnaires and Cognitive Behavioral Tasks. To explore potential mechanisms of NAC effects, subjects will complete a battery of trait measures at screening that assess various aspects of personality, including the Zuckerman-Kuhlman Personality Questionnaire, the Barratt Impulsiveness Scale and the Snaith-Hamilton Pleasure Sale (Patton et al., 1995; Snaith et al., 1995; Zuckerman and Kuhlman, 2000). As outlined in Table 2, subjects will also complete the Commodity Purchase Task, n-back Task, the Go/Stop Task and Money and Alcohol and Money Delay Discounting Tasks. These specific tasks measure several key facets of alcohol-related decision-making and impulsivity (MacKillop, 2016; Reynolds et al., 2008) and, along with the self-administration and subjective effects outcomes described above, model key stages of the addiction cycle (Litten et al., 2016b; MacKillop, 2016).

Commodity Purchase Tasks (Appendix A). Commodity tasks will be used to assess economic demand for alcohol and soda (Amlung et al., 2015; Bruner and Johnson, 2014; Murphy and MacKillop, 2006; Strickland and Stoops, 2017). In these task subjects are asked to indicate the hypothetical number of items

(e.g., one alcoholic drink) they would purchase at a range of monetary increments (e.g., $0.00 [free] to $15/drink). All choices are hypothetical and will not be purchased or administered. Data from the commodity purchase task will be analyzed using economic demand equations previously applied to purchase task data (e.g., Strickland et al., 2016). Primary outcomes of this task include elasticity of demand (α) and intensity of demand (Q_0).

n-Back (Appendix B). The n-Back task will be used to measure working memory and working memory capacity (Jaeggi et al. 2010). In this task, subjects are presented with a sequence of numbers and asked to indicate when the current stimulus matches the one from "n" steps earlier. Two settings will be used in this study, the 1-back and the 2-back (i.e., matching 1 and 2 stimuli back, respectively). The primary outcome of this task is the percentage of correct responses.

Go/Stop Task (Appendix C). A cued go/stop task will be used as a measure of response-inhibition (Miller et al., 1991). In this task, subjects must respond to target stimuli and withhold responses to non-target stimuli. During this task, go stimuli (e.g., a horizontal rectangle) will be followed by go target stimuli (i.e., a green horizontal rectangle) 80% of the time and followed by no-go stimuli (i.e., a blue horizontal rectangle) 20% of the time. Conversely, no-go stimuli (e.g., a vertical rectangle) will be followed by no-go target stimuli (i.e., a blue vertical rectangle) 80% of the time and go target stimuli (i.e., a green vertical rectangle) 20% of the time.

Hypothetical Delay Discounting (Appendix D). A 5-trial adjusting delay discounting task will be used to rapidly assess discounting rates for various commodities (Koffarnus and Bickel, 2014). In this task, subjects making a series of 5 choices between an immediately available, smaller reinforcer and a larger reinforcer at various delays. Versions of this task with monetary (e.g., $1000 delayed versus $500 now) and alcohol (e.g., $1000 of alcohol delayed versus $500 of alcohol now) commodities will be used. Subjects will be told that all choices are hypothetical. The primary outcome of this task is the discounting rate (k). Previous research has demonstrated that this measure provides rapid and accurate discounting rates across a range of commodities (Cox and Dallery, 2016; Koffarnus and Bickel, 2014; Strickland et al., 2017).

Data Analysis

Data will be analyzed as raw scores. Statistical significance refers to $p < .05$. Number of Drinks Chosen on the ALC self-administration procedure will be analyzed with a one-factor, repeated-measure ANOVA with NAC dose (0, 1.2 and 2.4 g/day) as the factor. A significant attenuation (i.e., decreased ALC self-administration) will be inferred if the main effect of NAC attains statistical significance in the ANOVA. Tukey's HSD *post-hoc* test will be used to make appropriate pair-wise comparisons between means if the ANOVA outcome attains statistical significance. Data from the subjective-effects questionnaires and safety/tolerability measures following administration of the sampling dose will be calculated as peak effect (i.e., the maximum score observed after ALC sampling). These data will be analyzed in the same fashion as ALC self-administration data. The influence of relevant biological (e.g., age) and behavioral variables (e.g., cigarette smoking, weekly ALC use) will be determined in initial correlation analyses and included as covariates in analyses should they be significantly associated with outcomes. Outcomes from trait questionnaires and cognitive behavioral tasks will also be correlated with self-administration, subjective effect and physiological data to explore the mechanisms by which NAC attenuates the effects of ALC.

