Cover Page

Study Short Title: Altering memories that increase risk of relapse in alcohol use disorders (Pro00073523)

NCT03732248

4/29/2019

A. Statement of Problem/Background:

The role of basic associative learning processes in addiction has been well established in both theory and research[1-8]. In alcohol use disorders (**AUD**), numerous pairings between alcohol-related cues (e.g., sight and smell of preferred beverage) and the reinforcing effects of alcohol can result in cues acquiring the ability to elicit a range of conditioned responses, most importantly craving and physiological reactivity/arousal. Since craving has been shown to be powerfully associated with relapse[9-14], it is clear that craving remains a major obstacle to successful abstinence. While some current pharmacotherapies for AUDs have craving dampening properties[15], it appears that individual differences greatly influence their efficacy[16-18]. Thus, the treatment of AUDs could be significantly advanced by increasing medication alternatives to address this important obstacle to abstinence. To this end, the medication to be tested in this pilot study specifically targets the memory processes that support cue-elicited craving.

New learning is said to become stable in memory via the process of consolidation[19-23]. Reconsolidation refers to a process in which retrieval of consolidated memories begets a period of instability during which the memories can either be strengthened or otherwise altered and then are restabilized in long-term storage[24-30]. Generally, reconsolidation begins with memory retrieval, which is initiated by the presentation of cues that elicit target memories. There is a large body of basic neuroscience research[27,31,32] demonstrating that reconsolidation of memories for both appetitively- and aversively-motivated learning can be pharmacologically disrupted, leading to a decrement in, or near eradication of, behavior supported by the original learning. By contrast, a small body of human fear conditioning studies, using the β-adrenergic antagonist propranolol as the disrupting agent, has yielded findings that parallel those of the animal literature[33-37]. Translational studies targeting clinical anxiety and addictive disorders are also few in number and have focused almost exclusively on propranolol as a disruptive agent. In the case of anxiety disorders, three published reports have yielded suggestive evidence of attenuated trauma-related memory in PTSD, as indicated by decreased emotional responsiveness and PTSD symptomatology[38-40]. Our research group was the first to study propranolol's effects on memories for important addiction related-learning. In that study of cocaine dependent individuals, we found a single administration of propranolol following memory retrieval (via cocaine cue presentation) resulted in attenuated cocaine craving and physiological reactivity during a test performed the following day[41]. Recently, a study[42] involving nicotine dependent smokers failed to find any effect of propranolol on smoking cue-elicited reconsolidation (i.e., no effect on physiological and emotional reactivity to smoking cues presented in a test the following day). These contrasting findings tentatively suggest that a single disrupting agent may not have uniform effects across all addictive disorders and that reconsolidation disruption in substance users might be more profitably pursued with a pharmacological agent other than propranolol.

Identification of an alternative disrupting agent can be guided by the existing basic neuroscience literature. Specifically, one prototypical agent that has been used in animal studies to demonstrate **disruption of reconsolidation (DoR)** is the protein synthesis inhibitor, anisomycin[26,43-45]. The robust DoR effects observed with anisomycin have not been replicated in humans because of concerns about toxicity[46]. However, there are three emerging lines of evidence suggesting that the FDA-approved, protein synthesis inhibitor **rapamycin (sirolimus)** may be as effective a disrupting agent as anisomycin. First, animal studies employing fear conditioning procedures have reported that either systemic or intra-amygdalar injection of rapamycin can substantially disrupt reconsolidation of contextual and discrete cue fear memory[47-49]. Second, it has been shown that systemic rapamycin administration following re-exposure to a drug-paired environment results in lasting (14 days) decrements in morphine-, cocaine- and alcohol-reinforced place preferences, which could not be reinstated with priming drug injections[50]. Additionally, a recent Nature Neuroscience report showed that either systemic or intra-amygdalar administration of rapamycin after retrieval of alcohol-related memories substantially impaired relapse to alcohol self-administration for up to 14 days; these effects were similar to those observed when anisomycin served as the DoR agent[51]. Lastly, a clinical study employing Vietnam era and post-Vietnam era war veterans has provided the first evidence that a 15-mg dose of rapamycin vs. placebo administered contiguously with recall (retrieval) of war-related trauma resulted in reduced PTSD symptom score at a 1-month follow-up assessment, albeit only in the post-Vietnam era war veterans and not at 3-month follow-up[52]. Importantly, the authors of the study reported that no adverse medical outcomes or side-effects occurred during the course of the study. Collectively, these studies indicate that systemic rapamycin (sirolimus) (i) can effectively disrupt memories for both fear-based and a broad range of drug-reinforced learning and that this effect may be long lasting and comparable to anisomycin-induced DoR, and (ii) may be able to attenuate clinically important memories in the absence of side-effects.

While rapamycin's exact mechanism of action is unknown, it is likely that it achieves DoR by inhibiting the mammalian target of rapamycin (mTOR) kinase, which regulates phosphorylation of a large number of intracellular targets responsible for protein synthesis and translation[53-57]. Since rapamycin is a safe medication with minimal side effects, there are no obstacles to initiating research with human participants. Accordingly, the

proposed research will evaluate the novel hypothesis that the strategic administration of rapamycin (sirolimus) can disrupt reconsolidation of memories for cue-elicited craving in AUD drinkers.

B. Primary Hypotheses to be Tested:

Primary aim and hypothesis:

Aim: Preliminarily evaluate (a) the safety and tolerability of a single 15 mg dose of rapamycin (sirolimus), and (b) the effects of post-retrieval rapamycin vs. placebo on craving and cue reactivity assessed 1 day and ≈10 days following a medicated **Retrieval** session.

Hypothesis: Side effects/adverse events will be low and indistinguishable among the rapamycin vs. placebo groups. Compared to placebo treated AUD individuals, rapamycin-treated AUD individuals will evidence less craving, emotion/arousal and physiological reactivity to alcohol cues presented during the (a) **Test** session (24-hr. post) and (b) **Follow-up** session (≈10 days post).

