YALE UNIVERSITY SCHOOL OF MEDICINE
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Research

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: Glutamate-opioid interactions in alcohol drinking behaviors			
Principal Investigator: Suchitra Krishnan-Sarin, Ph.D.		**Yale Academic Appointment:** Associate Professor	
Campus Address: S-208, 34 Park Street, New Haven, CT 06519			
Campus Phone: 203-974-7595	**Fax:** 203-974-7606	**Pager:**	**E-mail:** suchitra.krishnan-sarin@yale.edu
Protocol Correspondent Name & Address (*if different than PI*): Dana Cavallo, 34 Park Street, New Haven CT 06519			
Campus Phone: 203-974-7607	**Fax:** 203-974-7606	**E-mail:** dana.cavallo@yale.edu	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☒ **N**			**tment:**
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

SECTION II: GENERAL INFORMATION

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

a. Internal Location[s] of the Study:

☐ Magnetic Resonance Research Center (MR-TAC) ☐ PET Center
☐ YCCI/Church Street Research Unit (CSRU)
☐ Yale Cancer Center ☒ YCCI/Hospital Research Unit (HRU)
☐ Yale-New Haven Hospital ☐ YCCI/Keck Laboratories
☐ Specify Other Yale Location ☐ Cancer Data Repository/Tumor Registry

b. External Location[s]:

☐ APT Foundation, Inc. ☐ Haskins Laboratories
☒ Connecticut Mental Health Center ☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven ☒ Other Locations, Specify: CMU

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

☒ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC) Approval Date:

☐ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
☐ *Radioactive Drug Research Committee (RDRC) Approval Date:
☐ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☐ Magnetic Resonance Research Center PRC (MRRC-PRC) Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form

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

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities. 5 years

3. **Targeted Enrollment:** What is the number of subjects: 86 non-treatment-seeking, alcohol-dependent, family history positive heavy drinkers
   a. targeted for enrollment at Yale for this protocol? 86 participants
   If this is a multi-site study, what is the total number of subjects targeted across all sites?
   b. expected to sign the consent form? 172
   c. expected to complete some or all interventions for this protocol? 86

4. **Research Type/Phase: (Check all that apply)**

   a. Study Type
   ☒ Single Center Study
   ☐ Multi-Center Study
   Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☐
   ☐ Coordinating Center/Data Management
   ☐ Other:

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

   c. Area of Research: (Check all that apply) Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:
   ☒ Clinical Research: Patient-Oriented
   ☒ Clinical Research: Epidemiologic and Behavioral
   ☐ Translational Research #1 ("Bench-to-Bedside")
   ☐ Translational Research #2 ("Bedside-to-Community")
   ☐ Clinical Research: Outcomes and Health Services
   ☐ Interdisciplinary Research
   ☐ Community-Based Research

5. Is this study required to be registered in a public database? Yes ☒ No ☐
   If yes, where is it registered?
   Clinical Trials.gov registry ☒
   Other (*Specify*)

6. Will this research study utilize clinical care services at Yale New Haven Hospital or YMG?
   Yes ☒ No ☐

If yes, might these be billable to the subject, the sponsor, grant or other third party payer?
Yes ☐ No ☒
If you answered "yes", please register this study in the IDX/GE system at
http://www.yalemedicalgroup.org/pfs/forms/10000/NewStudyRequest.pdf

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

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

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? No

c. Will a novel approach using existing equipment be applied? No

If you answered "no" to question 7a, or "yes" to question 7b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

SECTION III: FUNDING, RESEARCH TEAM AND TRAINING

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

PI	Title of Grant	Name of Funding Source	unding	unding Mechanism
Suchitra Krishnan-Sarin, Ph.D.	Glutamate-opioid interactions in alcohol drinking behaviors	NIAAA	Internal X External	X Grant-M# 140141 Contract#
John Krystal	Center for Translational Neuroscience of Alcohol	NIAAA	Internal X External	X Grant-M# Contract#

2. **Research Team:** List all members of the research team. Indicate under the affiliation column whether the investigators or study personnel are part of the Yale faculty or staff, or part of the faculty or staff from a collaborating institution, or are not formally affiliated with any institution. **ALL members of the research team MUST complete Human Subject Protection Training (HSPT) and Health Insurance Portability and Accountability Act (HIPAA) Training before they may be listed on the protocol. See NOTE below.**

*****My signature here indicates that I have read, am in compliance with, and will continue to be in compliance with the HIC's Protocol-Specific Conflict of Interest policy and the University's policy on Conflict of Interest and Conflict of Commitment. NOTE: The HIC will remove from the protocol any personnel who have not signed the application and/or completed required training. A personnel protocol amendment will need to be submitted when training is complete or signature is provided.**

	Name	Signature ***	Protocol-Related COI?	Affiliation
Principal Investigator	Suchitra Krishnan-Sarin, Ph.D.		Yes ☒ No	Yale faculty
Role: Co-I	Stephanie O'Malley		Yes ☒ No	Yale faculty
Role: Project Manager	Dana Cavallo, Ph.D.		Yes ☒ No	Yale faculty
Role: Data Manager	Elaine LaVelle		Yes ☒ No	Yale staff
Role: Research Assistant	Nicholas Franco		Yes ☒ No	Yale staff
Role: Research Assistant	Tricia Dahl Thomas Liss		Yes ☒ No	Yale staff
Role: Postdoc	Krysten Bold			
Role: Research Assistant	Heather LaVallee Alissa Goldberg Maggie Mae Mell Michael Kleinberg Cameron DeLeone		Ye ☒ No	Yale staff
Role: Study Nurse	Denise Romano, FNP, APRN		Yes ☒ No	Yale staff
Role: Therapist	Grace Kong		Yes ☒ No	Yale staff
Role: Study physician	Julia Shi, M.D.		Yes ☒ No	Yale faculty
Role: Co-PI	John Krystal, Ph.D.		Yes ☒ No	Yale faculty
Role: Study physician	Jeanette Tetrault, M.D.		Yes ☒ No	Yale faculty

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

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

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

_____
PI Name (PRINT) and Signature Date

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

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

_____
Advisor Name (PRINT) a

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Department Chair's Assurance Statement

Do you know of any real or apparent institutional conflict of interest (e.g., Yale ownership of a sponsoring company, patents, licensure) associated with this research project?
☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?
☐ Yes, and I agree to submit the Protocol-Specific Conflict of Interest Disclosure Form.
☐ No

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

_____
Chair Name (PRINT) and Signature Date

_____
Department

YNHH Human Subjects Protection Administrator Assurance Statement

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

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:
- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to submit a Protocol-Specific Conflict of Interest Disclosure Form if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and had the support of the hospital for this research project.

_____
YNHH HSPA Name (PRINT) and Signature Date

SECTION V: RESEARCH PLAN

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

Development of new and effective strategies to treat alcoholism should be based on our understanding of the behavioral and neurochemical mechanisms mediating alcohol reward and drinking. Initiation and progression of alcohol-seeking behaviors involve complex interactions in the cortico-striatal circuitry between neurotransmitters like dopamine and opioids that signal reward in the nucleus accumbens and engage stimulus response habits in the dorsal striatum, as well as glutamatergic projections from the prefrontal cortex and basolateral amygdala to the nucleus accumbens that are involved in the reinstatement of alcohol drinking.

In HIC # 9912011476 and HIC # 0602001068, we examined the independent roles of dysfunctions in the glutamatergic and opioidergic circuits in mediating alcohol-drinking behaviors, using an alcohol drinking paradigm (ADP) for heavy drinkers developed by our group (O'Malley, Krishnan-Sarin et al., 2002). Using an NIH funded grant in HIC # 9912011476, we showed for the first time that the opioid antagonist naltrexone (NTX) significantly reduced drinking in drinkers with a positive family history (FH) of alcoholism (FHP; Krishnan-Sarin et al., 2007) but increased drinking in those without a FH of alcoholism (FHN), with no consistent changes in alcohol craving and stimulation. Similarly, interim analyses from HIC # 0602001068 indicate that the glutamate (NMDA) antagonist memantine (MEM) significantly reduced alcohol stimulation and craving with emerging, modest reductions in drinking (Krishnan-Sarin et al., 2009a) in FHP drinkers, with no consistent changes in FHN drinkers. This exciting evidence provides the first demonstration of distinctive roles for the glutamatergic and opioidergic systems in FHP drinkers; specifically, blocking opioid receptors with NTX appears to reduce stimulus response habits and alcohol drinking, while blocking NMDA receptor function with MEM appears to reduce alcohol reward.

In the current proposal we would like to conduct an important extension of this work. We will test the hypothesis that by targeting both opioidergic and glutamatergic mechanisms, one may synergistically target multiple neurochemical and behavioral processes, leading to optimal reduction of alcohol drinking. This pilot trial will be conducted in FHP drinkers in whom we have observed reduction in alcohol reward with MEM and in alcohol drinking with NTX. We will test the efficacy and tolerability of the combined use of MEM and NTX. We will also test the efficacy and tolerability of the combined use of N-acetyl cysteine and NTX. N-acetylcysteine (NAC), an acetyl pro-drug of cysteine is another agent which can be use to target the glutamatergic system. NAC is believed to produce its effects by stimulating the cysteine-glutamate exchanger and thus altering synaptic glutamate levels. A significant potential advantage of NAC is better tolerability, which may make it a better glutamate-altering agent to use in combination with NTX. Finally, we will also evaluate the predictive utility of impulsive responding, implicit alcohol motivational tendencies, and variations in genes for opiate receptors and for signal transduction proteins linking dopamine and glutamate receptors upon alcohol drinking behaviors.

The following specific aims will be tested:

Primary Aim 1: Evaluate the effects of pretreatment with a combination of naltrexone (NTX; 50 mg) and memantine (MEM; 20 mg) or N-acetylcysteine (NAC; 2400 mg) on drinking in FHP drinkers.

Primary Hypothesis: Combination treatment with NTX and MEM or NAC, when compared with NTX alone, will reduce number of drinks consumed during the self-administration period.
Exploratory hypothesis: Combination treatment with NTX and MEM will reduce time to first drink and increase the average time to consume each drink, during the self-administration phase.

Primary Aim 2: Evaluate the effects of pretreatment with a combination of naltrexone (NTX; 50 mg) and memantine (MEM; 20 mg) or NAC (2400 mg) on alcohol craving in FHP drinkers
Hypothesis: Combination treatment with NTX and MEM or NAC, when compared with NTX alone, will reduce alcohol craving (Yale Craving Scale) during the self-administration period
Exploratory hypothesis: Combination treatment with NTX and MEM will reduce craving in response to the alcohol cue presented just prior to the priming drink period.

Primary Aim 3: Evaluate the effects of pretreatment with a combination of naltrexone (NTX; 50 mg) and memantine (MEM; 20 mg) or NAC (2400 mg) on alcohol-induced stimulation in FHP drinkers.
Hypothesis: Combination treatment with NTX and MEM or NAC, when compared with NTX alone, will reduce alcohol-induced stimulation (Biphasic Alcohol Effects Scale) during the self-administration period.

Secondary Aim: Examine the tolerability of co-administration of NTX and MEM and NTX and NAC

Exploratory Aims: Examine the influence of innovative predictors of treatment response, including:

- Impulsive propensity and implicit alcohol associations
- Opioidergic (OPRM1) and signal transduction proteins that moderate the function of NR2B-containing NMDA receptors (Fyn kinase, STEP) genotypes
- Pavlovian to Instrumental transfer propensity

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

Alcohol use, abuse, and dependence are a huge public health problem in the United States and worldwide. Epidemiological evidence suggests that almost 17.6 million adults in the U.S. are abusing alcohol or are alcohol dependent (Grant et al., 2004), emphasizing the importance of developing optimal interventions for the treatment of this disorder. At present, three medications have been approved by the FDA in the U.S. for the treatment of alcohol dependence: disulfiram, naltrexone, and acamprosate. However, the treatments effects for these agents range from minimal to modest, and they are minimally utilized in clinical community settings (Garbutt et al., 1999; Krishnan-Sarin et al., 2009b). Therefore, there is a significant need for other medications, or combinations of medications, that are more efficacious in reducing alcohol use.

A cornerstone to the development of pharmacotherapy to reduce alcohol drinking rests in a detailed understanding of the neurotransmitters involved in mediating alcohol's effects. Alcohol influences a variety of neurotransmitters, such as opioids, dopamine, serotonin, gamma amino butyric acid, and glutamate, and each one of these neurotransmitter systems has been shown to play a role in many of alcohol's effects, including but not limited to alcohol reinforcement, craving, and withdrawal (Krishnan-Sarin et al., 2009; Krystal and Tabakoff, 2002). While most clinical trials have focused on single pharmacological agents with single mechanisms of action,

the above evidence suggests that it would be important to investigate the efficacy of combining drugs that influence multiple neurotransmitter systems. In fact the development of combination therapies that have minimal side effects and can be used to produce optimal reductions in alcohol drinking in habitual drinkers by targeting different behaviors is a major priority of treatment development (e.g., Kranzler, 2000).

Naltrexone and alcohol drinking:

Naltrexone's efficacy in reducing alcohol drinking is believed to be mediated through interactions between the endogenous opioid system and dopamine systems, specifically through antagonism of the mu-opioid receptors. The most commonly used dose of naltrexone of 50 mg/day has been shown to result in complete occupancy of the brain mu-opioid receptors (Weerts et al., 2008). Clinical trials indicate that treatment seeking drinkers who receive naltrexone at a dose of 50 mg/day in combination with a behavioral intervention have lower levels of relapse to drinking during the treatment period. However, it is important to note that not all the clinical trials conducted with naltrexone over the past decade have observed significant improvements in drinking treatment outcomes (e.g. Krystal et al., 2001), and two recent meta-analytical reports suggest that naltrexone has small to modest efficacy in preventing relapse to drinking (Bouza et al., 2004; Srisurapanont and Jarusuraisin, 2002). Moreover, while naltrexone is relatively well tolerated, the potential risk of hepatotoxicity at high doses requires caution in use in those with liver disease. A more recent multi-site trial which used a higher dose of naltrexone (100 mg/day) with medical management to enhance compliance found significant reductions of alcohol drinking with naltrexone, although again with a small effect size in the overall sample (Anton et al., 2006).