<u>*Power and Sample Size Considerations*</u>. We used the results of a prior meta-analysis of human laboratory studies of ALC self-administration and craving to determine an appropriate sample size for the present experiment (Hendershot et al., 2016). That meta-analysis demonstrated that naltrexone maintenance decreased the quantity of ALC consumed and self-reported craving relative to placebo with an average estimated effect size (i.e., Cohen's f) of approximately 0.27. Considering the overall design and use of three NAC doses, enrolling 25 subjects will provide sufficient power (power = 0.80, $\alpha = 0.05$, G*Power) to detect a medium effect size (f) of approximately 0.26 for attenuation of the effects of ALC as a function of NAC dose.

8. RESOURCES

This study will take place at University of Kentucky Laboratory of Human Behavioral Pharmacology (LHBP) and the Clinical Services Core (CSC) unit. Study visits will only be conducted on weekdays, but subjects will be provided with emergency contact information should they experience problems/AEs/SAEs on weekends. The LHBP and CSC are well equipped to conduct the necessary physiological monitoring and behavioral assessments. Dr. Rayapati is a psychiatrist who has worked extensively with individuals

with substance use disorders in both the clinical and research setting and he is the responsible medical investigator for this study. Dr. Hays is an adult psychiatrist who has worked extensively with individuals with substance use disorders, especially alcohol use disorder, in both the clinical and research setting and he is the back up medical investigator for this study. They will be available to attend to medical problems as well as any psychological or psychiatric issues that may arise. Drs. Stoops will provide scientific oversight for the study and have safely completed numerous human behavioral pharmacology studies. Overall, the study team and resources described above are well equipped to protect subjects and successfully implement, carry out and complete this study protocol.

9. POTENTIAL RISKS

The behavioral and physiological assessment procedures employed in this study are benign. The risks to the study subjects are those related to the ingestion of the drugs under study. All of the drugs to be administered in the proposed research have been administered safely to human subjects under controlled laboratory conditions. The relative safety, as well as the contraindications and possible side effects of these compounds are well known and documented. We, and others, have administered all the drugs and doses in prior studies without serious side effects. However, the administration of any drug involves some risks simply because individuals differ in their reactions to drugs. The main risk is that subjects will experience side effects that may be unpleasant.

Common side effects of ALC include drowsiness, slurred speech, headache, gastrointestinal upset, breathing difficulties, distorted vision and hearing, impaired judgment and decreased perception and coordination. More serious side effects following the chronic, unsupervised administration of much higher doses of ALC have occurred and include blackouts (memory lapses, where the drinker cannot remember events that occurred while under the influence), anemia, unconsciousness and coma. These side effects may be more frequent and larger in magnitude when testing the ALC with NAC.

Common side effects of NAC include nausea, vomiting, diarrhea and constipation. Less common side effects include rashes, fever, headache, drowsiness, low blood pressure and liver damage.

There is also the risk that others may see a subject's Protected Health Information (PHI). PHI is considered individually identifiable health information transmitted or maintained in any form (e.g., electronic means, on paper, or through oral communication) that relates to the past, present, or future physical or mental health conditions of an individual that may be used or disclosed. The following PHI will be collected as part of this project: names (e.g., individual, employer, relatives, etc.), address, telephone number, Social Security number, dates (e.g., birth, admission, discharge), medical record numbers, driver's license numbers, mental and physical health history, drug use history, results from mental and physical health screening, results from personality questionnaires and data from experimental measures.

The doses to be administered in the present experiment were chosen to minimize, if not eliminate, the chance of these side effects occurring since these side effects are related to dose. Thus, it is unlikely that subjects will experience side effects during the experimental protocol. All work proposed here will be conducted at the LHBP, under medical supervision. Side effects of the drugs are temporary, usually dissipating in less than 24 hours. The principal investigator on this project, Dr. Stoops, has had extensive experience for over 17 years administering therapeutic and supratherapeutic doses of drugs to subjects in an outpatient and inpatient setting and has never observed untoward effects. Dr. Stoops will train all staff on this project.

