Cover Page

Study Short Title: Enhancing Self-Regulation Among Smokers
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Enhancing self-regulation by altering memories that increase risk of relapse among smokers: A translational clinical neuroscience study of a novel medication

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I. Objectives/Specific Aims

Smoking-related nicotine dependence (ND) is the single greatest cause of addiction-related morbidity and mortality. Current pharmacological treatments have improved cessation rates but there is still considerable room for improvement. The impetus for the proposed translational HCC Team Science study (**Project 2**) comes primarily from recent neuroscience research demonstrating that the FDA-approved protein synthesis inhibitor rapamycin (henceforth sirolimus), administered after the reactivation (i.e., recall) of memories for learned associations, can result in disrupted memory reconsolidation. Importantly, the behavioral consequence of disrupted memory reconsolidation is diminished reactivity to stimuli/cues that previously controlled robust behavioral activation.

The well-known ability of smoking cues (e.g., sight/smell of cigarettes) to elicit craving and other bodily reactions in smokers attempting to quit has long been assumed to result from learned associations that develop over a smoker's extensive history of pairings between the cues and the appetitive pharmacological effects of nicotine. For smokers, the challenge of quitting requires that they deploy a range of self-regulatory processes (e.g., control the impulse to smoke) in order to sustain abstinence. Not surprisingly, encounters with smoking cues elicit strong cravings that represent a significant obstacle to successful self-regulation, which often elevates relapse risk. To address the threat to self-regulation and cessation posed by memories for these learned associations, the overarching goal of this project is to strategically employ sirolimus to attenuate smoking-related memories, thereby decreasing cue-elicited craving, physiological reactivity and smoking.

The proposed study will employ 58 ND treatment-seeking smokers who will be randomly assigned to receive either 15-mg of sirolimus or placebo (group n's=29) immediately after the first of two smoking cue exposure sessions scheduled to occur on consecutive days. The first session will serve as a **Retrieval** session during which smoking (e.g., handling and lighting of a cigarette) cue exposure will elicit retrieval and reconsolidation of smoking-related memories; the second session will be a **Test** session to examine the potential modulatory role of sirolimus on the reconsolidation of memories putatively elicited during the retrieval session. Participants will be required to refrain from smoking the night before (bedtime) their first laboratory (i.e., Retrieval) session and will remain abstinent from smoking 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 the retrieval session. Measures of subjective responses (i.e., craving) and physiological reactivity (i.e., heart rate & skin conductance) 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 7-days following completion of the test session. Treatment effects on self-report measures of smoking behavior during the 7-days preceding the Follow-up session will also be assessed.

Primary specific aim and hypothesis:

Aim: Evaluate the effects of post-retrieval sirolimus vs. placebo on measures of craving and cue reactivity administered 1 day and 1 week following the medicated **Retrieval** session.

Hypothesis: Compared to placebo treated smokers, sirolimus-treated smokers will evidence less craving, emotion/arousal and physiological reactivity to smoking cues presented during the (a) **Test** session and (b) **Follow-up** session.

Secondary specific aim and hypothesis:

Aim: Evaluate the effects of post-retrieval sirolimus vs. placebo on smoking behavior occurring during the one-week follow-up period.

Hypothesis: Compared to placebo-treated controls, sirolimus-treated smokers will evidence changes in smoking behavior during follow-up as indicated on multiple measures including (i) mean cigarettes per day, (ii) days to first cigarette, (iii) percent days non-smoking during follow-up.

Since most smoking relapse occurs in the first week of cessation, a pharmacological aid that decreases craving and cue reactivity may make the difference between successful self-regulation and cessation vs. failed self-regulation and relapse. Favorable outcomes from this study could lead to a new generation of treatment adjuncts that would be brief, easy to administer and cost effective. These treatments could also 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 nicotine addicted smokers. Because smoking is a primary causal factor in lung and other cancers, this translational, treatment-focused project has the potential to significantly advance **cancer prevention and control**.

II. Background

A. Significance:

Conceptual Overview: The role of basic associative learning processes in addiction has been well established in both theory and research[1-8]. In smokers, numerous pairings between smoking-related cues (e.g., pack of cigarettes) and the reinforcing effects of nicotine can result in cues acquiring the ability to elicit a range of conditioned responses, most notably craving and physiological arousal. Importantly, there is substantial evidence that craving is powerfully associated with smoking relapse[9-15]. One viable explanation for this association is that craving undermines the **self-regulatory processes** (e.g., impulse control, emotion regulation, distress tolerance[16-18]) necessary to initiate and maintain cessation. Consistent with this conceptualization are the findings of neuroimaging studies suggesting that failures to self-regulate may occur when connectivity between prefrontal control regions (e.g., lateral prefrontal cortex) and subcortical reward regions (e.g., ventral striatum) are diminished or otherwise impaired[17]. Given this conceptual model, there are at least two approaches by which impaired self-regulation could be treated: a) restore/strengthen prefrontal-subcortical connectivity (see Project 1), and b) diminish the motivational properties of smoking-related cues. **Project 2** will adopt the latter of these two approaches by using an existing FDA-approved medication rapamycin (hereafter sirolimus) to weaken memories for the learned associations between smoking-related cues and nicotine reward, thereby diminishing craving and smoking behavior (cf., Figure 1). The supporting evidence and rationale for the Project 2 is detailed below.

