Cover Page

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PROTOCOL TITLE: Targeting Foundational Memory Processes in Nicotine Addiction: A Translational Clinical Neuroscience Study of a Retrieval-Extinction Intervention to Reduce Craving and Smoking Behavior

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1.0 Objectives / Specific Aims

Recent research has shown that lasting reductions in drug seeking behavior can be achieved with a simple, dual element behavioral intervention known as **retrieval-extinction training or RET.** The first element of RET involves briefly presenting drug-associated cues in order to retrieve drug use memories. The second element, occurring after a brief interval, involves conducting extinction training (i.e., massed unreinforced exposure to drug-associated cues). It is argued that the initial retrieval of the memories prior to extinction training initiates a period of instability, which is followed by reconsolidation of the memories back into long-term storage. Extinction training during the period of instability is presumed to over-write the original cue->drug association with a cue->no drug association, thereby attenuating expression of drug-seeking behavior. A clinical translational study reported in Science showed, in heroin addicts, that craving and cue reactivity could be profoundly impaired by two sessions of RET; importantly, this effect was still evident 6-months post-intervention. Recently, we completed a NIDA-funded R21 designed to replicate and extend these findings in smokers. In this study (see JAMA Psychiatry, March 2017), one group of smokers (Group R-E) received two sessions of R-E training while a second 'no-retrieval' group (Group NR-E) received identical training except the retrieval cues were not smoking related. Results indicated that craving to both familiar and novel smoking cues was significantly lower for Group R-E vs. NR-E at 1-mo. post-treatment. Most importantly, Group R-E vs. NR-E reported a significant 25% reduction in cigarettes smoked per day (CPD) during the follow-up period. There was also trend level evidence of a slower latency to relapse in Group R-E. These findings are especially compelling given that the (i) only difference between the groups was two exposures to a 5-min 'retrieval' video with smoking (vs. neutral) content, and (ii) study was powered only to detect craving differences.

The overarching goal of the proposed study will be to replicate and extend these very encouraging initial findings by 1) increasing the dose of RET (3 vs. 2 sessions) so as to bolster treatment effects, 2) employing fMRI methods to identify patterns of brain activity (BOLD signal changes in regions of interest, ROIs, and functional connectivity) uniquely associated with R-E training and potentially predictive of treatment outcome, and 3) extending follow-up period to more completely assess the long-term effects of RET. Two groups of treatment-seeking smokers will remain abstinent overnight prior to undergoing a baseline cue reactivity assessment and fMRI session (only a portion of each group will undergo fMRI pre- and post-treatment. On their target-quit day, a three-day intervention phase will begin. On each day, Group R-E will receive RET whereas Group NR-E will first be administered retrieval cues that do not contain smoking content (NR) and then receive extinction training (E). The next day, all groups will undergo a cue reactivity assessment and fMRI session identical to baseline. This will end a four-day period of compensated abstinence designed to limit the possibility of reinforcing smoking memories via nicotine administration. Both groups will monitor their smoking behavior and undergo identical laboratory test sessions over a 6-month follow-up period. The aims/hypotheses are:

Aim 1: Assess the acute and long-term effects of R-E training on indices of smoking behavior/relapse.

H1) Relative to control, Group R-E will show decreased smoking (CPD) during follow-up.

Secondary H1) Relative to control, more participants in Group R-E will achieve a 60% smoking reduction (from baseline) during follow-up.

Aim 2: Assess RET effects (vs. baseline/control) on fronto-limbic-striatal circuitry function & behavior.

H2.a) Cue reactivity: Group R-E will evidence attenuated CR BOLD in medial prefrontal cortex (mPFC: dorsal anterior cingulate-dACC; ventromedial: vmPFC), limbic regions (amygdala, hippocampus) and ventral striatum. Greater reduction in BOLD response in regions of interest will predict decreased smoking.

H2.b): Group R-E will evince strengthened resting-state functional connectivity (rsFC) between mPFC regions and ventral striatum (frontostriatal circuits), reduced rsFC between mPFC and amygdala (frontolimbic); and the relative magnitude of change in rsFC will be inversely related to craving and smoking (e.g., CPD).

Aim 3: Assess the acute and long-term effects of R-E training on craving and cue reactivity (CR).

H3) Relative to control, Group R-E will evince lower craving/cue reactivity during post-treatment testing.

Positive findings from this study could significantly advance exposure-based treatment of smoking. The study may elucidate unique patterns of RET-induced brain activity and identify relationships between brain responses and clinical outcomes, the latter of which could inform treatment optimization. An RET treatment adjunct would be appealing to clinicians and consumers because it would be brief, low cost/burden, easily administered and amenable to use with first first-line medications (e.g., nicotine patch). Most importantly, RET would be easily adapted for treatment of other addicted populations, so successful completion of this study would lead to several immediate translational opportunities.

2.0 Background

Significance

Smoking occurs in approximately 19% of the US population and is responsible for an annual mortality rate of approximately 480,000 US citizens and an economic burden of $176 billion[1-5]. The suboptimal outcomes achieved with existing medications may owe in part to the fact that they do not address foundational learning-memory processes involved in smoking addiction. We recently completed a NIDA-funded study[6] that provided the first evidence that reductions in important smoking outcomes can be achieved with a brief behavioral intervention that targets smoking-related memory processes. The proposed study represents the next logical step in the replication and extension of these promising findings, the long-term goal of which is to improve treatment for the single greatest addiction-related public health problem.