To avoid potential drug interactions, subjects taking any prescribed medication chronically, except birth control or as described in the inclusion/exclusion criteria above, will be excluded. The medical personnel on this protocol will determine if it is safe for a potential subject to discontinue taking their medication during their participation.

There is some theoretical risk that subjects might choose to seek out sources of drugs they received experimentally and liked. However, this risk is minimal since all drugs are administered under blind conditions and in a setting that is not conducive to the development of dependence.

10. SAFETY PRECAUTIONS

Subjects are carefully screened (history and physical exam, routine labs such as CBC, complete metabolic panel and urinalysis, ECG and psychiatric assessment) to exclude those with potential increased risk of adverse effects, such as personal or family histories of heart disease, histories of seizure or head injury associated with more than a brief loss of consciousness, hypertension, psychosis, etc. During sessions subjects remain under careful medical observation and are monitored continuously by on-site

medical staff. Vital signs will be collected throughout the dosing period. Staff is familiar with the acceptable physiological parameters for these studies. In addition, Dr. Stoops has substantial experience administering medications to human subjects under a variety of dosing conditions. Lastly, female subjects are also given pregnancy tests prior to each session to ensure that we do not administer active medications to a pregnant woman.

Legal risks including loss of confidentiality: All intake documentation that contains personal information is handled separately from the actual data collected during the study. All information of a personal nature (intake assessments, medical test results) is kept locked either on password-protected computers or in secure filing cabinets all behind locked doors and accessible only to key personnel involved in the research. A Certificate of Confidentiality has been obtained from NIAAA.

11. BENEFIT vs. RISK

The degree of risk to which individual study subjects are exposed as a consequence of their research participation is slight. In contrast, the potential and probable benefits to be derived by society in general and by patients as a group appear to be considerable. The major benefits of this study are clinical and scientific ones related to the knowledge gained about putative medications for AUD. The data from this project will contribute to a better understanding of alcohol abuse and will ultimately contribute to the development of improved prevention, control and treatment procedures. Individual study subjects are expected to benefit personally from the medical and psychiatric evaluations and from referrals for medical and psychiatric treatment that are provided whenever appropriate. Overall, the risk/benefit ratio appears favorable and the conduct of this research seems well justified.

12. AVAILABLE ALTERNATIVE TREATMENTS

There are no available alternative treatments as this is not a treatment study. If subjects express the desire for treatment they will be given referrals for treatment and not be allowed to participate in this study.

13. RESEARCH MATERIALS, RECORDS AND PRIVACY

Urine and blood samples will be collected at screening prior to a subject's participation in the experimental protocol under another IRB approved protocol (Number 03-0509). These urine samples will be tested for the presence of a full range of drugs of abuse. Blood samples will be used for the laboratory chemistries. Females will also be given a pregnancy test at the time of screening (via the urine sample). Urine drug and pregnancy tests will be conducted prior to the conduct of each experimental session. Other data obtained from the subjects will involve subjective effects based on questionnaires, various computer-based tasks and non-intrusive staff observations and ratings. The consent form states that subject's confidentiality will be protected.

14. CONFIDENTIALITY

Identifying information will be stored in a separate, locked area from all other de-identified data and codes linking the two will be kept under lock and key or on password protected computers. Incidental materials containing subject identifiers will be shredded or incinerated. Identification and access of identified data/specimens will be available only to study investigators when it is detrimental to subject safety or the conduct of the research protocol. For example, if a subject has an adverse event, we will want to obtain a quantitative drug screen to identify whether there may have been illicit drug use while in the study versus a true adverse event related to the study procedures. In the future, data/specimens may be shared with non-UK affiliations in a HIPAA compliant manner.