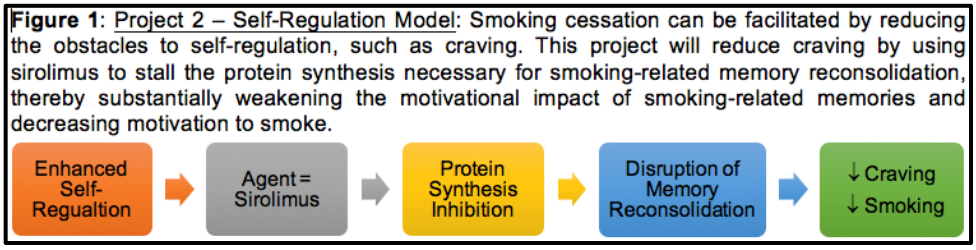

Figure 1: Project 2 – Self-Regulation Model: Smoking cessation can be facilitated by reducing the obstacles to self-regulation, such as craving. This project will reduce craving by using sirolimus to stall the protein synthesis necessary for smoking-related memory reconsolidation, thereby substantially weakening the motivational impact of smoking-related memories and decreasing motivation to smoke.

Scientific Premise: 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 a network of 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 memories 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; we found that a single dose 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]. However, a recent study[42] involving smokers failed to find any effect of propranolol on craving, emotional and physiological reactivity to smoking cues presented in a test performed 1-week post-treatment. These contrasting findings tentatively suggest that a single disrupting agent may not have uniform effects across all addictive disorders and that reconsolidation disruption in smokers might be more profitably pursued with a pharmacological agent other than propranolol.

One candidate agent that has been used in animal studies to demonstrate disruption of reconsolidation (DoR) is the protein synthesis inhibitor, anisomycin[26,43-45]. While robust DoR effects have been observed with anisomycin, they 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 (henceforth 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[47-50]. Second, it has been shown that systemic sirolimus 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[51]. Additionally, a recent Nature Neuroscience report showed that either systemic or intra-amygdalar administration of sirolimus 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[52]. Lastly, a clinical study employing Vietnam era and post-Vietnam era war veterans has provided the first evidence that a 15-mg dose of sirolimus 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[53]. 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 or intracranial 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 sirolimus' exact mechanism of action is unknown, it is likely that it achieves DoR by inhibiting the mammalian/mechanistic target of rapamycin (mTOR) kinase, which regulates phosphorylation of a large number of intracellular targets responsible for protein synthesis and translation[54-58]. 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, post-retrieval administration of sirolimus can disrupt reconsolidation of memories for cue-elicited craving in smokers.

B. Innovation:

The hypothesis being tested is highly innovative in that it draws on an established basic neuroscience phenomenon, disruption of memory reconsolidation (DoR) via protein synthesis inhibition, and seeks to document its potential treatment utility in nicotine addicted smokers. To our knowledge, no other research group is evaluating this highly innovative and potentially important hypothesis. Since the study focuses on the alteration of memory for appetitive addictions-related memory, it could lead to the development of an entirely new intervention strategy that will significantly advance relapse risk reduction in smokers. Positive findings from this study could stimulate treatment development for a broad spectrum of substance abusing populations. Accordingly, the proposed research fits well with NIDA's mission to stimulate clinical neuroscience research that focuses on the neurobiological mechanisms of addiction and its treatment.

The proposed research is also novel because it is translational: it uses basic neuroscience findings as a springboard for the development of novel medication that targets the dampening of the motivational properties of memories for cue-elicited learning involved in the etiology/maintenance of smoking-related ND. The proposed study also challenges an existing paradigm in that it focuses on altering memories for "pathological" learning that subserves smoking-related ND rather than the establishment of new, extinction-based learning to oppose it. The overarching hypothesis is that attenuation of these memories should decrease smoking-related behavior including craving and cue reactivity, thereby potentially enhancing smoking outcomes.

III. Inclusion and Exclusion Criteria/Study Population:

A total of 58 treatment seeking ND men and women smokers (29 per group), aged 18 or older, will be randomized into the study. Participants must smoke 10+ cigarettes/day for three or more years, have a Fagerström Test for Nicotine Dependence score ≥ 4, be willing to make a cessation attempt, and comply with reinforced abstinence requirements for the three laboratory sessions described below. Participants must (i) not be dependent on other substances (may meet criteria for abuse), (ii) willing to use appropriate birth control methods during study participation including 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, (iii) remain abstinent from alcohol and all non-prescription drugs prior to medication administration and testing sessions, (iv) not be undergoing other smoking cessation treatment (e.g., nicotine replacement, Chantix), (v) not be taking medications that may interact with the study medication or alter responding on any study measure, (vi) live with within a 50 mile radius of our research program and have reliable transportation, (vii) consent to fast for a two-hour period prior to medication administration, and (viii) consent to random assignment to rapamycin (sirolimus) vs. placebo conditions. Participants will be excluded if they (i) are undergoing other smoking cessation treatment (e.g Chantix, nicotine replacement), (ii) are pregnant, nursing, or of childbearing potential and not using birth control, (iii) present 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 (sirolimus) has known immunosuppressive properties, (vi) have a history of or current psychotic disorder, severe major depression as evidenced by active and profound psychomotor retardation and/or persistent and intense suicidal ideation, (vii) are currently taking anti-arrhythmic 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) are taking medications that could adversely interact with 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 with a current 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 trauma wounds, and (x) have any planned surgeries within the next month, including surgical dental procedures.