A. Scientific Premise:

i. Initial learning initiates a consolidation process consisting of a complex array of neurobiological processes that govern the gradual stabilization of memory into long-term storage/memory (LTM). Prior to consolidation, memories occupy a labile state and can be altered/disrupted by various means (e.g., protein synthesis inhibitors[7,8]). Relevant stimulus event(s) can prompt or retrieve memories back into an active state where they will again occupy a time-limited period of instability prior to being restabilized into LTM. This post-retrieval transition from destabilization and restabilization is known as reconsolidation and while it is known to have properties in common with consolidation[9-11], there are important differences[12] suggesting that reconsolidation is more than a recapitulation consolidation[13]. Arguably, the most important distinction between these two memory processes is that consolidation, by definition, affords only one opportunity to alter/disrupt important memory processes whereas reconsolidation offers many. Because the neurocognitive systems that govern learning/memory processes are central to the etiology of clinical disorders (e.g., substance abuse, PTSD), retrieval-induced memory lability is receiving increased attention as an intervention target.

ii. Neuropathophysiology of memory systems in drug addiction. Mechanistic research demonstrates that chronic use of addictive substances, including nicotine[14] produces neuroplasticity in the medial prefrontal cortex (mPFC)—a region that codes for the relevance of stimuli in the environment[15], medial temporal lobe (MTL) memory structures (e.g. limbic regions: amygdala, hippocampus[16]); and augments neural communication between the mPFC, MTL and the ventral striatum—a region involved in reward prediction and appetitive behavior[17]—henceforth fronto-limbic-striatal circuitry. Though the extant literature has reported on the effects of extinction training on fronto-limbic-striatal circuitry[18], there is a dearth of mechanistic studies on reconsolidation, in particular in the context of models of substance abuse.

iii. Memory Modulation and Implications for Addictive Behavior. Most basic neuroscience studies that have targeted reconsolidation have focused on two general strategies. The first strategy involves the use of pharmacological agents (e.g. β-adrenergic antagonists, propranolol) to disrupt or alter reconsolidation. To illustrate, our group has demonstrated that post-retrieval propranolol may dampen cocaine craving and cue reactivity in cocaine users[19]. Though propranolol's neural effects on addictive behaviors are largely unknown, propranolol has been shown to reduce amygdala[20,21] and mPFC[22] response to fear memories concomitant with reducing PTSD symptoms, providing evidence that disrupting reconsolidation is, at least partially, mediated through fronto-limbic circuitry.

The second strategy—the focus of this proposal, henceforth retrieval-extinction training or RET, is a behavioral intervention that involves brief cue-elicited retrieval followed shortly thereafter by extinction (i.e., unreinforced presentations of conditioned cues). It is postulated that the brief cue presentation(s) results in destabilization of target memories, which are then either disrupted or updated with new information via extinction training. The result is that memory-relevant behavior is either altered or eradicated. It is further proposed that RET is distinct from extinction in that the former directly alters the original memory whereas the latter produces new inhibitory learning[23], through frontostriatal pathways[24], that opposes the expression of the original memory. Because extinction leaves memory intact, the behavior associated with the memory can spontaneously recover after the passage of time, be renewed when cues are encountered in novel circumstances, or be reinstated following occurrence of the reinforcer used to establish the original learning[25-29]. For these reasons, extinction is generally disappointing as an addiction intervention[29,30].

The seminal works on RET were conducted by Monfils et al., (Science, 2009)[31] and Schiller et al., (Nature, 2010). These studies showed, in rats and humans, that RET could produce a loss of conditioned fear that was resistant to the known pitfalls of extinction. Since then, 18 published reports have yielded results consistent with the seminal study[32-49]. Collectively, these studies demonstrate the memory modulating effects of RET under varied testing conditions (tests of spontaneous recovery, renewal, reinstatement, etc.), across different species (human, rats and mice), using both aversive conditioning (shock, loud noise) and appetitive conditioning (food, alcohol, cocaine, morphine, heroin and nicotine) procedures. However, there are 12 reports of inconsistent findings and failures to replicate[50-61]. In many cases, these inconsistencies/failures appear to be explained by between-study methodological differences[43,54,62] and subject individual differences[46,49,63]. Additionally, in at least one of these studies the negative findings may have resulted from RET parameters that failed to initiate memory reconsolidation[60,62]. In two other reports, RET produced positive findings but similar effects were found in a control condition where the training regimen was reversed (i.e., ERT, extinction was conducted before rather than after retrieval)[55,59]. While this outcome appears to be at odds with the reconsolidation hypothesis, it is not necessarily so if one accepts the very real possibility that the ERT outcomes were the result of some process unrelated to memory reconsolidation. Regardless of the conceptual implications of these unexpected findings, they do not represent a challenge to the clinical-translational potential of RET. Lastly, it appears that RET may be especially suited to updating appetitive memories. Of the studies that have targeted memories for food- and drug-reinforced learning, there are 7 published reports of positive findings and only one with inconsistent findings. Moreover, the inconsistent study was one of the two studies where both RET and ERT attenuated reinstatement. As already noted, this is not so much a failure of RET as it was an unexpected

outcome in a control condition. In sum, the mixed findings in infrahuman and human laboratory studies of RET suggest that there are boundary conditions or minimum parameter thresholds that must be met to realize optimal levels of behavioral suppression. When this is achieved, RET appears to be a highly effective behavioral strategy for updating memories, especially those established via appetitive/drug reward.