Secondary aim and hypothesis:

Aim: Evaluate the effects of post-retrieval rapamycin vs. placebo on drinking behavior occurring during the ≈10-day follow-up period.

Hypothesis: Compared to placebo-treated controls, rapamycin-treated AUD individuals may evidence changes in drinking behavior during follow-up (approximately 9 days) as indicated on multiple measures including total number of standard drinks consumed over the ≈10-day follow-up period, mean number of standard drinks consumed per drinking day, % days drinking, time to first drink.

C. Research Plan:

i. Overview:

The proposed study will employ treatment-seeking AUD individuals who will be randomly assigned to receive either 15 mg of rapamycin (sirolimus) or placebo (group n's=9) immediately after the first of two alcohol cue exposure sessions scheduled to occur on consecutive days. The first session will serve as a **Retrieval** session during which alcohol (e.g., sight, smell and handling of preferred alcoholic beverage) cue exposure will elicit retrieval and reconsolidation of alcohol-related memories; the second session will be a **Test** session to examine the potential modulatory role of rapamycin on the reconsolidation of memories putatively elicited during the retrieval session. Participants will be required to refrain from drinking the day before their first laboratory (i.e., Retrieval) session and will remain abstinent from drinking until the completion of the second laboratory (i.e., Test) session. It is posited that changes in reactivity during the test session will reflect medication effects on memory reconsolidation that occurred following cue exposure in the Retrieval session. Subjective responses (i.e., craving) and physiological (heart rate & skin conductance) reactivity will be obtained before, during and after cue presentations in both sessions. The durability of any observed treatment effects will be assessed in a **Follow-up** session performed ≈10 days following completion of the initial Test session. Treatment effects on self-report measures of drinking behavior during the ≈10 days preceding the Follow-up session will also be assessed.

ii. Participants, Sample Size and Recruitment:

A total of 18 treatment seeking AUD men and women (9 per group), aged 18 or older, will be randomized into two groups. Participants must meet DSM-V criteria for AUD, drink ≥ 30 standard drinks per week, be willing to make an abstinence attempt, and comply with reinforced abstinence requirements for the three laboratory sessions described below. Participants must (i) not have another substance use disorder (other than nicotine), (ii) willing to use appropriate birth control methods (females) during study participation, (iii) remain abstinent from alcohol and all non-prescription drugs for a full day prior to medication administration and testing sessions, (iv) not be undergoing other medication treatment for AUD (e.g., naltrexone), and (v) not be taking medications that may interact with the study medication or alter responding on any study measure.

Sample Size. This pilot study is designed to yield an estimate of variance by calculating a 95% confidence interval for the pilot-derived squared sample standard deviation as well as estimates of mean responses and their differences. These estimates will be used to determine the necessary sample size needed to conduct a fully powered follow-up study (NIH R mechanism such as R21, R34, RO1).

Recruitment. Participants will be primarily recruited using the local online media (e.g., Craigslist), a recruitment strategy that has been very successful in our previous and ongoing studies. We will also recruit from the Medical University of South Carolina's (MUSC) Center for Drug and Alcohol Programs (CDAP). Additionally, we will be adding television advertisements as a form of recruitment.

iii. Screening, Consent, Baseline Cue Reactivity and General/Abstinence Assessment:

Screening, Consent, Baseline Cue Reactivity. A brief phone screening will preliminarily assess participant suitability via inclusion/exclusion criteria. Qualifying individuals will be scheduled for an in-person assessment session; they will be requested to remain abstinent from alcohol and other drug use beginning the day before this session. Upon arrival at MUSC, participants will undergo an IRB-approved informed consent procedure. After consent, all female participants will undergo a pregnancy test to confirm a negative test result. Any female

participant with a positive pregnancy test result will be immediately excluded from the study. Then, participants will undergo a baseline cue reactivity (BCR) assessment. The purpose of this assessment is to ensure that participants exhibit expected levels of responsive to alcohol cues (a necessary precondition for inclusion in this study). During the BCR assessment, participants will be shown two sets of three neutral picture cues on a video monitor (e.g. picture of a residential lawn, a telephone), followed by two sets of three alcohol picture cues related to their preferred alcoholic beverage (beer, liquor, or wine), and then finally two more sets of three neutral picture cues. Participants will score each set of cues on a scale from 0-100 indicating craving for alcohol. After each set of picture cues, participants will also be asked to provide an estimate of their desire for their favorite alcoholic beverage on a scale of 0-100. Inclusion in the study will occur if participants provide a mean craving rating of at least 30 during the alcohol picture cues, which must also be higher than the mean craving rating in response to the neutral picture cues. Those who do not qualify for inclusion will (1) receive $25 compensation for their time, (2) be provided the opportunity to receive treatment referral, and (3) be discontinued from further participation.

In the event that a participant experiences elevated craving at the end of the baseline session (i.e., report a post-session craving score ≥ 20% above baseline), they will be asked to remain in the SCTR Research Nexus until their craving subsides. A member of the research staff will be available to discuss management of craving/urges. This procedure will be used in all subsequent sessions as well.

General/Abstinence Assessment: Participants whose craving responses qualify them for inclusion in the study will undergo a general assessment of alcohol and other substance use and general psychiatric functioning. They will be administered the Mini-International Neuropsychiatric Interview for DSM-V (Version 7.0.0) or MINI[58-62], which is a measure of psychiatric functioning. The Timeline Follow-Back (TLFB; a calendar-based instrument with specific probes to obtain detailed information about substance use)[63] will be used to assess alcohol and other substance use during the three month period preceding study involvement. Participants will also undergo a general health screening. Then, MUSC SCTR Research nexus nursing staff will provide additional services including vitals, an EKG, and a blood sample. With the blood sample, a complete metabolic panel and a complete blood count (without differential) will be performed to ensure participants are medically fit to participate in the study.