Many investigations have attempted to understand the modest efficacy of naltrexone by evaluating its mechanism of action as well as understanding factors that predict treatment response to naltrexone. Naltrexone is believed to produce its effects by blocking the GABAergic neurons that tonically inhibit the dopamine neurons that project to the nucleus accumbens from the ventral tegmental area (see Johnson, 2008). From a behavioral perspective, early studies conducted in social drinkers observed that administration of a single dose of naltrexone (50 mg) followed by an alcoholic drink resulted in more sedation and less stimulating effects of alcohol (Swift et al., 1994), and that reduction in alcohol-induced stimulation was only observed in those who had a high risk of developing alcoholism (King et al., 1997). More recent examinations of naltrexone in alcohol-dependent drinkers using self-administration paradigms (Davidson et al., 1999; O'Malley et al., 2002; Drobes et al., 2003) and clinical trials (e.g. Richardson et al., 2008) suggest that the efficacy of naltrexone may be related to its ability to reduce craving or urge to drink. Myrick et al. (2008) observed that naltrexone reduced alcohol-cue-induced activation of the ventral striatum. Similarly, naltrexone reduced alcohol-induced stimulation, positive mood, and craving experienced in response to an intravenous dose of alcohol in alcohol-dependent drinkers (Ray and Hutchinson, 2007). However, our evidence from HIC # 9912011476 suggests that naltrexone-induced reduction in drinking in FHP drinkers is not accompanied by any consistent changes in alcohol craving or stimulation.

Evaluations of predictors of treatment response to naltrexone have identified many factors including the presence of a family history of alcoholism and variants of the mu-opioid receptor gene (OPRM1 gene). Our work in HIC # 9912011476 found that alcoholics with a family history of alcoholism appear to respond better, with greater reductions in alcohol consumption than those who have a negative family history of alcoholism (Krishnan-Sarin et al., 2007). Moreover, individuals who have a variant of the OPRM1 gene (or the gene for the mu-opioid

receptor where naltrexone produces its effects) have better response to naltrexone treatment in most (Oslin et al., 2003; Oroszi et al., 2009; Anton et al., 2008) but not all (Gelernter et al., 2007) clinical trials.

In summary, the opioidergic system is an important modulator of alcohol effects and the opioid antagonist naltrexone has been shown to be effective at reducing alcohol drinking. Existing evidence also suggests that naltrexone may reduce alcohol drinking by attenuating craving for alcohol and that variants of the OPRM1 gene and family history of alcoholism may predict treatment response. However, the overall efficacy of naltrexone in reducing alcohol drinking is rather modest, with most clinical trials documenting a medium effect size. Interestingly, in our earlier project (HIC # 9912011476), while we observed significant naltrexone-induced reductions in drinking in FHP drinkers, these changes were not accompanied by consistent changes in alcohol craving or alcohol reward. All the above evidence suggests that there is room for improvement and a great need for medication approaches that have greater efficacy in reducing alcohol use.

Glutamate and alcohol addiction:

The amino acid L-glutamate is the major excitatory amino acid neurotransmitter in the central nervous system. The glutamate system is involved in fast synaptic transmission as well as plasticity and higher cognitive functions. Glutamate produces its effects by binding to one of three different types of ionotropic postsynaptic receptors: the *N*-methyl-D-aspartate (NMDA) receptor, the alpha-amino-3-hydoxy-5-methylisoxazole-4-propionate (AMPA), and the kainate receptor (Parsons et al., 2005). Glutamate also produces some of its effects by binding to metabotropic glutamate receptors (mGluR's) located in the perisynaptic and presynaptic regions (Gass and Olive, 2008). Most important, glutamate is one of the primary neurochemical substrates of synaptic plasticity, and it is believed that neuroadaptations in glutamatergic transmission produced by chronic drug use leads to compulsive drug use (Gass and Olive, 2008; Koob et al., 2009; Kalivas et al., 2005).

Alcohol appears to have paradoxical effects on this system, with increases in extracellular levels of glutamate observed in a variety of brain regions, both after administration of low doses of alcohol as well as following withdrawal from chronic alcohol use. The NMDA receptor is one of the primary high-affinity targets for ethanol in the brain (Grant and Lovinger, 1995), and acute ethanol administration dose-dependently attenuates NMDA receptor function (Grant and Lovinger, 1995; Hoffman et al., 1990; Lovinger et al., 1989). Ethanol-induced modulation of NMDA receptor function is observed at doses of ethanol that produce intoxicating behavioral effects in animals (Woodward, 1999). NMDA receptors in many brain regions, including the cerebral cortex, septum, amygdala, hippocampus, locus coeruleus, cerebellum, and the VTA and nucleus accumbens, are sensitive to this effect of ethanol (see Gass and Olive, 2008). Preclinical studies indicate that chronic exposure to ethanol inhalation enhances glutamatergic synaptic transmission in the nucleus (Nie et al., 1993), suggesting that NMDA receptors are up-regulated by chronic ethanol exposure. During ethanol withdrawal, this glutamatergic up-regulation and subsequent enhanced Ca2+ influx contribute to ethanol withdrawal and associated neurotoxicity (Hoffman et al., 1990; Tsai et al., 1995; Tsai and Coyle, 1998). NMDA receptors also appear to be essential for developing tolerance to many of ethanol's behavioral effects (Rafi-Tari et al., 1996; Szabo et al., 1994; Wu et al., 1993). Ethanol also appears to inhibit the function of AMPA and KA receptors, but these receptors seem to be less sensitive to inhibition by ethanol, which is only observed at higher concentrations.

Preclinical drug discrimination studies indicate that NMDA antagonists have ethanol-like discriminative stimulus properties, particularly at higher training doses of ethanol (Grant and Columbo, 1993; Hundt et al., 1998). There have been some examinations of sub-anesthetic doses of NMDA antagonists in humans (Krupitsky et al., 2001; Krystal et al., 1998; Petrakis et al., 2004; Schultz and Soyka, 2000). Anecdotal reports from healthy human subjects indicate that NMDA antagonists like PCP (Luby et al., 1959) and ketamine (Krystal et al., 1994) produce ethanol-like subjective effects. Studies conducted in alcohol-dependent subjects reported that ketamine's euphoric effects were associated with sedation and cognitive impairment. Similar to the preclinical studies, lower doses of ketamine were judged to be more alcohol-like and similar to 1-2 standard drinks, while higher doses of ketamine were judged to be similar to 8-9 alcoholic drinks (Krystal et al., 1998; Krupitsky et al., 2001; Petrakis et al., 2004).

The above evidence suggests that the NMDA-related actions of ethanol may contribute to its behavioral effects. Therefore, drugs modulating this component of ethanol's effects should attenuate ethanol intoxication and reward. In support of this, extensive preclinical and molecular evidence suggests that NMDA receptor antagonists reduce operant responding for alcohol and alcohol self-administration (Bienkowski et al., 1999; Holter et al., 1996; Holter et al., 2000; Lin and Hubbard, 1995), deprivation-induced alcohol drinking (Vengeliene et al., 2005), cue-induced reinstatement of alcohol drinking (Backstrom and Hyttia, 2004; Bachteler et al., 2005), acquisition of conditioned place preference for alcohol (Biala and Kotlinska, 1999; Boyce-Rustay and Cunningham, 2004), and sensitization to the locomotor-stimulating effects of alcohol (Broadbent and Weitemier, 1999; Meyer and Phillips, 2003; Kotlinska et al., 2007).Acamprosate, or calcium acetyl homotaurinate, an NMDA receptor modulator (Harris, 2002; Pope and Lovinger, 2000), is FDA approved for the treatment of alcohol drinking, and has been shown in preclinical and clinical studies to reduce alcohol consumption (Chick, 2003; Heyse et al., 1998; Stromberg et al., 2001; Spanagel et al., 1996; Heyser et al., 1998; Vengeliene et al., 2007). However, more recent clinical evidence questions the efficacy of this agent in reducing alcohol drinking (Anton et al., 2006). Topiramate, another agent that targets the kainate and AMPA glutamatergic system as well as extrasynaptic GABA-A receptors (see review by Krystal et al., 2006), has also shown preliminary efficacy in reducing alcohol drinking in clinical RCT (Johnson et al., 2007; see review by Kenna and colleagues, 2009); however, the agent's adverse event profile may limit its utility.

Memantine:

Memantine is a non-competitive NMDA antagonist with secondary effects of blocking alpha-7 nicotinic receptors (Arcava et al., 2005; Zakharova et al., 2005). A significant advantage of memantine is it's selectivity as an NMDA antagonist, as well as its tolerability and lack of abuse liability, in contrast to some other agents like amantadine, ketamine, and dextromethorphan which also block NMDA receptors (Danysz et al., 1997; Kohler et al., 1997). There is extensive evidence of the safety and tolerability of memantine (Kavirajan, 2009), and it is FDA approved for the treatment of moderate to severe Alzheimer's disease. Unlike ketamine, memantine does not have profound perceptual effects. Memantine also increases intracortical inhibition, reduces intracortical facilitation (Schwenkreis et al., 1999) and enhances vigilance in healthy elderly volunteers (Schultz et al., 1996), with no significant effects on on mood, attention, or immediate and delayed recall (Schugens et al., 1997). Memantine was well tolerated in randomized double-blind, placebo-controlled trials for patients with moderately severe to severe dementia and moderate to severe Alzheimer's disease, as well as vascular dementia (Lundbeck, 2002; Reisberg et al., 2003; Winblad and Poritis, 1999). The most common adverse events observed in clinical trials are diarrhea, insomnia, dizziness, headache, constipation, and confusion (Jarvis and Figgit,

2003). Memantine is recommended in doses of up to 20 mg/day for the treatment of dementia but has also been evaluated at doses of up to 40 mg/day in placebo-controlled trials of neuropathy (Forrest labs, unpublished data). Importantly, evidence from our HIC # 0602001068 project also indicates that this agent was well tolerated in FHP drinkers (see preliminary section).

In regard to alcohol effects, preclinical evidence suggests that memantine attenuates alcohol-withdrawal-induced neurotoxicity in hippocampal neurons (Stepanyan et al., 2008), potentiates the ataxic effect of alcohol (Chen and Holmes, 2009), and reduces alcohol drinking in a schedule-induced polydipsia paradigm (Escher and Mittleman, 2006). Bisaga and Evans (2004) observed that memantine (in doses of 15 and 30 mg) was well tolerated and reduced alcohol craving in moderate social drinkers and resulted only in mild dissociative effects when combined with alcohol. Memantine, in doses of 20 mg/day and 40 mg/day, produced dose-dependent decrease in cue-induced alcohol craving in recently abstinent, alcohol-dependent participants (Krupitsky et al., 2007). Two open-label trials with memantine, at a dose of 20 mg/day, reported improvement in alcohol-related dementia (Cheon et al., 2008) and reduction in alcohol drinking and urges to drink (Arias et al., 2007). More recent evidence from a small 16 week, double-blind, placebo-controlled trial of a 40 mg/day dose in alcohol-dependent drinkers did not demonstrate any significant benefits for memantine (Evans et al., 2007); while the results of this trial raise questions about the efficacy of memantine as a stand-alone agent, it is also important to note that this trial did not examine differences in efficacy by family history of alcoholism.

Pilot evidence from our ongoing project suggests that memantine's effects are related to dose as well as family history of alcoholism; specifically, we observed that in FHP drinkers, memantine reduced alcohol-induced stimulation and alcohol craving at both the 20 mg/day and 40 mg/day doses with emerging evidence for modest reductions in drinking at the 40 mg/day dose. In contrast, memantine did not have any effects on alcohol stimulation, craving, or drinking behaviors in FHN drinkers at either of the doses examined.

N-acetyl cysteine:

N-acetylcysteine (NAC) is an N-acetyl pro-drug of the naturally occuring amino acid cysteine. This agent is FDA-approved for use as a mucolytic agent for bronchopulmonary disorders (e.g. Grandjean et al., 2000) and as an oral or intravenous antidote to treat acetaminophen poisoning (Smilkstein et al., 1988). NAC is a precursor of glutathione synthesis and is available worldwide in intravenous, oral and nebulizer forms and is also sold over the counter in health food stores. NAC has been found to stimulate cysteine-glutamate exchange, thereby increasing non-synaptic glial release of glutamate (e.g. Baker et al., 2003) which then stimulates inhibitory presynaptic metabotropic glutamate autoreceptors to decrease vesicular release of glutamate. This mechanism is believed to mediate the reduced reinstatement of drug seeking behavior observed in animal models with the use of this agent (Baker et al., 2003; Madayag et al., 2007; Moran et al., 2005). Kalivas and colleagues (2008) have postulated that since drug self-administration down regulates the cysteine-glutamate exchanges (Kau et al., 2008) the upregulation of the exchanger via NAC administration may directly normalize drug-induced changes.
While there have been no tests of the influence of NAC on alcohol behaviors there is extensive evidence with other substances. For example, NAC reduces cocaine use in cocaine dependent rodents (e.g. Madayag et al., 2007; Moran et al., 2005). Preliminary clinical trials suggest that NAC is well tolerated with minimal side effects and that it decreases cocaine craving and use in cocaine dependent humans (Larowe et al., 2006; Mardikian et al. 2007). More recent evidence suggests that NAC restores the ability to induce long-term-potentiation and long-term-depression in cocaine treated rats, possibly by indirectly stimulating mGluR2/3 and mGluR5 receptors

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(Moussawi et al., 2009). Similarly encouraging results have been observed in preclinical work with opioid dependence (Zhou and Kalivas, 2008) and clinical studies involving obsessive compulsive disorder (Lafleur et al., 2006), pathological gambling (Grant et al., 2007) and nicotine dependence (Knackstedt et al., 2008).