Determining Eligibility. A brief phone screening will preliminarily assess participant suitability via inclusion/exclusion criteria. Qualifying individuals will be scheduled to undergo an IRB-approved informed consent procedure. After consent, all female participants will undergo a urine pregnancy test to confirm a negative test result. Any female participant with a positive urine pregnancy test result will be immediately excluded from the study. Participants will also provide a urine sample for a urine drug screen, a breath sample for a breathalyzer, and breath sample to measure carbon monoxide in the participants' breath. Any participant found to be dependent on other substances or have a CO breath sample indicating a nonsmoker (< 3ppm) will be excluded from the study. (Participants can continue in the study if they test positive for cannabis/marijuana use, but not if they meet criteria for moderate or severe substance use disorder for cannabis/marijuana.) The remainder of the in-person assessment will consist of a comprehensive assessment of substance use, nicotine dependence and general psychiatric functioning. It will include the following: Fagerström Test for Nicotine Dependence (FTND)[61]; Mini-International Neuropsychiatric Interview or MINI from the DSM-V[62,63]. The Timeline Follow-Back (TLFB; a calendar-based instrument with specific probes to obtain detailed information about substance use)[64] will be used to assess smoking and other substance use (a) during the three month period preceding to study involvement, and (b) during the week preceding the Follow-up laboratory session. All participants will undergo a history and physical exam. This includes an electrocardiogram and blood chemistries, which will be performed by the SCTR Research Nexus. 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.

IV. Number of Subjects

We expect to recruit 58 participants, equally allocated to treatment and placebo groups during the active treatment phase of the study. While literature is lacking on studies of the proposed treatment, we used data from our previous and ongoing work to derive an estimated standard deviation of 2.1 for single-item craving rating. Using this estimate and fixing power at 80% and α=.05, we can expect to detect a minimum mean craving rating difference between the two treatment groups of 1.7 with 25 participants randomized to each treatment group (n=50). Although some attrition is expected, the proposed study uses a participant compensation strategy (see below) that is designed to minimize attrition. This strategy was based on one used in our previous NIDA-funded study of treatment-seeking smokers[59]. In this study, we found 5% attrition occurs between two contiguous laboratory sessions where abstinence was required and 15% attrition at 2-week follow-up. Extrapolating to the proposed study, we are confident that 29 participants randomized to each study group (n=58) will provide sufficient power to evaluate expected group differences in this proof of concept study.

V. Setting

The research team will be conducting the research at the Research Nexus and the Addictions Studies suite of MUSC. Both locations have individual rooms for privacy. The Research Nexus has a specific room set up for collecting skin conductance and heart rate from the participants while simultaneously allowing the research assistant to monitor them.

VI. Recruitment Methods

Participants will be primarily recruited using local media (e.g., print, online, radio), a recruitment strategy that has been very successful in our previous and ongoing studies of ND smokers. We may also elect to recruit from the Medical University of South Carolina's (MUSC) Center for Drug and Alcohol Programs (CDAP).

VII. Consent Process

The study PI, Co-I or other research staff will obtain informed consent. The informed consent form includes a detailed description of the study procedures, along with statements regarding participants' rights to withdraw from the procedure at any time without consequences. The informed consent form will be explained to participants in easy-to-understand language, and participants will be instructed to read the form carefully prior to

signing it. Consent will be documented by the signature of the participant on the informed consent agreement, accompanied by the signature of the individual obtaining the consent.

VIII. Study Design/Methods

A. Screening and Diagnostic/Abstinence Assessment:

Screening. All participants will be screened in two parts. First through a phone screen followed by an in-person screening. The screenings are both detailed above in the "determining eligibility" section.

Smoking and Other Drug Abstinence Assessment. Participants will be required to remain abstinent from smoking beginning at bedtime on the night before the (first) **Retrieval** cue exposure session and remain abstinent through the completion of the (second) **Test** session. Participants failing to meet the CO criterion at the Retrieval session will be allowed to reschedule their participation one time; however, if a participant fails the abstinence assessment on the second day, they will be dropped from the study because abstinence violators would be different from abstainers (a) with respect to the reconditioning of the putative learning/memory processes under study, and (b) amount of time elapsing between the Retrieval and Test sessions. Lastly, there will be no abstinence requirement for the 7-day period between the Test and Follow-up sessions; however, participants will be required to abstain from smoking starting at bedtime the night before the **Follow-up** session.

Briefly, participants will be informed that their participation (and associated compensation) in any given laboratory session will necessitate that they provide a breath sample that conforms with a CO criterion of $\leq$ 10 ppm. The full schedule of compensation is described in section F below.

Additionally, abstinence from alcohol and other drug use prior to all laboratory sessions will be confirmed via expired breath alcohol (BAC) and urine drug screen (UDS), respectively. A positive test will result in rescheduling/termination as described above for smoking; however, participants may test positive for cannabis/marijuana and continue to participate.

B. Cue Reactivity Session Measures:

(This team has extensive experience using all listed measures).

Self-Report Measures: The key measure that will be used to quickly and unobtrusively assess smoking craving is: single-item verbal report of subjective craving to smoke. Prior to any stimulus presentation, participants will be trained to provide a verbal report of a numeric value between 0 (none) and 20 (extreme) that best represents their current level of craving ("My urge to smoke right now is"). In addition to single item self-reports, brief multi-item assessments of craving to smoke and emotion will be performed: The Questionnaire of Smoking Urges-Brief (QSU)[65] is a 10-item self-rating questionnaire for assessment of reward-related and withdrawal-related craving. The Mood Form[66] is a 9-item immediate assessment of current negative and positive emotional states.

