Study Title: Kappa-PET imaging and naltrexone in alcohol drinking behaviors

ClinicalTrials.gov Identifier: NCT01625611

Document Date: 5/6/2019

YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1

Please refer to the HIC website for application instructions and information required to complete this application. The Instructions are available at http://www.yale.edu/hrpp/forms-templates/index.html. Submit the original application and two (2) copies of all materials including relevant sections of the grant which funds this project (if applicable) to the HIC.	**HIC OFFICE USE ONLY**	
	DATE STAMPED-RECEIVED	PROTOCOL NUMBER 1011007710

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: Kappa-PET imaging and naltrexone in alcohol drinking behaviors			
Principal Investigator: Suchitra Krishnan-Sarin, Ph.D.		**Yale Academic Appointment:** Associate Professor	
Campus Address: 34 Park Street, S-208, 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) ☒ **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Does the principal investigator, co-investigator, or any research team member obtaining consent, or any of their family members (spouse, child, domestic partner) have an incentive or interest, financial or otherwise, that may be viewed as affecting the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? See Disclosures and Management of Personal Interests in Human Research http://www.yale.edu/hrpp/policies/index.html#COI

Yes X No

If yes, list names of the investigator or person obtaining consent:

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

b. External Location[s]:

- ☐ APT Foundation, Inc.
- ☒ Connecticut Mental Health Center
- ☐ Veterans Affairs Hospital, West Haven
- ☐ Haskins Laboratories
- ☐ John B. Pierce Laboratory, Inc.
- ☒ 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 56 non-treatment-seeking heavy drinkers, family history positive and negative and 10 healthy controls

   a.targeted for enrollment at Yale for this protocol? 66 participants
   If this is a multi-site study, what is the total number of subjects targeted across all sites?
   N/A
   b. expected to sign the consent form?
   136
   c. expected to complete some or all interventions for this protocol? 66

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**
☐ 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://ycci.yale.edu/comply/billing_idxge.html

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, use of the IDS pharmacy

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	Funding	Funding Mechanism
Suchitra Krishnan-Sarin, PhD	Kappa-PET imaging and naltrexone in alcohol drinking behaviors	NIAAA	☐ ☒ Federal ☐ State ☐ Non Profit ☐ Industry ☐ Other For Profit ☐☒ Other	☐☒ Grant-M#1R01AA01998001 ☐ Contract# Contract ☒ Pending ☐ Investigator/Department Initiated ☐ Sponsor Initiated Other, Specify:

IRB Review fees are charged for projects funded by Industry or Other For-Profit Sponsors. Provide the Name and Address of the Sponsor Representative to whom the invoice should be sent. ***Note: the PI's home department will be billed if this information is not provided.***

Send IRB Review Fee Invoice To:

Name:
Company:
Address:

2

NOTE: The HIC will remove from the protocol any personnel who have not completed required training. A personnel protocol amendment will need to be submitted when training is completed.

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) and Signature Date

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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

For HIC Use Only

_____ _____

Date Approved **Human Investigation Committee Signature**

SECTION V: RESEARCH PLAN

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

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

Naltrexone (NTX), a non-specific opioid antagonist that binds dose-dependently to mu, delta, and kappa opioid receptors, has been shown to be efficacious in the treatment of alcohol dependence [1]. Understanding how NTX reduces drinking is needed to help optimize NTX treatment and identify important new targets for the development of new pharmacotherapeutic agents to treat alcohol dependence. Recent evidence suggests that kappa opioid receptors (KOR"s) may play an important, but complex, role in mediating alcohol drinking behavior. This proposal brings together a translational team of scientists to examine the *in vivo* biochemistry of NTX in heavy drinkers (HD), before and during treatment with NTX, via high resolution PET imaging of the KOR and relate this to clinical outcomes of NTX efficacy.

Using a laboratory-based human Alcohol Drinking Paradigm (ADP; [2]) that we pioneered, we have shown that family history (FH) of alcoholism may moderate (encode the direction of) response to NTX [3]. Specifically, pretreatment with a 100 mg/day dose of NTX was associated with lower drinking in the ADP in heavy drinkers with a positive FH (FHP), whereas the same dose correlated with higher drinking for those with a negative FH (FHN). What could be the underlying biochemistry? Existing evidence suggests that, at a dose of 50 mg/day, mu-opioid receptors are saturated, and therefore unlikely to determine variability in NTX responsivity [4], pointing to possible roles for delta and kappa opioid receptors. At 100 mg/day, NTX should antagonize KOR, raising the possibility that the differences we observed in the two cohorts of drinkers may be related to differences in KOR. KOR levels in clinical populations can be examined using PET imaging, however, until recently, such an examination was heretofore prohibited by the lack of an appropriate tracer. [11C]LY2795050 (referred to as [11C]-PKAB at Yale PET Center) is a selective, high affinity, KOR antagonist tracer with favorable kinetics for imaging KOR and drug occupancy *in humans* [5]. The new tracer has excellent test/retest reproducibility and is well-suited to quantitating KOR levels in striatal and extra-striatal regions.

This proposal unites neuroimagers, experts in conducting and analyzing human PET studies with behavioral scientists, experts in developing and testing treatments for alcoholism. We will conduct the first-ever *in vivo* PET imaging study of KOR and NTX occupancy in two cohorts of HD (18 FHP and 18 FHN) and relate our imaging findings to FH and alterations of drinking behavior by NTX in the ADP. Through this work, we will advance our understanding of (a) the degree of involvement of the kappa system in reducing drinking in humans by NTX, (b) the contribution of FH to this relationship, and (c) refine the use of a new PET imaging tracer. In so doing, we will inform the development of future targeted pharmacotherapies for alcoholism and lay the groundwork for other important investigations into the KOR/dynorphin system *in vivo*.

Primary (P), Secondary (S), and Auxiliary (A) Specific Aims:

P1. ***Occupancy of KOR by NTX and drinking*:** To determine the degree to which occupancy of KORs by a 100 mg/day dose of NTX mediates (influences the strength of) responsivity to NTX treatment in all heavy drinkers. It is expected that KOR occupancy will significantly mediate the relationship between NTX treatment and responsivity. „Responsivity", measured via ADP, is the change in number of drinks consumed at the baseline ADP vs. NTX ADP.

P2. ***Family History of Alcoholism as a moderator:*** To determine whether the relationship between NTX responsivity and occupancy of KOR is different in FHP vs. FHN heavy drinkers.

P3. To determine if baseline KOR availability differs between heavy drinkers and healthy control socialdrinkers

S1. ***Baseline KOR differences***

a. To determine if baseline levels of KOR differ between FHP and FHN heavy drinkers.
b. To determine if baseline KOR level is related to either baseline drinking or responsivity to NTX.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

S2. ***Identify and validate a reference region*** (area devoid of specific binding) for our new KOR tracer. A reference region would simplify PET analysis.

S3. ***Optimize the scan duration*** for clinical populations being imaged with the new KOR tracer.

Shorter scans would increase compliance and decrease discomfort to subjects.

A1.***Relationship to other behavioral measures:*** To determine if occupancy of KORs is related to measures of alcohol craving, stimulation or sedation, assessed in the ADP.

We hypothesize that (1) degree of NTX occupancy of KOR during treatment will be correlated with the degree of responsivity to NTX in all heavy drinkers and (2) FHP heavy drinkers, who respond positively to NTX, will have lower baseline KOR levels when compared with FHN heavy drinkers.

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 abuse is one of the leading causes of disability in the United States. At present, three medications are approved by the FDA for the treatment of alcohol dependence: disulfiram, naltrexone, and acamprosate. However, the efficacy of these agents ranges from minimal to modest, and they are under-utilized in clinical community settings [6, 7]. There is a pressing need for novel medications that are more effective in reducing alcohol use.

Development of new pharmacotherapeutic approaches to treating alcohol dependence should be based on our understanding of the behavioral and neurochemical mechanisms mediating alcohol drinking, as well the efficacy of agents known to reduce alcohol drinking. Naltrexone (NTX) is a non-specific opioid antagonist that has been shown to have minimal-modest efficacy in treating alcohol dependence. Clinical trials indicate that treatment-seeking heavy drinkers who receive NTX at a dose of 50 mg/day [1, 8] or 100 mg/day [9], have lower levels of relapse to drinking during the treatment period than do those receiving placebo. Subpopulations of alcohol dependent patients may respond better to NTX [10] and family history (FH) of alcoholism is emerging as an important predictor of NTX response [3, 11].

Our earlier work, using a laboratory-based alcohol drinking paradigm (ADP) revealed that NTX responsivity was altered by the presence or absence of a FH of alcoholism; specifically NTX treatment was associated with lower drinking in those with a positive FH (FHP) and actually with higher drinking in those with a negative FH (FHN) [3]. Developing a better pharmacological understanding of this differential response could help enhance the efficacy of NTX treatment. Naltrexone"s ability to reduce alcohol drinking is believed to be mediated, in part, through its effects on the endogenous opioid system, specifically through dose dependent antagonism of mu, delta and kappa opioid receptors. Weerts and colleagues [4] have shown that the most commonly used dose of naltrexone, 50 mg/day, results in almost complete occupancy (saturation) of mu-opioid sites in the brain with variable, partial occupancy of delta opioid receptor sites. This evidence suggests that the differential efficacy of NTX is unlikely to be mediated by mu-opioid binding but could be related to variability in binding to other (delta or kappa) opioid receptor sites. While some investigations have focused on the delta system [4], to date, no one has examined the relationship of kappa opioid receptors (KOR) to alcohol drinking or NTX responsivity in human alcoholics.

Emerging evidence suggests an important, but complex, role for the KOR system in alcohol drinking. Dynorphin is the endogenous opioid ligand that binds to both mu and KORs [12]. In general, activation of KORs is aversive. Animals will not self-administer dynorphin, administration of dynorphin results in conditioned place aversion in animals, and dynorphin

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

activation of KOR results in decreased dopamine release in brain reward areas [13, 14]. The potential role of KORs in mediating alcohol drinking behavior has been the focus of several studies in animals and humans. Mice that lack the KOR drink more alcohol and have greater release of dopamine in response to alcohol [15]. Rodents with an increased propensity toconsume alcohol have lower dynorphin levels in several brain regions that are involved in the control of alcohol drinking [16-18]. In humans, the presence of the OPRK1 allele (which decreases expression of KOR) is associated with increased risk of alcoholism [19]. The above evidence suggests that differences in the status of the KOR system may mediate differences in propensity to drink alcohol. The current proposal will be the first to compare KOR occupancy in FHP and FHN drinkers and correlate KOR levels with drinking in the ADP.

The effect of stimulating or inhibiting the KOR opioid system on alcohol drinking appears to depend, in part, on the prior history of alcohol exposure. In alcohol naïve animals, stimulation of KOR by agonists decreases alcohol drinking [20], while inhibition of KOR by antagonists increases alcohol drinking [21]. Chronic alcohol exposure dysregulates the dynorphin/KOR system; specifically, dynorphin levels are enhanced and number of KOR are reduced [22, 23]. The aversive effect of dynorphin/KOR activation is decreased in individuals whose dynorphin/KOR system has been chronically stimulated, such as those with chronic pain or those with a long history of polysubstance abuse, which may reflect a reduced number or binding of KOR"s [24, 25]. In contrast to the above evidence, in alcohol dependent animals, KOR antagonists reduce alcohol drinking [26], suggesting that chronic alcohol drinking also alters the KOR system. The above evidence suggests that differences in the KOR system may mediate differential responsivity to NTX.