15. PAYMENT

Twenty-five (≈8 women) non-treatment-seeking subjects with AUD will complete the study. Subjects, recruited from the community, will earn $40/session and a $40/session completion bonus if they finish the protocol. Subjects may also make up to $24/session during the ALC self-administration phase if they do not choose to drink any of the alcohol available. To maximize medication adherence, a contingency management approach will be used wherein subjects will receive a monetary bonus each day that medications are administered per protocol as verified by Wisepill®. Payments will start at $5 and increase by $2 each consecutive day of dosing adherence. Subjects will receive the Wisepill® device packed with the appropriate doses in the mornings of Day 1, 13 and 25 of participation (see Table 1). They will receive $10 for coming to pick up doses. The total possible earnings during the course of the trial are $707 if every appointment is kept/dose is taken as scheduled and no alcohol is chosen during the self-administration phase. We believe that this strategy is a relatively inexpensive investment that, if successful, will ensure the power and scientific integrity of a future large-scale clinical trial.

16. COSTS TO SUBJECTS

There will be no cost to the subject for participating. Costs for the screening procedures (i.e., medical history questionnaire, physical examination including laboratory chemistries (blood chemistry screen, complete blood count, urinalysis) and a psychiatric examination will be paid by the Laboratory of Human Behavioral Pharmacology.

17. DATA AND SAFETY MONITORING

Data Monitoring Plan. Data will be collected using a computerized data collection and management system. This system automates the collection of the subjective-effect and physiological data, which ensures the accuracy and completeness of data collection. The data are stored in a unique file on the hard drive of the computer and are printed after all the tasks are completed. Paper-and-pencil task data are verified by staff and double-entered into excel spreadsheets after each session. In all instances, the data files do not contain the name of the subject; but instead, a unique four-digit number identifies each subject. A computer file linking the unique number with the subject's name will be kept on a stand-alone, password-protected computer. Data files for experimental tasks and physiological measures from each experimental session will be manipulated and combined into a single electronic spreadsheet for each subject by the PI. Data for all subjects will then be combined into a single electronic spreadsheet and separated by experimental measure for statistical analysis using SPSS (IBM Corporation, Armonk, NY).

The primary outcome for this study will be Number of Drinks during ALC self-administration. Secondary outcome measures will include craving, BALs, physiological and subjective response to ALC, self-reported ALC use in the natural environment during maintenance, as well as performance on cognitive-behavioral tasks, and the Udvalg for Kliniske UndersØgelser (UKU) Side Effects Rating Scale.

As noted above, the majority of data are collected using an automated computer system, which increases the accuracy and completeness of data collection and ensures the validity and integrity of the data. Hand written questionnaires and tasks are double-entered and verified by staff prior to analysis. The initial data manipulation described above will be conducted twice and compared. The quality of manipulated data and data analyses will be monitored by random inspection by the PI and/or Co-Investigators.

Safety Monitoring Plan. Potential subjects will provide information regarding their ALC and drug use history and undergo an extensive physical and psychiatric health screening to determine their eligibility and safety of their participation. Potential subjects must meet DSM-5 criteria for AUD and must present with a urine sample positive for ethyl glucoronide at the time of screening. Any potential subject with a history of clinically significant physical disease, current physical disease (e.g., impaired cardiovascular functioning, histories of seizure, head trauma or CNS tumors), or current or past histories of psychiatric disorder, other than AUD or tobacco use disorder, will be excluded from research participation. Females must be using an effective form of birth control in order to participate and must not be pregnant. Methods for monitoring AEs will include observations by the medical and research staff, spontaneous report by the subjects, regular measurement of cardiovascular indices and use of the UKU Side Effects Rating Scale and subjective-effects questionnaires. Subjects will not receive study drug if they have any signs or symptoms that may contraindicate its administration (e.g., heart rate and blood pressure outside of predetermined range).

All AEs occurring during the course of the study will be collected, documented and reported to the PI. The occurrence of AEs will be assessed daily for the duration of participation and during the follow-up visits at 2 and 4 weeks following discharge. Each week a study investigator will review the AE forms from the previous week for events that were reported as new or continuing. The study investigators will follow all AEs to the point of a satisfactory resolution. Subjects may be withdrawn from the study if the medically responsible investigator determines it is the best decision in order to protect the safety of the subject. All AEs will be assessed to determine if they meet criteria for a serious AE.

Serious AEs, as defined by the FDA, will be systematically evaluated daily for the duration of participation and during the follow-up visits at 2 and 4 weeks following discharge. Any serious AE, whether or not related to the study drug, will be reported to the IRB, NIAAA, and the FDA. The initial serious AE report will be followed by submission of a completed serious AE report to all three institutions.