In the interest of harmonizing measures across projects, we will obtain several additional measures including the Cognitive Emotion Regulation Questionnaire (CERQ), Profile of Mood States (POMS), Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ), Barratt Impulsiveness Scale (BIS) and the Toronto Alexithymia Scale (TAS-20).

Physiological Measures: Heart rate and skin conductance will be measured as indices of physiological arousal during each cue exposure session. 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.

C. Smoking Cue Reactivity Stimuli:

Because we have been successful eliciting smoking craving in previous work with ND smokers[67], we will adopt a similar strategy here. In particular, smoking cues will consist of the handling and lighting of the participant's preferred brand of cigarettes. Participants will be instructed to (a) look at the cigarette pack/lighter, (b) take one cigarette from the pack and hold it in hand as they normally do, (c) handle/smell the cigarette, (d) pick up the lighter and flick the lighter without actually lighting the cigarette, and (e) light the cigarette without smoking and then extinguish it. This exposure sequence will take approximately 4 minutes.

D. Randomization, Rapamycin Dosing, Preparation and Administration:

Randomization. Stratified block randomization will be used to assign participants to groups while balancing treatment assignment based on gender and Fagerström Test for Nicotine Dependence (FTND) score. Both are known to influence smoking cue reactivity and/or behavior. Dependence will be balanced on FTND score ≤5 or >5. This method, when used with smaller block sizes, is appropriate for small study sample sizes[68] and has been used in a number of previous and ongoing studies by our research group.

Sirolimus 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[69-71]. Additionally, sirolimus adequately crosses the blood brain barrier, with an estimated blood to brain ratio of 1:3[72]. 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[73]. 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 $\mu g/L*h$[74]. 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 the proposed study. The pivotal trials used to gain approval for sirolimus in transplantation had an arm that received a loading dose of 15 mg orally during the perioperative period[75-77].

Lastly, we recently received (January 2018) both Investigational New Drug (IND) approval from the FDA and MUSC IRB approval for a similar study involving a single 15-mg administration of sirolimus in individuals who misuse alcohol.

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

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

E. Laboratory Session Procedures:

Session Preparation. Abstinence will be assessed (see above **A**, 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 measures 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 smoking-related memories. To achieve this objective, participants will be exposed to the smoking cues as described above (view, handle, light and extinguish the cigarette). A craving rating will be obtained midway through the smoking cue exposure (i.e., the inquiry will be made by research staff via headphones). Immediately following smoking 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 and on one final occasion at 50-min post-medication (see GENERAL PROCEDURES TABLE above). Although HR and SC will be collected continuously during the smoking 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. Approximately one hour after medication administration, participants will have blood drawn for the purpose of determining sirolimus levels; this will permit subsequent assessment of the association between blood levels of sirolimus and primary/secondary outcomes. Immediately after the blood draw, participants will be asked to complete a brief questionnaire that asks whether or not they thought they received study medication or placebo. Lastly, the participant will be reminded of the smoking abstinence requirement and instructed not to smoke or use other substances (abstinence serves to mitigate the confounding reconditioning effects of smoking on the reconsolidation processes initiated in this session).

GENERAL PROCEDURE TABLE	Measurement				
	Single-Item Craving	HR	SC	Mood Form	QSU
Measurement Occasion					
Baseline Measures	X	X	X	X	X
During Smoking Cues	X	X	X		
Immediate Post-Smoking Cues	X	X	X	X	X
10-, 20-, 30-, 40- and 50-Min Post	X	X	X	X	X

Table Notes: HR=heart rate; SC=skin conductance; QSU=Questionnaire of Smoking Urges-Brief; Monitoring of Side Effects Scale (MOSES) will be administered during the 50-min post assessment of the cue exposure sessions. Timeline Follow-Back assessment of cigarette use over the intervening week (if participant fails to complete smoking diary) will be done at the end of Follow-up session.

Test session 2 procedure. This session provides an opportunity to test the hypothesis that rapamycin will acutely attenuate craving and physiological reactivity to smoking cues. 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 smoking diary in which to record the occurrence of any smoking behavior over the week follow-up period (abstinence is not required during the week preceding Follow-up session). Finally, the experimenter will provide compensation and schedule the Follow-up cue reactivity session.

Follow-up session procedure. This session affords the opportunity to assess the maintenance of any effects observed in Test session 2 and the effects of treatment on smoking behavior. In the Follow-up session, participants will experience the same session preparation and Retrieval session 1 procedures as described above with the following exceptions. First, no medication will be administered. Second, smoking diary data will be collected (research staff will perform a TLFB assessment of smoking behavior if participants do not present with their smoking dairy). Finally, debriefing will take place to address questions or concerns that participants may be experiencing (blinding will be maintained). Referral for additional cessation treatment will be provided.

F. 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 hired and 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 18 months and plan to allow two months for final data cleaning/reduction, analysis and manuscript preparation (albeit manuscript preparation will begin sooner). At a recruitment rate of approximately 2-3 participants per month, we should have no difficulty in completing the study in the two-year timeframe. With regard to recruitment milestones, we anticipate being able to recruit approximately 26 ND smokers in the 8-month recruitment period of the first year and the remaining 32 participants in the 10-month recruitment period of the 2nd year.