A significant advantage of this agent is the fact that it has been safely used for several decades in adults and children (Mucomyst Package insert) and in studies evaluating long-term treatments with this agent for chronic bronchitis have found that it was cost-effective to use and generally well tolerated with mild, most commonly gastrointestinal adverse effects (Grandjean et al., 2000). Memantine, on the other hand, is associated more side effects; the tolerability of NAC relative to memantine is a strength.

In the proposed study, we will test the influence of NAC on alcohol drinking and alcohol-induced behaviors including stimulation, sedation and craving using the same ADP as our ongoing memantine study. We propose to use a dose of 2400 mg/day of NAC which was observed to be safe and tolerable in cocaine dependent participants (e.g. Larowe et al., 2006).

Why combine memantine/NAC and naltrexone?

Habitual alcohol use in alcohol-dependent heavy drinkers may be dependent not just on continued alcohol reward but also on conditioned incentive processes, like cue-induced craving and automated motivational tendencies, which are mediated by complex interactions between different neurochemical processes (Johnson et al., 2008; Krishnan-Sarin et al., 2009; Koob and Le Moal, 2008). Therefore, alcohol drinking in habitual, alcohol-dependent drinkers could be optimally reduced by combined treatment with agents that influence different behavioral and neurochemical effects of alcohol. Modulation of ventral striatal dopaminergic function by various neurotransmitters including glutamate, GABA, and opioids plays a crucial role at every stage of the addiction process, including initiation, maintenance, and relapse (Vengeliene et al., 2008). While it is very difficult to pinpoint which system contributes most to transition from initiation to compulsive alcohol use, it is widely believed that agents which target these systems may work synergistically to reduce ventral striatal activity and thus reduce reward and associated processes.

There is considerable evidence of direct and indirect interactions between the glutamate and opioid systems in mediating reward. The glutamatergic system has been shown to play a very important role in addiction to opioids like methadone (see review by Guo et al., 2009). The mu-opioid receptor system is an important modulator of glutamatergic function in rat central amygdala neurons (Zhu and Pan, 2005), and NMDA and mu-opiate receptors are co-localized in the nucleus accumbens (Gracy et al., 1997) and nucleus tractus solitarius (Huang et al., 2000). Mu-receptor antagonists have been shown to reduce glutamatergic response to acute alcohol administration in the nucleus accumbens (Nie et al., 1993). More recent evidence also suggests that chronic alcohol use may lead to increased recruitment of functional mu- and delta-opioid receptors on glutamate neurons of the central nucleus of the amygdala that may be involved in maintaining conditioned place preference for alcohol (Zhu et al., 2009).

The above evidence suggests that two agents that might work synergistically to reduce alcohol drinking behaviors are glutamatergic agents like memantine/NAC and naltrexone. This hypothesis is directly supported by the work of Kuzmin and colleagues (2008), who examined the effects of naltrexone in combination with memantine on operant alcohol self-administration in rats. The results of this investigation suggest that memantine at a dose of 1 mg/kg significantly enhanced the effect of a low dose of naltrexone (0.1 mg/kg) in inhibiting alcohol self-administration. This

evidence suggests that memantine, when combined with low-dose naltrexone, may block alcohol reinforcement and that this combination may have therapeutic value in the treatment of alcohol drinking.

We propose that combining naltrexone with memantine/NAC will target distinctive mechanisms underlying alcohol-drinking behavior. The combination will optimally suppress ventral striatal dopaminergic activity, improve striatal functioning, and restore control over alcohol-drinking behavior, and lead to optimal reduction in alcohol-drinking behaviors.

3. **Research Plan:** Provide an orderly scientific description of the study design and research procedures as they directly affect the subjects.

This project will use a within-subjects, crossover design to examine the effects of naltrexone with or without memantine or NAC on drinking and other alcohol-related behavioral measures in alcohol-dependent or abusive heavy drinkers with a positive family history of alcoholism (FHP). Non-treatment-seeking heavy drinkers will participate in three ADP lab sessions, occurring prior to medication and after the first and second weeks of medication treatment. They will also participate in two follow-up appointments at one week and one month after participation. The baseline ADP will be used to familiarize subjects with the laboratory procedures and reduce order effects in the subsequent ADP sessions. Each ADP will involve consumption of a priming alcoholic drink (0.03 mg%) at 3 pm followed by three one-hour choice drinking periods. During each hour subjects will choose between 4 alcoholic drinks (0.015 mg% each) or equivalent monetary rewards ($3 per drink). During each 6-8-day medication outpatient treatment period (up to 13 days), subjects will receive naltrexone and be randomized to receive either 20 mg of memantine, 2400 mg of NAC or placebo, and medication doses will be tapered up and adverse events recorded on a daily basis.

The study will consist of five phases: 1) **Phase 1 (Screening):** We obtain informed consent and evaluate eligibility for the study using clinical assessments and physical exams, if eligible, participants will be scheduled for the rest of the study, 2) **Phase 2 (Baseline):** Participants will complete some memory and mood tasks, participate in the PIT task, and complete a baseline alcohol drinking paradigm (ADP), 3) **Phase 3 (Med 1):** The first 6-8day outpatient medication phase during which participants will come into our clinic every day to take the medication combination they have been assigned and participate in the second ADP on the last day of medication, (participants may be asked to take the medication for up to 13 days, based on scheduling for their overnight stay) Also, in the event that a medication appointment will be missed because the participant is unable to come to the clinic the following day, he/she will be given an extra dose of medication to take home for the next day. The participant will be called the next day as a reminder to take the medication and will also be asked about any adverse events. 5) **Phase 4 (Washout):** At least 6-8 day washout phase (length of washout based on hospital scheduling) and 6) **Phase 5 (Med 2):** Participants will be randomized to receive another medication combination for 6-8 days which will culminate in the third ADP (again, this is based on scheduling for the overnight at the hospital and can be extended to 13 days). In the event that a participant is unable to make it to one of the overnight visits, and needs to discontinue one of the medication periods early due to personal circumstances, we would like to have the option of restarting the medication. We do not believe that this will increase risk to participants since they would have already taken the medications during an earlier phase. It is essential that participants complete all parts of the study for us to be able to use the data to evaluate the effects of combining naltrexone and memantine on drinking behaviors.

Once all phases are complete, participants will be scheduled for a 1-week follow up appointment. We will record any remaining adverse events and assess drinking behavior. A clinical psychologist will talk to all participants about their drinking behavior and if they are interested will provide them with treatment referrals. The one-week and one-month follow-up sessions will be conducted with all participants, even those who drop out during the course of the study.

Recruitment:

Participants will be recruited through specific advertisements placed in local newspapers, postings and promotional materials (e.g., magnets) distributed in community locations (bars, alcohol/coffee shops, grocery stores), and advertisements on free community TV channels. We have also had success with recruiting participants from postings on Craigslist and social networking sites. We have created a Facebook page for our study and have also developed our own study URL, www.paidalcoholstudy.com, which links subjects to confidential surveys that assess some preliminary eligibility criteria and provides preliminary information about the project. We have had a lot of interest in our URL and survey, and most people who access the survey report hearing about the study through the internet (86.6%) with a minority reporting that they saw flyers in the community (7%). The results of the survey are then accessed by the research staff, and individuals who seem eligible are contacted. Individuals who call in response to ads and flyers contact us directly. Subjects will also be referred to this study by research staff on protocol #0903004912. Detailed procedures on the referral process are outlined in protocol #0903004912 and have been approved by the Yale HIC.
Each potential participant's eligibility will be assessed by a research staff member who will then contact them and set them up for the initial intake appointment. We do not anticipate any problems with recruiting these subjects since their profile is similar to that of the participants we have been recruiting for our ongoing projects and we have been quite successful in our recruitment efforts. Informed consent will be obtained from all subjects, and they will be screened by a physician for contraindicating medical or psychiatric conditions and will receive complete physical and laboratory workup, including EKG.

Justification for Drinking Criteria:

We will recruit alcohol-dependent or abusive heavy drinkers who consume between 20-65 drinks per week for women and 25-70 drinks per week for men. The lower limits reflect alcohol consumption that just exceeds the WHO Brief Intervention Study Group guidelines (WHO guidelines) for non-hazardous drinking, which are no more than 4 drinks/day 4-5 days per week for men (20 drinks) and no more than 3 drinks/day 5 days per week for women (15 drinks). The upper limits were chosen to ensure that we chose subjects whose typical drinking quantities would be unlikely to exceed the amount of alcohol that would be available to them for possible administration during the laboratory session. These criteria are similar to the ones being used in our ongoing work with memantine in HIC # 0602001068.

Justification of Age Criteria and for Excluding Children and Adolescents Under 21 Years of Age:

We will not use subjects under 21 years of age in this study. First and foremost, this is an alcohol self-administration study in which we will be offering drinkers an opportunity to consume alcohol, and in the State of Connecticut the legal age limit for drinking alcohol is 21. Secondly, subjects

below the age of 21 have probably been drinking alcohol for a shorter duration of time and will have different patterns of drinking from older drinkers, which may affect drinking during the self-administration paradigm and confound study results. The upper age limit was determined using our pilot data (see Krishnan-Sarin et al., 2007) which indicates that the majority of the subjects who participated in our ongoing study were in this age range.

Justification for choice of memantine dose:

We will evaluate the effects of 20 mg/day of memantine. Most clinical trials conducted with memantine in patients with dementia have used doses of 10 or 20 mg/day. Also, memantine has been used in doses of 15 and 30 mg/day in moderate, non-dependent drinkers (Bisaga and Evans, 2004) and has been shown to reduce cue-induced craving in doses of 20 and 40 mg/day in alcohol-dependent drinkers (Krupitsky et al., 2007). But most important, our choice of this dose is based on our most recent analyses of our final data, which suggests that 20 mg of memantine reduced alcohol-induced stimulation and also seemed to reduce alcohol craving and drinking. The dose of memantine will be tapered up slowly to reduce incidence of adverse events. We designed these procedures based on our experience with using memantine in the current HIC # 0602001068 project. Our data from this protocol indicates that the total number of adverse events experienced by participants receiving either the 40 mg or 20 mg dose of memantine did not differ from those receiving placebo.

Justification for choice of naltrexone dose:

We will use naltrexone in a dose of 50 mg/day. This is the dose of naltrexone that is FDA approved for the treatment of alcohol dependence and has been most commonly used in most clinical trials (Anton et al., 2005; O'Malley et al., 1992; Volpicelli et al., 1992) and laboratory studies (Davidson et al., 1999; O'Malley et al., 2002; Anton et al., 2004). This dose also produces complete blockade of the mu-opioid receptors (Weerts et al., 2008). The 100 mg/day dose has also shown efficacy in many recent clinical trials (e.g., Anton et al., 2006), and our own preliminary evidence suggests that this dose may produce greater reduction in drinking in FHP drinkers than the 50 mg dose. However, in the proposed "proof of concept" trial we did not want to run the risk of missing the signal for a synergistic effect of memantine or NAC by combining it with a dose of naltrexone, which had a large effect of its own. Moreover, now that we are combining two medications we want to use a lower dose of naltrexone to minimize side effects from the combined pharmacotherapy.

Justification for choice of N-acetyl cysteine dose:

N-acetylcysteine (NAC) is an N-acetyl pro-drug of the naturally occuring amino acid cysteine. This agent is FDA-approved for use as a mucolytic agent for bronchopulmonary disorders (e.g. Grandjean et al., 2000) and as an oral or intravenous antidote to treat acetaminophen poisoning (Smilkstein et al., 1988). We propose to use a dose of 2400 mg/day of NAC which was observed to be safe and tolerable in cocaine dependent participants (e.g. Larowe et al., 2006).

Procedures

Recruitment, Baseline Assessments, Physical Exam and PIT Task

Potential subjects will be recruited as described earlier. Participants will be scheduled for an appointment at the Substance Abuse Center in the Connecticut Mental Health Center or at the

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Substance Abuse Treatment Unit, New Haven, CT, where informed consent will be obtained prior to any other procedures. Then the intake battery will be administered to assess eligibility for the study. At this intake appointment FH of alcoholism will be assessed using the FHAM (see assessments section, below). The research assistant will schedule a physical exam (including EKG) and routine laboratory work, and hepatic, kidney, and thyroid function tests will be conducted by an APRN. A detailed medical history will be obtained from all subjects and pregnancy tests for all females. Subject characteristics and medical history will be reviewed by the PI to ensure that the subject meets all the eligibility criteria. The subjects will then be scheduled to meet with the study physician (Dr. Julia Shi), who will also review their eligibility for the study and review their medical history information and EKG. If eligible to participate, and if they have also consented to participate in the genetic study, we will draw plasma samples for this purpose. For those participants without the use of a phone or cell phone and may be difficult to reach for the next 20 appointments after the initial intake, we will provide them with a disposable study cell phone to enhance communication and attendance at all appointments. Urine will be collected to test for drugs and ethylglucuronide, a metabolite of ethyl alcohol that will be used as a biomarker of alcohol consumption at baseline and the ADP sessions.