The proposed project would be the first to assess the potential contribution of the KOR system to individual differences in naltrexone-mediated changes in alcohol drinking using an established ADP in alcohol dependent, heavy drinkers (HD). Additionally, the current proposal will be the first to examine KOR levels and NTX responsivity in FHP and FHN heavy drinkers; this will help us understand if the differential responsivity to NTX observed in FHP and FHN drinkers is related to KOR occupancy by NTX.

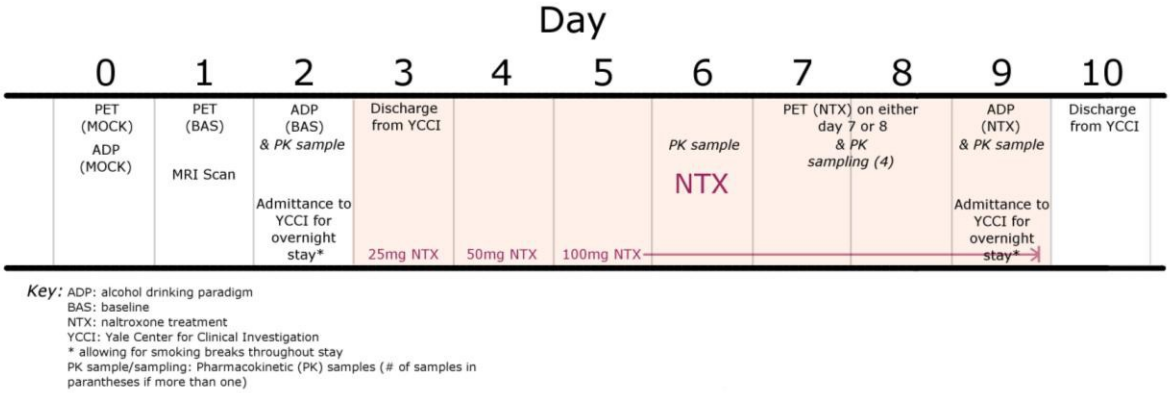
Our project will investigate a provocative finding in alcohol treatment research [3]. Namely, that FH of alcoholism may influence (or be a marker of an underlying trait that influences) the response of heavy drinkers (HD) to NTX. Simply put, not every HD responds favorably to NTX but lower levels of drinking can be achieved and risk of relapse is diminished in some. It is essential for clinical treatment and future drug development to understand how NTX works. Preclinical results have implicated kappa in *changes* in alcohol drinking, but the directionality of these changes are complex. We propose that the differential efficacy of naltrexone may be relate to differences in occupancy of kappa opioid receptors by naltrexone. This translational project will test this hypothesis. The results of this project could not only help maximize treatment effectiveness of naltrexone but also result in identification of new medication treatment targets for more optimal reduction of alcohol drinking.

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

A total of 56 HD subjects will be tested; equal numbers of FHP and FHN subjects will be recruited. Data from the first year's subjects ("initial cohort" perhaps specify how many will be used for this purpose) will be analyzed prior to scanning the remaining subjects in order to address aims S2 and S3 (identification and validation of a reference region; reduction in scan duration) which are addressed below in PET methods.

Study design: Because we are interested in investigating the implications of baseline KOR, as well as Occupancy of KOR by NTX, we must perform 2 PET scans on each subject; one at

baseline and one following 5-11 days of NTX pretreatment. Similarly, because we are interested in relating responsivity to occupancy of KOR by NTX we must make two assessments of drinking behavior using the ADP; one at baseline the day before or after the first PET scan and one following 5-11 days of treatment with NTX. The post PET scan and post ADP will ideally take place on Day 6 or Day 7 of medication, but we will allow up to 11 days of medication for scheduling issues and the possibility that the appointments will be within this time frame. All



In designing the study, we had to balance various competing interests and we provide justifications below:

a. Lack of Placebo and use of within subjects design: We considered including a placebo control. But then in order to control for "order" effects, we would have had to randomize placebo and NTX conditions AND allow a significant washout period between medication conditions, especially for those receiving NTX first. This would have led to problems with retention and increased the sample size. We also considered a between subjects design but this would have also increased sample size and costs. Therefore, in order to contain costs and increase feasibility, we decided to use a within subjects design examining a change from baseline, and configured the overall duration of participation to be as close to a single week as possible. While our proposed design could still have "order" effects we do not believe that it will influence our ability to detect an initial signal.

b. Use of Mock ADP and PET sessions: To minimize the possible stress and novelty associated with a first PET scan and the ADP sessions, as time and scheduling permits, we will conduct a mock PET scan and a walk-through of the ADP procedures prior to the baseline PET and ADP. During the mock PET session, the subject will lie in a replica of the PET scanner, be told when the scanner is being "turned on" and when blood samples are "being drawn". During the mock ADP session, participants will be exposed to the HRU rooms in YNHH where the ADP's are conducted and will be walked through the ADP procedures.

c. Timing of PET scan: The preliminary PET test/retest data suggest that BP_{ND} for our new KOR tracer may be higher in the afternoon (Figure 6). (Note: This *cannot be* explained by a carryover effect of a high-mass injection of tracer in the morning (test) scan, since that would cause BP_{ND} to be lower in the second scan.) We speculate that this effect could be due to diurnal fluctuations in dynorphin level. Regardless, we will control for it by scheduling each scan at approximately the same time of day (around mid-day).

d. Dose, Timing of NTX and PK samples: We will taper subjects up to a NTX maintenance dose of 100 mg/day. 100 mg/day is the dose of NTX that has shown efficacy in many recent clinical trials [9], and our own preliminary evidence suggests that this dose may produce the greatest *reduction* in drinking in FHP drinkers but *increased* drinking in FHN drinkers. We considered testing more doses of NTX but we felt that this would not have been justified and would have been too costly. NTX dose will be administered at approximately 10 am each

day. All participants will start with 25 mg/day and be tapered up to 100 mg by day 3 of medication. We will obtain 2 blood samples for pharmacokinetic (PK) analysis during the post PET scan to confirm that steady state levels of NTX were in effect during these procedures. These PK samples will be tested for NTX and its major active metabolite, 6(beta)-naltrexol.

e. Tobacco Use and Smoking breaks: In our experience, 50% of alcoholics who participate in our projects smoke cigarettes or use other tobacco products. In order to avoid nicotine withdrawal, on the ADP days, participants will be given smoke breaks until about an hour prior to the start of the ADP. We will not allow them to smoke during the ADP since this could not only alter their craving for alcohol but could also pose a potential risk (since YNHH is a nonsmoking facility and the participant would have to be walked out for a smoke break while intoxicated). On the PET days, we will allow regular smoke breaks up until about two hours prior to PET scans. It is very unlikely that the smoking on the morning of the scans would cause kappa receptors to upregulate. Since these individuals have been smoking and drinking regularly, we expect that any receptor regulation from smoking probably happened a long time ago. Further, if there is any acute dynorphin release associated with a smoking break, it will occur sufficiently early relative to the start of the PET scan to minimize any residual effect of transiently elevated dynorphin on our measurements of KOR level (BP_{ND}).

Recruitment:

All heavy drinker participants will meet DSM-IV criteria for alcohol abuse or dependence, will be drinking 25-70 drinks/week for men and 20-65 drinks per week for women and will report not being abstinent more than 3 days per week. All other criteria, designed to protect subjects from risks of participation are described in the human subjects section and are similar to those used by our group in earlier projects [2, 3, 28].

Participants will be recruited through advertisements in local newspapers, community TV channels and postings in community locations (bars, alcohol/coffee shops, grocery stores). We have also had success with recruiting participants from postings on Craigslist and social networking sites like Facebook as well as our study URL, www.paidalcoholstudy.com, which links subjects to confidential surveys that assess some preliminary eligibility criteria and provides preliminary information about the project. Each potential participant"s eligibility will be assessed over the phone by the research assistant who will then 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.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

We will recruit 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. We will also recruit healthy controls to match the demographics and background of the heavy drinkers, including family history of alcoholism; these controls will be light social drinkers consuming less than or equal to 20 drinks per week for women, and less than 25 drinks per week for men in the past 90 days and not meet past or current criteria DSM-IV abuse or dependence criteria for the past 5 years. The remainder of the exclusion criteria will be similar to the heavy drinkers.

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 [3] which indicates that the majority of the subjects who participated in our ongoing study were in this age range.

Justification for choice of naltrexone dose:

We will use naltrexone in a dose of 100 mg/day. The 100 mg/day dose has shown efficacy in many recent clinical trials [9], and our own preliminary evidence suggests that this dose may produce greater reduction in drinking in FHP drinkers than the 50 mg dose.

Procedures:

Recruitment, Baseline Assessments, Physical Exam: 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 at the Substance Abuse Center in the Connecticut Mental Health Center, New Haven, CT, where informed consent will be obtained and eligibility will be assessed. At this appointment, DSM-IV criteria (SCID, [41], Family history of alcoholism (FHAM; [42]) and drinking over the past 90 days (TLFB; [43, 44]) will be assessed. 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 session. The research assistant will schedule a physical exam (including EKG) and routine laboratory work, and hepatic, kidney, and thyroid function tests and pregnancy tests for all females. Subject characteristics and medical history will be reviewed by the PI's and the study physician Dr. Julia Shi, medical director of the Central Medical Unit) to ensure that the subject meets all the eligibility criteria. If eligible to participate and if they have also consented to participate in the genetic study they will have additional blood drawn at this time. We plan to study the genes that might be related to the medication's effects on drinking behavior. In addition, we may study other genes. Since all of the genes cannot be anticipated at this time, we will store the samples to study in the future. These samples will only be used for the following purposes: to learn about relationships between genes and characteristics of yours that relate to alcohol drinking and to learn about natural variation of genes between different groups of individuals. These samples will be de-identified and stored in a -70 freezer until they are transported to Dr. Joel Gelernter's lab. Eligible participants will be scheduled for the study procedures (as

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

described in figure 9) and will be reminded to not use any illicit drugs or other medications throughout the duration of the study.

Practice ADP session: All eligible participants will be scheduled to participate in a practice ADP. Before participation in the first ADP, participants will be shown the HRU room where the ADP"s will be conducted, walked through all the procedures of the ADP, and given information on what to bring and not to bring on the day of the ADP. Healthy controls will not complete the ADPs.

Mock PET scan: As time and scheduling permits, we will conduct a mock PET scan prior to the baseline PET and ADP. During the mock PET session, the subject will lie in a replica of the PET scanner, be told when the scanner is being "turned on" and when blood samples are "being drawn".

Medication Administration: Eligible participants will receive NTX for a five to eleven day period. The dose of NTX will be tapered up over the first four days as shown in figure 9 above. Participants will come into our clinic on a daily basis to take their medications and provide reports on drinking behavior and adverse events. In the event that a participant 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 or PET This arrangement will be determined by the investigator and her staff based on the participant's other obligations and compliance. Healthy controls will not take any medication.

Alcohol Drinking Paradigm: The procedures for all the alcohol-drinking sessions will be similar. Participants will be told not to consume alcohol starting at 5 pm the night before and arrive at the Hospital Research Unit (HRU) of Yale New Haven Hospital, New Haven, at 9:00 am. We will first assess breath alcohol levels and conduct urine drug tests. If the urine drug tests are positive and/or breath alcohol levels are 0.05 or greater, then the session will be rescheduled. Participants will be given the opportunity to taste and drink two chosen beverages (of which they will choose one for the 2 ADPs; see below). These procedures will make them more comfortable drinking in the HRU room and will also help reduce order effects in the current design.