G. Participant Compensation:

The compensation strategy for this study is designed to (i) provide remuneration for participant time and effort, (ii) maintain adherence to laboratory session schedule, and (iii) minimize abstinence violation and participant attrition. We have used this compensation strategy in a previous study (noted above) involving treatment-seeking smokers and have had very high levels of compliance with smoking and other substance abstinence.

Participants will be compensated as follows: Screening and assessment = $50.00; Lab sessions 1 & 2 (Retrieval and Test) = $75.00 each; 1-week Follow-up session = $100.00. Maximum compensation for participation is $300.00.

IX. Data Management

Primary Hypothesis. Mixed effects models will be used to test the hypothesized group differences at the Test (acute effect) and 1-week Follow-up (maintenance effect) laboratory sessions. 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). Other self-report measures (e.g., QSU, affect ratings, etc.) and HR/SC measures will be analyzed in a similar manner. Additional measures of craving, such as Area Under the Curve (AUC) and/or maximum within-session decrement in craving may be considered as outcomes. It may also be of interest to fit a covariance pattern model, analyzing craving over time, while accounting for treatment and session (Retrieval, Test & Follow-up Sessions). Secondary Hypothesis. Treatment group differences on various indices of smoking behavior (time to first cigarette, % days abstinent, CPD) will be assessed using general linear regression models while controlling for potential covariates. Continuous outcomes (% days abstinent, CPD) will be analyzed using normal linear models while time to first cigarette will be assessed using time to event models for efficacy analysis (Log-rank, Cox PH Models). Binary outcomes will be assessed using Logistic regression models. While only large group differences are likely to be statistically detectable, analysis of smoking behavior is consistent with the interventional focus of this research and will provide efficacy estimates to inform sample size estimation for a larger study.

X. Provisions to Monitor the Data to Ensure the Safety of Subjects

Data and Safety Monitoring Board. A Data and Safety Monitoring Board (DSMB) will be created to ensure the safety of participants and the validity and integrity of data collected during the project. The board will consist of Drs. Hugh Myrick, Bryan Tolliver and Karen Hartwell, all board-certified addiction psychiatrists, and Royce Sampson, R.N., MSN, the Director of the Regulatory Core for the CTRC. The board will meet as needed to review the adverse events related to the study, and recommendations for appropriate action to maintain a favorable safety profile will be made. The board will also ensure that all serious adverse events have been followed to resolution, and that the appropriate agencies (including the IRB and internal funding agency) have been informed of the event.

Adverse Event Monitoring

Adverse events will be monitored throughout the study and all events will be followed to resolution or stabilization. All serious adverse events will be collected and reported immediately to the IRB and the internal funding agency (Hollings). Serious adverse events related to rapamycin (sirolimus) will be reported to the FDA. A serious adverse event is one that meets any of the following criteria:

1. Fatal or life threatening
2. Requires or prolongs inpatient hospitalization
3. Results in persistent or significant disability/incapacity
4. Congenital anomaly
5. Medical event that may jeopardize the participant or require intervention to mitigate serious outcome
6. Cancer
7. Overdose
8. Results in the development of drug dependency or abuse.

XI. Risks to Subjects

Rapamycin (Sirolimus; Rapamune®) Dosage 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 in the main application, there was a dosing precedent established in the clinical literature. Specifically, the previously noted study by 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.

In addition to the precedent set by the Suris et al study, there is strong animal and clinical pharmacokinetic (PK) and pharmacodynamic (PD) data to demonstrate the proposed 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 administration1-3

Additionally, sirolimus adequately crosses the blood brain barrier, with an estimated blood to brain ratio of 1:34. In a clinical PK study in health males, an 8-mg/m2 one-time oral dose produced a whole blood Cmax of 115.2 ng/mL at 1.0 hour after ingestion. This corresponds to an approximate peak brain concentration of 38.4 ng/mL60. 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 day5. 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 AUC0-24h of 270□12 □g/L*h6. By using the trapezoidal rule, the AUC0-□ in rats for an 8 mg/kg dose would approximate 350 □g/L*h. This dose of 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 period6.

Other Considerations. Participants may experience some craving and arousal with exposure to smoking cues. However, the length of each cue exposure is brief, and no participants among the several hundreds tested with similar procedures in our previous research in this area reported feelings of prolonged distress. Therefore, participant risk is considered minimal, and there are no relevant alternative procedures that would be more advantageous for participants. If a participant is intolerant of the interview or cue reactivity procedures, the participant will be withdrawn from the study and appropriate therapeutic measures (brief intervention performed by senior investigator, appropriate clinical referral, etc) will be undertaken. In the event that a participant experiences elevated craving at the end of a cue reactivity 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. Confidentiality will be maintained at all times. Participants who would like research staff to discuss their study participation with their personal therapist will be required to sign a release of information form.

Protection Against Risk. Participants will be informed of the potential side effects of rapamycin/sirolimus and will be closely followed by either a study physician, Pharm.D. or other member of the research team. Rapamycin (sirolimus) administration will occur in a fully staffed clinical environment (MUSC's CTRC). Drs. Saladin, Gray or Brady will monitor all study participants for psychiatric stability, and Drs. Gray, Taber and Brady will monitor for medical stability. Adverse Events (AEs) will be assessed by the medical clinician at all visits using the Monitoring of Side Effects Scale (MOSES). The medical clinician will be available 24/7 by phone/pager, and study staff will have phone contact with participants at least once during the follow-up period. Adverse events will be coded at all visits using Medical Dictionary for Regulatory Activities (MedDRA) rules, and entered into a database. For each study meeting, study staff will prepare a summary of all AEs, including their severity and presumed relation to study medication. The PI will review this at the weekly study meeting (or before if more urgent).