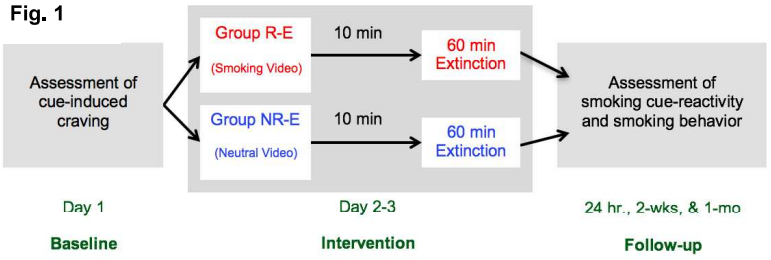
Only two published studies have used RET as treatment with a clinical population. One[64], employed RET with spider phobics, and reported significant clinical benefits in both the RET and control groups. However, the equivalent outcomes were likely due to either a failure to induce reconsolidation or implicit fear reactivation in both groups. The other study, by Xue et al (Science, 2012)[35], was a well-controlled assessment of RET's effects on craving and cue reactivity in a sample of inpatient heroin addicts. Results indicated that the R-E group evidenced substantially attenuated heroin craving and cue reactivity compared to controls (one control received retrieval involving non-drug cues and one control received extinction training after reconsolidation was complete). Remarkably, these effects persisted for 6 months post-treatment. Our NIDA-funded R21 was built on these impressive initial findings, and specifically examined the effects of RET on the craving, cue reactivity and smoking behavior of cigarette smokers.

B. Scientific Premise: Results of NIDA-funded R21 (R21DA035993)

As in the Xue et al study, our study (published JAMA Psychiatry[6], March 2017) employed a 'retrieval' video cue to putatively initiate the memory destabilization that defines reconsolidation; the video was followed 10-min later by extinction training (Group R-E in **Fig. 1**). The reconsolidation hypothesis suggests that RET should result in the original memory being updated with information that is contrary to the cue->reward contingency established via innumerable bouts of smoking (i.e., cue->no reward). We hypothesized that RET would attenuate craving and cue reactivity to both familiar and novel smoking cues presented during test sessions that occurred 24-hr., 2-weeks and 1-month following intervention. We preliminarily hypothesized that smoking behavior (e.g., cigarettes per day or CPD) would be reduced during the 1-month follow-up.

i. Overview of study design & procedures. Treatment-seeking, nicotine dependent smokers were assessed for baseline levels of craving and cue reactivity (Day 1, **Fig. 1**) following monetary reinforced overnight smoking abstinence. Participants were then randomized to one of two groups: Group R-E (n = 44) vs. Group NR-E (n = 43). All participants set their cessation attempt to begin the night before the first of three consecutive laboratory sessions (**Fig. 1**: Days 2-3 and the 24-hr test). Participants received monetary incentives to remain smoking abstinent. The first two sessions involved administration of the intervention. During these sessions, R-E participants first viewed a 5-min 'retrieval' video containing smoking content and then, 10 minutes later, received 1-hour of 'extinction' training in which they received four sequences of video, picture and in vivo smoking cues. NR-E participants received the same treatment as group R-E except that they viewed a 'No Retrieval' video that has non-smoking, neutral content (**Fig. 1**). Thus, the only difference between groups was the 5-minute video that preceded the extinction phase, which served to retrieve memories prior to reconsolidation (or not). The third session, performed 1-day later, was a test session that involved exposure to the smoking video cues and to a series of novel smoking picture cues, the latter of which assessed generalization of RET effects. The remaining two test sessions, performed 2-weeks and 1-month after RET, were identical to the first test session except (a) smoking abstinence was not compensated, (b) participants used diaries to collect daily smoking and craving data, and (c) breath samples were collected to provide CO corroboration of self-report smoking/abstinence.

Fig. 1



ii. Results: Groups R-E and NR-E were comparable on all demographic and smoking-related measures at baseline, including mean years of smoking. They were also comparable on non-cue-elicited and smoking cue-elicited craving obtained during the baseline cue reactivity session. Our main results focused on three areas: (a) subjective craving (via the Craving Questionnaire, CQ[65], a brief, 4-item self-report questionnaire; min=1 and max=5), (b) cigarettes smoked per day during follow-up, (c) Expired breath CO level during follow-up testing. **Fig. 2a** depicts the mean craving rating during the three test sessions. In test sessions, after controlling for covariates (time between visits, sex, age when regular smoking began and baseline craving), Group R-E shows a trend towards lower craving than Group NR-E at the 24-hr. and 2-week test sessions, which then fully emerges into a significant effect at 1 mo. ($p = 0.039$, $d = 0.44$; novel cues: $p = 0.038$, $d = 0.44$). This difference appears to be due to a continued downward trajectory in the craving of Group R-E as compared to a stalling of craving decline in Group NR-E. This suggests RET effects on craving emerge over a relatively long time frame. Given the constraints on follow-up duration imposed by the R21 mechanism, the durability of RET effects on craving remains unknown.

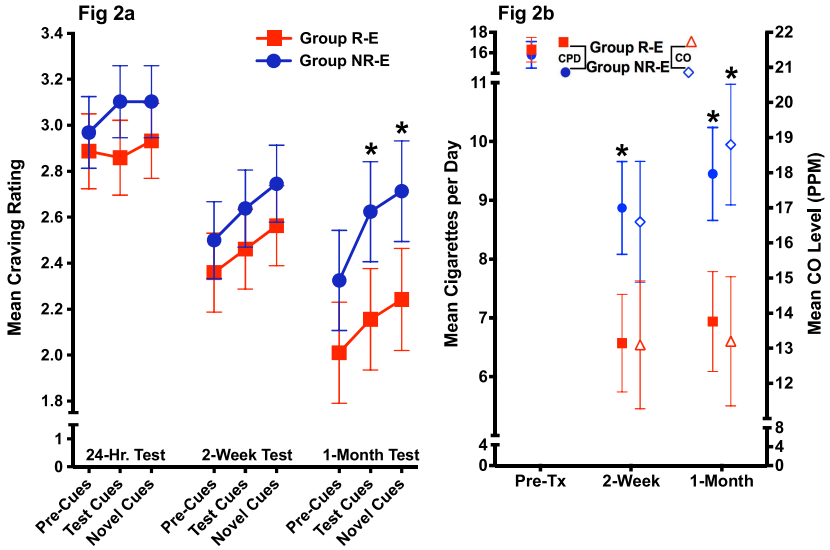
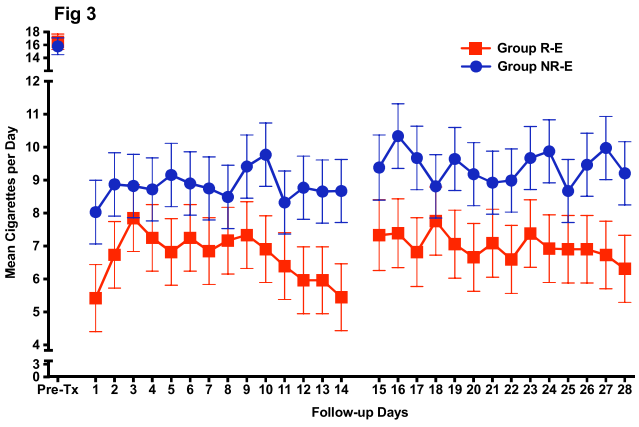