The ADP will be conducted in a private room in the HRU. It 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.

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.

Priming dose (PD) period

The PD of alcohol will be provided at 3 pm to model a ''lapse'' situation and subjects will have 5 minutes to drink it. A 40 minute absorption period will follow during which the alcohol craving (AUQ; [45]), stimulation/sedation (BAES; [46]) and physiological effects (heart rate, blood pressure, breath alcohol levels) of this priming dose of alcohol will be monitored every 10 minutes.

Alcohol self-administration (SA) periods

Following the PD, participants will be exposed to three one-hour SA periods designed to model a

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

"relapse" situation. During each SA period they will be permitted to drink up to four alcoholic drinks designed to raise Blood Alcohol Level (BAL) 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. Number of drinks that each participant consumes during this period will serve as the primary outcome for this portion of the experiment.

- *Beverage content and mixers*

The YNHH Investigational Pharmacy will calculate the alcohol dose for each participant. The PD dose will be designed to raise blood alcohol levels to 0.03 mg% and will be based on the formula specified by Watson [47] which takes into account gender, weight, and age of the subject. The subsequent drinks provided in the SD blocks will 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, non-caffeinated, 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 favorite alcohol and mixer earlier that day. Specifically, they will be asked to choose two mixed drinks of their favorite alcohol and mixer to go with it from a list that will be provided to them at the intake appointment. The mixers on this list have been chosen to be equicaloric and to not contain any carbonation (which could alter absorption of alcohol). Subjects will be asked to taste both mixed drinks on the morning of the baseline ADP and pick the one that they would like to use for both ADP's.

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 get dinner and will stay in the hospital overnight and will be discharged between 6 and 7 am the next day.

Assessments during and after the three choice periods: During the second, third and fourth hour of the laboratory session alcohol craving (AUQ; [45]) and stimulation/sedation (BAES; [46]) and breath alcohol levels (assessed using Alco-Sensor 3; Intoximeter, St. Louis, MO following rinsing of mouth) will be assessed every thirty minutes. The range of assessments, however, is limited to avoid interfering with the evaluation of drinking behavior. Changes in craving and stimulation/sedation can then be correlated to KOR occupancy. Breath alcohol levels will be used to confirm that there are no differences in alcohol absorption between subjects.

Scheduling of the ADP and PET sessions requires a lot of coordination and requests are made in advance. Given the consequences that occur when a participant does not arrive for a scheduled appointment, we are asking a secondary participant who is scheduled for a later session to serve as a standby participant in case the primary scheduled participant has a conflict for the ADP and/or PET scan. If the scheduled participant arrives, we will pay the standby participant $50 for showing up and ask them to come back at a later date to complete their scheduled appointments. However, if the scheduled participant does not arrive, the standby participant will still be paid $50 and will be invited to complete the appointments scheduled for the original participant.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

In most instances, participants will be scheduled the PET scan the morning after the ADP session; however, due to issues that could occur with the PET scanner and chemistry on the morning of the scan, participants will need to be rescheduled. In the event that a participant, who is a heavy drinker, needs to be rescheduled, they will be asked if they are willing to spend the evening before the rescheduled PET scan at the HRU to ensure that they meet the BAC requirements the next morning and can complete the PET scan. They will be admitted to the HRU between 3:00 and 4:00 pm and be discharged early the next morning to be escorted to the PET Center. The healthy controls will not be invited to spend the night at the HRU since they do not meet criteria for heavy drinking.

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 [48]. As previously done, this intervention will be based on the principles of Miller's Motivational Enhancement Therapy (MET) [49]. We will also provide them with the NIAAA brochure, "Rethinking Drinking."

PET Methods:

Data Acquisition: PET scans will be conducted on the Siemens High Resolution Research Tomograph (HRRT), the world"s highest sensitivity and resolution human brain PET scanner, with a practical resolution of 2.5-3 mm [50]. The Yale University PET center has conducted more than 400 human scans on the HRRT with 20 different tracers. The excellent spatial resolution of the HRRT will be critical in discerning small structures [51]. The HRRT is a list- mode machine so we can bin up the dynamic data anyway we choose. We will create a graduated series of time-frames stating with 30 second frames and lengthening to 5 minutes.

Hardware motion correction using the Vicra system (NDI Systems, Waterloo, Ontario) will be performed on an event-by-event basis. Arterial blood samples will be acquired via a continuous withdrawal device (PBS-101, Veenstra Instruments, Joure, The Netherlands) for the first 5-10 minutes of the scan and manually thereafter, and radioactive metabolites identified by HPLC. Plasma concentration of free parent tracer over time will be used for subsequent kinetic modeling analysis. Emission images will be reconstructed iteratively with built-in corrections for attenuation, normalization, scatter, randoms, deadtime and subject motion [27].

PK samples: Two PK samples will be collected during the NTX PET session. Days 5-11 were chosen for the NTX PET scan to build some flexibility into scheduling but maximize the likelihood that NTX levels would be at equilibrium, which will be confirmed by PK analyses. We will also look (on a population basis) for a relationship between occupancy of KOR and NTX (and 6(beta)-naltrexol) levels and interaction with FH of alcoholism.

ROI analysis and parametric image generation: After data reconstruction with all corrections, a new summed image (0-10 min) will be registered to the subject's T1-weighted MR image, which will be registered to an MR template so that PET and MR images are in the same MNI space. ROI"s, including the 13 examined in the Preliminary data section in Figures 4 and 6 will be taken from a template (Anatomical Automatic Labeling (AAL) for SPM2).

Possible to dispense with arterial sampling, 2T model fitting?: Preliminary results suggest that the

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

cerebellum may be an appropriate reference region. That is, it may be devoid of KOR. It certainly has low specific binding compared to the other regions assayed. The excellent agreement between BP_{ND} estimated by the 2T model and by SRTM certainly suggests that in healthy controls, we can assess BP_{ND} at baseline with a reference region model using the cerebellum as the reference region. If this turns out to be generally true (e.g., in larger samples, in heavy drinkers), then we can justifiably dispense with arterial sampling and analyze all data via reference tissue models (such as SRTM and SRTM2). As mentioned in Preliminary PET studies, to validate the absence of KOR in the cerebellum, under multiple scan conditions (baseline and NTX) in clinical populations, we will have to show that V_T of cerebellum does not change between baseline and NTX (i.e., there is no specific binding to block).

Possible to shorten scan duration?:
Preliminary results also indicate that we could shorten the scan duration from 2 hrs (used in our preliminary scans) to 1 ½ hrs without any loss of accuracy or precision.

Analysis of Initial Cohort: Data from the first 6 FHP and 6 FHN subjects scanned in year one of the project (24 scans total) will be analyzed to determine whether or not a reference region exists for [11C]LY2795050 ([11C]-PKAB) and whether or not the scan duration can be shortened without loss of precision of the data. To be sure that a reference tissue model is appropriate in our study, two findings are required. The V_T of the putative reference region must be unchanged by blocking and the endpoint, BP_{ND}, estimated by the reference region method must agree with the BP_{ND} calculated using an AIF. If the BP_{ND} values from 2T and SRTM continue to show excellent agreement, as applied to *both* baseline and NTX scans, then we may choose to discontinue arterial sampling thereafter and rely solely on a reference region model for analysis. After a preliminary analysis of the initial cohort, we will determine the acquisition protocol for the remaining 24 subjects.

Modeling of PET data to yield BP in regions of interest and parametric images
1. Standard kinetic modeling approaches: With plasma input function measurements in and, we can compare multiple standard methods of estimating our desired endpoints (BP_{ND} at baseline and difference in BP_{ND} from baseline to NTX) to determine the best model or technique [52] that yields the lowest variance in the estimates. Tentatively based on preliminary data, we expect to use the cerebellum as the reference region for reference region methods but we will continue to investigate the possibility of another (better) reference region.
2. Measuring occupancy does not depend on displacement, time of day:
The two primary imaging endpoints (B_{avail} and $Occup^{Kappa}$) depend on our ability to measureBP_{ND}. It is important to note that the half-life of NTX is very long (10.5 hrs in the blood at 100 mg oral dose) and subjects would have been on 100 mg for at least 2 days before the NTX scan. Therefore, we can expect that NTX in the brain will be at a nearly constant level prior to and during the NTX-scan. Thus, we are not measuring displacement of [11C]LY2795050 ([11C]-PKAB) but rather, the effect of a pre-blocking (i.e., a measurement at a second steady state). This is an important distinction. The latter does not depend on the displaceability of the tracer (a kinetic issue), and thus, we have not addressed it in our preliminary data. To be safe, however, we will scan each subject near mid-day for both baseline and NTX scans. Given that the half-life of NTX is 10.5 hours and that it drops ~5% per hour, we will maintain the delay from morning oral dose of NTX to time of scan for each subject at 2 hours which should coincide with the time of peak NTX concentration in the blood.

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

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

this center.

b)SCID: The Structured Clinical Interview for DSM-IV (SCID) [41] 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 [42]. 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 [38]. 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) [45]*:* 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 psycho physiological scaling methods. In her work on individual differences in the ability to taste bitterness, Dr. Bartoshuk and colleagues [35] used magnitude- matching procedures [35] 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) [36], 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 [39]. 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.*

e)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 [30] withdrawal [29]

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

and is used extensively in research on alcohol withdrawal [31, 32].

f) 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 [56]. This combination correlates at .87 with the Full Scale IQ.

g) 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.

Self-Reports:

a) The Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) [57, 58] 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 (version 11) [59]: This 30 item self-report instrument provides a trait measure of impulsiveness and yields four scores: a total score, non-planning activity, cognitive impulsivity, and motor impulsivity. Cronbach alpha coefficients range from .79-.83.

Laboratory Measures:

a) 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).

b) *Cued Go-No Go task (Abroms et al., 2006)* This task will be used to examine the ability to Inhibit prepotent responses. Subjects are presented with 250 trials over 20 minutes in which a go or a no-go target is preceded by a cue; the orientation of which cues the probability that a go or no-go target will be displayed. Dependent measures include: number of failures to inhibit responding and speed of responding. Alcohol has been shown to impair the ability to inhibit responses This computerized task assesses a component of impulsivity related to rapid, unplanned reactions to stimuli before completing information processing.

h) Biphasic Alcohol Effects Scale [46]: This 14 item self-report, adjective rating scale will be used to Measure the stimulant and sedative effects of alcohol during the priming dose during ADP 2. This instrument has been found to be sensitive to memantine and naltrexone's effects on alcohol intoxication [63, 61, 64].

j) Ratings of Drinking Behavior During the Alcohol Self-Administration Period: Subjects will be videotaped during the alcohol self-administration portion. 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.

k) 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. Blood samples will be stored at -4°C and will be analyzed using gas chromatographic techniques at the HRU Laboratory.

l) Psychophysiological Measures: These will include heart rate and blood pressure monitored

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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.

l) Cold Pressor Task (Edens & Gil, 1995): To evaluate the ability of participants to deal with a physiological stressor and to examine pain thresholds in relation to medication effects and drinking behavior. Subjects will be informed that the experimenters are investigating the body's physiological response to cold water during nicotine withdrawal. Subjects will be asked to immerse their right arms into ice water maintained at 3-4^{o}C. Subjects are asked to raise their left hand when they begin to feel pain (pain threshold) and to remove their hand from the water when they can no longer tolerate the pain (pain tolerance). While their hand is immersed in water subjects are also asked to rate their pain on an analog scale of 0-100.