Participants will be required to fast (not eat any solid food; water consumption is acceptable and encouraged) two hours prior to the first study visit because food may influence pharmacokinetics of rapamycin. Participants will also be required to remain abstinent from drinking beginning the day preceding the initial in-person assessment session and the day preceding the (first) **Retrieval** cue exposure session and remain abstinent through the completion of the (second) **Test** session. Participants failing to meet the breathalyzer criterion (0.0) at the Assessment or Retrieval session will be allowed to reschedule their participation one time; however, if a participant fails the breathalyzer assessment on the second day, they will be dropped from the study. Lastly, there will be no abstinence requirement for the ≈10-day period between the Test and Follow-up sessions; however, participants will be required to abstain from drinking starting the day before the **Follow-up** cue exposure session. The Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised (CIWA-Ar) will be used to assess alcohol withdrawal at each study visit. Lastly, other drug use will be assessed via urine drug screen (UDS). A positive test will result in rescheduling/termination as described above for drinking (with the exception of cannabis/marijuana).

iv. Laboratory Session Measures: (Our research team has extensive experience using all of the following measures).

Self-Report Measures: The key measure that will be used to quickly and unobtrusively assess alcohol craving is: single-item verbal report of subjective craving to drink. Prior to any stimulus presentation, participants will be trained to provide a verbal report of a numeric value between 0 (none) and 100 (extreme) that best represents their current level of craving ("My craving/urge to drink right now is"). In addition, particpants will fill out two forms. The Mood Form[64] (9-items) which will be used to assess immediate assessment of current negative and positive emotional states. And a cue exposure rating form (CDMS) which will be used to asses various reactions to cue exposure.

Physiological Measures: Heart rate (HR) will be collected via two electrodes along the bottom of the participant's ribcage, rather than on the participant's forearm, to minimize movement artifacts. Skin conductance (SC) will be recorded using Ag/AgCl electrodes attached to the second phalanx of the first and third fingers of the non-dominant hand. HR and SC signals will be amplified using ECG 100c and GSR 100c Biopac Modules and interfaced with the Biopac MP100 data acquisition system.

v. Laboratory Session Stimuli:

Alcohol Cues. Because we have been successful eliciting alcohol craving in our previous work with AUD individuals[65,66] we will adopt a similar strategy in the proposed study. In particular, the alcohol cues will consist of the sight and smell of the participant's preferred alcoholic beverage. The beverage will be placed close to the participant's nose with the bottle of alcohol placed in their line of sight. Participants will be asked to smell and

visually inspect the beverage for one minute, after which they will be able to handle and smell the drink for an additional minute. This alcohol cue exposure sequence will occur in each of the three laboratory sessions (i.e., Retrieval, Test and Follow-up).

vi. Randomization, Rapamycin Dosing, Preparation and Administration:

Randomization. Stratified block randomization will be used to assign participants to groups while balancing treatment assignment on gender. This method, when used with smaller block sizes, is appropriate for small study sample sizes[67] and has been used in a number of previous and ongoing studies by our research group.

Sirolimus (Rapamycin; Rapamune®) Dosage Rationale and Potential Side Effects. The decision to employ a 15-mg dose of rapamycin (sirolimus) in the proposed study was based on the following rationale. First, as noted above, Suris et al, 2013 employed 15 mg of rapamycin to disrupt trauma-related memories in combat veterans. The study results suggested, in a subsample of non-Vietnam era veterans, that 15 mg of rapamycin vs. placebo administered in conjunction with trauma memory reactivation was associated with decreased PTSD symptomatology at a 1-month follow-up assessment. Importantly, they also documented that this dose was not associated with any adverse medical consequences or side effects.

There is strong animal and clinical pharmacokinetic (PK) and pharmacodynamic (PD) data to demonstrate the proposed 15-mg dosing regimen will demonstrate clinically relevant outcomes. Results from a number of clinical PK studies demonstrate that sirolimus is rapidly absorbed after oral ingestion, with peak whole blood concentrations occurring 1.0 to 1.3 hours after administration[68-70]. Additionally, sirolimus adequately crosses the blood brain barrier, with an estimated blood to brain ratio of 1:3[71]. In a clinical PK study in health males, an 8 mg/m^2 one-time oral dose produced a whole blood Cmax of 115.2 ng/mL at 1.0 hours after ingestion. This corresponds to an approximate peak brain concentration of 38.4 ng/mL[60]. Additionally, the organ transplant animal and clinical data strongly support the proposed dosing strategy. Studies conducted in rats using sirolimus to prevent organ rejection utilized dosing ranges between 0.5 to 50 mg/kg per day[72]. However, maximum pharmacodynamic activity with sirolimus monotherapy in rat kidney and heart transplant models was demonstrated with a dose of 8 mg/kg intravenously. This dose correlated with a mean $AUC_{0\text{-}24h}$ of 270 ± 12 $\mu g/L*h$[73]. By using the trapezoidal rule, the $AUC_{0\text{-}\infty}$ in rats for an 8 mg/kg dose would approximate 350 $\mu g/L*h$. This dose of rapamycin (sirolimus) also demonstrated maximal lymphocyte protein kinase inhibition at 2 hours post-dose (i.e., maximal protein synthesis inhibition), with return of full function occurring at 12 to 24 hours post-dose. Therefore, based on the above noted literature, a one-time 15-mg dose should produce substantial protein kinase inhibition in the brain within 1-1.3 hours of administration, while also being well-tolerated, with mild-transient side effects. The current clinical use of sirolimus in organ transplantation also supports using the 15-mg oral dose in this proposed study, as it is commonly given as a loading dose of 15 mg orally during the perioperative period[73].

The recently-updated labeling for sirolimus (dated 1/2018) includes warnings regarding health consequences associated with sirolimus use. These warnings include increased susceptibility to infection and possible development of lymphoma due to the medication's immunosuppressive properties. The warnings that are potentially applicable to this protocol also include hypersensitivity reactions, angioedema, impaired or delayed wound healing, increased risk of opportunistic infections, and embryo-fetal toxicity.