Fig. 2b depicts the mean self-report CPD smoked (left axis, solid symbols) and expired CO levels obtained (right axis, empty symbols) at Baseline, 2-week and 1-month follow-up. Group R-E evidenced lower mean CPD than Group NR-E at both 2-week and 1-month follow-up (adjusted for baseline CPD, sex, years of regular smoking, and negative affect; $p = 0.024$, $d = 0.50$). Further, expired CO level was lower in Group R-E vs. NR-E at 1-month follow-up (adjusted for negative affect and race; $p = 0.033$, $d = 0.47$). Thus, the self-report smoking and CO findings indicate that RET confers a smoking reduction advantage over extinction training alone. Specifically, Group R-E evidenced a 25% greater reduction in smoking than group NR-E. **Fig. 3** depicts group level CPD smoked each day post-treatment. The figure clearly shows that RET had immediate effects on smoking and that the group difference persists unabated over the follow-up. In fact, this figure, together with the CO outcomes, suggests the possibility that the effect magnitude may be increasing during the latter 2-week period of follow-up. Lastly, there was trend level evidence that a greater percentage of participants in Group R-E (51.5%) vs. NR-E (25.6%) achieved a 60% reduction in smoking (from baseline) during follow-up (risk ratio (RR)=1.62 (0.98-2.67), p=.06; At 2-week and 1-month follow-up time points, the p's=.18 and .04, respectively)



We believe these results are very impressive, especially when one considers the modest difference in treatment between the two groups (i.e., a 5-minute smoking- vs. neutral-content

video that preceded extinction trials). Cast in terms of the overall difference in duration of stimulus exposure, Group R-E received 8% more exposure to smoking cues than did Group NR-E (10-min of smoking video exposure / 130-min total cue exposure). Remarkably, this resulted in Group R-E evidencing (i) significantly lower craving in response to both familiar and novel smoking cues, albeit only at the 1-month post-treatment test session, and ii) a significantly greater reduction (25%) in self-reported cigarettes smoked per day, which was CO verified 1-month post-treatment. Preliminarily, there also was suggestive evidence that more Group R-E participants achieved a stringent smoking reduction threshold (i.e., 60% reduction from baseline). Collectively, these findings suggest that clinically meaningful reductions in craving and smoking behavior can be achieved via an exposure-based intervention in which brief, smoking-related memory retrieval precedes protracted smoking cue exposure/extinction. Though tempting to pursue these initial findings by proposing a clinical trial examining the effects of RET in combination with a first-line pharmacotherapy, we believe that this would be premature. Instead, the findings presented here encourage careful replication, incremental extension and measured consideration of potential mechanism(s) subserving RET.

C. Proposed Study: Replication, Extension and Mechanism

Several aspects of the R21 findings have directly informed our decision-making concerning the 'next steps' outlined in the proposed project. One next step relates specifically to the findings on the behavioral outcomes. Although the R21 was powered only to assess the effects of RET on craving and cue reactivity, it yielded strong evidence that the intervention can reduce smoking and possibly impede relapse to baseline 'heavy' smoking. Given the clinical importance of these findings, we believe it is essential that the proposed project have a sample size large enough to ensure sufficient power to detect clinically meaningful treatment effects on smoking reduction.

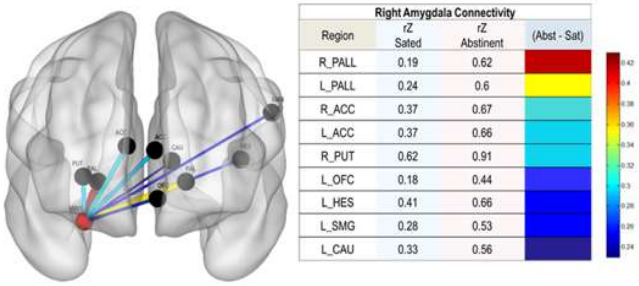
Another 'next step' pertains to how we might increase the treatment signal observed in Group R-E. Increasing the number of sessions of RET (dose) is a logical strategy for achieving this end. While there is little research addressing this strategy, a cross-study comparison of two infrahuman studies does offer some cause for optimism. Unpublished work by our colleagues Greg Sartor and Gary Aston-Jones[33] has shown that 1 session of RET was insufficient to reduce renewal of cocaine self-administration. By contrast, Xue et al., 2012[35] found that 8 sessions could reduce renewal of cocaine self-administration (as well as spontaneous recovery and reinstatement). In the present case, there are legitimate practical reasons why we should not excessively increase the number of RET sessions (e.g., participant burden). A realistic incremental dosing could be achieved by adding a single additional session of RET. This would represent a 50% increase in dosing while at the same time maintaining the practical potential of RET and allaying concerns that too many exposures to the retrieval cues might compromise their ability to initiate memory destabilization (addressed further in Alternative Design considerations.).