Guidelines have been developed for managing and reporting of AEs, including serious adverse events (SAE; defined as any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others). If an AE is non-serious (self-limited with no intervention needed), no further action will be necessary. However, in the case of a serious, unresolved event, an AE follow-up log will be completed. The first member of the research team to become aware of a SAE will notify the study physician and PI immediately. Study staff will record the information on SAE Notification Form. The clinician will forward hard copies of the complete report (SAE Notification Form, Concomitant Medication Log, and AE Log) to Dr. Saladin, who will, in turn notify the IRB and DSMB about the SAE. If the event is "Serious, Unexpected and Associated" (an SAE is considered unexpected if it is not described in the Package Insert), Dr. Saladin will complete Food and Drug Administration (FDA) Form 3500A and will forward it to the FDA. Dr. Saladin also will inform the IRB about the SAE. In all of these reviews and reports, strict patient confidentiality will be maintained.

The instrumentation used for physiological recordings meets all safety standards for non-invasive recordings, and participants are located out of reach of any AC-powered devices in the laboratory.

All sessions will be conducted under the supervision of trained personnel. In the unlikely event that crisis intervention is necessary, senior staff will be available to evaluate the participant and provide an intervention or referral.

All participants will be fully informed that they may withdraw from the experiment at any time without penalty. All participant records will be kept in a locked filing cabinet, and confidentiality of all materials will be maintained. Offices also will be locked when not in use.

To ensure confidentiality, participant data will be number coded, and only the investigators will have access to the master lists of codes. All participant records will be kept in an office that will be locked at times when not in use. The research staff understands the importance of maintaining confidentiality. This method of maintaining confidentiality has been used for several years by our research group and has been effective.

XII. Potential Benefits to Subjects or Others

Possible risks to study participants include distress, craving and arousal as a result of exposure to smoking cues. Additionally, adverse reactions to rapamycin (sirolimus) are possible (though this medication is known to be very well tolerated at the proposed single dose, as noted above). Benefits include detailed assessments of smoking and other substance use; participants will also receive appropriate professional referral as dictated by the outcome of their clinical/diagnostic assessment. Moreover, participants may benefit from their experience during the cue reactivity procedure and these benefits may include a potential decrease/elimination of craving to smoking cues and/or smoking. The minimal risks are reasonable in relation to the benefits to be gained from the investigation. The clinical assessment, cue reactivity procedures and/or medication interventions may provide important information that can guide treatment for future patients with nicotine dependence. This information can be provided to participants' health care providers (with signed consent) if individual participants make this request. In addition, understanding the mechanisms underlying memories for, and responses to, smoking cues is important for the development of more effective treatment for nicotine dependence. Thus, the benefits of this study outweigh the risks.

Importance of the Knowledge to be Gained. This study may provide important information that can improve treatment for individuals with nicotine dependence. More specifically, this research could lead to the development a new class of therapeutic agents to be used in the treatment of nicotine dependence. It is also possible that rapamycin (sirolimus) may be shown in the future to be similarly beneficial in the treatment of persons with other substance use disorders (i.e., cocaine, methamphetamine, marijuana, etc.).

XIII. Future Directions

If sirolimus exerts the expected disruptive effect on memory reconsolidation, then attenuated craving and other cue-elicited reactions should be observed during subsequent presentations of the smoking-related cues. Additionally, it is possible that reduced craving and cue reactivity will translate into changes in smoking behavior. Favorable outcomes from this study would set the stage for a larger, phase II RCT within the context of a NIH program project grant and could lead to a new generation of brief and strategic neuropharmacological interventions that substantially improve cessation rates by reducing craving-related obstacles to self-regulation. This type of intervention could be especially important early during a quit attempt when craving-related risk of relapse is greatest. It could also become a complementary treatment paradigm that would operate synergistically with the current generation of pharmacological and behavioral interventions to reduce the cancer burden posed by this most prevalent form of addiction. More broadly, this project fits within our larger Team Science application, which proposes a translational framework to identify/develop (Projects 1 & 2) or apply (Project 3) novel, evidence-based treatments that either (1) directly target processes subserving self-regulation (Project 1) or target obstacles to self-regulation (Projects 2 & 3) in the interest of advancing smoking cessation. We believe this collection of studies will put us in strong position for future development of program project funding.