3. **Statistical Considerations:** Describe the statistical analyses that support the study design. Outcomes for this study will be collected using scannable forms (Teleforms). The scanned forms will be processed through the Teleforms software and exported to a database on a secure computer. Error checking and data validation will occur weekly and any problems will be queried and resolved immediately. Drs. Krishnan-Sarin will receive monthly data quality reports to check for completeness and accuracy of key demographic and prognostic variables, as well as rates of recruitment, retention, and follow-up. Statistical tests will be conducted using SAS v9.1 and SPSS v16 (or later) and will be performed at 2-tailed alpha level of 0.05

Primary Aim 1. Occupancy of KOR by NTX and drinking. This aim seeks to determine the degree to which occupancy of kappa by NTX mediates responsivity to NTX in heavy drinkers. Responsivity to NTX is defined as the change in the total number of drinks consumed in the NTX ADP versus the baseline ADP. Tests for mediation will be conducted using the regression strategies described initially Baron and Kenny [53], and the more recent elaboration by Kraemer et al. [54]. The proposed mediator (i.e., the potential mechanism of change), occupancy of KORs, is measured as the fractional change in BP_{ND} before and after treatment with NTX. The analyses will determine whether occupancy is associated with a) NTX treatment, and b) NTX responsivity.

Primary Aim 2. Family History of Alcoholism as a moderator: This aim seeks *t*o determine if the relationship between NTX responsivity and occupancy of KOR is altered by FH status. FHP and FHN will be compared on NTX responsivity and occupancy of KOR. Tests for moderation will be conducted using the strategies described by Baron and Kenny [53], and the elaborated by Kraemer et al. [54]. The primary tests will be whether there is a significant FH by dose interaction on NTX response and occupancy of KOR.

Secondary Aim 1. Baseline KOR differences. This aim seeks to determine if baseline levels of KOR differ between FHP and FHN heavy drinkers, and are related to either drinking in baseline or NTX ADP. FHP and FHN groups will be compared using chi-square tests for categorical variables, and using t-tests or Mann-Whitney tests for continuous variables. All continuous variables will be examined for adherence to the normal distribution using normal probability plots and Kolmogorov-Smirnov tests. If normality is not satisfied and transformations do not help with achieving normality, alternative analytic strategies will be considered such as generalized estimating equations or nonparametric methods. The relationship between baseline KOR and drinking will be examined via Pearson product-moment correlations.

Secondary Aims 2 and 3. Scan duration and reference region. We will look for correlations between BP_{ND} estimates based on 2T model with full AIF and all data vs alternative models and data ranges. Group variances in baseline KOR at or lower than 2T estimates will permit adoption of simpler models.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

Auxiliary Aim 1. Kappa and behavioral indicators relevant for alcoholism. This aim will examine the correlations between occupancy of KOR and alcohol craving, stimulation and sedation. A series of Spearman rank-order and Pearson correlation analyses will be conducted for this aim.

Power analysis: The sample size was based on the power calculations for the hypotheses of (a) reduced drinking in the NTX 100 condition, and (b) power to detect an interaction by FH. Power calculations were generated using G*Power software Version 3.0 [55]. Effect sizes were estimated based on the preliminary FH and NTX effects on drinking behavior in the ADP described in [6] and in our preliminary studies. The effect size for the effect of FH is .91. To detect this effect size at alpha=.05 with 80% power, a total of 16 participants would be required for each arm. Therefore the proposed sample size of n=18 per arm would be sufficient to detect differences in drinking between FHP and FHN at the 100 mg dose of naltrexone.

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

Are there any investigational devices used or investigational procedures performed in a YNHH Operating Room? ☐ Yes ☒ No *If Yes, please be aware of the following requirements*:

a. A YNHH OR New Product/Trial Request Form must be completed. Please contact the OR Materials Manager, Chris Baillargeon, at 203-688-8912 for more information on this requirement;

b. Your request must be reviewed and approved by the Operating Room New Technology Committee before patients may be scheduled; and

c. The notice of approval from the OR New Technology Committee must be submitted to the HIC for the protocol file.

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).
   Naltrexone is an FDA approved drug that is used in the treatment of alcoholism and opioid addiction.
   [C-11]LY2795050 ([C-11]PKAB) PET radiotracer to image kappa opioid receptor, Production of [C-11]LY2795050 ([C-11]PKAB) will be performed with the permission of Yale-New Haven Hospital RDRC

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**

i. What is the Investigational New Drug (IND) or Investigational Device Exemption (IDE) **number** assigned by the FDA? Production of [C-11]LY2795050 ([C-11]PKAB) will be performed with the permission of Yale-New Haven Hospital RDRC

ii. For IDE"s: Did the FDA approve this IDE as a Category A (experimental/investigational) or as a Category B (non-experimental/investigational)? Not applicable.

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: N/A

i. Is the intention of the investigation to report to the FDA as a well controlled study in support

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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) eview 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.

Naltrexone:

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.

[C-11]LY2795050 ([C-11]PKAB)

Dosimetry:

A total of 4 human subjects (2 M, 2 F, 73 ± 16 kg) underwent whole body dynamic PET scanning on the Siemens ECAT HR+ scanner following iv bolus injection of [^{11}C]PKAB

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

(injected activity = 282 ± 68 MBq). Subjects were scanned for 150 to 170 min in a sequence of 12 or 14 passes with 8, 9 or 11 bed positions according to their sizes, covering each subject from top of the head to mid-thigh. Scans were reconstructed and visually inspected for organ activity concentrations exceeding background level. Included organs were liver, kidneys, urinary bladder contents, heart, gallbladder, brain, spleen, thyroids, gonads and red marrow (femur). Regions of interest were drawn on these organs and mean activity values computed in order to form time activity curves of activity concentration (kBq/cm^3).

Within-pass decay correction was removed to reflect actual activity in each organ, and cumulated activity (Bq*hr/cm^3) computed as trapezoid sums for data from the scan plus physical decay for the tail portions after the scan period. These were multiplied by the organ volumes of a standard 70 kg reference mathematical phantom, normalized by subject mass / 70 kg and then normalized to injected activity to obtain organ residence times (also called N), (hr). These were entered into Olinda software to obtain absorbed doses in all organs. Electron doses to the stomach were adjusted to reflect the fact that activity was observed in stomach wall rather than contents.

Absorbed doses derived from the 70 kg reference man phantom were expressed as mean ± standard deviation across the 4 subjects. Under RDRC exposure limits as specified in CFR 361.1, (For single study, 5 rem per organ or 3 rem to selected organs undergoing rapid cell division, whichever is less) gallbladder is the dose-limiting organ with single study dose of 26 mCi. Applied to the 55 kg adult female phantom (Table 2), gallbladder is still the critical organ, with single study dose limit of 23 mCi.

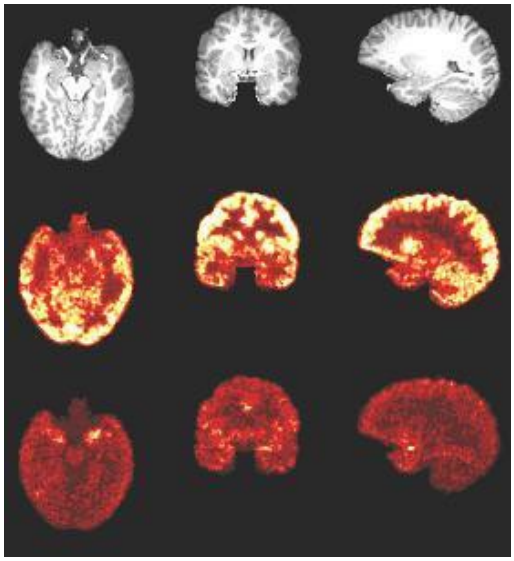


Figure 2. Top row: MRI images of an individual subject. Middle row:0-10 min summed PET image. Bottom: 60-120 min summed PET **image**. Left column: axial slice; middle column: coronal slice; right column: sagittal slice. The amygdala is bright on all three views at late time. The cingulate cortex is apparent on the coronal image at late time.

PET Imaging Results –Imaging of KOR Sites

Introduction

The preliminary data in this section represent our initial experience with the new PET tracer [^{11}C]LY2795050 ([C-11]-PKAB) in humans and monkeys. A number of opioid receptor radiotracers are currently available for PET imaging in humans: [^{11}C]carfentanil(mu-specific), [^{11}C]/[^{18}F]diprenorphine (nonspecific), [^{11}C]buprenorphine (nonspecific), [^{18}F]cyclofoxy (mu, kappa), and [^{11}C]methylnaltrindole (delta) [33]. None of these existing tracers is selective for KOR. So far, the results with [^{11}C]LY2795050 ([C-11]-PKAB) suggest that it is taken up consistent with the known distribution of KOR [34-36]; It also appears to have good contrast to background properties, good test/retest reproducibility, and there may be a reference region with little to no KOR.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

Occupancy of KOR by NTX – or simply "occupancy" - is measured as a percentage change in $\mathbf{BP_{ND}}$ from the baseline condition to the drug condition (in our case, a week of NTX treatment). If we assume that neither the $\mathbf{K_D}$ nor the $\mathbf{f_{ND}}$ of the tracer change in an individual between scans, then occupancy can be seen to be the fractional change in available receptors due to the drug.

Total Volume of Distribution, V_T, is a related concept to BP. V_T is the equilibrium ratio of the concentration of parent tracer in tissue to parent tracer in plasma.

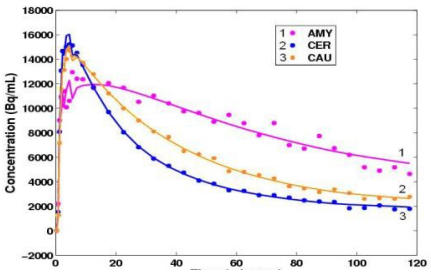


Figure 3. Selected regional time activity curves for a single subject; amygdala (AMY, pink, „1"), cerebellum (CER, blue, „2"), and caudate (CAU, orange, „3"). Smooth curves through data represent fits of 2T model. Metabolite-corrected arterial plasma samples were used for input function.

b. In vitro results

Radioligand competition assays were conducted to assess the *in vitro* binding affinities of LY2795050 to opioid receptors. LY2795050 is a KOR antagonist and displays high binding affinity and great selectivity to KOR *in vitro* (K_i = 1.37, 87. 3, and 475 nM, respectively, for kappa, mu, and delta receptors) [38].

c. Test/retest imaging in humans

Seven healthy volunteers, age = 26 ± 6 (5 males and 2 females), participated in a test-retest study of [^{11}C]LY2795050 ([C-11]-PKAB), an antagonist PET radiotracer for the KOR. Each subject received two bolus injections of up to 20 mCi of radioactivity and mass dose of ≤ 10 µg for each injection. The injected activity dose was 13.0 ± 4.6 mCi, specific activity was 0.59 ± 0.22 mCi/nmol at time of injection, and injection mass was 8.8 ± 1.2 µg across all subjects. Arterial (plasma) input function (AIF) curves were prepared from arterial samples taken during the study. Metabolite fraction was applied to yield an input curve for the native tracer only. BP_{ND} throughout the brain (calculated two different ways) was used as the study endpoint. Further details of our standard image processing methods are given below under „PET methods".

d. Distribution of [^{11}C]LY2795050 ([C-11]-PKAB) in the brain

Figure 2 shows the early- and late-time distributions of [^{11}C]LY2795050 ([C-11]-PKAB) in the brain. The early image is an average of the 0-10 min data (middle row); it reflects initial entry into the brain via blood flow. The late image is an average of the 60-120 min data (bottom row) and reflects preferential retention of the tracer by brain regions containing specific binding sites for the tracer. Rank order of activity uptake was as follows: amygdala (AMY) > cingulate cortex (CIN) > globus pallidus (PAL) ~ putamen (PUT) > temporal cortex (TEM) ~ frontal cortex (FRO) > hippocampus (HYP) ~ caudate (CAU) > cerebellum (CER). The distribution of tracer at late time agrees with the known distribution of kappa receptors in the brain [34-36].

e. Time-activity curves: for use with kinetic models

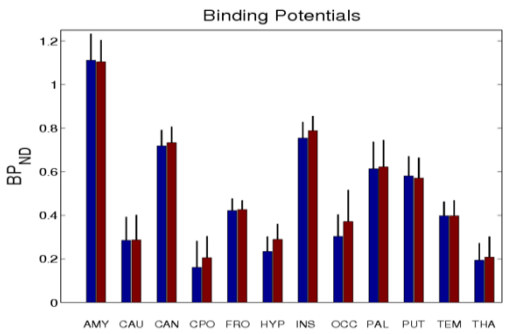


Figure 4. Regional estimates of BP_{ND} by 2T model (blue bars) and by SRTM (red bars). Agreement between models is high and variability across subjects (error bars are SD = 7 - 8%) is quite good for neuroreceptor tracers.