However, many of these health consequences were observed with regular sirolimus use rather than a one-time dose, as will be the case in this study. Participants who are not willing to use an effective form of birth control during the course of the study and for twelve weeks after will be excluded from participating given the risk of embryo-fetal toxicity. Female participants will be pregnancy tested twice (including once on the same day as medication administration) and immediately excluded if the result is positive in order to avoid the risk of embryo-fetal toxicity. Participants will receive a maximum of 15mg of sirolimus under medically supervised conditions.

Rapamycin Preparation & Administration. Rapamycin (15-mg sirolimus) and placebo will be compounded, packaged and dispensed by the MUSC Investigational Drug Service (IDS). The IDS will work with the study Statistician (Baker) to oversee the randomization procedures for the study. Immediately after the cue exposure in the Retrieval session, 15 mg of rapamycin (sirolimus) or a matching placebo will be administered.

vii. Laboratory Session Procedures:

Session Preparation. Abstinence will be assessed (see above **iii**, Abstinence Assessment) at each laboratory session visit. If abstinence is confirmed, participants will be escorted to MUSC's CTRC waiting room where they will remain until the laboratory is ready for cue exposure administration (approximately noon). Next, they will be escorted to the laboratory where they will sit quietly/read in order to acclimate to the environment until the cue exposure procedures begin. Following the acclimation period, HR and SC sensors will be placed, sound-attenuating headphones will be fitted, and baseline assessments of subjective and physiological measures will be collected (see general procedures table below).

Retrieval session (1) procedure. The primary objective of this session is to elicit the retrieval of alcohol-related memories. To achieve this objective, participants will be exposed to the alcohol cues as described above (sight, smell and handling of alcohol cues). A craving rating will be obtained midway through the alcohol cue exposure (i.e., the inquiry will be made by research staff via headphones). Immediately following the alcohol cue presentation, participants will provide the craving rating and complete the other study measures. Participants will receive medication immediately after the measures have been obtained. Collection of all study measures will occur every 10-min after medication administration with the final occasion at 50-min post-medication (see general procedures table to the left). Although HR and SC will be collected continuously during the alcohol cue exposure, only single time point measures will be collected thereafter. Possible medication side effects will be assessed using the Monitoring of Side Effects Scale (MOSES) and will be managed by a study physician (Gray) if necessary. Participants will be asked to complete a brief questionnaire that asks whether or not they thought they received study medication or placebo (blind effectiveness assessment). Lastly, the participant will be reminded of the drinking abstinence requirement and instructed not to drink or use other substances (abstinence serves to mitigate the confounding reconditioning effects of drinking on the reconsolidation processes initiated in this session). The participants will remain at the CTRC for a total of three hours after medication administration in order to permit continuous assessment of potential medication side-effects.

GENERAL PROCEDURE TABLE

Measurement Occasion	Single-Item Craving	HR	SC	Mood Form	CDMS
Baseline Measures	X	X	X	X	X
During Alcohol Cues	X	X	X		
Immediate Post-Alcohol Cues	X	X	X	X	X
10-, 20-, 30-, 40- and 50-Min Post	X	X	X	X	X

Test session (2) procedure. Test session 2 will be identical to session (1) with the following exceptions. First, no medication will be administered. Second, at the end of the session, participants will receive a drinking diary in which to record the occurrence of any drinking behavior over the ≈10-day follow-up period (abstinence is not required during the ≈10 days preceding Follow-up session). Finally, the experimenter will provide compensation and schedule the Follow-up cue reactivity session.

Follow-up session (3) procedure. The Follow-up session will be identical to Test session (2) with the following exceptions. First, drinking diary data will be collected (research staff will perform a TLFB assessment of drinking behavior if participants do not present with their dairy). Second, debriefing will take place to address questions/concerns. Recommendations for additional treatment will be provided if desired.

viii. Participant Compensation:

Participants will be compensated as follows: Screening and assessment = $50.00; Retrieval, Test and Follow-up sessions = $50.00. Maximum compensation for participation is $200.00.

ix. Risks to Subjects:

1. Study medication: The most common side effects of rapamycin (occurring in 30% or more of patients using rapamycin to prevent rejection of kidney transplants) include swelling in the arms or legs, elevated triglyceride level, high blood pressure, high cholesterol, increased creatinine level, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, joint pain, pain, and low platelet count. Other side effects, such as stomatitis (swelling of and sores in the mouth), nausea, viral infection of the nose and throat, acne, chest pain, upper respiratory tract infection, headache, dizziness, and pain in the muscles, were reported in 20% or more of patients taking rapamycin for a lung disease known as lymphangioleiomyomatosis. Sirolimus use has been associated with rare serious side effects, including increased risk of infection(s), lymphoma (lymphatic cancer), allergic reactions, angioedema (swelling of the area under the skin), delayed wound healing, and embryo-fetal toxicity (as reported in studies with animals). The rare serious side effects of this medication were observed in studies where participants were taking rapamycin (sirolimus) daily for 2-3 years. In this study, the medication will be administered once under medically supervised conditions. Female participants will be tested for pregnancy twice, including once on the day of medication administration, to minimize the potential for harm to an unborn baby.
2. Interviews: The interviews that the participant will undergo during the course of the study involve no specific risks or discomforts beyond those of a standard clinical interview situation, such as feeling upset at the review of psychiatric status, boredom, or fatigue. If a question makes a participant uncomfortable the participant may refuse to answer it without fear of penalty (i.e. loss of compensation or study dismissal).
3. Experimental treatment: Participants will not receive any FDA-approved medications for the treatment of alcohol use disorder for the duration of their study participation (about 3-4 weeks).
4. Placebo: If a participant is in a group that receives a placebo, the participant's condition will go without active treatment for the duration of the study. (approximately 3-4 weeks)