Literature Cited

1. Childress AR, Ehrman RE, Rohsenow DJ, Robbins SJ, O'Brien CP. Classically Conditioned Factors in Drug Dependence. In: Lowinson JH, Ruiz P, Millman RB, eds. *Substance Abuse A Comprehensive Textbook* 2nd ed. Baltimore: Williams & Wilkins; 1992:56-69.
2. Eikelboom R, Stewart J. Conditioning of Drug-Induced Physiological Responses. *Psychological Review.* 1982;89(5):507-528.
3. Niaura RS, Rohsenow DJ, Binkoff JA, Monti PM, Pedraza M, Abrams DB. Relevance of cue reactivity to understanding alcohol and smoking relapse. *Journal of Abnormal Psychology.* 1988;97(2):133-152.
4. Wikler A. Conditioning processes in opioid dependence and in relapse. In: Wikler A, ed. *Opioid dependence: Mechanisms and treatment*. New York: Plenum Press; 1980.
5. Hogarth L, Balleine BW, Corbit LH, Killcross S. Associative learning mechanisms underpinning the transition from recreational drug use to addiction. *Ann N Y Acad Sci.* 2013;1282:12-24.
6. Hyman SE. Addiction: a disease of learning and memory. *Am J Psychiatry.* 2005;162(8):1414-1422.
7. Robbins TW, Ersche KD, Everitt BJ. Drug addiction and the memory systems of the brain. *Ann N Y Acad Sci.* 2008;1141:1-21.
8. Robbins TW, Everitt BJ. Limbic-striatal memory systems and drug addiction. *Neurobiol Learn Mem.* 2002;78(3):625-636.
9. Piasecki TM. Relapse to smoking. *Clin Psychol Rev.* 2006;26(2):196-215.
10. Weiss F. Neurobiology of craving, conditioned reward and relapse. *Curr Opin Pharmacol.* 2005;5(1):9-19.
11. Ferguson SG, Shiffman S. The relevance and treatment of cue-induced cravings in tobacco dependence. *J Subst Abuse Treat.* 2009;36(3):235-243.
12. Herd N, Borland R, Hyland A. Predictors of smoking relapse by duration of abstinence: findings from the International Tobacco Control (ITC) Four Country Survey. *Addiction.* 2009;104(12):2088-2099.
13. Killen JD, Fortmann SP. Craving is associated with smoking relapse: findings from three prospective studies. *Exp Clin Psychopharmacol.* 1997;5(2):137-142.
14. Shiffman S, Gwaltney CJ, Balabanis MH, et al. Immediate antecedents of cigarette smoking: an analysis from ecological momentary assessment. *J Abnorm Psychol.* 2002;111(4):531-545.
15. Shiftman S, Paty JA, Gwaltney CJ, Dang Q. Immediate antecedents of cigarette smoking: an analysis of unrestricted smoking patterns. *J Abnorm Psychol.* 2004;113(1):166-171.
16. Duckworth AL, Gendler TS, Gross JJ. Situational Strategies for Self-Control. *Perspect Psychol Sci.* 2016;11(1):35-55.
17. Heatherton TF. Neuroscience of self and self-regulation. *Annu Rev Psychol.* 2011;62:363-390.
18. Roos CR, Witkiewitz K. A contextual model of self-regulation change mechanisms among individuals with addictive disorders. *Clin Psychol Rev.* 2017;57:117-128.
19. McGaugh JL. Memory--a century of consolidation. *Science.* 2000;287(5451):248-251.
20. Alberini CM. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? *Trends in Neuroscience.* 2005;28(1):51-56.
21. Dudai Y. The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol.* 2004;55:51-86.
22. Schafe GE, LeDoux JE. Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *J Neurosci.* 2000;20(18):RC96.
23. Schafe GE, Nader K, Blair HT, LeDoux JE. Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends Neurosci.* 2001;24(9):540-546.
24. Dudai Y. Reconsolidation: the advantage of being refocused. *Curr Opin Neurobiol.* 2006;16(2):174-178.
25. Lee JL, Di Ciano P, Thomas KL, Everitt BJ. Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron.* 2005;47(6):795-801.
26. Sara SJ. Retrieval and reconsolidation: toward a neurobiology of remembering. *Learning and Memory.* 2000;7(2):73-84.
27. Tronson NC, Taylor JR. Molecular mechanisms of memory reconsolidation. *Nat Rev Neurosci.* 2007;8(4):262-275.
28. Alberini CM. *Memory reconsolidation.* London ; New York: Elsevier Academic Press; 2013.
29. Alberini CM, Ledoux JE. Memory reconsolidation. *Curr Biol.* 2013;23(17):R746-750.
30. Nader K, Einarsson EO. Memory reconsolidation: an update. *Ann N Y Acad Sci.* 2010;1191:27-41.
31. Finnie PS, Nader K. The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. *Neurosci Biobehav Rev.* 2012;36(7):1667-1707.