After subjects are determined to be eligible, they will participate in the Pavlovian Instrumental Transfer (PIT) Task. This task will use a sucrose reinforcer, and so measure PIT as a general tendency and not an alcohol specific tendency. In this part of the study, subjects will be asked to taste different stimuli. The stimuli will be delivered as liquids. Stimuli will be stored in a refrigerator and brought to room temperature before use. Liquids will be delivered with our custom designed gustometer built by Dr. Small (in collaboration with colleagues in the Pierce shop). This set up has been used successfully by Dr. Small's lab for the past 9 years (Small et al. 2003; Small et al. 2004; Veldhuizen et al. 2007; Small et al. 2008; Veldhuizen et al. 2010; Veldhuizen et al. 2011; Veldhuizen et al. 2011; Veldhuizen et al. 2012). In brief, the liquid delivery consists of a computer running E-Prime, controlling a series of programmable syringe pumps with 60ml syringes and beverage tubing attached. New tubing and syringes are used for each subject. Our stimuli will contain either a taste (juice or bitter) or a neutral solution. All solutions will be prepared in the laboratory using commercially available tastes. The tastes might include sucrose, glucose, sodium chloride, citric acid, quinine hydrochloride, sodium bicarbonate, potassium chloride, capsaicin, and artificial sweeteners such as aspartame, acesulfame potassium, and sucralose. The juice will consist of three different flavors of Gatorade that the subjects will choose.

Alcohol-Drinking Paradigm

The procedures for all the alcohol-drinking sessions will be similar. Participants will be asked to arrive at the Hospital Research Unit (HRU) of Yale New Haven Hospital, New Haven, at 9 am. They will be told not to consume any alcohol after 10 pm on the previous evening. We will assess breath alcohol levels upon arrival and conduct urine drug tests. If the breath alcohol levels are positive but below 0.05 and decreasing, then the participant will be allowed to continue. If the urine drug tests are positive and/or breath alcohol levels are 0.05 or greater, then the session will be rescheduled. The drinking sessions will be conducted in a private room in the HRU. The participants will stay in these rooms from time of admission to discharge.

Table1 below provides a detailed timeline of the procedures. Participants will complete the impulsivity and alcohol association tasks at the beginning of the lab session, and this data will be used to examine medication effects and relationship to drinking behaviors. The alcohol-drinking session will start at 3 pm with the priming dose period, which will be followed by three one-hour drinking periods (4-5 pm, 5-6 pm, and 6-7 pm), and will conclude at 7 pm.

a. *Exposure to alcohol cue:* Ten minutes prior to the start of the priming dose (PD) period (i.e., 2:50 pm), the research assistant will walk into the participant's room with the glasses, the alcohol, and the mixers. The research assistant will then proceed with the mixing of the priming drink of alcohol in front of the participant. **When done, he/she will leave the drink on the table, instruct the participant not to consume the priming drink until they are told to do so and leave the room.** At 2:59 pm the research assistant will walk back into the room and ask the subject to report on how much alcohol craving they are experiencing. At 3 pm the research assistant will walk back into the room and tell the participants that they have five minutes to consume the PD of alcohol and will then again leave the room, and the PD period will commence.

b. *Priming dose (PD) period:* The PD of alcohol that is provided at 3 pm will contain a dose of 80 proof alcohol designed to raise blood alcohol levels to 0.03 mg% of alcohol, and subjects will have 5 minutes to drink it. The purpose of the PD is to provide a standard dose for evaluating the effects of medications on the responses to a standard drink of alcohol and to model a "lapse" situation.

c. *Absorption Period and Ratings of Ethanol Effects*: A 50-minute absorption period will follow during which the subjective and physiological effects of this priming dose of alcohol in combination with memantine will be monitored. Using this procedure, we will be able to closely monitor changes in subjective effects (alcohol craving, stimulation/sedation) during the rising and falling limbs of the blood alcohol curve.

d. *Alcohol self-administration (SA) periods:* Following the PD, participants will be exposed to three one-hour SA periods designed to model a "relapse" situation. During each SA period they will be permitted to drink up to four alcoholic drinks designed to raise BALs by 0.015 mg% of alcohol, or to receive cash (equivalent to the price of each drink that is not consumed). The first SA period will begin at 4 pm, when the research assistant will take 4 prepared drinks into the room along with a "tab" sheet worth $12. The participant will be informed that these 4 drinks will be available to him/her for the next 60 minutes (i.e., until 5 pm). S/he can choose either to drink or to keep the money; each drink will cost $3. For example, if the participant chooses to drink only one drink in the next one hour, s/he will earn $9. The money will be given to them the next morning before they leave the hospital. The second and third SA periods will begin at 5 pm and 6 pm, respectively, and will be similar to the SA period. Thus, participants can choose to consume up to 12 additional drinks over this 3-hour period or to receive up to $36 to take home the next morning.

e. *Beverage content and mixers:* The YNHH Investigational Pharmacy will calculate the alcohol dose for each participant. In the priming drink, the dose will be designed to raise blood alcohol levels to 0.03 mg% and will be based on the formula specified by Watson (1989) which takes into account gender, weight, and age of the subject. The subsequent drinks provided in the SD blocks are designed to raise BAL by 0.015 mg% each, using the same formula. The alcohol doses will be delivered to the HRU unit and any unused doses will be returned to the pharmacy.

Alcoholic beverages administered during this study will consist of 1 part 80 proof liquor of the subject's choosing to 3 parts mixer chosen from a selection of equicaloric, noncaffeinated, non-carbonated drinks. The research assistant will prepare the drinks

using the alcohol doses prepared by the YNHH pharmacy. Participants would have already chosen their mixers on an earlier day. Specifically, they will be asked to choose two mixers and choice of alcohol (vodka, rum etc.) from a list that will be provided to them at the intake appointment. Based on our previous experience, we have found that subjects prefer being offered a choice of alcoholic beverages and mixers, thereby providing a closer approximation to their own drinking experiences. The pharmacy will prepare 2 syringes (5cc each) of the specified liquor(s) and deliver them to the HRU the morning of or the day before the subject's ADP#1. The liquors will be mixed with the subject's chosen mixers, same ratio as drinking session (e.g. 1 part liquor to 3 parts mixer). The subject will sample each drink once settled into their room and will be instructed to swish it around in their mouth and then spit it out. Once the subject has made their selection, we'll call the pharmacy to confirm their choice. Frozen plastic cubes will be used to chill each drink without diluting them and the prepared drinks will be covered with saran wrap to avoid any evaporation of alcohol during the drinking period. Drinks will be prepared ten minutes prior to each drinking hour.

f. *Assessments during and after the three choice periods:* During the second, third and fourth hour of the laboratory session, drinking behavior will be videotaped for later analysis, and craving and stimulation/sedation will be assessed every thirty minutes. **The range of assessments, however, is limited to avoid interfering with the evaluation of drinking behavior.** In order to monitor blood alcohol levels we will draw three ml of blood from the subjects every 10 minutes during the first hour (i.e., at 2:10, 2:20, 2:30, 2:40, and 2:50). After the priming dose, blood draws (3 ml) for blood alcohol determination will occur every 60 minutes (i.e., at 4, 5, 6, 7, and 8 pm), regardless of whether the participant chooses an alcoholic beverage. This frequency of assessments provides a sensitive assessment of blood alcohol levels (as was demonstrated in our naltrexone study) without unduly disturbing the subject's drinking behavior. These changes can then be correlated to the subjective changes produced by alcohol at these same time points.

Table 1

PERIOD	TIMING	PROCEDURES
ADP	10 am	Breath alcohol levels, AUQ, YCS, TLFB, SAFTEE, CADSS, Vitals Administer last doses of medications
	11 am	Approach Avoidance Task, Impulsivity tasks; Self report and Laboratory measures
	12 pm	Lunch and Smoke Break for smokers
	1 pm	Insertion of IV for blood alcohol draws
	2 pm	Start of baseline; AUQ, YCS, BAES, Vitals; Smoke break for smokers
	2:30 pm	AUQ, YCS, BAES, Vitals
	2:45pm	YCS
	2:50 pm	Alcohol cue exposure to preparation of priming drink (PD)
	2:59 pm	YCS
	3 pm	Priming dose period: Instruct participant to consume PD (0.03g/dl) in five minutes

	3:10 pm	Blood alcohol, AUQ, YCS, BAES
	3:20 pm	Blood alcohol, AUQ, YCS, Vitals
	3:30 pm	Blood alcohol, AUQ, YCS, BAES
	3:40 pm	Blood alcohol, AUQ, Vitals
	3:50 pm	Blood alcohol, AUQ, YCS, BAES
	4 pm First tray	1st Self-administration (SA) period: Bring in first tray of 4 drinks (each designed to raise BAL by .015 g/dl) and drinking tab ($12); Instruct participants on choice of drinks versus money.
	4:30 pm	AUQ, YCS, BAES
	5 pm Second tray	Blood alcohol, AUQ, BAES, Vitals @ 4:55 pm 2nd SA period: Remove first tray and bring in second tray: Present participant with 4 drinks (each will raise BAL by .015 g/dl) and drinking tab ($12); repeat instructions
	5:30 pm	AUQ, YCS, BAES
	6 pm Third tray	Blood alcohol, AUQ, BAES, Vitals @ 5:55 pm 2nd SA period: Remove second tray and bring in third tray: Present participant with 4 drinks (each will raise BAL by .015 g/dl) and drinking tab ($12); repeat instructions
	6:30 pm	AUQ, YCS, BAES
	7 pm	Blood alcohol, AUQ, BAES, YCS, Vitals
	7:30 pm	Dinner
	8 pm	Breath Alcohol, AUQ, YCS, BAES, Vitals
	9 pm	Breath Alcohol, AUQ, YCS, BAES, Vitals
	10 pm	Breath alcohol, AUQ, YCS, BAES, Vitals
Discharge	7 am	Breath alcohol, AUQ, YCS, Vitals, CADSS, BPRS

g. End of alcohol self-administration period and overnight stay in HRU: The alcohol administration portion of the study will end at 7:05 pm. Following this, breath alcohol levels and craving will be assessed every 30 minutes until the subject's breath alcohol level falls below 0.02. Participants will then be given dinner and will stay in the hospital overnight. This overnight stay will serve multiple purposes: 1) to ensure that subjects do not continue to drink following exposure to alcohol in this paradigm, 2) to ensure that they are safe and are not discharged while still intoxicated, and 3) to motivate subjects to drink during the session by ensuring that they do not have an opportunity to have access to more alcohol later that same day. The next morning participants will be assessed using craving and withdrawal measurements, they will provided with breakfast, and they will then be discharged between 8 and 9 am.

Study Medication (Naltrexone/Memantine/NAC) Administration and Stabilization: 6-8 Days

All subjects will receive naltrexone treatment and be randomly assigned to receive either memantine 20 mg, NAC 2400 mg or placebo for seven days as outpatients. The randomization schedule will be generated by the Data Management and Biostatistics Component statisticians and provided to the pharmacist. They will take the final dose of 50 mg NTX + 20 mg MEM or 2400 mg NAC or placebo, when they will complete the alcohol self-administration paradigm described below. Doses of naltrexone and memantine will be tapered up as follows

GROUP	Day 1	Day 2	Day 3	Day 4	Day 5	Days 6-8 (up to 13)
Naltrexone	25 mg	50 mg	50 mg	50 mg	50 mg	50 mg
Memantine/ NAC/placebo	0/0/0 mg	0/0/0 mg	5/600/0 mg	10/1200/0 mg	20/1200/0 mg	20/2400/0 mg

Subjects will come to our clinic each day to take their medications at 10 am. During these appointments they will complete assessments. These procedures will 1) ensure compliance with medication administration, 2) monitor side effects during naltrexone and memantine/nacetylecysteine dose escalation, and 3) monitor tolerability of naltrexone, memantine, and NAC co-administration. ***During this period, subjects will be asked to continue normal drinking behavior similar to the procedures used in our preliminary studies.***

In the event that a subject cannot come to the clinic daily, other arrangements will be made, such as giving the participant more than one dose of their medication at a time and using a daily phone call as both a reminder to take the medication and as a means to monitor adverse events. We will allow this up until the final two doses of medication prior to the ADP. This arrangement will be determined by the investigator and her staff based on the participant's other obligations and compliance.

Follow-up Appointments

Subjects will participate in a one-week follow-up appointment during which drinking over the past week will be determined using TLFB techniques and any remaining adverse events will be monitored. At this appointment a brief motivational intervention will be provided to encourage the subject to address their alcohol problem and an immediate referral to treatment will be made if subjects are interested. Even though the subjects participating in this study are not seeking treatment for their drinking, we feel that their participation in this project provides us with a "teaching moment" to address their drinking behavior. We have found that similar brief advice resulted in decreases in alcohol-drinking behavior and increased motivation to quit drinking (Sinha et al., 1997). As previously done, this intervention will be based on the principles of Miller's Motivational Enhancement Therapy (MET) (Miller et al., 1992). We will provide subjects with personalized feedback regarding their physical exam and laboratory findings, and on the influence of drinking on their health. We will also review with them the potential benefits of quitting drinking. If interested, subjects will also be given the option of participating in one of our alcohol treatment studies. We will also provide them with the NIAAA brochure "Rethinking Drinking."

Subjects will also participate in a one-month follow-up appointment during which information about their drinking since their prior appointment will be assessed using the Time-Line Follow-Back Interview. This interview provides us with another opportunity to provide subjects with additional information about treatment if they are interested. This data will also be used to evaluate the risks and benefits for subjects participating in this protocol (O'Malley et al., 2002; Anton et al., 2004).