Estimates of kinetic parameters require regional tracer concentration values over time. Automatic regions-of-interest (ROI) were applied (see PET methods) to the dynamic emission images to generate time-activity curves (TACs) in 13 ROI"s. The time-activity curves (TAC) were fitted to the 4-parameter, 2-tissue compartment model (2T) using the metabolite-corrected input function. Examples of fitted TACs are shown in Figure 3. All of the regions were fit well by the 2T model. The rapid efflux from the cerebellum compared to the other regions suggests that the cerebellum has little to no specific binding and is therefore a possible reference

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

region. However, the definitive identification of a reference region must be based on displacement experiments.

f. Regional distribution of desired endpoint: KOR at baseline (BP_{ND})

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

We computed BP_{ND} in each ROI two ways. One method requires the use of AIF and the other does not. From the fits of the TAC data via 2T model with AIF, we calculated BP_{ND} as the relative difference in volumes of distribution between the target ROI and the putative reference region, in this case, cerebellum: (V_T(ROI) – V_T (CER)) / V_T(CER). BP_{ND} was also computed using the Simplified Reference Tissue Model (SRTM) using the cerebellum as the reference region. The regional distribution of BP_{ND} based on 2T and SRTM is shown in Figure 4. Figure 4 shows that the methods are in close agreement and the variability, by region, across the cohort is small.

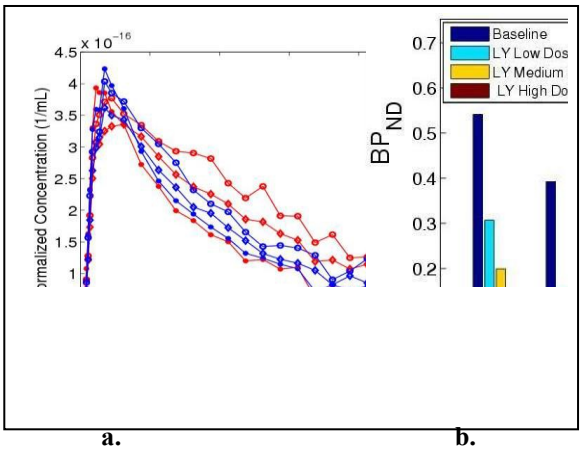


Figure 5 **a.** Effect of pre-block with unlabeled KOR antagonist drug. Baseline TACs for cerebellum (CER), caudate (CAU), and frontal cortex (FRO) (solid circles, open circles, diamond, all in red). Curves after pre-block (blue) all show reduced specific binding and are nearly indistinguishable from CER at baseline. Curves are from one animal and are normalized to injected activity dose. **b**. BP_{ND} is reduced dose-dependently according to dose of specific KOR antagonist in both CAU and FRO region.

g. Pre-blocking of [11C]LY2795050 ([C-11]-PKAB): can we detect drug occupancy at kappa sites?

We can verify that uptake and retention of [11C]LY2795050 ([C-11]-PKAB) is due to specific binding to KOR by looking at pre- blocking studies with well-characterized (unlabeled) drugs. Pre- blocking studies were performed in 3 rhesus monkeys. The experiments were similar to what is being proposed herein to measure occupancy of KOR by NTX. In the preliminary studies, rhesus monkeys were given iv, 1mg/kg naloxone, a non-selective KOR antagonist, or varying doses of LY2456302, a selective kappa antagonist developed by an industry partner. Example results for the blocking studies are shown in two parts of Figure 5. Figure 5a: At baseline, regions such as CAU and FRO appear to have more uptake than CER. Under pre-block with the specific KOR antagonist, TACs for two target regions (CAU and FRO) are nearly identical to the putative reference region (CER) in the unblocked case because they reflect only (the same) non- displaceable binding. The data from these two experiments on the same animal have been normalized by injected activity. The behavior of the regions shown is typical. These data are suggestive, but not definitive proof that CER is a valid reference region (See "SRTM vs. 2T", immediately below for criteria). Further evidence of specific binding in CAU and FRO is shown in 5b. BP_{ND} declines in a dose-dependent manner as the specific KOR antagonist dose is increased from low to high.

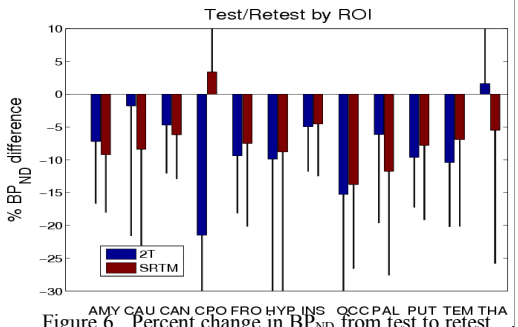


Figure 6. Percent change in BP_{ND} from test to retest scans for both 2T (blue bars) and SRTM (red bars) models. Each region is based on the average of 7 subjects. Test/retest variability is actually slightly better for SRTM estimates than for 2T.

h. SRTM vs 2T: Can we use a reference region model?

A reference region model such as SRTM has a number of practical advantages, if its use is appropriate. Its use obviates the need for arterial sampling, thus decreasing the risk to the patient and simplifying the PET experiment. Figure 4 shows that estimation of BP_{ND} via SRTM and 2T is in good agreement and that use of the reference region method would be justified. In this case, CER was used as the reference region. The correlation based on all regionsover all subjects was BP_{ND} (SRTM) = 1.01*BP_{ND}(2T), R^2 = 0.98,

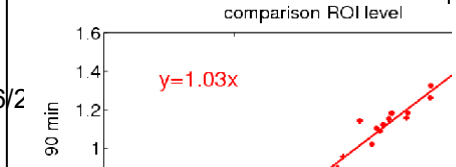


Figure 7. Correlation between estimates of BP_{ND} using either a full 120 min dataset (X-axis) or only 90 minutes (Y-axis) of TAC

which confirms near-perfect agreement. Note: this agreement between the 2T and SRTM does not *validate* the CER as a true reference region. It indicates a valuable fact: the two estimates of BP_{ND} are consistent (they might both be biased for different reasons). For many purposes, consistency may be sufficient to argue for adoption of the SRTM or other reference method. For the reference region to be validated (i.e., shown to be devoid of specific binding), we must also show that V_T of the region does not change in the presence of a pre-blocking drug such as NTX.

i. Percent change between „test" and „retest" scans: How small an effect can we detect?

Small test/retest variability is essential for us to be able to detect small diferences between conditions. For each subject, the test-retest variability was measured by computing the ratio of the difference between binding potentials to their means:

$$\frac{100*(BP_{ND}(TEST) - BP_{ND}(RETEST))}{[\ ½\ (\ BP_{ND}(TEST) + BP_{ND}(RETEST)\ )\ ].}$$

The mean test-retest variability indices were 8.2% (2T) and 7.2% (SRTM) indicating that BP_{ND} values increased during the retest scan (conducted in the afternoon of the same day) compared to the test scan, across all regions. Figure 6 shows the test/retest comparison as an average value over subjects for all regions.

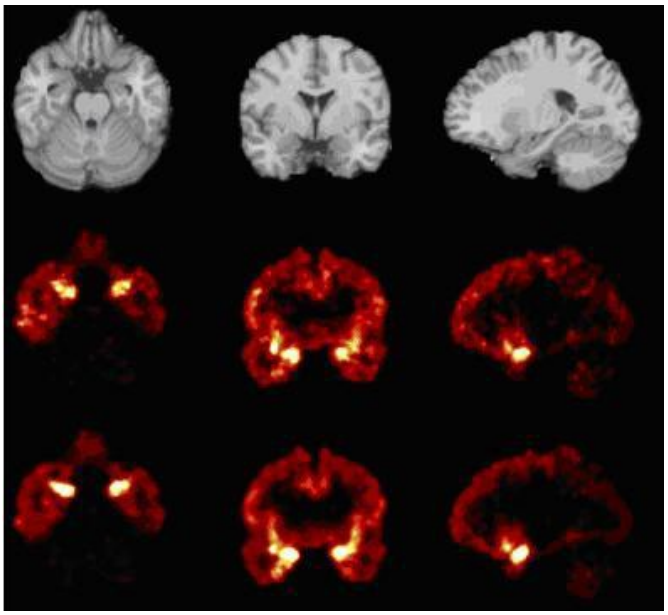


Figure 8. Top row: MRI images (axial, coronal, sagittal) of individual subject. Middle row: Corresponding BP_{ND} images estimated using SRTM Bottom: Corresponding BP_{ND} images estimated using SRTM2. They are slightly less noisy than SRTM, as would be expected.

j. 90 min vs 120 min results: Can we shorten the scan?

A 1 ½ hr PET scan is slightly less burdensome for the subject than a 2 hr scan and given the fairly rapid kinetics of the tracer, it seems worth considering the shorter scan. Therefore, we compared results of BP_{ND} estimates from the SRTM model using the full 120 min worth of scan data and using only 90 minutes of data. The answers were nearly identical. The correlation plot between the two families of estimates is shown in Figure 7. We conclude from this exercise that we need only to scan for 90 minutes in each scan session. A shorter scan time could improve subject compliance.

k. BP_{ND} images

One advantage of having a kinetically well-behaved tracer, such as [^{11}C]-LY2795050 ([C-11]-PKAB), with sufficient signal to noise in the TACs for each pixel, is that we can produce pixel-by-pixel parametric images of BP_{ND}. Figure 8 shows BP_{ND} (created with two variants of the SRTM model) for one of the subjects in our test/retest cohort. The middle row in the figure presents three canonical views of the BP_{ND} estimated by SRTM; the bottom row repeats the same views of BP_{ND} images estimated via SRTM2 which imposes a constraint that the apparent efflux rate parameter, k2" be the same at each voxel. The constraint has the effect of reducing the variance in the image which may further aid our ability to detect slight differences between patient populations or conditions. The images show that there is discernible specific binding of tracer in cortical areas and maximal specific binding in the amygdala and cingulate cortex.

Summary: As we have shown above, we have developed and validated a specific KOR ligand that has high specificity and sensitivity and that can be combined with our established ADP to evaluate the hypotheses that are central to the current proposal.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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

Production of [C-11]LY2795050 ([C-11]PKAB) will be performed with the permission of Yale-New Haven Hospital RDRC

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.