In the event that a subject either withdraws from the study or the investigator decides to discontinue a subject due to a serious AE, the subject will have appropriate follow-up medical monitoring. Monitoring will continue until the problem requiring hospitalization has resolved or stabilized with no further change expected, is clearly unrelated to the study medications, or results in death. Outcome of serious AEs will be

periodically reported to NIAAA. A summary of the serious AEs that occurred during the previous year will be included in the annual progress report to NIAAA.

18. SUBJECT COMPLAINTS

Subjects may at any time ask study personnel questions about the study procedures or make complaints. All staff will be aware to notify Drs. Stoops, Rush, Lile, Hays, or Rayapati about any subject concern or complaint as it arises. Subjects will be allowed the opportunity to discuss any concerns or questions with an investigator promptly, in person and in confidence. It should be noted, however, that subjects will be told that some concerns and complaints will not be kept private such as an adverse event, protocol deviation or threat to the safety of subjects or integrity of the research study. In these cases, all information will be made available to the Principal Investigator in order to determine any further course of action. Dr. Hays or Rayapati will also communicate with the nursing or laboratory staff on at least a weekly basis in order to discuss any concerns regarding particular subjects or with respect to the conduct of the study.

19. RESEARCH INVOLVING NON-ENGLISH SPEAKING SUBJECTS OR SUBJECTS FROM A FOREIGN CULTURE Not Applicable.

20. HIV/AIDS RESEARCH POLICY Not applicable.

21. PI SPONSORED FDA-Regulated Research Not applicable.

Appendix A

Commodity Purchase Task

Example Instructions for Subjects

This is a series of questions designed to assess choices for alcohol across changes in price. This information is entirely for research purposes. All questions about purchasing are completely hypothetical (pretend).

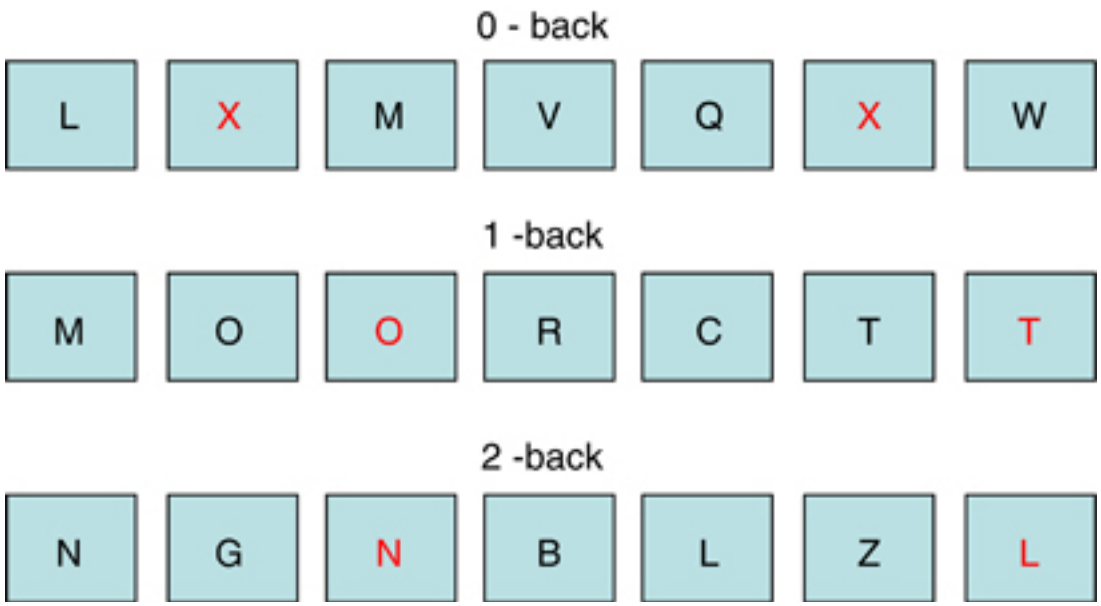
Imagine a TYPICAL DAY during which you drink alcohol. The following questions ask how much alcohol you would consume if it cost various amounts of money. The alcohol is your preferred brand and type (e.g., beer, wine, liquor). Assume that each drink is a standard drink (i.e., 12 oz. beer, 5 oz. wine, 1.5 oz. shot alone or in mixed drink). Assume that you have the same income that you have now and NO ACCESS to any alcohol products than those offered at these prices. In addition, assume that you would consume all the alcohol you purchase on that day; that is you cannot save or stockpile any for a later date.