Assessments

a) Socio-demographic/General Information: At intake, demographic data, medical history, and family psychiatric history will be assessed with interviews and self-report forms that provide data on age, race, socioeconomic status, marital status, educational and occupational levels, and significant medical history. These are adapted from previous diagnostic and clinical studies at this center.

b) SCID: The Structured Clinical Interview for DSM-IV (SCID) (First et al.,1996) will be used to determine psychiatric diagnoses. This interview assesses DSM-IV current and lifetime psychiatric diagnoses for anxiety, mood, psychotic, alcohol and substance use, somatoform, and eating disorders.

c) Psychiatric Family History by Interview, the FHAM: As a source of pedigree information, the psychiatric status (including substance abuse/dependence, mood disorder, ASPD, etc.) of all first- and second-degree biological relatives will be obtained from each subject (including parents) using the family history method (FHAM-Family History Assessment Module) developed by COGA. DSM-IV criteria will be used to diagnose all biological family members. The FHAM is a reliable method for obtaining family history information and the specificity and sensitivity of the FHAM for the diagnosis of substance dependence is quite good (Rice et al., 1995). We will administer the FHAM in three steps. First, the structure of the family pedigree is drawn and reviewed with the informant. Next, psychiatric screening questions are asked about all relatives in the pedigree. Then, based on the responses to the screening questions, symptom checklists are completed for each first-degree relative, spouse, or other relative well known to the informant.

d) Time-Line Follow-Back Assessment Method: This interview procedure will be used to obtain quantity/frequency alcohol consumption data for each day during the 90-day period prior to the study, during the outpatient stabilization period, and during the three-month follow-up (Sobell and Sobell, 1992). Subjects are given a blank calendar covering the time interval to be re-constructed and are asked to reconstruct retrospectively their drinking behavior over that interval. The process is facilitated by establishing anchor points (e.g., holidays, anniversaries, major national events, etc.). It can be scored to provide the number of days on which various levels of consumption occurred. The time-line method has good test-retest reliability and good validity for verifiable events. It has been used in numerous studies to compare pre- to post-treatment drinking.

e) Craving Measures:

Alcohol Urge Questionnaire (AUQ) (Bohn et al., 1995)*:* The AUQ is an 8 item questionnaire, derived from a larger 49 item "Questionnaire of Alcohol Urges," that assesses *desire for a drink, expectation of positive effect from drinking,* and *inability to avoid drinking if alcohol was available.* The AUQ is a reliable and valid scale for the measurement of self-reported alcohol urges, and scores have been shown to be strongly related to alcohol dependence severity (as measured by ADS scores) and to cognitive preoccupation with alcohol. Its brevity and time frame for ratings (i.e., right now) makes it suitable for administration during the alcohol drinking period.

Yale Craving Scale (YCS): We have been collaborating with Linda Bartoshuk to develop this craving measure based on psychophysiological scaling methods. In her work on individual differences in the ability to taste bitterness, Dr. Bartoshuk and colleagues (Marks et al., 1988) used magnitude-matching procedures (Stevens and Marks, 1980) in which participants matched the intensity of perceived bitterness to sounds. By doing so, the problem of differences in how labels (e.g., very strong) are applied was circumvented by making the comparison to a standard that is unrelated to taste. The resulting scale, Labeled Magnitude Scale (gLMS) (Bartoshuk , 2002), has been extended to measure hedonic ratings for foods, and we have adapted it for rating craving for tobacco and alcohol. It is not subject to the ceiling effects that often occur in craving research (Sayette et al., 2000). We have been collecting craving data using this scale in our ongoing projects and the findings from this scale have been found to parallel those obtained using the Alcohol Urge questionnaire. *A significant advantage of this scale is that following completion of baseline training to match perceived intensity of craving to the perceived brightness from the sun, each assessment timepoint only consists of a single visual analog scale of craving, making it very easy to administer.*

f) Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar): This is a modified, shorter version of the Clinical Institute Withdrawal Scale for Alcohol which is equally efficient and reliable as the original scale without a significant loss in accuracy. The CIWA-Ar is a 10 item scale that contains alcohol withdrawal signs and symptoms that include nausea/vomiting, tremor, headache, anxiety, agitation, orientation, sweating, and auditory, visual, and tactile disturbances. This tool often guides the clinical management of alcohol (Sullivan et al., 1991) withdrawal (for a review, see Saitz and O'Malley, 1997) and is used extensively in research on alcohol withdrawal (Sellers et al., 1991; Saitz et al., 1994).

g) Psychiatric and mood symptoms: We will assess changes in these symptoms during Days 1-13 using:
Positive and Negative Syndrome Scale (PANSS) (Chapman et al.,1980): A clinician-rated general assessment measure used to measure psychosis (positive symptoms), emotional or motivational deficits (negative symptoms), and thought disorder.
Clinician–Administered Dissociative States Scale (CADSS) (Bremner et al., 1998): A 19 item self-report questionnaire with eight observer ratings designed to assess perceptual, behavioral, and attentional changes that occur during dissociative experiences from NMDA receptor antagonists (Krystal et al., 1994).

h) Intellectual Functioning: In order to obtain an assessment of general intellectual functioning and a reference point for other cognitive assessments, we will complete the two subtest short-form of Block Design and Information from the WAIS-III (Weschler, 1997). This combination correlates at .87 with the Full Scale IQ.

i) Impulsivity and Automatic Motivational Measures: Change from occasional to compulsive drug use may be less dependent on positive reinforcement and more dependent on implicit processes that automatically evaluate the motivational significance of the alcohol cue for affect regulation. Such processes may include "fast" impulsive responses which automatically orient the individual to either approach or avoid the stimulus and "slower," more reflective processes involving conscious deliberation and emotion regulation. We will assess using impulsive responding using measures which encompass several clinically relevant core components such as rapidity of response, degree of planning, and disregard of future consequences. Additionally, we will also measure implicit alcohol-affect associations as well as individual variations in the ability of incentive stimuli to control behavior. We will evaluate changes in impulsivity and implicit alcohol associations at baseline and then after each medication period. Pavlovian to Instrumental transfer task will be assessed at baseline only.

j) Padua Inventory (Sanavio, 1988): consists of 60 items describing common obsessional and compulsive behavior and allows investigation of the topography of such problems in normal and clinical symptoms.

k) Chronic Stress Scale (CSS) (Turner et al., 1995): This measure is used to assess a person's perception of ongoing and enduring sources of stress in their life conditions. The chronic stress scale (CSS) includes a list of 51 items about common life conditions and situations (e.g., financial issues, work, marriage and relationship, parental, family, social life). The interviewer reads each item aloud and asks the respondent to reply with not true (0), somewhat true (1), or very true (2).

Self Reports:

a) *The Behavioral Inhibition System/Behavioral Activation System* (BIS/BAS) (Carver and White, 1994; Gray, 1981) will assess behavioral activation and inhibition, which have been proposed as biological systems underlying behavior and affect. This measure has been shown to have good convergent, discriminant, and predictive validity in which it measures sensitivity rather than the person's typical experience. The BIS will be used to tap the

component of impulsivity related to decreased sensitivity to the negative consequences of behavior.

b) *Barratt Impulsiveness Scale (BIS)(version 11)* (Patton et al.,1995)*:* This 30 item self-report instrument provides a trait measure of impulsiveness and yields four scores: a total score, nonplanning activity, cognitive impulsivity, and motor impulsivity. Cronbach alpha coefficients range from .79-.83.

c) *Short Inventory of Problems (SIP*) [153a]: A brief version of the Drinker Inventory of Consequences (DrInC), this is a 15-item test that measures physical, social, intrapersonal, impulsive, and interpersonal consequences of alcohol consumption. Subjects indicate whether each item occurred in the previous 12 months.

d) *Self Rating of Effects of Alcohol (SRE)* [153b]: This 5-item self-report contains questions related to the number of drinks required for up to four different effects early in the drinking career.

e) *Alcohol Expectancy Questionnaire (AEQ)* [153d]: This 68-item questionnaire is an empirically derived self-report form designed to measure the degree to which individuals expect alcohol to produce a variety of general and specific positive effects.

f) *Negative Alcohol Expectancy (NAEQ)* [153e]: This 60-item self-report provides assesses the current level of motivation to restrain/stop drinking and the constituent components of the current level of motivation. The NAEQ also identifies negative expectancies that may serve as a deterrent and represent motivation to stop or restrain drinking.

g) *Sensation Seeking Scale (SSS)* [153f]: 40-item self report measures individual differences in optimal levels of stimulation and arrival.

h) *Depression Anxiety Stress Scale (DASS)* [153g]: This 42-item self-report which measures the negative emotional states of depression, anxiety and stress.

g) *Biphasic Alcohol Effects Scale (BAES)*(Martin et al., 1993): This 14 item self-report, adjective rating scale will be used to measure the stimulant and sedative effects of alcohol during the priming dose on Day 7. This instrument has been found to be sensitive to memantine and naltrexone's effects on alcohol intoxication (pilot date, Kranzler et al., 2000; Reynolds et al., 2004; Swift et al., 1994).

h) *Childhood Trauma Questionnaire (*Bernstein, et al. 1994): This 28-item self-report inventory that provides brief, reliable, and valid screening for histories of abuse and neglect. It inquires about five types of maltreatment - emotional, physical, and sexual abuse, and emotional and physical neglect. Also included is a 3 item Minimiziation/Denial scale for detecting false-negative trauma reports.

i) *Drinking Motives Questionnaire (DMQ)*(Cooper et al. 1992): contains 15 reasons why people might be motivated to drink alcoholic beverages. Participants rate on a 4-point scale how frequently each of the 15 listed reasons motivate them to drink alcoholic beverages. The measure yields three scale scores reflecting different motives for drinking alcohol.

j) *Brief Self-Control Scale (Tangney et al., 2004):* provides a rapid brief assessment of self-control and regulation.

k) *Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ)(*Torrubia et al., 2001)*:* consists of 48 yes-no items that included questions about habitual behaviours in response to cues of punishment, frustrative non-reward and novel stimuli as well as stimuli related to reward and approach-related tendencies.

l) *The Self-Report Habit Index (SRHI) (*Verplanken & Orbell, 2003).*:* a 12-item index of habit strength developed on the basis of features of habit; that is, a history of repetition, automaticity (lack of control and awareness, efficiency), and expressing identity.

m) *UPPS Impulsive Behavior Scale* (Whiteside & Lynam, 2003): a 45-item self-report questionnaire which distinguishes four facets of impulsivity: urgency, lack of premeditation,

lack of perseverance, and sensation-seeking. It is scored on a 4-point scale from Strongly Agree to Strongly Disagree.

Laboratory Measures:

a) *BART (*Lejuez et al., 2002): This is a computerized behavioral task which measures risk-taking propensity by having the subject press a button to inflate a balloon. The more the balloon is inflated, the greater the reward, however, if the balloon pops, the reward is eliminated. The subject must balance potential gain against potential risk of loss.

b) *Experiential Discounting Task* (EDT) (Reynolds and Schiffbauer, 2004): This delay-discounting task exposes participants to choice consequences during test administration. The EDT involves multiple blocks of choices, one for each delay. Choices are made between a standard amount that is delivered immediately and is certain and a probable amount that is delayed and uncertain. The EDT is sensitive to various levels of alcohol dosing (i.e., between 0 and 0.8g/kg) (Reynolds et al., 2006).

c) *Alcohol approach-avoidance task* (AAT) (Wiers et al., 2009): We chose to use the AAT to assess automatic alcohol-affect associations since it has been used to show that heavy drinkers with a G allele of the OPRM1 gene have stronger automatic approach tendencies for alcohol. The AAT is an alcohol variety of the Approach Avoidance task developed by Rinck and Becker (2007) and measures approach bias for alcohol related stimuli. The subject pushes or pulls computer presented stimuli according to a content irrelevant feature, the tilt of the stimulus. Pushing the joystick gradually decreases stimulus size, while pulling gradually increases stimulus size. The zooming feature also generates a sense of approach or avoidance (Neumann, 2000). Reinout Wiers will provide consultation to our group on the AAT.

d) *The Implicit Association Test (IAT)* (Greenwald et al., 2003): This task measures implicit affective asssociations with the alcohol. Using a computerized sorting task, individuals simultaneously classify two target conditions, 'alcohol' (ie., wine, beer, pint, vodka, whiskey, wine cooler) versus 'soft drink' (coca cola, juice, orange soda, root beer, sparkling water, 7-up) , and two affective categories relevant to drinking, 'pleasant' (ie. talkative, excited, cheerful, happy, funny, lively), versus 'unpleasant' (i.e., nauseous, listless, awful, miserable, sad, annoying). IAT effects will be calculated with the D600 scoring algorithm. Internal consistency for biploar alcohol-related affective IAT was .79 in (Houben, in press); the bipolar IAT predicted drinking above explicit measures and outperformed five other variants of the IAT(e.g., unipolar positive, uniplor negative IAT).

e) *A Pavlovian Instrumental Transfer Test (PIT)* will be completed to examine individual variation in the ability of incentive stimuli to control behavior. This task will use a sucrose reinforcer, and so measure PIT as a general tendency and not an alcohol specific tendency. Prior studies have explored the neural mechanisms of PIT with monetary (Talmi et al., 2008) and juice reinforcement (Bray et al., 2008). PIT involves three distinct training phases: (1) a Pavlovian phase, (2) an instrumental learning phase and (3) a transfer phase. Multiple trials occur in each phase.

g) Ratings of Drinking Behavior During the Alcohol Self-Administration Period: Subjects will be videotaped during the alcohol self-administration portion of the study on Day 6. These videotapes will be rated by two independent raters who will indicate the onset and offset of each sip of alcohol. Using this data, dependent measures will be constructed including time until the first sip and average time to consume each drink.

h) Blood Alcohol Levels: Blood samples will be drawn to measure plasma levels of blood alcohol (BAC) during the priming dose and during the alcohol self-administration paradigm on day 6. Blood samples will be stored at -4°C and will be analyzed using gas chromatographic techniques at the HRU Laboratory.

i) Psychophysiological Measures: These will include heart rate and blood pressure monitored using a Critikon Dinamap while skin temperature will be measured using Yellow Springs Instruments 4600 precision thermometer. The cuff of the Dinamap will be on the subject's dominant arm while the probe of will be attached to the middle finger of the subject's non-dominant arm. These data will be further used to examine the safety of using the medication combination during alcohol self-administration.

4. **Statistical Considerations:** Describe the statistical analyses that support the study design.