5. Randomization: The experimental treatment participants receive may prove to be less effective or to have more side effects than other study treatment(s) or other available treatments.
6. Pregnancy: We do not know if the study drug will affect mother's milk or an unborn fetus. Therefore, breast-feeding and pregnant women are not allowed to take part in the study. If a potential participant is pregnant or becomes pregnant, there may be risks to the embryo or fetus that are unknown at this time. Women who can become pregnant must take a pregnancy test before the start of the study. Women who are not currently pregnant but could become pregnant must continue to use an effective birth control method (such as a diaphragm, condoms with spermicide, surgical sterilization, oral contraceptives, an intra-uterine contraceptive device, or complete abstinence from sexual intercourse) beginning prior to the study and continuing until 12 weeks after receiving the study medication. Participants will be advised not father a child while on this study as the treatment may indirectly affect an unborn child. If a participant is sexually active and are at risk of causing a pregnancy, then the participant and his female partner(s) must use a method to avoid pregnancy that works well (such as a diaphragm, condoms with spermicide, surgical sterilization, oral contraceptive, or an intra-uterine contraceptive device) or the participant must not have sex. Unless a participant cannot have children because of surgery or other medical reasons, the participant must be using an effective form of birth control before starting the study. The participant must also agree to continue to use an effective form of birth control for 12 weeks after taking the study drug.
7. Exposure to alcohol cues: Exposure to cues may produce some craving for alcohol or other discomfort. However, this discomfort is usually brief and the participant will be in the safety of an alcohol-free laboratory environment. Although previous studies do not show increased risk of alcohol craving or relapse after cue exposure, this possibility cannot be completely ruled out.
8. Alcohol withdrawal: During the study, the participant will be asked to abstain from alcohol for three days, beginning the day before the first visit until the end of the second visit. You will also be asked to abstain the day before the final visit. This has the potential to cause symptoms of withdrawal such as tremors, sweats, and anxiety.
9. Blood drawing: The risks of drawing blood include temporary discomfort from the needle stick, bruising, and possible infection. Fainting could occur.
10. Electrocardiogram (ECG): The ECG procedure may cause some mild discomfort during the placement and removal of the leads to and from the skin. You may also experience some local irritation, redness, or burning in the areas where the leads are attached.
11. Confidentiality: There is a risk of loss of confidentiality of personal information as a result of participation in this study. Please refer to confidentiality section for detailed description of confidentiality protections for all participants.
12. Unknown Risks: The experimental treatments may have unknown side effects. The researchers will let participants know if they learn anything that might make the participants change their minds about participating in the study.

x. Data Analysis:

Primary Hypothesis. The proportion of participants experiencing any adverse events will be reported and compared across treatment conditions using a Chi-Squared test statistic and overall adverse event counts and severity will be compared between groups using a simple Poisson modeling process. In the case of cue-elicited craving, mixed effects models will be used to preliminarily evaluate the hypothesized group differences and variance across the Test session measurement (24-hr. acute effect) and again at the 10-day Follow-up (maintenance effect) laboratory session. Other self-report measures (e.g., affect ratings) and HR/SC measures will be analyzed in a similar manner. Additional functional forms of the response measures, such as Area Under the Curve (AUC) and/or maximum within-session decrement in craving may be assessed to understand how treatment differences may occur. Although not specifically powered, models will be developed to additionally assess the effects of potential covariates (e.g., age) on the single-item craving outcomes and possible effect modification (through interaction terms).

Secondary Hypothesis. Treatment group differences on various indices of drinking behavior (total number of standard drinks consumed over the 10-day follow-up period, mean number of standard drinks consumed per drinking day, % days drinking, time to first drink) will be assessed using general linear regression models. Continuous outcomes (% drinking days) will be analyzed using normal linear models while time to first drink will be assessed using time to event models for efficacy analysis (Log-rank, Cox PH Models). While only large group differences are likely to be statistically detectable, analysis of drinking behavior is consistent with the overarching 'proof-of-concept' theme of this research and will provide efficacy estimates to inform a power-based sample size determination for a larger study.

xi. Timeline:

Since we have ongoing studies with a variety of substance using populations, an active recruitment network and extensive experience using cue reactivity paradigms, we anticipate start up to occur in approximately 4-months. During this period, research staff will be trained, the protocol will be IRB approved at MUSC, an IND application will be approved by the FDA, and laboratory procedures and database(s) will be established.

We will actively recruit participants for 7 months and plan to allow one month for final data cleaning/reduction, analysis and report/manuscript preparation (albeit manuscript preparation will begin sooner). Submission of a proposal for a more extensive controlled trial if the results are promising will occur as soon as possible. At a recruitment rate of approximately 2-3 AUD participants per month, we should have no difficulty in completing the study in a one-year timeframe.

D. Inclusion/Exclusion Criteria:

i. Inclusion

Participants must (i) be treatment-seekers who are willing to remain abstinent from alcohol and drugs of abuse during specified periods of their study participation, (ii) meet criteria for alcohol use disorder according to DSM-V criteria and drink at least 30 standard drinks per week, (iii) be able to provide informed consent and function at an intellectual level sufficient to allow accurate completion of all assessment instruments, (iv) use one of the following methods of birth control: oral contraceptives, barrier methods (diaphragm or condoms with spermicide or both), surgical sterilization, use of an intra-uterine contraceptive device, or complete abstinence from sexual intercourse, (v) live within a 50-mile radius of our research program and have reliable transportation, (vi) consent to remain abstinent from alcohol and all non-prescription drugs prior to medication administration and testing sessions, (vii) consent to fast for a two-hour period prior to medication administration, and (viii) consent to random assignment to the rapamycin vs. placebo conditions.