   The starting material for the production of [C-11]PKAB / [C-11]LY2795050 will be provided by Eli Lilly and company. Manufacturing of [C-11]LY2795050 ([C-11]PKAB) will be carried out at the Yale PET Center.

   [^{11}C]Carbon dioxide is produced by bombardment of a target containing a mixture of nitrogen and 0.5 – 1% oxygen with accelerated protons. [^{11}C]CO_2 is then converted to [^{11}C]HCN using an automated chemistry device from GE Medical Systems. Reaction of [^{11}C]HCN with the iodo precursor LY2806578 under Pd(0)-catalyzed conditions provides the radiolabeled benzonitrile intermediate [C-11]LY2893110, which is hydrolyzed under basic conditions and in the presence of hydrogen peroxide to produce the radioactive drug product [C-11]LY2795050 ([C-11]PKAB). The product is purified by semi-preparative HPLC and formulated with a mixture of ethanol and saline (final ethanol concentration less than 10%). The final product solution is filtered through a 0.22 um membrane filter for terminal sterilization before its release for dispensing and IV administration.

   The carbon-11 labeled drug product [C-11]LY2795050 ([C-11]PKAB) has a radioactive half-life of 20 min and has been demonstrated to be stable for at least 65 min after preparation and formulation. An expiration time of 60 min post-formulation will be assigned to the drug product.

   For each batch of the radioactive drug product [C-11]LY2795050 ([C-11]PKAB) that will be used for human study, pyrogen and sterility tests will be performed. Pyrogen testing will be conducted in house at the Yale PET Center using FDA-approved method. Sterility test will be performed by the Yale Microbiology Laboratory.

   The radioactive drug product [C-11]LY2795050 ([C-11]PKAB) will be produced according to the local Drug Master File and to local quality control procedures in effect at the Yale PET Center. Production of [C-11]LY2795050 ([C-11]PKAB) will be performed with the permission of Yale-New Haven Hospital RDRC

5. **Use of Placebo:** ☒ **Not applicable to this research project**
   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.
      No placebo
   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.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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:** Describe how potential subjects will be identified, contacted and recruited.

56 heavy drinkers, drinking on at least 4 days per week (20-45 weekly standard drinks for women and 25-70 for men), an equal number of family history positive and family history negative (as specified by the FHAM) will be recruited for the study. All subjects will be 21-65 years of age, physically healthy non-treatment-seeking heavy drinkers. Volunteers will be recruited through advertisements in local newspapers, postings in community locations (bars, coffee shops, grocery stores) and advertisements on free community TV channels. Additionally, 25 healthy controls who report drinking socially will be recruited to match the demographics and background of the heavy drinkers, including family history of alcoholism; these controls will be light social drinkers consuming less than or equal to 20 drinks per week for women, and less than 25 drinks per week for men in the past 90 days and not meet past or current criteria DSM-IV abuse or dependence criteria, for the past 5 years. The remainder of the exclusion criteria will be similar to the heavy drinkers.

1. Indicate recruitment methods below. 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):	☒ Clinicaltrials.gov Registry (do not send materials to HIC)		

2. **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, some of the subjects If yes, describe the nature of this relationship.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

No

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

3. **Subject Population** Provide a detailed description of the targeted involvement of human subjects for this research project.
   56 healthy heavy drinkers who are not currently seeking treatment for their drinking behavior, between 21-65 years of age, family history positive or family history negative. Also, 10 healthy controls between 21-65 years of age matched on demographics including family history of alcoholism.

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

   Inclusion criteria:

   Ages 21-65

   Able to read English at 6th grade level or higher and to complete study evaluations

   Meet DSM-IV criteria for alcohol abuse or dependence (assessed using the SCID)

   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

   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:

   Individuals who are seeking alcohol treatment or have been in alcohol treatment within the past 6 months

   DSM-IV dependence criteria for other substances, other than nicotine in the past year

   Positive for opiates, cocaine, benzodiazepines, or barbiturates at more than one appointment

   Quantitative marijuana levels going up from PE to MR appointment

   Regular use of psychoactive drugs including anxiolytics and antidepressants

   Psychotic or otherwise severely psychiatrically disabled

   Medical conditions that would contraindicate the consumption of alcohol

   Medical conditions that would contraindicate the use of naltrexone such as hepatic dysfunction

   Any history of neurological trauma or disease, seizures, head injury, brain tumor, delirium, or hallucinations, or hepatic, cardiovascular, metabolic, endocrine, or gastrointestinal disease

   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.

   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

   Subjects who report disliking spirits will be excluded because hard liquor will be provided during the alcohol administration components of the study.

   Subjects who have taken any investigational drug within 3 weeks immediately preceding

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

admission to the treatment period
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 cause excessive sedation.
Subjects who have donated blood within the past six weeks
Subjects with a history of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).

Additionally, subjects who meet the following imaging exclusion criteria will not be included in this study:
Subjects who suffer from claustrophobia.
Subjects with MRI-incompatible implants and other contraindications for MRI, such as pace-maker, artificial joints, non-removable body piercings, tattoos larger than 1 cm in diameter, claustrophobia, etc.
Subjects who have received a diagnostic or therapeutic radiopharmaceutical within 7 days prior to participation in this study.
Participation in other research studies involving ionizing radiation within one year of the PET scans that would causes the subject to exceed the yearly dose limits for normal volunteers.
Subjects with history of IV drug use which would prevent venous access for PET tracer injection.
Severe motor problems that prevent the subject from lying still for PET and MR imaging.
Subjects who complain of chronic pain (e.g., as the result of rheumatoid arthritis)

4.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

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

5. **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

5.a. 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 the names of all members of the research team who will be obtaining consent/assent. Nicholas Franco, Dana Cavallo, Tricia Dahl, Thomas Liss, Suchitra Krishnan-Sarin, Evan Morris, Alissa Goldberg.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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 enroll only 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

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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____
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 survey monkey so it wouldn"t be practical to obtain authorization to use this data.

i. 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 naltrexone, administration of alcohol, the blood draw during the physical exam and alcohol drinking period, and the PET and MRI scans.

1. MRI:

MR carries a risk for subjects who are claustrophobia or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR. Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

2. Risks Associated with Radiation:

The Yale-New Haven Hospital Radiation Safety Committee (RSC) will review the use of radiation in this research study, and no subjects will be enrolled until RSC approval is obtained. This research study involves exposure to radiation from [C-11]PKAB PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only. The total amount of radiation an individual subject will receive in this study is from **2** injections with a total of ≤ 40 mCi from **[C-11]PKAB** plus transmission scans of the brain.

Although each organ will receive a different dose, the amount of radiation exposure subjects will receive from this study is equal to an effective dose of 1.44 rem for a total of ≤ 40 mCi of [C-11]PKAB in 2 injections (approx. 20 mCi each). That is, a total of 1.44 rem for 2 PET scans is the amount of radiation exposure that a subject will receive from the study. This calculated value is used to relate the dose received by each organ to a single value.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

The amount of radiation subjects will receive in this study is below the dose guidelines established by the FDA and monitored by the Yale-New Haven Hospital Radiation Safety Committee for research subjects. This guideline sets an effective dose limit of 5 rem per year.

3. 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.

4. 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

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

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.

5. 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.

6. Risks Associated with Blood Drawing and IV line Insertion:
Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during this study, include screening laboratories, will be approximately 19 tablespoons. This is not expected to have any serious negative effects on a study participant.

Subjects will have approximately 30 cc of blood drawn at the intake appointment to determine liver and kidney functioning and for genetics testing and a total of 60 cc of blood during the 2 ADPs. 180 cc will be taken at the PET scans and an additional 15cc in PK samples will be drawn during the NTX PET scan. Therefore, the total amount of blood drawn during the study (30+60+180+15= 285cc) is 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.

7. Risks Associated with Use of an Arterial Catheter:
On the PET scan day, a radial arterial catheter will be inserted. Arterial sampling may be associated with mild-to-moderate pain or bruising at the puncture site. In rare instances blocking of the artery, poor healing, hematoma, inflammation, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during arterial catheter placement. If for some reason, an arterial catheter cannot be inserted, after no more than three attempts per arm by a trained staff member, per the Yale University Positron Emission Tomography Center, the scan will be rescheduled for another day and the participant will still be paid $50 for his/her time. At the rescheduled scan, the physician will try to insert an arterial catheter again and the scan will be conducted regardless of

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

whether or not the catheter was inserted.

For two days following the placement of the arterial line, participants will be instructed to check their wrist/arm daily. The study team will also be in touch with them daily either via phone, or in person, during this two-day period. If they experience any excessive pain, tenderness, swelling, redness, drainage, skin color changes, numbness, pins and needles, or decreased strength in the arm that had the catheter, they are instructed call the study team immediately (203-464-6015). They can also contact the study PI Dr. Krishnan-Sarin (860-575-9895), the PET center physician, Dr, David Matuskey (203-370-1403), or the study physician, Dr. Julia Shi (203-781-4640).

8. 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).

9. 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.

10. Laboratory Tasks: The computer tasks (, EDT and Cued Go-No-Go) as well as the cold water task can produce some uneasiness at the time of the task. The hand kept in the very cold water can be uncomfortable. However, once the task is over, there is very little anxiety that carries over, thus posing minimal risk. Moreover, the participants will have total control over the task and can stop performing it if he/she gets too uncomfortable. There is the possibility that the desire to drink may occur in participants during the task, which may linger after the end of the task.

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

1) 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 will be issued medcards which allow health professionals to find out more about Naltrexone by calling the CMHC pharmacy. Subjects will also be constantly monitored for troublesome side

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

effects during the course of the outpatient and inpatient periods.

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.

2) 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, ifa 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 after taking study medication. Given the cost of the scan and the hospital visit, 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 if they think you are not responding well to the medication.

3) 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.

4) Risks Associated with Blood Drawing:

The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel under sterile conditions. To avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.

5) Risks Associated with Use of an Arterial Catheter**:**

Risks of radial artery cannulation are minimized by having the procedure performed by an experienced physician. Pain is minimized by local anesthesia. Bleeding is prevented by local pressure applied for a minimum of 15 minutes after catheter removal. Subjects will have their hand and finger blood supply examined after arterial cannulation and again

following catheter removal. Also, subjects will be asked to abstain from aspirin and other NSAIDs for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal. Subjects will be provided a 24 hour emergency physician telephone number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. Nurses will provide the subjects an instruction sheet documenting problems to watch for and procedures to follow should such problems occur. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

6) Risks Associated with Radiation:

The dose of radiation will be submitted for approval to the Yale-New Haven Hospital Radiation Safety Committee (Y-NHH RSC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals. Subjects will be asked about their previous radiation exposure and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study subjects will be notified to the study doctor.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study.

7) MRI:

Magnetic resonance imaging (MRI) scans (3 T) will be collected in each subject to co-register PET and MRI for image analysis. Within four weeks of the PET study, an MRI will be acquired at the Yale University MRI Center. In the event that the PET does not occur within six months of the MRI, subjects will be asked to perform a repeat MRI. Subjects will be taken through a ferromagnetic metal detector before entering the scan room. The acquisition sequence is a 3D fast spoiled grass (FSPGR) MR pulse sequence with an IR prep of 300 ms. (TE= 3.3 ms, flip angle=17 degrees; slice thickness= 1.2 mm) optimized for delineating gray matter/white matter/CSF boundaries. The small voxel size (0.93 X 1.2 X 0.93 mm) provides high-resolution volumetric images. MR images provide a matching anatomical atlas for creating individualized region-of-interest templates for each subject.