All outcomes will be summarized descriptively and assessed for normality prior to analysis using normal probability plots and Kolmogorov test statistics. Transformations or nonparametric analyses will be performed as necessary. In the mixed models described below, the correlation between repeated measures on an individual will be modeled using random effects and/or structured variance-covariance matrices. The best-fitting variance-covariance structure will be determined by information criterion. The mixed-effects approach is advantageous in that it is unaffected by randomly missing data and allows greater flexibility in modeling the correlation structure of repeated-measures data (Gueorguieva and Krystal, 2004). In the models below, we will test for order effects, although any such effects should be greatly minimized with the inclusion of the baseline session. All tests will be two-sided and considered statistically significant at alpha=.05. Significance levels for secondary comparisons will be adjusted for multiple tests using the Bonferroni correction, basing the adjustment on the number of conceptually related statistical tests within each hypothesis.

Aim 1: Total number of drinks consumed during the self-administration period will be compared among treatment conditions using linear mixed models with treatment (NTX vs. NTX+MEM/NAC) included as a within-subjects factor. In these models, we will also consider baseline drinking, as assessed by using the 90 day TLFB obtained at intake, as a potential covariate. Non-significant covariates will be dropped for parsimony. A significant treatment effect, where total number of drinks is reduced during combination NTX+MEM/NAC treatment compared to NTX alone, will be supportive of our hypothesis. Secondary measures of alcohol-drinking typology such as average time to consume each drink and inter-sip interval will be analyzed using similar models as described above, whereas time to first drink during the ad-lib drinking period will be analyzed using survival models with subject-specific frailties to account for correlation of the repeated survival outcomes within individuals.

Aim 2: Scores on the Yale Craving Scale will be the primary outcome in this aim and will represent the dependent variable in a linear mixed model evaluating combination treatment effects of NTX and MEM/NAC on alcohol craving. The model will include treatment (NTX vs, NTX+MEM/NAC) and time (see study time points, Table 1) as within-subjects explanatory factors, and random subjects effects. The interaction between treatment and time will be modeled and interpreted using graphical displays and appropriate post-hoc tests. We anticipate a significant treatment by time interaction explained by greater reductions in craving over time among combination NTX+MEM/NAC compared to NTX alone. A secondary measure of craving, the Alcohol Urge Questionnaire, will be analyzed using the same model described above. We will also explore the influence NTX vs. NTX+MEM/NAC on YCS alcohol craving in response to the alcohol cue exposure just prior to the start of the priming drink period using a similar model.

Aim 3: Alcohol-induced stimulation, measured by the Biphasic Alcohol Effects Scale (BAES), will be analyzed using the same model described for alcohol craving in Aim 2. A significant treatment by time interaction, explained by reduced alcohol stimulation to due to combination

NTX+MEM/NAC treatment, compared to NTX alone, will be supportive of our hypothesis. A secondary analysis will conduct similar comparisons on the sedation scores obtained using the BAES.

Exploratory Aims

Exploratory analyses will evaluate the quantity and quality of adverse events observed with NTX+MEM/NAC treatment. We will also explore the effects of NTX and NTX+MEM/NAC treatment on the reaction time outcomes of Implicity Association Task and the alcohol Approach-Avoidance Task, as well as the behavioral and self-report impulsivity measures. Exploratory analyses will also examine impulsive propensities and responses on the PIT task, as predictors of treatment response. We will also evaluate potential correlations between the behavioral effects of alcohol and impulsivity measures as well as the presence of glutamate (STEP/FYN) and opioid (OPRM1) gene polymorphisms.

Power Analysis

This is a pilot trial and we will use the data to determine initial tolerability and effect size estimates for future trials. We will recruit a total of 30 completers in this trail, 10 in each group.

SECTION VI: RESEARCH INVOLVING DRUGS, DEVICES, BIOLOGICS & PLACEBOS

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

   Memantine, or 1-amino-3, 5-dimethyladamantane hydrochloride, is a moderate affinity noncompetitive NMDA antagonist with voltage-dependent binding characteristics. FDA approved as a pharmacotherapy for the treatment of moderate to severe Alzheimer's disease.

   Naltrexone is an FDA approved drug that is used in the treatment of alcoholism and opioid addiction.

   N-acetyl cysteine is FDA approved for use as a mucolytic agent for bronchopulmonary disorders (e.g. Grandjean et al., 2000) and as an oral or intravenous antidote to treat acetaminophen poisoning (Smilkstein et al., 1988). NAC is a precursor of glutathione synthesis and is available worldwide in intravenous, oral and nebulizer forms and is also sold over the counter in health food stores.

All protocols which utilize a drug, device or biologic **not** approved by, but regulated by, the FDA must provide the following information: ☒ **Not applicable to this research project**

The new agent being used in this protocol, NAC, is freely available over the counter in health food stores and has been used off-label safely for a wide number of indications at the doses proposed. Therefore, we do not feel we would need an IND for this protocol.

i. What is the Investigational New Drug (IND) or Investigational Device Exemption (IDE) **number** assigned by the FDA?
ii. For IDE's: Did the FDA approve this IDE as a Category A (experimental/investigational) or as a Category B (non-experimental/investigational)?
iii. Who holds the IND or IDE?

The clinical investigation of a drug product that is lawfully marketed in the United States may be exempt from the requirements for filing an IND. If there is no IND and an exemption is being sought, complete the following: Exempt

i. Is the intention of the investigation to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug? ☐ Yes ☐ No
ii. If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, is the intention of the investigation to support a significant change in the advertising for the product? ☐ Yes ☐ No
iii. Does the investigation involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product? ☐ Yes ☐ No
iv. Will the investigation be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56)? ☐ Yes ☐ No
v. Will the investigation be conducted in compliance with the requirements regarding promotion and charging for investigational drugs? ☐ Yes ☐ No

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

Memantine has been used in the treatment of patients with Parkinson's disease [48], dementia [49-51] and neurogenic bladder dysfunction in spasticity [52]. The drug was also found to be stimulating or vigilance-enhancing in comatose patients [53]. Memantine induces open-channel blockade of NMDA receptors and has been observed to be partially trapped in NMDA receptor channels. There have been a number of studies of memantine in healthy volunteers. Memantine has been shown to increase intracortical inhibition and reduce intracortical facilitation [54] and found to have no significant effects on mood, attention or immediate and delayed verbal and visuospatial memory [55]. Memantine has vigilance-enhancing effects in health elderly volunteers [56] but no effects on a series of perceptual and psychomotor tasks, including temporal discrimination, reaction time, critical flicker fusion frequency and signal detection [57]. Memantine is completely absorbed from the GI tract and maximum plasma concentrations occur between 3-8 hours after oral administration. It is 100% bioavailable after oral administration and has been shown to have a linear pharmacokinetic profile that is not influenced by age, sex or food (Forest Labs, data on file). Approximately 80% of the agent circulates as unchanged parent drug, the mean terminal elimination half-life is 60-100 hours and it is eliminated renally [43]. Memantine was well tolerated in randomized double-blind, placebo-controlled trials for patients with moderately severe to severe dementia, moderate to severe Alzheimer's disease as well as vascular dementia [43, 58, 59]. The most common adverse events observed in clinical trials are diarrhea, insomnia, dizziness, headache, constipation, and confusion [60]. In patients with severe renal impairment or hepatic problems the use of memantine has not been systematically evaluated and is not recommended.

Naltrexone, an opioid antagonist, is widely used in the treatment of opioid addiction and more recently has been found to be beneficial in the treatment of alcoholism. Numerous studies have found naltrexone use to be safe and rarely associated with toxicity or severe side effects. The most frequent reported side effects are gastrointestinal in nature. Those include epigastric pain,

nausea and vomiting. Other, less frequent side effects include nervousness, dizziness, headaches, blurred vision, low energy, fatigue, sleepiness, joint and muscle pain and insomnia. Hepatotoxicity, the most serious potential side effect, has been shown in studies using very high doses of naltrexone (1400 to 2100 mg per week). At the doses used in this study naltrexone has not been reported to produce hepatotoxic effects. However, we will monitor liver function tests prior to the study and exclude individuals with evidence of significant hepatocellular injury (AST, ALT >3x normal established in pregnant and nursing women, they will be excluded from participation. Naltrexone can also precipitate or exacerbate opiate withdrawal, as a results subjects with abuse or dependence on opiates will be excluded from the study on the basis of self-report and urine drug screens.

Since naltrexone is an opiate antagonist, alternative nonopioid methods of analgesia can be used. In an emergency situation requiring opioids, the amount of opioids necessary for analgesia may be greater than usual, and the resulting respiratory depression may be deeper and more prolonged. As a result, a rapidly acting analgesic which minimizes respiratory depression is preferred and the amount of the analgesic administration titrated to the needs of the patient in a setting equipped and staffed for cardiopulmonary resuscitation. As a result, subjects will be given a card showing that they may be receiving naltrexone. This card will provide detailed information to medical personnel describing the special precautions necessary in the event that the subject should require pain management. In addition, this card will have a code number on it that can be used to identify which medication the subject is on. A phone number of the pharmacy and for the physician on call at the Connecticut Mental Health Center will be listed on the card in the event of an emergency in which it is necessary to determine whether the subject is on active naltrexone.

NAC (also known as Acetylcysteine) has been safely used for several decades in adults and children as a long-term treatment for chronic bronchitis. It has been found to be cost-effective to use and generally well tolerated with mild, most commonly gastrointestinal adverse effects. This dose of NAC (2400 mg) was observed to be safe and tolerable in cocaine dependent participants (e.g. Larowe et al., 2006).

3. **Source:**
   a) Identify the source of the drug, device or biologic to be used.
   All study medications will be purchased from appropriate vendors through the Investigational Pharmacy of YNHH.

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

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

5. **Use of Placebo: x Not applicable to this research project**
   This is not a treatment trial. We need a placebo condition to identify the individual versus combined effects of naltrexone and the glutamatergic agents (memantine or NAC).

Provide a justification which addresses the following:

a. Describe the safety and efficacy of other available therapies (if any). This is not a treatment trial or a treatment seeking population of heavy drinkers

b. State the maximum total length of time a participant may receive placebo while on the study. 16 days
c. Address the greatest potential harm that may come to a participant as a result of not receiving effective therapy (immediate or delayed onset.) Not a treatment seeking population
d. Describe the procedures that are in place to safeguard participants receiving placebo. Not a treatment seeking population

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

If yes, is the use of the controlled substance considered:
☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.
☐ Non Therapeutic: Note, the use of a controlled substance in a non therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See Instructions for further information.

7. **Continuation of Drug Therapy After Study Closure** ☒ **Not applicable to this project**
Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended? ☐ Yes ☐ No
If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

SECTION VII: HUMAN SUBJECTS

1. **Recruitment Procedures:** How will potential subjects be identified, contacted and recruited? Attach copies of any recruitment materials that will be used.

☒ Flyers ☒ Internet/Web Postings ☒ Radio
☒ Posters ☐ Mass E-mail Solicitation ☐ Telephone
☐ Letter ☒ Departmental/Center Website ☒ Television
☐ Medical Record Review ☐ Departmental/Center Research Boards ☒ Newspaper
☐ Departmental/Center Newsletters ☒ Web-Based Clinical Trial Registries
☒ Other (describe): use of promotional materials in community (e.g., magnets)
☒ Clinicaltrials.gov Registry (do not send materials to HIC)

1a. **Assessment of Current Health Provider Relationship for HIPAA Consideration:**
Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐ Yes, all subjects
☐ Yes, some of the subjects
☒ No
If yes, describe the nature of this relationship.

2. **Subject Population** Provide a detailed description of the targeted involvement of human subjects for this research project.

86 healthy heavy drinkers who are not currently seeking treatment for their drinking behavior, between 21-55 years of age.

3. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion? How will eligibility be determined, and by whom?

Inclusion criteria:

1) Ages 21-55
2) Able to read English at 6th grade level or higher and to complete study evaluations
3) Meet DSM-IV criteria for alcohol dependence or abuse (assessed using the SCID)
4) Family history criteria (assessed using the FHAM; see Assessment section)
   Family history positive subjects: At least one first-degree relative with alcoholism as determined by the FHAM
   Family history negative subjects: No first degree relative with alcoholism and no 2nd degree relative with alcoholism unless the participant cannot answer details about the 2nd degree family member's drinking consequences on the FHAM.
   (Average weekly alcohol consumption of standard drinks of at least 25-70 drinks for men and 20-65 drinks for women)
5) No more than 3 days abstinence/week in order to maximize the likelihood that subjects will choose to drink during the laboratory sessions

Exclusion criteria:

1) Individuals who are seeking alcohol treatment or have been in alcohol treatment within the past 6 months
2) Current DSM-IV dependence criteria for other substances, other than nicotine
3) Positive test results at more than one baseline appointment on urine drug screens conducted for opiates, cocaine, benzodiazepines, and barbiturates
4) Regular use of psychoactive drugs including anxiolytics and antidepressants
5) Psychotic or otherwise severely psychiatrically disabled; scores above 2 SD of the mean on the Wisconsin Psychosis Proneness scale
6) Medical conditions that would contraindicate the consumption of alcohol
7) Medical conditions that would contraindicate the use of naltrexone such as hepatic dysfunction
8) Medical conditions that contraindicate the use of memantine or NAC such as asthma, seizures, kidney disease, repeated urinary tract infections, or liver disease
9) Individuals taking medications that might interact with memantine, including amantadine or rimantadine; dextromethorphan; carbonic anhydrase inhibitors such as acetazolamide, dicholrphenamide, or methazolamide; or potassium citrate
10) Any history of neurological trauma or disease, delirium, or hallucinations, or hepatic, cardiovascular, metabolic, endocrine, or gastrointestinal disease
11) Subjects who at any intake appointment have a Clinical Institute Withdrawal Assessment Scale score of 8 or greater, or who report any history of significant or repeated alcohol withdrawals will be excluded from the study and referred for standard alcohol detoxification. This is to reduce the likelihood that subjects enrolled in the study will experience withdrawal symptomatology if they reduce their drinking.
12) Women who are pregnant, nursing, or refuse to use a reliable method of birth control; urine pregnancy tests will be completed at intake and prior to administration of alcohol
13) Subjects who report disliking spirits will be excluded because hard liquor will be provided during the alcohol administration components of the study.
14) Subjects who have taken Naltrexone within 3 weeks immediately preceding admission to the treatment period, all other inverstigational drugs will require a 4 week washout period.