ii. Exclusion

Participants will be excluded if they (i) are undergoing other alcohol cessation treatment (such as Antabuse or Naltrexone), as this may confound results in the present study and because of potential/unknown interactions with study medications, (ii) are pregnant, nursing, or of childbearing potential and not using birth control, (iii) have evidence of or a history of significant endocrine, cardiovascular, pulmonary, renal, or neurological disease, as these conditions may affect heart rate or skin conductance measurement, (iv) have significant liver impairment (as indicated by alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) values that are three times the upper limit of normal) as rapamycin (sirolimus) is hepatically metabolized, (v) have an existing infection or immune system disorder, as rapamycin has known immunosuppressive properties, (vi) have a history of or current psychotic disorder, severe major depression (i.e. active and profound psychomotor retardation, persistent and intense suicidal ideation) or bipolar affective disorder as these may compromise both data integrity and the participant's ability to safely complete the study, (vii) currently take anti-arrythmic agents, psychostimulants, or any other agents known to interfere with heart rate and skin conductance monitoring, (viii) have known or suspected hypersensitivity to macrolide compounds (such as rapamycin/sirolimus), (ix) currently take medications that could adversely interact with the study medication, including but not limited to significant inhibitors of CYP2D6 or CYP3A4 (voriconazole, fluconazole, itraconazole, erythromycin, clarithromycin, diltiazem, verapamil, etc.), or significant inducers of CYP3A4, such as anticonvulsants (carbamazepine, phenobarbital, phenytoin, etc.) and antibiotics (rifabutin, rifapentine, etc.), (x) have a history of thrombocytopenia, idiopathic thrombocytopenia purpura (ITP) or have a platelet count of less than 100,000 cells per mm3, (xi) have any unhealed wounds, including but not limited to oral ulcers, foot ulcers, or recent surgical or traumatic wounds, (xii) have any planned surgeries within the next month, including surgical dental procedures, or (xiii) have a history of complicated alcohol withdrawal symptoms (including, but not limited to, symptoms such as seizures, hallucinations, and high blood pressure).

E. Significance of the Project and Relationship to ARC Research Goals:

Significance. AUDs are arguably the second greatest (behind smoking) addiction-related public health problem[74,75]. Craving is a central feature of AUD and it remains one of the primary catalysts for relapse to misuse. Therefore, any incremental gains made in the treatment of alcohol craving and drinking behavior would translate into significant reductions in the public health burden posed by AUD. To this end, favorable outcomes from this and subsequent studies could lead to a new generation of treatment adjuncts that would be brief, easy to administer and cost effective. These interventions could become a complementary treatment paradigm, operating synergistically with the current generation of pharmacotherapies and/or cognitive behavioral treatment approaches to reduce cue-elicited craving in AUD. Furthermore, since learning/memory processes are a primary etiological variable in all addictions, it seems evident that reconsolidation-based interventions could be devised for many other vulnerable addicted populations (i.e., cocaine, opiates, marijuana, etc.).

Relationship to ARC Research Goals: The central mission of the ARC is treatment development and the proposed pilot directly aligns with this mission in that it seeks to provide preliminary empirical support for a novel

pharmacotherapy that targets the memory processes that undergird AUDs. Another theme of the ARC is translational science, where cross fertilization between basic and clinical science serves to advance the treatment of AUDs. The proposed project derives from this perspective in that it is empirically and conceptually based on a robust basic neuroscience literature in memory reconsolidation. The ARC's emphasis on multidisciplinary collaborative science is also evident in the proposed pilot. Specifically, this project would be the first campus-wide effort to bring together investigators from addiction sciences and transplant medicine to advance medications development in the area of alcohol-related addiction. Lastly, this pilot project is conceptually congruent with the ongoing clinical components of the ARC (Anton & Schacht and Hanlon & Prisciandaro) in that it targets novel treatment development of alcohol cue-elicited craving.

F. Pathway to Extramural Funding:

The first step in a pathway to extramural funding is a strong research team. Drs. **Saladin** (PI, Clinical Psychologist, Dept. of Health Sciences and Research), **Taber** (Co-I, PharmD., Department Surgery, Transplant Medicine) and **Gray** (Medical Monitor, Psychiatrist, Dept. of Psychiatry and Behavioral Sciences), together with statistician Nathaniel **Baker** (Co-I, Department of Public Health Sciences), have all the necessary research and clinical expertise/skills to safely, efficiently and successfully execute the proposed pilot study. Additionally, the extramural funding potential for this project is high because the central hypothesis is based on a highly reproducible neuroscience phenomenon (memory reconsolidation) that has endured more than four decades of intense empirical scrutiny[28]. Thus, the combination of the strong research team and a solid, empirically-based hypothesis provide the context for a successful scientific endeavor. Positive findings from this pilot could lead either to a developmental/exploratory study, R21/R34, or to a larger scale clinical trial, RO1 (depending on the magnitude of the pilot findings).

LITERATURE CITED

1 Childress, A. R., Ehrman, R. E., Rohsenow, D. J., Robbins, S. J. & O'Brien, C. P. in *Substance Abuse A Comprehensive Textbook* eds J. H. Lowinson, P. Ruiz, & R. B. Millman) 56-69 (Williams & Wilkins, 1992).

2 Eikelboom, R. & Stewart, J. Conditioning of Drug-Induced Physiological Responses. *Psychological Review* **89**, 507-528 (1982).

3 Niaura, R. S. *et al.* Relevance of cue reactivity to understanding alcohol and smoking relapse. *Journal of Abnormal Psychology* **97**, 133-152 (1988).

4 Wikler, A. in *Opioid dependence: Mechanisms and treatment* (ed A. Wikler) (Plenum Press, 1980).

5 Hogarth, L., Balleine, B. W., Corbit, L. H. & Killcross, S. Associative learning mechanisms underpinning the transition from recreational drug use to addiction. *Ann N Y Acad Sci* **1282**, 12-24 (2013).

6 Hyman, S. E. Addiction: a disease of learning and memory. *Am J Psychiatry* **162**, 1414-1422 (2005).

7 Robbins, T. W., Ersche, K. D. & Everitt, B. J. Drug addiction and the memory systems of the brain. *Ann N Y Acad Sci* **1141**, 1-21 (2008).

8 Robbins, T. W. & Everitt, B. J. Limbic-striatal memory systems and drug addiction. *Neurobiology of Learning & Memory* **78**, 625-636 (2002).