To minimize risks, each subject will fill out the Yale Magnetic Resonance Research Center MRI Safety Questionnaire before the study. Only subjects who fulfill the criteria by this questionnaire will be eligible for the study. In addition, subjects will remove all metal (watch, hair pins, jewelry) and change into scrubs immediately prior to the study and pass through the metal detector in the MRRC before entering the MRI room.

Subjects will be watched closely throughout the MR study. Some people may feel

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

uncomfortable or anxious. If this happens, the subject may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will ask subjects to tell the research staff if they have them.

There are some risks with an MR study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting a subject. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once subjects are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet. If the subject has any metallic prostheses/implants they will be excluded from the study.

This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The primary investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator or consulting physician will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that a subject receives based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

3. **Data and Safety Monitoring Plan:** Replace with new HIC text for data safety and monitoring plan form hrpp website, document: 420FR1DSMPTemplate.pdf (use text for moderate risk studies) 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? NA

c.Data and Safety Monitoring Plan:

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Either the principal investigator, or the Yale HIC have the authority

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed moderate for the following reasons: (choose those that apply)
1. We do not view the risks associated with the Naltrexone as minimal.
2. We do not view the risks associated with the combined use of Naltrexone and alcohol as minimal.
3. Given the now established safety and validity of the current Naltrexone in our prior work, we do not view the proposed studies as high risk.
4. Given our experience with the combined co-administration Naltrexone and alcohol, 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, Suchitra Krishnan-Sarin, PhD, 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
X Yale New-Haven Hospital Radiation Safety Committee (Y-NHH-RSC)
☐ Quality Assurance and Compliance and Safety Committee (QUACS)
☐ National Institutes of Health
☐ Food and Drug Administration (Physician-Sponsored IND #_____)
☐ Medical Research Foundation (Grant_____)

The principal investigator, Suchitra Krishnan-Sarin, PhD, 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.

d. For multi-site studies for which the Yale PI serves as the lead investigator: NA

i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
ii. What provisions are in place for management of interim results?
iii. What will the multi-site process be for protocol modifications?

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? [X] Secure Server

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?

Identifiable research data, including recruitment and screening information and code keys, are stored on a secure database located on the internal PET Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library with 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to those members of the research team who have direct contact with the research subjects, such as physicians, research nurses, and technologists. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid, and authentication is performed using Yale"s central authentication server. Users always access research data through the random identifier only. Direct identifiers belonging to subjects who withdraw from the study will be stripped from the key.

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?

Procedures to ensure confidentiality follow the regulations and policies of Yale School of Medicine. The security measures in 4d will continue to be in place to protect study data.

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, study personnel and the Department of Psychiatry Business Office

h. Which external or internal individuals or agencies (such as the study sponsor, FDA, QUACS, SSC, etc.) will have access to the study data?

Internal: Principal investigators and their respective research teams, YCCI Hospital Research Unit, CMU, PET and MRI centers, Y-NHH Radiation Safety Committee (RSC) and Yale University RSC.

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 with high resolution PET imaging before and after naltrexone treatment will shed new light on the actions of NTX and the dynorphin/KOR system in alcohol drinking behavior. An additional benefit to subjects is that they will be offered feedback about 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.

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, the amount and schedule of payments, and the conditions for receiving this compensation.

Heavy Drinkers: 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 $50 for the initial interview, $50 for the physical examination, $250 for the first PET scan, $450 for the second PET scan and $50 for the MRI. Subjects will also receive $150 for participating in the first drinking paradigm, $10 per day (up to 11 days) for transportation to our clinic for daily medication dose, $170 for taking medication on all days, $250 for participating in the second lab drinking paradigm, and $30 for completing a one week follow up. In addition, all subjects will have the opportunity to earn an extra $36 (depending on their drink consumption) during each of the ADP sessions and $30 for computer assessments at each of the ADP sessions. Additionally, in the case that the first arterial line cannot be placed at the first PET scan, participants will be paid $50 and rescheduled. In the event that a participant is asked to come back in for a repeat lab appointment (bloodwork or EKG) he/she will be paid $25. In the event that the participant serves as a standby for ADP/PET 1, he/she will be paid $50. In the event that a participant needs a repeat MRI, he/she will be paid $50. In the event that a participant needs to complete an extra overnight visit on the evening prior to a rescheduled PET

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

scan, he/she will be paid $100. That is a possible total of $2017, for attending and participating in all appointments.

An additional benefit to subjects is that they will be offered feedback about 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.

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-subject 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.

Healthy controls: Healthy controls will be paid $50 for the initial interview, $50 for the physical examination, $250 for the PET scan, and $50 for the MRI. Additionally, in the case that the first arterial line cannot be placed at the PET scan, participants will be paid $50 and rescheduled. In the event that a participant is asked to come back in for a repeat lab appointment (bloodwork or EKG) he/she will be paid $25. In the event that the participant serves as a standby for a PET scan, he/she will be paid $50. In the event that a participant needs a repeat MRI, he/she will be paid $50. That is a possible total of $575 for attending and participating in all appointments.

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? Treatment may be provided by Yale- New Haven Hospital or any health care provider chosen by the study subjects.
c. Are there any limits to the treatment being provided? Limits to treatment may be imposed by the insurance carrier of the study subjects.
d. Who will pay for this treatment? The study participant or their insurance carrier will be expected to pay for the cost of the treatment. No additional financial compensation for injury or lost wages is available.
e. How will the medical treatment be accessed by subjects? The study team will provide assistance to the subjects in accessing medical treatment through referrals, or the study subjects may choose to access treatment on their own.

References

1. O'Malley, S.S., A.J. Jaffe, G. Chang, R.S. Schottenfeld, R.E. Meyer, and B. Rounsaville, *Naltrexone and coping skills therapy for alcohol dependence. A controlled study.* Arch Gen Psychiatry, 1992. 49(11): p. 881-7.
2. O'Malley, S.S., S. Krishnan-Sarin, C. Farren, R. Sinha, and M.J. Kreek, *Naltrexone decreases craving and alcohol self-administration in alcohol-dependent subjects and activates the hypothalamo-pituitary-adrenocortical axis.* Psychopharmacology (Berl),

2002. 160(1): p. 19-29.
3. Krishnan-Sarin, S., J.H. Krystal, J. Shi, B. Pittman, and S.S. O'Malley, *Family history of alcoholism influences naltrexone-induced reduction in alcohol drinking.* Biol Psychiatry, 2007. 62(6): p. 694-7.
4. Weerts, E.M., Y.K. Kim, G.S. Wand, R.F. Dannals, J.S. Lee, J.J. Frost, and M.E. McCaul, *Differences in delta- and mu-opioid receptor blockade measured by positron emission tomography in naltrexone-treated recently abstinent alcohol-dependent subjects.* Neuropsychopharmacology, 2008. 33(3): p. 653-65.
5. Huang, Y., W. Williams, G. Tomasi, M.-Q. Zheng, J. Olsen, M. Tabriz, R.E. Carson, Y.S. Ding, J. Tauscher, and A. Neumeister, *Imaging Kappa Opioid Receptor in Humans with a Novel Antagonist Tracer, [11C]LY2795050.* Annual Meeting of Society of Nuclear Medicine, 2010. Abstract(Submitted).
6. Garbutt, J.C., S.L. West, T.S. Carey, K.N. Lohr, and F.T. Crews, *Pharmacological treatment of alcohol dependence: a review of the evidence.* Jama, 1999. 281(14): p. 1318-25.
7. Krishnan-Sarin, S., S. O'Malley, and J.H. Krystal, *Treatment Implications: Using Neuroscience to Guide the Development of New Pharmacotherapies for Alcoholism.* Alcohol Research & Health, 2008. 31(4): p. 400-407.
8. Volpicelli, J.R., A.I. Alterman, M. Hayashida, and C.P. O'Brien, *Naltrexone in the treatment of alcohol dependence.* Arch Gen Psychiatry, 1992. 49(11): p. 876-80.
9. Anton, R.F., S.S. O'Malley, D.A. Ciraulo, R.A. Cisler, D. Couper, D.M. Donovan, D.R. Gastfriend, J.D. Hosking, B.A. Johnson, J.S. LoCastro, R. Longabaugh, B.J. Mason, M.E. Mattson, W.R. Miller, H.M. Pettinati, C.L. Randall, R. Swift, R.D. Weiss, L.D. Williams, and A. Zweben, *Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial.* Jama, 2006. 295(17): p. 2003-17.
10. Gueorguieva, R., R. Wu, B. Pittman, J. Cramer, R.A. Rosenheck, S. O'Malley S, and J.H. Krystal, *New insights into the efficacy of naltrexone based on trajectory-based reanalyses of two negative clinical trials.* Biol Psychiatry, 2007. 61(11): p. 1290-5.
11. Monterosso, J.R., B.A. Flannery, H.M. Pettinati, D.W. Oslin, M. Rukstalis, C.P. O'Brien, and J.R. Volpicelli, *Predicting treatment response to naltrexone: the influence of craving and family history.* Am J Addict, 2001. 10(3): p. 258-68.
12. Kosterlitz, H.W., A.D. Corbett, and S.J. Paterson, *Opioid receptors and ligands.* NIDA Res Monogr, 1989. 95: p. 159-66.
13. Mucha, R.F. and A. Herz, *Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning.* Psychopharmacology (Berl), 1985. 86(3): p. 274-80.
14. Shippenberg, T.S., A. Zapata, and V.I. Chefer, *Dynorphin and the pathophysiology of drug addiction.* Pharmacol Ther, 2007. 116(2): p. 306-21.
15. Kovacs, K.M., I. Szakall, D. O'Brien, R. Wang, K.Y. Vinod, M. Saito, F. Simonin, B.L. Kieffer, and C. Vadasz, *Decreased oral self-administration of alcohol in kappa-opioid receptor knock-out mice.* Alcohol Clin Exp Res, 2005. 29(5): p. 730-8.
16. Fadda, P., S. Tronci, G. Colombo, and W. Fratta, *Differences in the opioid system in selected brain regions of alcohol-preferring and alcohol-nonpreferring rats.* Alcohol Clin Exp Res, 1999. 23(8): p. 1296-305.
17. Marinelli, P.W., K. Kiianmaa, and C. Gianoulakis, *Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats.* Life Sci, 2000. 66(20): p. 1915-27.
18. Winkler, A. and R. Spanagel, *Differences in the kappa opioid receptor mRNA content in distinct brain regions of two inbred mice strains.* Neuroreport, 1998. 9(7): p. 1459-64.
19. Edenberg, H.J., J. Wang, H. Tian, S. Pochareddy, X. Xuei, L. Wetherill, A. Goate, T. Hinrichs, S. Kuperman, J.I. Nurnberger, Jr., M. Schuckit, J.A. Tischfield, and T. Foroud,