The above findings also point to the need to fully characterize the magnitude and duration of effects induced by RET. Specifically, the R21 findings suggest the possibility that the craving differences between the groups may have just begun to emerge at the 1-month time point. Also, while the group difference on self-report smoking was present throughout follow-up, CO corroboration occurred only at 1-month, suggesting that the difference in smoking may have become more firmly established during the latter 2-weeks of follow-up. The trend level data on the proportion of participants that achieved a 60% reduction from baseline smoking suggests the possibility that a difference might have emerged with sufficient time. Accordingly, the proposed study will have a substantially extended follow-up period (6-months) in order to better characterize the long-term effects of RET on craving and smoking behavior. Also, if the plan to increase the amount of RET has the expected effect(s), then an extended 6-month follow-up may permit a more thorough assessment of larger and lasting treatment effects.

Although not explicitly suggested by the R21 findings, it seems critical that our 'next step' study should endeavor to identify the neural mechanisms that mediate RET effects on craving and smoking reduction. The extant literature on mechanism involved in modifying associative memories has primarily focused on extinction training. Evidence from infrahuman and human research in aversive and appetitive extinction learning indicates that distinct MTL structures (i.e., hippocampus and amygdala)[68] interact with the inhibitory frontostriatal (vmPFC-ventral striatum)

pathway in a dissociable manner to modulate conditioned stimulus-unconditioned stimulus (CS-US) associations. The amygdala is necessary for extinction learning—potentially through a habituation process[69]—whereas the hippocampus is necessary for memory integration and updating[70].

To date, only four human fMRI studies have examined the neural underpinnings of RET, all in human fear conditioning. First, Agren et al (2012)[40] followed fear conditioning with RET where extinction training either occurred within the reconsolidation window (10-min. post-retrieval) or after reconsolidation was complete (i.e. 6 hrs. post retrieval; control group). An fMRI scan was performed 3 days later to assess neural correlates of renewal-induced fear. Relative to the control, the group receiving RET in the reconsolidation window exhibited less fear that was associated with attenuated BOLD response in the amygdala and weaker functional connectivity between amygdala (seed) and hippocampus and mPFC (i.e. pregenual anterior cingulate). Astonishingly, in a follow-up study[45] of the same participants, the attenuated amygdala-mediated fear response persisted over 18 months. In a third study by this group[48], spider phobics who underwent RET exhibited (i) reduced fear-elicited basolateral amygdala activity during a subsequent test, and (ii) greater behavioral approach to spider cues (relative to controls who received extinction/exposure outside the reconsolidation window). Lastly, Schiller and colleagues (2013)[39] overlaid fMRI in a repeated measures (pre/post) fear conditioning paradigm to examine the effects of RET on PFC and amygdala response to CS paired with shock (CS+). Compared to control conditions, RET reduced fear responding (skin conductance response) and normalized BOLD response in mPFC to the CS+, similar to levels observed in the CS- control condition. These findings suggest that RET may attenuate CS+ potency by reducing both amygdala responsivity to conditioned cues and the strength of frontro-amygdala functional connectivity. Although these findings come from fear paradigms, they are highly relevant to smoking as our preliminary data (**Fig. 4**) demonstrate that smoking abstinence strengthens rsFC between amygdala, mPFC and striatum. Though speculative, RET effects on smoking may occur through fronto-amygdala-striatal circuitry and confer therapeutic benefit by reducing the salience of drug-cue memories among smokers making a quit attempt.



Right Amygdala Connectivity

Region	rZ Sated	rZ Abstinent	(Abst - Sat)
R_PALL	0.19	0.62	
L_PALL	0.24	0.6	
R_ACC	0.37	0.67	
L_ACC	0.37	0.66	
R_PUT	0.62	0.91	
L_OFC	0.18	0.44	
L_HES	0.41	0.66	
L_SMG	0.28	0.53	
L_CAU	0.33	0.56	

Fig. 4: Amygdala-cortico-striatal rsFC: rsFC was assessed in smokers (*N*=18) while sated and following 24hrs smoking abstinence. Compared to sated, the effects of abstinence on rsFC were examined using whole brain analyses. Abstinence strengthened rsFC connectivity between the amygdala, striatum and mPFC ($p<.05$).

Although there are no published neuroimaging studies on the effects of RET on appetitive responding, we have recently published data[71] demonstrating that, consistent with preclinical models of frontostriatal mediated extinguished drug seeking[18], the strength of rsFC in a frontostriatal pathway (Nucleus Accumbens: NAcc /ventral striatum – vmPFC) among abstinent smokers is associated with less craving (R^2 = .5) and CO verified reduction in smoking (R^2 = .25) over 3 ½ days of monetary reinforced abstinence. Despite this mechanistic account of extinction learning and RET in aversive conditioning paradigms, systems-level clinical neuroscience findings on the effects of RET on the neural substrates of addictive behavior is nonexistent, thus representing a significant gap in the literature. The proposed study is an initial effort to fill this gap.