9 Weiss, F. Neurobiology of craving, conditioned reward and relapse. *Curr Opin Pharmacol* **5**, 9-19 (2005).

10 Dolan, S. L., Rohsenow, D. J., Martin, R. A. & Monti, P. M. Urge-specific and lifestyle coping strategies of alcoholics: relationships of specific strategies to treatment outcome. *Drug Alcohol Depend* **128**, 8-14 (2013). PMC3505227

11 Ramirez, J. & Miranda, R., Jr. Alcohol craving in adolescents: bridging the laboratory and natural environment. *Psychopharmacology (Berl)* **231**, 1841-1851 (2014). PMC4127892

12 Hulse, G. K., Ngo, H. T. & Tait, R. J. Risk factors for craving and relapse in heroin users treated with oral or implant naltrexone. *Biol Psychiatry* **68**, 296-302 (2010).

13 Sinha, R., Garcia, M., Paliwal, P., Kreek, M. J. & Rounsaville, B. J. Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. *Archives of General Psychiatry* **63**, 324-331 (2006).

14 Killen, J. D. & Fortmann, S. P. Craving is associated with smoking relapse: Findings from three prospective studies. *Experimental and Clinical Psychopharmacology* **5**, 137-142 (1997).

15 Monti, P. M. *et al.* Naltrexone's effect on cue-elicited craving among alcoholics in treatment. *Alcohol Clin Exp Res* **23**, 1386-1394 (1999).

16 Ray, L. A. & Hutchison, K. E. Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. *Arch Gen Psychiatry* **64**, 1069-1077 (2007).

17 Ray, L. A., Meskew-Stacer, S. & Hutchison, K. E. The relationship between prospective self-rating of alcohol sensitivity and craving and experimental results from two alcohol challenge studies. *J Stud Alcohol Drugs* **68**, 379-384 (2007).

18 Monterosso, J. R. *et al.* Predicting treatment response to naltrexone: the influence of craving and family history. *Am J Addict* **10**, 258-268 (2001).

19 McGaugh, J. L. Memory--a century of consolidation. *Science* **287**, 248-251 (2000).

20 Alberini, C. M. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? *Trends in Neuroscience* **28**, 51-56 (2005).

21 Dudai, Y. The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol* **55**, 51-86 (2004).

22 Schafe, G. E. & LeDoux, J. E. Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *J Neurosci* **20**, RC96 (2000).

23 Schafe, G. E., Nader, K., Blair, H. T. & LeDoux, J. E. Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends Neurosci* **24**, 540-546 (2001).

24 Dudai, Y. Reconsolidation: the advantage of being refocused. *Curr Opin Neurobiol* **16**, 174-178 (2006).

25 Lee, J. L., Di Ciano, P., Thomas, K. L. & Everitt, B. J. Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron* **47**, 795-801 (2005).

26 Sara, S. J. Retrieval and reconsolidation: toward a neurobiology of remembering. *Learning and Memory* **7**, 73-84 (2000).

27 Tronson, N. C. & Taylor, J. R. Molecular mechanisms of memory reconsolidation. *Nat Rev Neurosci* **8**, 262-275 (2007).
28 Alberini, C. M. *Memory reconsolidation*. (Elsevier Academic Press, 2013).
29 Alberini, C. M. & Ledoux, J. E. Memory reconsolidation. *Current biology : CB* **23**, R746-750 (2013).
30 Nader, K. & Einarsson, E. O. Memory reconsolidation: an update. *Ann N Y Acad Sci* **1191**, 27-41 (2010).
31 Finnie, P. S. & Nader, K. The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. *Neurosci Biobehav Rev* **36**, 1667-1707 (2012).
32 Das, R. K., Freeman, T. P. & Kamboj, S. K. The effects of N-methyl D-aspartate and B-adrenergic receptor antagonists on the reconsolidation of reward memory: a meta-analysis. *Neurosci Biobehav Rev* **37**, 240-255 (2013).
33 Kindt, M., Soeter, M. & Vervliet, B. Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat Neurosci* **12**, 256-258 (2009).
34 Soeter, M. & Kindt, M. Dissociating response systems: erasing fear from memory. *Neurobiology of Learning & Memory* **94**, 30-41 (2010).
35 Soeter, M. & Kindt, M. Erasing fear for an imagined threat event. *Psychoneuroendocrinology* **37**, 1769-1779 (2012).
36 Schwabe, L., Nader, K. & Pruessner, J. C. beta-Adrenergic blockade during reactivation reduces the subjective feeling of remembering associated with emotional episodic memories. *Biol Psychol* **92**, 227-232 (2013).
37 Schwabe, L., Nader, K., Wolf, O. T., Beaudry, T. & Pruessner, J. C. Neural signature of reconsolidation impairments by propranolol in humans. *Biol Psychiatry* **71**, 380-386 (2012).
38 Brunet, A. *et al.* Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *J Psychiatr Res* **42**, 503-506 (2008).
39 Brunet, A. *et al.* Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials. *J Clin Psychopharmacol* **31**, 547-550 (2011).
40 Poundja, J., Sanche, S., Tremblay, J. & Brunet, A. Trauma reactivation under the influence of propranolol: an examination of clinical predictors. *Eur J Psychotraumatol* **3** (2012). 3402116
41 Saladin, M. E. *et al.* A double blind, placebo-controlled study of the effects of post-retrieval propranolol on reconsolidation of memory for craving and cue reactivity in cocaine dependent humans. *Psychopharmacology (Berl)* **226**, 721-737 (2013). PMC3637960
42 Pachas, G. N. *et al.* Single dose propranolol does not affect physiologic or emotional reactivity to smoking cues. *Psychopharmacology (Berl)* **232**, 1619-1628 (2015). PMC4404704
43 Suzuki, A. *et al.* Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci* **24**, 4787-4795 (2004).
44 Nader, K., Schafe, G. E. & Le Doux, J. E. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature* **406**, 722-726 (2000).
45 Abel, T. & Lattal, K. M. Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol* **11**, 180-187 (2001).
46 Rudy, J. W., Biedenkapp, J. C., Moineau, J. & Bolding, K. Anisomycin and the reconsolidation hypothesis. *Learn Mem* **13**, 1-3 (2006).
47 Blundell, J., Kouser, M. & Powell, C. M. Systemic inhibition of mammalian target of rapamycin inhibits fear memory reconsolidation. *Neurobiology of Learning and Memory* **90**, 28-35 (2008).
48 Parsons, R. G., Gafford, G. M. & Helmstetter, F. J. Translational control via the mammalian target of rapamycin pathway is critical for the formation and stability of long-term fear memory in amygdala neurons. *J Neurosci* **26**, 12977-12983 (2006).
49 Mac Callum, P. E., Hebert, M., Adamec, R. E. & Blundell, J. Systemic inhibition of mTOR kinase via rapamycin disrupts consolidation and reconsolidation of auditory fear memory. *Neurobiology of Learning & Memory* (2013).
50 Lin, J. *et al.* Rapamycin prevents drug seeking via disrupting reconsolidation of reward memory in rats. *Int J Neuropsychopharmacol* **17**, 127-136 (2014).
51 Barak, S. *et al.* Disruption of alcohol-related memories by mTORC1 inhibition prevents relapse. *Nat Neurosci* **16**, 1111-1117 (2013). 3725202
52 Suris, A., Smith, J., Powell, C. & North, C. S. Interfering with the reconsolidation of traumatic memory: sirolimus as a novel agent for treating veterans with posttraumatic stress disorder. *Ann Clin Psychiatry* **25**, 33-40 (2013).