32. Das RK, Freeman TP, Kamboj SK. The effects of N-methyl D-aspartate and B-adrenergic receptor antagonists on the reconsolidation of reward memory: a meta-analysis. *Neurosci Biobehav Rev.* 2013;37(3):240-255.
33. Kindt M, Soeter M, Vervliet B. Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat Neurosci.* 2009;12(3):256-258.
34. Soeter M, Kindt M. Dissociating response systems: erasing fear from memory. *Neurobiol Learn Mem.* 2010;94(1):30-41.
35. Soeter M, Kindt M. Erasing fear for an imagined threat event. *Psychoneuroendocrinology.* 2012;37(11):1769-1779.
36. Schwabe L, Nader K, Pruessner JC. beta-Adrenergic blockade during reactivation reduces the subjective feeling of remembering associated with emotional episodic memories. *Biol Psychol.* 2013;92(2):227-232.
37. Schwabe L, Nader K, Wolf OT, Beaudry T, Pruessner JC. Neural signature of reconsolidation impairments by propranolol in humans. *Biol Psychiatry.* 2012;71(4):380-386.
38. Brunet A, Orr SP, Tremblay J, Robertson K, Nader K, Pitman RK. Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *J Psychiatr Res.* 2008;42(6):503-506.
39. Brunet A, Poundja J, Tremblay J, et al. Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials. *J Clin Psychopharmacol.* 2011;31(4):547-550.
40. Poundja J, Sanche S, Tremblay J, Brunet A. Trauma reactivation under the influence of propranolol: an examination of clinical predictors. *Eur J Psychotraumatol.* 2012;3.
41. Saladin ME, Gray KM, McRae-Clark AL, 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).* 2013;226(4):721-737.
42. Pachas GN, Gilman J, Orr SP, et al. Single dose propranolol does not affect physiologic or emotional reactivity to smoking cues. *Psychopharmacology (Berl).* 2015;232(9):1619-1628.
43. Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci.* 2004;24(20):4787-4795.
44. Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature.* 2000;406(6797):722-726.
45. Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol.* 2001;11(2):180-187.
46. Rudy JW, Biedenkapp JC, Moineau J, Bolding K. Anisomycin and the reconsolidation hypothesis. *Learn Mem.* 2006;13(1):1-3.
47. Blundell J, Kouser M, Powell CM. Systemic inhibition of mammalian target of rapamycin inhibits fear memory reconsolidation. *Neurobiology of Learning and Memory.* 2008;90(1):28-35.
48. Parsons RG, Gafford GM, Helmstetter FJ. 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.* 2006;26(50):12977-12983.
49. Mac Callum PE, Hebert M, Adamec RE, Blundell J. Systemic inhibition of mTOR kinase via rapamycin disrupts consolidation and reconsolidation of auditory fear memory. *Neurobiol Learn Mem.* 2013.
50. Zubedat S, Akirav I. The involvement of cannabinoids and mTOR in the reconsolidation of an emotional memory in the hippocampal-amygdala-insular circuit. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology.* 2017;27(4):336-349.
51. Lin J, Liu L, Wen Q, et al. Rapamycin prevents drug seeking via disrupting reconsolidation of reward memory in rats. *Int J Neuropsychopharmacol.* 2014;17(1):127-136.
52. Barak S, Liu F, Ben Hamida S, et al. Disruption of alcohol-related memories by mTORC1 inhibition prevents relapse. *Nat Neurosci.* 2013;16(8):1111-1117.
53. Suris A, Smith J, Powell C, North CS. Interfering with the reconsolidation of traumatic memory: sirolimus as a novel agent for treating veterans with posttraumatic stress disorder. *Ann Clin Psychiatry.* 2013;25(1):33-40.
54. Tang SJ, Reis G, Kang H, Gingras AC, Sonenberg N, Schuman EM. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc Natl Acad Sci U S A.* 2002;99(1):467-472.
55. Raught B, Gingras AC, Sonenberg N. The target of rapamycin (TOR) proteins. *Proc Natl Acad Sci U S A.* 2001;98(13):7037-7044.
56. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004;18(16):1926-1945.

57. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. Translational control of long-lasting synaptic plasticity and memory. *Neuron.* 2009;61(1):10-26.
58. Li J, Kim SG, Blenis J. Rapamycin: one drug, many effects. *Cell Metab.* 2014;19(3):373-379.
59. Germeroth LJ, Carpenter MJ, Baker NL, Froeliger B, LaRowe SD, Saladin ME. Effect of a Brief Memory Updating Intervention on Smoking Behavior: A Randomized Clinical Trial. *JAMA Psychiatry.* 2017;74(3):214-223.
61. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict.* 1991;86(9):1119-1127.
62. 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).
63. 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).
64. Sobell LC, Sobell MB. Timeline followback: A technique for assessing self-reported ethanol consumption. In: Allen J, Litten RZ, eds. *Measuring alcohol consumption: Psychological and Biological Methods* Totowa, NJ: Humana Press; 1992:41-72.
65. Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine Tob Res.* 2001;3(1):7-16.
66. Diener E, Emmons RA. The independence of positive and negative affect. *Journal of personality and social psychology.* 1984;47(5):1105-1117.
67. LaRowe SD, Saladin ME, Carpenter MJ, Upadhyaya HP. Reactivity to nicotine cues over repeated cue reactivity sessions. *Addict Behav.* 2007;32(12):2888-2899.
68. Hedden SL, Woolson RF, Malcolm RJ. Randomization in substance abuse clinical trials. *Subst Abuse Treat Prev Policy.* 2006;1:6.
69. Brattstrom C, Sawe J, Jansson B, et al. Pharmacokinetics and safety of single oral doses of sirolimus (rapamycin) in healthy male volunteers. *Ther Drug Monit.* 2000;22(5):537-544.
70. Mahalati K, Kahan BD. Clinical pharmacokinetics of sirolimus. *Clin Pharmacokinet.* 2001;40(8):573-585.
71. Stenton SB, Partovi N, Ensom MH. Sirolimus: the evidence for clinical pharmacokinetic monitoring. *Clin Pharmacokinet.* 2005;44(8):769-786.
72. Meikle L, Pollizzi K, Egnor A, 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.* 2008;28(21):5422-5432.
73. Calne RY, Collier DS, Lim S, et al. Rapamycin for immunosuppression in organ allografting. *Lancet.* 1989;2(8656):227.
74. Barten MJ, Streit F, Boeger M, et al. Synergistic effects of sirolimus with cyclosporine and tacrolimus: analysis of immunosuppression on lymphocyte proliferation and activation in rat whole blood. *Transplantation.* 2004;77(8):1154-1162.
75. Groth CG, Backman L, Morales JM, et al. Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. *Transplantation.* 1999;67(7):1036-1042.
76. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. *Lancet.* 2000;356(9225):194-202.
77. Rapamune (sirolimus) [package Insert]. Philadelphia, PA: Wyeth Pharmaceuticals Inc.; 2017.