15) Subjects who report any use during the 30 days prior to randomization of the following: anxiolytics, beta blockers, central nervous system stimulants, hypnotics, non-therapeutic doses of neuroleptics and antidepressants, drugs with psychotropic activity, or drugs which cause excessive sedation
16) Subjects who have donated blood within the past six weeks

Additional PIT exclusion criteria:
17) Known (history of) taste or smell dysfunction
18) History of oral nerve damage
19) Food allergies or sensitivities (for example nuts, lactose, artificial sweeteners)
20) A diagnosis of diabetes
21) Chronic use of medication that may affect taste
22) Conditions that may interefere with gustatory or olfactory perception (colds, seasonal allergies).

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

3.b. If yes, will identifiable health information be collected during this screening process and retained by the research team? ☒ Yes ☐ No

4. **Subject Classifications: Check off all classifications of subjects that will be invited to enroll in the research project.** Will subjects, who may require additional safeguards or other considerations, be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

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

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

SECTION VIII: CONSENT/ ASSENT PROCEDURES

1. **Consent Personnel:** List all members of the research team who will be obtaining consent/assent.
Nicholas Franco, Dana Cavallo, Tricia Dahl, Thomas Liss, Suchitra Krishnan-Sarin

2. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.
At the start of the intake session, all subjects will receive an explanation of the study including its risks, benefits, and procedures, and will be given an opportunity to withdraw from the study. Following the resolution of any questions, the subject will be asked to sign the consent form, if he/she agrees to participate.

3. **Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.
   Subjects with limited decision making capacity will not be enrolled in this study.

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

5. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. Translated copies of all consent materials must be submitted for approval prior to use.
   Due to the intensity and complexity of the design of this study we will only enroll English speaking subjects.

6. **Waiver of Consent:** Will you request either a waiver of consent, or a waiver of signed consent, for this study? If so, please address the following:

   ☒ **This section is not applicable to this research project**

   Waiver of consent: (No consent form from subjects will be obtained.)

   a. Does the research pose greater than minimal risk to subjects? ☐ Yes ☐ No
   b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
   c. Why would the research be impracticable to conduct without the waiver?
   d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

   Waiver of **signed** consent: (Verbal consent from subjects will be obtained.)

   ☒ **This section is not applicable to this research project**

   a. Would the signed consent form be the only record linking the subject and the research? ☐ Yes ☐ No
   b. Does a breach of confidentiality constitute the principal risk to subjects? ☐ Yes ☐ No
      OR
   c. Does the research pose greater than minimal risk? ☐ Yes ☐ No **AND**
   d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

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

8. **Request for waiver of HIPAA authorization:** (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only)

   Choose one: For entire study:_____For recruitment purposes only: X

   i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data- Data is collected over the phone and through Qualtrix so it wouldn't be practical to obtain authorization to use this data.

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

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

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

SECTION IX: PROTECTION OF RESEARCH SUBJECTS

1. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.
The major potential risks in this study are related to administration of alcohol, memantine, N-acetyl cysteine, naltrexone, and the blood draw during the physical exam and alcohol drinking period.

1) Memantine:
An NMDA antagonist, has been approved by the FDA for the treatment of moderate to severe Alzheimer's disease in the US. Numerous studies have found memantine to be safe and associated with few side effects. In a study of 252 patients, 17% of memantine-treated patients and 10% of placebo-treated patients discontinued treatment because of adverse events during 28-weeks of treatment. The most common side effects occurring with equal frequency in memantine and placebo groups included agitation, urinary incontinence, urinary tract infections, insomnia and diarrhea. Side effects occurring at a higher rate in memantine-treated patients included dizziness (7%), headache (6%), constipation (6%), confusion (6%), insomnia (less than 1%) and hallucinations (less than 1%). Since memantine could also cause drowsiness or dizziness, participants will be told to use caution when driving, operating machinery or performing other hazardous activity. Memantine is in FDA category B which means that it is not expected to be harmful to an unborn baby but since there has been minimal use of this agent in a young patient population (such as the one in this proposal) we have chosen to be cautious and exclude women who are pregnant or nursing. Such individuals could not be included in the current proposal anyway since we are administering alcohol to all subjects. The major route of memantine elimination is renal and therefore the use of this drug in patients with renal impairment is not recommended. There is also some question about its use in those with seizure disorders of liver disease. Since liver disease is often seen in alcohol-dependent subjects we are proposing to exclude individuals with kidney disease, liver disease or seizure disorder.

Since memantine is an NMDA antagonist and other NMDA agents like PCP and ketamine are known to have some abuse liability, various studies have evaluated the abuse liability of memantine. In both rat and monkey drug-discrimination studies, memantine did partially substitute for PCP but with significant decreases in rates of responding [165, 166]. In the monkey studies, memantine had weak reinforcing potential only at higher doses and in the clinical trials with memantine there were no evidence of drug seeking behavior. Therefore it does not appear likely that memantine has significant potential for abuse. As is our practice with other similar studies, all subjects will be given a card showing that they may be receiving memantine. This card will provide detailed

information to medical personnel about the drug. In addition, this card will have a code number on it that can be used to identify which medication the subject is on. A phone number of the pharmacy and for the physician on call at the Connecticut Mental Health Center will be listed on the card in the event of an emergency in which it is necessary to determine whether the subject is on active memantine.

2) N-acetyl cysteine

N-acetyl cysteine is FDA approved for use as a mucolytic agent for bronchopulmonary disorders (e.g. Grandjean et al., 2000) and as an oral or intravenous antidote to treat acetaminophen poisoning (Smilkstein et al., 1988). It is also used for chest pain (unstable angina), bile duct blockage in infants, amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Alzheimer's disease, allergic reactions to the anti-seizure drug phenytoin (Dilantin), and an eye infection called keratoconjunctivitis. It is also used for reducing levels of a type of cholesterol called lipoprotein (a), homocysteine levels (a possible risk factor for heart disease) and the risk of heart attack and stroke in patients with serious kidney disease.

Some people use N-acetyl cysteine for chronic bronchitis, chronic obstructive pulmonary disease (COPD), hay fever, a lung condition called fibrosing alveolitis, head and neck cancer, and lung cancer. It is also used for treating some forms of epilepsy; ear infections; complications of kidney dialysis; chronic fatigue syndrome (CFS); an autoimmune disorder called Sjogren's syndrome; preventing sports injury complications; radiation treatment; increasing immunity to flu and H1N1 (swine) flu; and for detoxifying heavy metals such as mercury, lead, and cadmium.

N-acetyl cysteine is also used for preventing alcoholic liver damage; for protecting against environmental pollutants including carbon monoxide, chloroform, urethanes and certain herbicides; for reducing toxicity of ifosfamide and doxorubicin, drugs that are used for cancer treatment; as a hangover remedy; for preventing kidney damage due to certain X-ray dyes; and for human immunodeficiency virus (HIV).

N-acetyl cysteine is safe for most adults. It can cause nausea, vomiting, and diarrhea or constipation. Rarely, it can cause rashes, fever, headache, drowsiness, low blood pressure, and liver problems.

3) Naltrexone

Naltrexone has been shown to have an effect on the embryo in the rat and the rabbit when given in doses approximately 140 times the human therapeutic dose. Naltrexone, an opioid antagonist, is widely used in the treatment of opioid addiction and more recently has been found to be beneficial in the treatment of alcoholism. Numerous studies have found naltrexone use to be safe and rarely associated with toxicity or severe side effects. The most frequent reported side effects are gastrointestinal in nature. Those include epigastric pain, nausea and vomiting. Other, less frequent side effects include nervousness, dizziness, headaches, blurred vision, low energy, fatigue, sleepiness, joint and muscle pain and insomnia. Hepatotoxicity, the most serious potential side effect, has been shown in studies using very high doses of naltrexone (1400 to 2100 mg per week). At the doses used in this study naltrexone has not been reported to produce hepatotoxic effects. However, we will monitor liver function tests prior to the study and exclude individuals with evidence of significant hepatocellular injury (AST, ALT >3x normal established in pregnant and nursing women, they will be excluded from participation. Naltrexone can also precipitate or exacerbate opiate withdrawal, as a results subjects with abuse or dependence on opiates will be excluded from the study on the basis of self-report and urine drug screens.

Since naltrexone is an opiate antagonist, alternative nonopioid methods of analgesia can be used. In an emergency situation requiring opioids, the amount of opioids necessary for analgesia may be greater than usual, and the resulting respiratory depression may be deeper and more prolonged. As a result, a rapidly acting analgesic which minimizes respiratory depression is preferred and the amount of the analgesic administration titrated to the needs of the patient in a setting equipped and staffed for cardiopulmonary resuscitation. As a result, subjects will be given a card showing that they may be receiving naltrexone. This card will provide detailed information to medical personnel describing the special precautions necessary in the event that the subject should require pain management. In addition, this card will have a code number on it that can be used to identify which medication the subject is on. A phone number of the pharmacy and for the physician on call at the Connecticut Mental Health Center will be listed on the card in the event of an emergency in which it is necessary to determine whether the subject is on active naltrexone.

4) Alcohol:

A number of medical conditions could potentially be worsened by acute alcohol administration (e.g., liver disease, cardiac abnormality, pancreatitis, diabetes, neurological problems, and gastrointestinal disorders). As a result, subjects with medical problems as revealed by physical exam and laboratory findings will be excluded from the study.

Alcohol may also cause nausea in high doses; however, nausea is not expected at the dose being used in this sample of heavy drinkers. Subjects will not be drinking to levels more than they typically consume in their own drinking context and with the exception of the priming dose, they determine the amount of alcohol consumed.

Another area of potential risk to subjects under the influence of alcohol involves their safety during the experimental procedures. Although impairment of gross motor coordination in heavy drinkers is rare at the alcohol dose used in this study, all subjects will be under the supervision of the experimenters to prevent possible accidents such as falls. Subjects will not leave the laboratory during the self-administration procedure. By staying in the YCCI or CNRU overnight, the possibility that the subject might leave the session and continue to drink alcohol thereby placing themselves at risk for accidents is prevented.

Alcohol is a reinforcing agent, which may cause changes in behavior including repetitive or excessive alcohol consumption. Because of this, the administration of alcohol to alcoholics in treatment could potentially impede the progress of their recovery. In addition, the administration of alcohol to sober alcoholics living in the community presents a possible risk of relapse. As a result, we will be recruiting **non-**abstinent **non-**treatment seeking alcoholics in keeping with the National Advisory Council on Alcohol Abuse and Alcoholism's (1989) recommended guidelines on ethyl alcohol administration. At completion of the study, we will make a serious and concerted effort to link the subject with treatment for their alcohol problems. This will be done by giving the subject objective feedback about the fact that their drinking exceeds standards for avoiding hazardous drinking, providing a brief one session motivational intervention for their drinking, and by arranging for alcohol treatment services if they are interested. In our previous and ongoing work, several participants quit drinking and many others reduced their drinking in the three months following this intervention (Sinha R, Krishnan-Sarin S, Farren C, O'Malley SS. Naturalistic follow-up of drinking behavior following participation in an alcohol administration study. *Journal of Substance Abuse Treatment* 1999; 17: 159-62).

5) Interactions of Memantine and Alcohol:

There are no known interactions of co-administering memantine and alcohol. In a study by Bisaga and Evans [40] in moderate drinkers, memantine by itself produced some increase in dissociative symptoms which were further slightly increased by alcohol. Since, in this study,

participants will be taking memantine on an outpatient basis, we will monitor via face-to-face daily contact for side effects from memantine.

6) Interactions of NAC and Alcohol:
There are no known interactions of co-administering NAC and alcohol. Since, in this study, participants will be taking NAC on an outpatient basis, we will monitor via face-to-face daily contact for side effects from NAC.

7) Interactions of Naltrexone and Alcohol:
There are no known risks to contraindicate the administration of alcohol to subjects on naltrexone. It has been shown that pharmacokinetics properties of naltrexone and ethanol are not altered on simultaneous administration of both agents.

8) Intravenous Access:
Insertion of an intravenous catheter involves risk for hematoma at the site of the venous puncture. Very rarely, venous puncture can also result in a blood clot or infection.

9) Blood and Urine Collections:
Screening blood and urine collections are performed primarily as safeguards to subjects and should add no risks other than those normally associated with these procedures. Subjects will have approximately 40 cc of blood drawn at the intake appointment to determine liver and kidney functioning, 30 cc of blood for the genetics portion (see below) and during the fourth phase (self-administration) of the study we will draw approximately 30 cc of blood. Therefore, the total amount of blood drawn during the study (100 mls or approx. 3.4 oz) is well within the HIC guidelines of 450 cc within eight research weeks and the blood loss poses minimal risk in healthy subjects. We will advise subjects against donating blood for six weeks following study participation.

10) Rating Scales and Questionnaires:
These are all noninvasive and should add no risk. The major disadvantages are the time taken to complete them, and possible breach of confidentiality. Our past experience with these measures indicates that they are acceptable to subjects. Careful efforts aimed at maintaining confidentiality will be made.

11) Alcohol Withdrawal:
We will not ask participants to alter their drinking behavior during their participation in the study. However, there is always the possibility that some participants may reduce or stop drinking during the outpatient period while on the study medication. Therefore, we will inform that that some individuals who reduce or stop their drinking can experience alcohol withdrawal symptoms such as mild agitation, anxiety, restlessness, tremor, loss of appetite and difficulty sleeping or even more severe (but rare) symptoms like extreme restlessness, nervousness, disorientation, confusion, hallucinations (hearing and seeing things that are not there) and seizures, but these are extremely rare. We will monitor them daily during their visits to our clinic and will also inform them that if they experience worsening of withdrawal symptoms (CIWA > 8) we may have to hospitalize them and give them medications that are typically used to treat and manage withdrawal including benzodiazepines, such as chlordiazepoxide (librium), and other medications such as carbamazepine (tegretol).