5.0 Inclusion and Exclusion Criteria/Study Population

Admission into the study is open to men and women, ages 25 to 65, and to all racial and ethnic groups. Based on the published R21 report (on which this study is based), we anticipate we will screen (by telephone or in person) approximately 316 potential participants. A brief phone screening will assess participant suitability via inclusion/exclusion criteria. Qualifying individuals will be scheduled to undergo (i) informed consent, (ii) demographic information collection, and (iii)

comprehensive clinical assessment of smoking behavior/nicotine dependence, general psychiatric functioning, and substance use. The instruments employed in this assessment are the same as those used in the R21; they are widely cited and have strong psychometric properties. Assessments include the: a) Smoking History Form[99], a 20-item interview that solicits detailed information about smoking history, including initiation and duration; b) Fagerström Test for Nicotine Dependence (FTND[100]), a brief measure of nicotine dependence; c) Wisconsin Inventory of Smoking Dependence Motives (WISDM-68[101]), a multidimensional assessment of nicotine dependence; d) Contemplation Ladder[102] to measure readiness to quit; e) Wisconsin Predicting Patients' Relapse questionnaire (WPPR[103]) which assesses relapse potential and may serve as covariate in the analysis; f) Michigan Nicotine Reinforcement Questionnaire (MNRQ[104]) which assesses both positive and negative reinforcement from smoking; g) Beck Depression Inventory (BDI[105]); and h) the Mini-International Neuropsychiatric Interview or MINI[106] which assesses general psychiatric functioning and other alcohol/substance dependence (per inclusion/exclusion criteria). Quantity and frequency assessment of smoking and other substance use in the three months prior to study involvement will be assessed using the Timeline Follow-Back (TLFB[107]).

Inclusion Criteria

a) Participants must be able to provide informed consent and function at an intellectual level sufficient to allow accurate completion of all assessment instruments.
b) Fluency in English.
c) Participants must meet DSM-V criteria for current nicotine dependence, be a daily cigarette smoker of 10 or more cigarettes/day for a minimum duration of three years and have an expired carbon monoxide (CO) concentration of ≥ 10 ppm (to confirm inhalation).
d) Participants must live within a 50-mile radius of the research facility and have reliable transportation.
e) Participants must be treatment seeking and be willing to make a quit attempt beginning the night before the four consecutive daily laboratory sessions (i.e., three intervention sessions and the test session performed 24-hours after the third intervention session).
f) Participants must be willing to (a) be overnight smoking abstinent (CO verified) prior to the baseline smoking cue reactivity and fMRI assessment, and (b) make a cessation attempt and be smoking abstinent (CO verified) over the four-day period that corresponds to the three intervention sessions and the test session. Violation of the CO criterion for abstinence will require rescheduling if it occurs on the first intervention day; a violation of the CO criterion on the 2nd/3rd intervention day or the 24-hr test session will not result in discontinuation of study participation (participants will be made aware at study outset that full compensation during the intervention and 24-hr test session is contingent on meeting the CO criterion).
g) Participants must be willing to submit to a breathalyzer (alcohol) assessment and urine drug screen (for benzodiazepines, amphetamines, methamphetamine, cocaine, marijuana and opiates) and produce a negative test result on (a) the day of the baseline smoking cue reactivity assessment, and (b) each of the four consecutive days that correspond to the three intervention sessions and the test session.
h) Participants must be willing to submit saliva samples for the purposes of determining cotinine levels (as a means of biochemically verifying self-report abstinence, changes in smoking behavior).
i) Participants must consent to random assignment to the R-E and NR-E conditions/groups.
j) Participants must not use smokeless tobacco.
k) Participants must agree to forego any other medication or behavioral treatment for smoking cessation during their enrollment in this study (with the exception of a referral to the SC Quitline). Participants use of nicotine products such as e-cigs and nicotine patch will be

assessed over the course of the study). Additional treatment referrals will be provided at the end of the study.

l) 20/20 vision with corrective lenses.

Exclusion Criteria

a) Participants with current/active (untreated) psychotic disorder, current major depressive disorder (severe), bipolar affective disorder or a severe anxiety disorder as these conditions would likely interfere with their ability to fulfill the requirements for successful participation (e.g., provide accurate interview data, complete study assessments, attend scheduled laboratory visits, etc.).
b) Participants meeting DSM-V criteria for substance dependence (other than nicotine) within the past 60 days.
c) Participants who are unwilling or unable to maintain abstinence from alcohol and other drugs of abuse (benzodiazepines, amphetamines, methamphetamine, cocaine, marijuana and opiates) in order to comply with Inclusion Criterion g above.
d) Participants currently taking β-blockers, anti-arrhythmic agents, psychostimulants or any other agents known to interfere with heart rate, skin conductance or blood pressure responses.
e) Use of any pharmacotherapy or psychotherapy for smoking cessation at study entry.
f) Past head injury or primary neurological disorder associated with MRI abnormalities, including dementia, MCI, brain tumors, epilepsy, Parkinson's disease, or demyelinating diseases.
g) Any contraindication to MRI (this only applies to the participants in fMRI groups)
h) ECT in last 6 months.
i) Among females, pregnancy at the general clinical assessment session or either of the fMRI sessions will be exclusionary. They must agree to notify the study staff if they become pregnant during the study.

Inclusion of Women and Minorities

Women and minorities will be recruited in proportions similar to those attained in the R21 on which this project is based. Specifically, the sample will include approximately 52% African American, 46% Caucasian, and 2% other racial/ethnic groups (Asian, Native American, Pacific Islander, Hispanic) and 36% women (the small expected number of other racial/ethnic groups reflects the relatively small numbers of these minority groups in Charleston). There are no sex/gender, racial or ethnic groups that are excluded from this study and outreach programs for recruiting these group members as participants are not needed. Although no gender/race-related hypotheses are proposed in this study, exploratory analyses involving these attributes will be performed.