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

A regulatory variation in OPRK1, the gene encoding the kappa-opioid receptor, is associated with alcohol dependence. Hum Mol Genet, 2008. 17(12): p. 1783-9.
20. Lindholm, S., M. Werme, S. Brene, and J. Franck, *The selective kappa-opioid receptor agonist U50,488H attenuates voluntary ethanol intake in the rat.* Behav Brain Res, 2001. 120(2): p. 137-46.
21. Mitchell, J.M., M.T. Liang, and H.L. Fields, *A single injection of the kappa opioid antagonist norbinaltorphimine increases ethanol consumption in rats.* Psychopharmacology (Berl), 2005. 182(3): p. 384-92.
22. Lindholm, S., K. Ploj, J. Franck, and I. Nylander, *Repeated ethanol administration induces short- and long-term changes in enkephalin and dynorphin tissue concentrations in rat brain.* Alcohol, 2000. 22(3): p. 165-71.
23. Rosin, A., S. Lindholm, J. Franck, and J. Georgieva, *Downregulation of kappa opioid receptor mRNA levels by chronic ethanol and repetitive cocaine in rat ventral tegmentum and nucleus accumbens.* Neurosci Lett, 1999. 275(1): p. 1-4.
24. Pande, A.C., R.E. Pyke, M. Greiner, S.A. Cooper, R. Benjamin, and M.W. Pierce, *Analgesic efficacy of the kappa-receptor agonist, enadoline, in dental surgery pain.* Clin Neuropharmacol, 1996. 19(1): p. 92-7.
25. Walsh, S.L., B. Geter-Douglas, E.C. Strain, and G.E. Bigelow, *Enadoline and butorphanol: evaluation of kappa-agonists on cocaine pharmacodynamics and cocaine self-administration in humans.* J Pharmacol Exp Ther, 2001. 299(1): p. 147-58.
26. Walker, B.M. and G.F. Koob, *Pharmacological evidence for a motivational role of kappa-opioid systems in ethanol dependence.* Neuropsychopharmacology, 2008. 33(3): p. 643-52.
27. Carson, R.E., W.C. Barker, J.S. Liow, and C.A. Johnson, *Design of a motion-compensation OSEM list-mode algorithm for resolution-recovery reconstruction for the HRRT*, in *2003 Ieee Nuclear Science Symposium, Conference Record, Vols 1-5*, S.D. Metzler, Editor. 2004. p. 3281-3285.
28. McKee, S.A., E.L. Harrison, S.S. O'Malley, S. Krishnan-Sarin, J. Shi, J.M. Tetrault, M.R. Picciotto, I.L. Petrakis, N. Estevez, and E. Balchunas, *Varenicline reduces alcohol self-administration in heavy-drinking smokers.* Biol Psychiatry, 2009. 66(2): p. 185-90.
29. Saitz, R. & O'Malley, S. S. (1997). Pharmacotherapies for alcohol abuse. Withdrawal and treatment. *Medical Clinics of North American*, 81, 881-907.
30. Sullivan, J., Sykora, K., Schneiderman, J., Naranjo, C. A., & Sellers, E. M. (1991). Assessment of Alcohol Withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CiWA-Ar). *British Journal of Addictions*, 84, 1353-1357.
31. Sellers, E. M., Higgins, G. A., Tomkins, D. M., Romach, M. K. & Toneatto, T. (1991). Opportunities for treatment of psychoactive substance use disorders with serotonergic medications. *Journal of Clinical Psychiatry*, 52, 49-54.
32. Saitz, R., Mayo-Smith, M. F., Roberts, M. S., Redmond, H. A., Bernard, D. R., Calkins, D. R., et al. (1994). Individualized treatment for alcohol withdrawal. A randomized double-blind controlled trial. *JAMA*, 272, 519-23.
33. Chapman, L.J., Edell, W.S., & Chapman, J.P. (1980). Physical anhedonia, perceptual aberration, and psychosis proneness. *Schizophrenia Bulletin*, 6, 639-653.
34. Marks, L. E., J. Stevens, C., Bartoshuk, L., Gent, J. F., Rifkin, B., & Stone, V. K. (1988). Magnitude matching: The measurement of taste and smell. *Chemical Senses*, 13, 63-87.
35. Stevens, J. C. & Marks, L. E. (1980). Cross-modality matching functions generated by magnitude estimation. *Perception and Psychophysics,* 27, 379-389.
36. Bartoshuk, L. M. (2002). Labeled scales (e.g. category, Likert, VAS) and invalid cross-group comparisons. What we have learned from genetic variation in taste. *Food Quality and Preference*, 14, 125-138.
37. Bremner, J.D., Krystal, J.H., Putnam, F.W., Sothwick, S.M., Marmar, C., Charney, D.S.,

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

et al. (1998). Measurement of dissociative states with the Clinician-Administered Dissociative States Scale (CADSS). *Journal of Traumatic Stress*, 11, 125-136.
38. Sobell, L. C. & Sobell, M. B. (1992). Timeline follow-back: a technique for assessing self-reported ethanol consumption. *Measuring Alcohol Consumption: Psychosocial and Biochemical Methods*. J. Allen and R. Z. Litten. Totowa, NJ, Humana Press, Inc.: 41-72.
39. Sayette, M. A., Shiffman, S., Tiffany, S. T., Niaura, R. S., Martin, C. S., & Shadel, W. G. (2000). The measurement of drug craving. *Addiction,* 95 (Suppl2), 189-210.
40. Krystal, J.H., Webb, E., Cooney, N., Kranzler, H. R., & Charney, D. S. (1994). Specificity of ethanol like effects elicited by serotonergic and noradrenergic mechanisms. *Archives of General Psychiatry*, 51, 898-911.
41. First, M.B., R.L. Spitzer, M. Gibbon, and J.B.W. Williams, *Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version, Patient Edition*. 1996, New York: Biometrics Research, New York State Psychiatric Institute.
42. Rice, J.P., T. Reich, K.K. Bucholz, R.J. Neuman, R. Fishman, N. Rochberg, V.M. Hesselbrock, J.I. Nurnberger, Jr., M.A. Schuckit, and H. Begleiter, *Comparison of direct interview and family history diagnoses of alcohol dependence.* Alcohol Clin Exp Res, 1995. 19(4): p. 1018-23.
43. Cooper, A.M., M.B. Sobell, L.C. Sobell, and S.A. Maisto, *Validity of alcoholic's self-reports: duration data.* Int J Addict, 1981. 16(3): p. 401-6.
44. Maisto, S.A., L.C. Sobell, A.M. Cooper, and M.B. Sobell, *Comparison of two techniques to obtain retrospective reports of drinking behavior from alcohol abusers.* Addict Behav, 1982. 7(1): p. 33-8.
45. Bohn, M.J., D.D. Krahn, and B.A. Staehler, *Development and initial validation of a measure of drinking urges in abstinent alcoholics.* Alcohol Clin Exp Res, 1995. 19(3): p. 600-6.
46. Martin, C.S., M. Earleywine, R.E. Musty, M.W. Perrine, and R.M. Swift, *Development and validation of the Biphasic Alcohol Effects Scale.* Alcohol Clin Exp Res, 1993. 17(1): p. 140-6.
47. Watson, T.E., *Total body water and alcohol levels: updating the fundamentals*, in *Human Metabolism of Alcohol*, K.E. Krow and R.D. Batt, Editors. 1989, CRC Press: Florida. p. 41-66.
48. Sinha, R., S. Krishnan-Sarin, C. Farren, and S. O'Malley, *Naturalistic follow-up of drinking behavior following participation in an alcohol administration study.* J Subst Abuse Treat, 1999. 17(1-2): p. 159-62.
49. Miller, W.R., A. Zweben, C.C. DiClimente, and R.G. Rychtarik, *Motivational Enhancement Therapy manual: A clinical research guide for therapists treating individuals with alcohol abuse and dependence*. 1992, Rockville, MD: National Institute on Alcohol Abuse and Alcoholism.
50. de Jong, H.W., F.H. van Velden, R.W. Kloet, F.L. Buijs, R. Boellaard, and A.A. Lammertsma, *Performance evaluation of the ECAT HRRT: an LSO-LYSO double layer high resolution, high sensitivity scanner.* Phys Med Biol, 2007. 52(5): p. 1505-26.
51. Heiss, W.D., B. Habedank, J.C. Klein, K. Herholz, K. Wienhard, M. Lenox, and R. Nutt, *Metabolic rates in small brain nuclei determined by high-resolution PET.* J Nucl Med, 2004. 45(11): p. 1811-5.
52. Gallezot, J.D., N. Nabulsi, A. Neumeister, B. Planeta-Wilson, W.A. Williams, T. Singhal, S. Kim, R.P. Maguire, T. McCarthy, J.J. Frost, Y. Huang, Y.S. Ding, and R.E. Carson, *Kinetic modeling of the serotonin 5-HT(1B) receptor radioligand [(11)C]P943 in humans.* J Cereb Blood Flow Metab, 2010. 30(1): p. 196-210.
53. Baron, R.M. and D.A. Kenny, *The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations.* J Pers Soc Psychol, 1986. 51(6): p. 1173-82.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019

54. Kraemer, H., G. Wilson, C. Fairburn, and W. Agras, *Mediators and moderators of treatment effects in randomized clinical trials.* Archives of General Psychiatry, 2002. 59: p. 877-883.
55. Faul, F., E. Erdfelder, A.-G. Lang, and A. Buchner, *G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences.* Behavior Research Methods, 2007. 39: p. 175-191.
56. Weschler, D. (1997). Weschler Adult Intelligence Scale- Third Edition. San Antonio: Psychological Corporation.
57. Carver, C.S. & White, T. (1994). Behavioral Inhibition, Behavioral Activation, and Affective Responses to Impending Reward and Punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology*, 67, 319-333.
58. Gray, J.A. (1981). A critique of Eysenck's theory of personality. In H.J. Eysenck (Ed.): *A model for personality*. Springer-Verlag: Berlin.
59. Patton, J.H., Stanford, M.S., & Barratt, E.S. (1995). Factor structure of the Barratt impulsiveness scale. *Journal of Clinical Psychology*, 51, 768-774.
60. Lejuez, C.W., Read, J.P., Kahler, C.W., Richards, J.B., Ramsey, S.E., Stuart, G.L., et al. (2002). Evaluation of a behavioral measure of risk taking: the Balloon Analogue Risk Task (BART). *Journal of Experimental Psychology. Applied*, 8, 75-84.
61. Reynolds, B., Richards, J. B., & de Wit, H. (2004). *Ethanol effects on experiential and hypothetical measures of delayed discounting in humans*. Poster presented at the 30th Annual Association for Behavior Analysis Convention, Boston, MA.
62. Reynolds, B., Richards, J. B., & de Wit, H. (2006). Acute-alcohol effects on the Experiential Discounting Task (EDT) and a question-based measure of delay discounting. *Pharmacology, Biochemistry, and Behavior,* 83, 194-202.
63. Kranzler, H.R., 2000. Pharmacotherapy of alcoholism: gaps in knowledge and opportunities for research. *Alcohol and Alcoholism,* 35, 537−547.
64. Swift, R. M., Whelihan, W., Kuznetsov, O., Buongiomo, G. & Hsuing, H. (1994). Naltrexone-induced alterations in human ethanol intoxication. *American Journal of Psychiatry*, 151, 1463-1467.
65. Levine, J. & Schooler, N.R. (1986). SAFTEE: a technique for the systematic assessment of side effects in clinical trials. *Psychopharmacology Bulletin, 22, 343-381.*
66. Abroms, B.D., Fillmore, M.T., & Marczinski, C.A. (2003). Alcohol-induced impairment of behavioral control: effects on alteration and suppression of pre-potent responses. *Journal of Studies on Alcohol, 64,* 687-695.
67 Edens, J. L. & Gil, K. M. (1995). Experimental induction of pain: Utility in the study of clinical pain. *Behavior Therapy, 26,* 197-216.

APPROVED BY THE YALE UNIVERSITY IRB 5/6/2019