12) Genetic Bloods:

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Donation of 30 ml of blood for genetics studies will be part of every Informed Consent document. All participants will be given the option of participating in the genetic portion of the study. Subjects will be informed of possible risks and benefits of participation in genetics research, will be informed of the potential genomewide scope of the genotyping effort, and will be informed that their DNA samples will be retained indefinitely (banked). Participants will also be given the option of withdrawing from the study after blood samples have been obtained; alternatives provided will include destruction or de-identification of sample. A Certificate of Confidentiality from NIAAA has been obtained to further protect the confidentiality of this information."

13) PIT task:
There are no known risks associated with consumption of any of the odors or liquids that subjects will encounter. All are commercially available products that subjects will have likely encountered before. Subjects with food allergies or sensitivities (for example nuts, lactose, artificial sweeteners, red food dye) will be excluded.

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

1) Memantine:
Effective screening will exclude all subjects who would be at greater risk for complications because of medical, neurological or psychiatric illnesses. Individuals currently dependent on other drugs will be screened out. Subjects maintained on memantine study medication will be issued "keyed" cards which allow health professionals to break the double blind by calling, the Yale-New Haven pharmacy switchboard which answers 24 hours a day. Subjects will also be monitored during daily medication visits for troublesome side effects during the course of the 6 day outpatient period. Although memantine is in FDA category B, there have been few trials where it has been administered to younger women. Therefore, the following precautions will be taken for women: 1) urine pregnancy tests will be performed at intake, prior to starting the medication, and on the day of the alcohol self-administration session. Pregnant or nursing women will be excluded from participation, and encouraged to seek advice about the risk of heavy drinking, encouraged to seek alcohol treatment and if interested referred to other cessation programs; 2) women must agree to use a reliable method of birth control while they are in the study. They will be asked to alert the principal investigator if she departs from her birth control plans or if, in spite of adherence to these plans, she thinks she might be pregnant.

2) Naltrexone:
Effective screening will exclude all subjects who would be at greater risk for complications because of medical, neurological or psychiatric illnesses. Individuals currently dependent on other drugs will be screened out. Subjects who are using opiates will be excluded to avoid any possibility of the subjects experiencing naltrexone precipitated opiate withdrawal. The risk of hepatotoxicity will be minimized by excluding subjects with a history of cirrhosis or significantly elevated liver enzyme tests. Subjects maintained on naltrexone study medication will be issued "keyed" cards which allow health professionals to break the double blind by calling the CMHC pharmacy. Subjects will also be constantly monitored for troublesome side effects during the course of the 12 day outpatient and three days inpatient period.
Given the uncertain effects of naltrexone during pregnancy, the following precautions will be taken for women: 1) urine pregnancy tests will be performed at intake, and pregnant or nursing women will be excluded from participation, and encouraged to seek advice about the risk of heavy drinking, encouraged to seek treatment and if interested referred to other cessation programs; 2) women must agree to use a reliable method of birth control while they are in the

study and to alert the principal investigator if she departs from her birth control plans or if, in spite of adherence to these plans, she thinks she might be pregnant.

3) NAC:
Effective screening will exclude all subjects who would be at greater risk for complications because of medical, neurological or psychiatric illnesses. Individuals currently dependent on other drugs will be screened out. There is a concern that N-acetyl cysteine might cause bronchospasm in people with asthma, so participants will be excluded if they have asthma. Given the uncertain effects of NAC during pregnancy, the following precautions will be taken for women: 1) urine pregnancy tests will be performed at intake, and pregnant or nursing women will be excluded from participation, and encouraged to seek advice about the risk of heavy drinking, encouraged to seek treatment and if interested referred to other cessation programs; 2) women must agree to use a reliable method of birth control while they are in the study and to alert the principal investigator if she departs from her birth control plans or if, in spite of adherence to these plans, she thinks she might be pregnant.

4) Alcohol Challenges:
The alcohol challenges will be conducted by personnel experienced in alcohol challenge research. As described above, all subjects will be under supervision to prevent possible accidents. At the end of the challenge session all subjects will be kept in the HRU where they will stay overnight to prevent the possibility that they would continue drinking after the session and place themselves at risk of accidents. Although we have never had a subject chose to leave a session early, should a subject insist on leaving the research setting prematurely, we will provide transportation back to their residence. This contingency is explicitly addressed in the consent form. Clearly, subjects are free to discontinue the experiment at any time. However, if a subject chose to discontinue participation after alcohol has been administered we will require them to stay in the HRU until their blood alcohol level is below 0.04 and they will then be provided with a ride home. Furthermore, at the Principal Investigator's discretion, a participant will be discontinued if he/she does not drink any of the choice drinks at ADP 1. This absence of drinking creates a floor effect and does not allow for evaluation of change in drinking behavior at ADP 2 and ADP 3 after taking study medication. Given the cost of the hospital visits, it does not seem reasonable to continue participants whose data will not be meaningful. Participants will be told that the study doctor may discontinue his/her participation based on data collected at the first ADP.

5) Research Records:
Right to privacy for participation in this research will be protected through anonymous coding of data and proper storage of research records. Access will be limited to the PI and her designates involved in the study. A certificate of confidentiality has been obtained from NIAAA. Safeguards include screening by experienced professionals in order to ensure that the inclusion and exclusion criteria are met before patients are entered in the study, including physical exam and laboratory tests.

6) PIT task:
We will screen all subjects both on the telephone and when they arrive for their appointment to ensure that they do not have food allergies, which may interfere with the study. Researchers will keep in close communication with subjects while they are completing this study, and subjects experiencing discomfort can discontinue the study.

3. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? This protocol is a moderate risk protocol and therefore requires a data safety and monitoring plan.
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? N/A
    c. Data and Safety Monitoring Plan

1) **Personnel responsible for the safety review and its frequency:**
We will be accessing the Data and Safety Monitoring Board (DSMB) developed for the Center for Translational Neuroscience on Alcoholism (John Krystal, PI). The DSMB is multi-disciplinary and includes representatives with expertise in the primary components of the proposed trial. The following individuals will be on the DSMB as voting members:

Robert Swift, MD, PhD., Prof Psych (Brown)/ASOS Res Provid. VAMC Chmn, DSMB
Robert Stout, PhD., Director, Decision Sci. Int., Statistician, DSMB
Howard Zonana, MD., Dir, Dept Psychiatry Ethics Committee, IRB Rep, DSMB
Lisa Newton, PhD., Prof. Applied Ethics, Fairfield Univ. Ethicist, DSMB
This DSMB will follow the operational guidelines outlined in the YCCI OR CNRU plan for DSMB.

We hope to recruit subjects at a rate of about 1-2 per month, thus, the DSMB will review safety reports two times a year. More frequent meetings will be scheduled if indicated by interim findings.

2) **The risks associated with the current study are deemed moderate for the following reasons:**
Given the now established safety and validity of the current medications in our prior work, we do not view the proposed studies as high risk.
Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3) **Attribution of Adverse Events:**
Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator, Dr. Suchitra Krishnan-Sarin according to the following categories:
a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4) **Plan for Grading Adverse Events:**

The following scale will be used in grading the severity of adverse events noted during the study:
1. Mild adverse event
2. Moderate adverse event
3. Severe

5) **Plan for Determining Seriousness of Adverse Events:**
Serious Adverse Events:
In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it:
1. is life-threatening
2. results in in-patient hospitalization or prolongation of existing hospitalization
3. results in persistent or significant disability or incapacity
4. results in a congenital anomaly or birth defect OR
5. results in death
6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, or
7. adversely affects the risk/benefit ratio of the study
An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the HIC or HSC is necessary.

6) **Plan for reporting serious AND unanticipated AND related adverse events, anticipated adverse events occurring at a greater frequency than expected, and other unanticipated problems involving risks to subjects or others to the HIC or HSC.**
The investigator will report the following types of adverse events to the HIC or HSC: a) serious AND unanticipated AND possibly, probably or definitely related events; b) anticipated adverse events occurring with a greater frequency than expected; and c) other unanticipated problems involving risks to subjects or others.
These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC or HSC within 48 hours of it becoming known to the investigator, using the appropriate forms found on the website.

7) **Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Center for Clinical Investigation Research Subject Advocates (RSAs), Cancer Center's Quality Assurance, Compliance and Safety Committee (QUACS) Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies**

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

X All Co-Investigators listed on the protocol.

X **Yale Center for Clinical Investigation Research Subject Advocates (RSAs)**

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□ Quality Assurance and Compliance and Safety Committee (QUACS)

X National Institutes of Health

□ Food and Drug Administration (Physician-Sponsored IND #_____)

□ Medical Research Foundation (Grant_____)

The principal investigator, Dr. Krishnan-Sarin, will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

4. **Confidentiality & Security of Data:**

a. What protected health information about subjects will be collected and used for the research? Name, address, telephone number, email address SS #, and birth date will be collected from subjects

b. How will the research data be collected, recorded and stored? Research will be collected in a private room by a research assistant, recorded in binders, and stored in a locked, secure location.

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

d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during the subject participation in the study? Study data will be password protected on secured server, and paper data will be kept in a locked, secure location.

e. What mechanisms are in place to ensure the proper use and continued protection of these data after the subject participation in the study has ceased? Study data will continue to be kept in a locked, secure location after each subject's participation has ceased.

f. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured. Study data will be archived in a secure storage facility, data will not be destroyed.

g. Who will have access to the protected health information? (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, QUACS, SSC, etc.) The PI and study personnel. A CMHC record containing PHI will also be kept in a locked file at CMHC.

h. Which external or internal individuals or agencies (such as the study sponsor, FDA, QUACS, SSC, etc.) will have access to the study data? Principal investigator and her research team, YCCI Hospital Research Unit and CMU and CMHC.

i. If appropriate, has a Certificate of Confidentiality been obtained? A certificate has been obtained from NIAAA

j Are there any mandatory reporting requirements? (Incidents of child abuse, elderly abuse, communicable diseases, etc.) Child abuse, elder abuse and intent to harm self or others.

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

This study will not directly benefit the participants. The results of this laboratory-based drinking paradigm will provide help advance knowledge in the area of development of pharmacotherapies for alcohol drinking. An additional benefit to subjects is that they will be offered feedback about

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their drinking and active referral to treatment for their alcohol problems should they so desire. Most alcohol abusers have impairments in their psychosocial functioning as a result of their drinking and can benefit from treatment. The proposed study may be a conduit for some to receive treatment and for others to reduce their drinking on their own. Although the direct benefit is not great for subjects, given the potential benefit to developing effective treatments for alcoholism, the risk-benefit ratio appears favorable.

Furthermore, the results of this laboratory-based drinking paradigm will provide an important initial signal regarding the potential efficacy of the combination of naltrexone and memantine or Nacetylcysteine in reducing alcohol drinking. There is a great need for the development of new agents and combination of agents to treat alcohol dependence, particularly in subpopulations of drinkers. This laboratory paradigm will also provide information regarding the mechanism of action of these agents and thus significantly contribute to the literature.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?
   NA-This is not a treatment study. However, participants will be provided with a motivational interview upon completion of the inpatient and outpatient portions of this study to provide feedback on their drinking. If they request a referral for treatment to cut back or quit drinking at this time, we will provide them with referral options.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects and the conditions for receiving this compensation.
   Because this study may not have direct benefits to the individual participant, subjects will be offered payment for their participation. Subjects will have the opportunity to receive up to $2103 for completing all phases of the study. Payment for the screening interview will be $50, and they will receive an additional $50 for the physical examination; we have found that payments for these appointments encourages attendance as scheduled. Subjects will also receive $50 for the PIT task, $150 for participating in the first ADP, up to $160 for taking medication during Med 1 (6-8 days, up to 13 days if necessary), $200 for participating in the second ADP, $40 for complying with the 6-8 day washout procedures, up to $160 for taking medication during Med 2 (6-8 days, up to 13 days if necessary), $250 for participating in the third alcohol-drinking paradigm, and a bonus of $200 for completing all 3 drinking paradigms given the within-subjects design. Subjects will earn an additional $10 per day for transportation during phases 2, 3 and 5. Subjects will also receive an additional $10 for keeping each scheduled appointment, once they are enrolled (after the physical exam) for a total of $190. Subjects will also have the opportunity to earn an extra $36 during each of the self- administration periods as well as $75 for computer tasks done at the outpatient and inpatients appointments. Lastly, they will earn $30 for completing each of the two follow-up appointments. This progressively increasing payment structure was developed in order to motivate subjects to complete all phases of the study, since obtaining complete data in this within-subjects crossover study is the key to achieving the goals of the project. We are trying to avoid early drop out by using increasing incentives for completion of the entire study. Subjects will be reimbursed $20 for valet parking at Yale New Haven Hospital for overnight stays during the ADP's. They will also be compensated $25 if they are asked to come back for any repeat lab work (blood or EKG).
   If a participant comes in to be consented and decides, after learning more about the study, he/she is not interested in participating, we will discontinue the intake appointment and compensate you $10 for his/her time.

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

4. **In Case of Injury:** This section is required for any research involving more than minimal risk.
   a. Will medical treatment be available if research-related injury occurs? Yes, but injury is unlikely
   b. Where and from whom may treatment be obtained? At YNHH
   c. Are there any limits to the treatment being provided? No
   d. Who will pay for this treatment? The participant or their insurance carrier will be expected to pay for the cost of the treatment.
   e. How will the medical treatment be accessed by subjects? Nurses at HRU or ED at YNHH

References

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