Inclusion of Children

The sample recruited in the proposed study will include smokers between the ages of 25 and 65 years. We will exclude smokers younger than 25, including children, because we want participants to have smoking histories long enough to ensure they have a well-established network of smoking-related memories. Furthermore, in the R21 on which this proposal is based, the sample included no children (<18 years) and only 3 individuals under the age of 25. We believe that excluding younger smokers will more closely align the proposed project with the R21 and enhance homogeneity of sample with respect to smoking-related memory. Lastly, excluding individuals under the age of 25 should mitigate any concerns about the potential confounding effects of brain development in younger participants.

6.0 Number of Subjects

A total of 166 male and female participants with smoking-related nicotine dependence who are interested in making a cessation attempt (treatment seeking) will be enrolled.

7.0 Setting

As a member of the scientific community at MUSC, Dr. Saladin will have available the resources described below. Of special note is the Addictive Behaviors Research Laboratory (ABRL) and the Translational Research of Addiction and Integrative Neuroscience Laboratory (TRAIN Lab). These laboratories are where the research staff will administer the: (1) comprehensive clinical assessment, (2) baseline cue reactivity (non-fMRI), (3) laboratory-based intervention (e.g., retrieval-extinction training) sessions, and (4) laboratory-based test sessions (24-hours, 2-weeks and 1-month, 6-weeks, 2-months, 3-months and 6-months after the third intervention session).

Laboratory Spaces:

(1) The Addictive Behaviors Research Laboratory (ABRL) located within one block from the Department of Health Sciences and Research. The ABRL is specifically designed to serve as a human laboratory research facility in which laboratory-based cue reactivity studies could be performed with nicotine, alcohol and other drug addicted populations. The ABRL consists of five rooms, including a participant testing room, an experimental control room, an office, a kitchen, and a waiting room. The participant testing room is ventilated so that cigarette smoke can be evacuated. The walls, floor and ceiling of this space are sound attenuated to ensure that participants are acoustically isolated during the administration of study procedures. The adjacent experimental control room contains a computer (Apple) and psychophysiological measurement equipment (Biopac MP150 Data Acquisition Module, and bio-amplifiers) necessary for blood pressure, heart rate and skin conductance data collection.

(2) Translational Research of Addiction and Integrative Neuroscience Laboratory (TRAIN Lab) is adjacent to the Center for Biomedical Imaging (CBI, described below) and is located within one block of the Department of Health Sciences and Research. The research team has access to approximately 2000 square feet of laboratory space that includes an experimental cognitive testing room, one cue reactivity assessment space (to be used in the proposed study) and one room that is equipped with a ventilation system that allows for indoor smoking; a control room that is equipped with three workstations to monitor each experimental room, and a Dell Precision T3610 for online quality control and data analysis. Equipment includes smoking topography and smoke delivery devices, an infrared eye tracking system, a BIOPAC system including HR and respiratory amps, computer systems for electronic questionnaire administration, and expired air CO monitors. An additional BIOPAC system will be purchased and dedicated to this project; it will reside in the cue reactivity assessment room. The purchased system will have the capacity to collect heart rate and skin conductance data.

Imaging Facilities:

The Center for Biomedical Imaging (CBI), which is located adjacent to the TRAIN laboratory, has more than 4,500 sq. ft. of space at 30 Bee Street, as well as approximately 9,000 sq. ft. of office space in a new Bioengineering Building. The space at 30 Bee Street is the main facility for human imaging research and houses the Siemens 3T PRISMA MRI scanner that will be used in the proposed study. The scanner is also equipped with integrated fMRI paradigm presentation equipment. The scanner operates with a 100% mandate for research use and is covered by a master research agreement with Siemens Medical. The site also contains an image analysis laboratory and bioengineering facility along with subject interview and changing rooms. Researchers also have access to a clinical Siemens 1.5T and 3T Verio MR scanners, located within the Radiology Department in the Clinical Sciences Building.

8.0 Recruitment Methods

Participants will be recruited using multiple sources, primarily through advertising in local media via television, newspaper, flyers, Craig's List, Internet sites and by word of mouth among participants. Additionally, we will advertise with the Project Quit collaborative which has been IRB approved on several other smoking study protocols. Our R21 recruited 88 participants in 20 months, or 4.4 participants per month. This recruitment goal was achieved with a single research assistant and with very minimal advertising; in fact, there were times when recruitment was slowed because there were too many calls and eligibility assessments for our single staff member to manage. In the proposed study we plan to recruit 166 participants over 44 months, approximately 3-4 per month (approximately 1 per month will undergo fMRI procedures). This is entirely feasible given that we will have (a) two full-time research staff and a post-doc to manage the flow of participants, (2) an advertising budget that permits a two-fold increase in advertising beyond what we had in the R21 (i.e., it will permit advertising on two local TV stations as well as print advertising), and (3) two identically outfitted lab spaces (Addictive Behaviors Research Laboratory, ABRL and Translational Research of Addiction and Integrative Neuroscience, TRAIN Laboratory) in which to administer the intervention and test procedures (see Setting section for details).

9.0 Consent Process

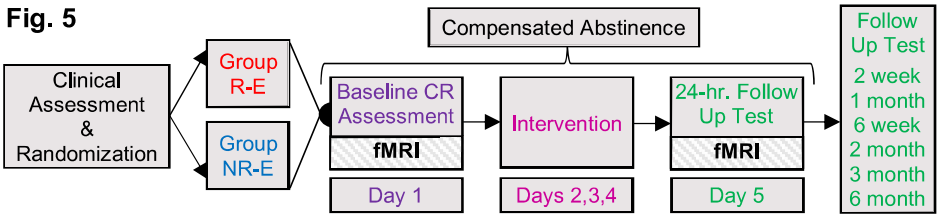
Participants who qualify based on the phone screening will be scheduled for the clinical assessment. Informed consent will be obtained from all participants at the start of this assessment. Research staff will describe study procedures, methods, risks/discomforts, and benefits of participating to potential participants.