53 Tang, S. J. *et al.* A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc Natl Acad Sci U S A* **99**, 467-472 (2002). 117583
54 Raught, B., Gingras, A. C. & Sonenberg, N. The target of rapamycin (TOR) proteins. *Proc Natl Acad Sci U S A* **98**, 7037-7044 (2001). 34619
55 Hay, N. & Sonenberg, N. Upstream and downstream of mTOR. *Genes Dev* **18**, 1926-1945 (2004).
56 Costa-Mattioli, M., Sossin, W. S., Klann, E. & Sonenberg, N. Translational control of long-lasting synaptic plasticity and memory. *Neuron* **61**, 10-26 (2009).
57 Li, J., Kim, S. G. & Blenis, J. Rapamycin: one drug, many effects. *Cell Metab* **19**, 373-379 (2014). 3972801
58 Sheehan, D. *et al.* 1-24 (Version 25.20.20) (Authors, Florida & Paris, 2003).
59 Sheehan, D. *et al.* The Mini International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnaostic psychiatric interview. *Journal of Clinical Psychiatry* **59 (suppl 20)**, 22-33 (1998).
60 Amorim, P., Lecrubier, Y., Weiller, E., Hergueta, T. & Sheehan, D. DSM-IH-R Psychotic Disorders: procedural validity of the Mini International Neuropsychiatric Interview (MINI). Concordance and causes for discordance with the CIDI. *Eur Psychiatry* **13**, 26-34 (1998).
61 Lecrubier, Y. *et al.* The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. *European Psychiatry* **12**, 224-231 (1997).
62 Sheehan, D. V. *et al.* The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. *European Psychiatry* **12**, 232-241 (1997).
63 Sobell, L. C. & Sobell, M. B. in *Measuring alcohol consumption: Psychological and Biological Methods* eds J. Allen & R. Z. Litten) 41-72 (Humana Press, 1992).
64 Diener, E. & Emmons, R. A. The independence of positive and negative affect. *J Pers Soc Psychol* **47**, 1105-1117 (1984).
65 Saladin, M. E. *et al.* PTSD symptom severity as a predictor of cue-elicited drug craving in victims of violent crime. *Addictive Behaviors* **28**, 1611-1629 (2003).
66 Saladin, M. E., Drobes, D. J., Coffey, S. F. & Libet, J. M. The human startle reflex and alcohol cue reactivity: effects of early versus late abstinence. *Psychol Addict Behav* **16**, 98-105 (2002).
67 Hedden, S. L., Woolson, R. F. & Malcolm, R. J. Randomization in substance abuse clinical trials. *Subst Abuse Treat Prev Policy* **1**, 6 (2006). 1436001
68 Brattstrom, C. *et al.* Pharmacokinetics and safety of single oral doses of sirolimus (rapamycin) in healthy male volunteers. *Ther Drug Monit* **22**, 537-544 (2000).
69 Mahalati, K. & Kahan, B. D. Clinical pharmacokinetics of sirolimus. *Clin Pharmacokinet* **40**, 573-585 (2001).
70 Stenton, S. B., Partovi, N. & Ensom, M. H. Sirolimus: the evidence for clinical pharmacokinetic monitoring. *Clin Pharmacokinet* **44**, 769-786 (2005).
71 Meikle, L. *et al.* Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci* **28**, 5422-5432 (2008). 2633923
72 Calne, R. Y. *et al.* Rapamycin for immunosuppression in organ allografting. *Lancet* **2**, 227 (1989).
73 Barten, M. J. *et al.* Synergistic effects of sirolimus with cyclosporine and tacrolimus: analysis of immunosuppression on lymphocyte proliferation and activation in rat whole blood. *Transplantation* **77**, 1154-1162 (2004).
74 Grant, B. F., Chou, S., Saha, T. D. & et al. Prevalence of 12-month alcohol use, high-risk drinking, and dsm-iv alcohol use disorder in the united states, 2001-2002 to 2012-2013: Results from the national epidemiologic survey on alcohol and related conditions. *JAMA Psychiatry* (2017).
75 HHS. U.S. Department of Health and Human Services (HHS). (Facing Addiction in America: Surgeon General's Report on Alcohol, Drugs, and Health, Washington (DC), 2016).