Example Questions for Subjects (for the Alcohol Purchase Task)

Price/Drink	# Drinks Purchased
Free	
$0.02	
$0.05	
$0.13	
$0.25	
$0.50	
$1.00	
$2.00	
$3.00	
$4.00	
$5.00	
$6.00	
$7.00	
$8.00	
$9.00	
$10.00	
$11.00	
$12.00	
$13.00	
$14.00	
$15.00	

Appendix B

n-Back Trial Diagram (Figure from Borgwardt et al. 2012)



APPENDIX C

Traditional Cued Go/No-go Task

Cued RT

Set-up

- Select Cued RT Task – FB
- Press space bar to begin task

Instructions to Participant:

This is a reaction time task that I would like you to perform. While you are performing the task you sit in front of the computer screen just as you are doing. You place your index finger on the green dot (?) key.

Presented on the screen will be rectangular boxes that are standing upright or lying flat. The boxes are empty when they first appear on the screen. If the box turns green then you are to press the green dot (?) button as quickly as possible. If the box turns blue then no response is required.

Now, before the box appears, you will see a plus sign in the middle of the screen. It serves as a fixation point so that you know where to focus your attention on the computer screen. After the plus sign disappears, a box will appear on the screen. Again, if the box turns green, respond as quickly as possible by pressing the green dot (?) key. If the box turns blue then no response is required.

To help you respond quickly, the computer will display how fast you are pressing the key when the green target appears. Once you respond to a green target, the screen will show the amount of time it took for you to make a response. So lower numbers are better. If you accidentally respond to a blue target, the screen will say “Incorrect Response.”

*** There are four 30-second breaks programmed into the task

Appendix D

5-Trial Adjusting Delay Discounting Task (Table from Koffarnus and Bickel 2014)

The below table describes the outcomes for the 5-trial task. For each of the 5 choices (i.e., No.), the subject is asked if the would prefer the immediate or delayed reinforcer. The delay to the delayed choice is systematically increased or decreased based on previous trial choice (i.e., Delay Choice; increases if delay is chosen, decreases if immediate is chosen). The primary outcome is k as labeled in the table below.

Table 1
Parameters of the Possible Individual Choice Trials in the 5-Trial Adjusting Delay Task

			ED_{50} (days) if last choice is:		*k* if last choice is:	
Index	Delay choice	No.	Immediate	Delayed	Immediate	Delayed
1	1 hr	5	0.04167	0.05893	24.0	17.0
2	2 hr	4				
3	3 hr	5	0.1021	0.1444	9.79	6.93
4	4 hr	3				
5	6 hr	5	0.2041	0.3062	4.90	3.27
6	9 hr	4				
7	12 hr	5	0.4330	0.7071	2.31	1.41
8	1 day	2				
9	1.5 days	5	1.225	1.732	0.816	0.577
10	2 days	4				
11	3 days	5	2.450	3.464	0.408	0.289
12	4 days	3				
13	1 week	5	5.292	8.573	0.189	0.117
14	1.5 weeks	4				
15	2 weeks	5	12.12	17.15	0.0825	0.0583
16	3 weeks	1				
17	1 month	5	25.28	43.05	0.0396	0.0232
18	2 months	4				
19	3 months	5	74.56	105.4	0.0134	0.00949
20	4 months	3				
21	6 months	5	149.1	210.9	0.00671	0.004741
22	8 months	4				
23	1 year	5	298.2	516.5	0.00335	0.00194
24	2 years	2				
25	3 years	5	894.7	1265.	0.00112	0.000791
26	4 years	4				
27	5 years	5	1633.	2310.	0.000612	0.000433
28	8 years	3				
29	12 years	5	3579.	5368.	0.000279	0.000186
30	18 years	4				
31	25 years	5	7748.	9131.	0.000129	0.000110

Note. ED_{50} = Effective Delay 50%.