Research staff will use an electronic consent process with the MUSC IRB's eConsent and HIPAA templates in the RedCAP system, which is an easy process for participants to navigate. No hyperlinks will be used to convey additional study information. Participants will view the electronic consent and HIPAA forms onsite in a private room with research staff using an iPad tablet. Participants will be given the opportunity to ask questions about the study protocol and time to understand the electronic consent process before signing the informed consent document and HIPAA form. Research staff will include a signature line at the end of the consent document and HIPAA form, and participants will provide a digital signature using their fingers. The use of digital signatures ensures that participant signatures can be verified as legitimate, uniquely identify the signer, and are under reasonable control of the signer. Participants will be emailed a copy or receive a printed copy (based on participant preference) of the signed consent document and HIPAA form. South Carolina state law allows for electronic informed consent for research. Research staff will use the electronic consent process with all participants; however, a paper-based consent process will be used in instances where the technology becomes unavailable or per participant request.

10.0 Study Design / Methods

i. Design Overview: Following initial contact with study staff, participants will be scheduled for a clinical assessment/randomization session, after which they will be scheduled for their baseline assessment session (see **Fig. 5**) in which baseline levels of smoking-related craving and cue reactivity (CR) will be assessed in a CR lab. After the initial CR assessment, a subsample of participants (n=68;

Fig. 5



34/group) will receive a second CR assessment in the fMRI scanner whereas the remaining participants (n=98; 49/group) will receive the equivalent CR experience except in the CR lab. Participants will be required to remain overnight abstinent (CO verified) for the baseline CR session; monetary incentives will reinforce compliance with abstinence. This abstinence requirement ensures that baseline data will be collected under the same conditions of abstinence that will prevail for the subsequent four sessions (3 intervention and 1, 24-hr. test). Following the baseline CR assessment, participants will plan their cessation attempt to begin the night before the first of four consecutive daily laboratory sessions (Days 2, 3, 4 & 5 in **Fig. 5**), again with monetary incentives for abstinence throughout (**Fig. 5** under compensated abstinence). The purpose of abstinence during these four sessions is to minimize the likelihood of strengthening smoking memories with nicotine reinforcement via at-home smoking. Group R-E will then undergo 3 daily sessions of RET consisting of 5-min 'retrieval' smoking video followed 10 minutes later by 1-hour of 'extinction' training consisting of four sequences of smoking-related cues (same as in R21). The first test session will be performed 24-hrs after completion of the third RET session; it will be identical to the baseline CR session (i.e., a participant that received lab-based CR followed by fMRI CR at baseline, will receive the same arrangement in the 24-hr. test) with the exception that the lab-based CR will be assessed to both familiar (as in baseline session) and novel smoking cues. The conclusion of the 24-hr. test session also marks the end of compensated abstinence. Follow-up (lab-based cue reactivity) test sessions will occur at the following time points: 2-weeks, 1-month, 6-weeks, 2-months, 3-months, and 6-months. All test sessions will involve assessment of CR to both familiar and novel smoking cues. Participants will use smoking diaries to record daily smoking behavior throughout the follow-up period. A 'no-retrieval' control group, Group NR-E, will receive the same treatment as above except the 'retrieval' video will contain neutral, not smoking content. Both groups will be treated identically beginning with the 24-hr test session through study completion. All follow-up test sessions (**Fig. 5**, last box) will be identical to the first (24-hr) test session except (a) there will be no compensation for abstinence, (b) there will be no fMRI lab session, (c) participants will use diaries to collect daily information about smoking and craving, and (d) salivary cotinine will be collected to permit biochemical verification of self-report smoking/abstinence.

ii. Participants: A total of 166 treatment-seeking men and women smokers (83 per group), **aged 25 to 65**, will be enrolled. While we recruited smokers between the age of 21-65 in the R21, we have narrowed the age range in the proposed study in the interest of mitigating concern about the potential confounding effects of brain development in younger participants. Also, since more than 80% of smokers begin smoking before age 21[77], employment of this age range will ensure, even at the lower bound, that we are recruiting smokers with sufficiently long smoking histories to have well-established smoking-related memories. This age range also ensures the findings will be generalizable to the vast majority of smokers. Additional inclusion requirements are: a) smoke 10+ cigarettes/day for three or more years, b) be willing to make a cessation attempt, c) remain overnight abstinence prior to the baseline smoking cue reactivity assessment, and d) agree to comply with the reinforced abstinence regimen in place during the four laboratory sessions (3 intervention & 1 test). Prospective participants will be excluded if they meet DSM criteria for a psychotic disorder, severe major depression, bipolar affective disorder, substance dependence (other than nicotine) or have a neurological disorder/injury (e.g., dementia, brain tumor, Parkinson's disease, head injury).

iii. Sample Size Estimation. A sample of 166 participants assigned equally to 2 treatment groups will provide 80% power to detect clinically relevant reductions in smoking behavior while optimizing power (≥80%) to detect meaningful effects across the remaining hypotheses. Hypothesis 1a: In the R21 analysis, a greater reduction in CPD was seen in Group R-E as compared to Group NR-E (**Fig. 2b**: Cohen's d=0.5). Since small studies are more likely to report larger effects than larger trials[78,79], we have designed this study to have at least 80% power to detect an effect of treatment on CPD with a 10% reduction from that observed in the R21 (d=0.45). In the proposed study, we will measure CPD at each of 6 follow-up time points (**Fig. 6**). Under the assumption of independent observations, a total sample size of 78 participants randomized to each group will provide 80% power ($\alpha < .05$) to detect an effect size of d=0.45 between Group R-

E vs. control groups. However, the sample size in a repeated measures design is reduced when assuming a 1st order autoregressive error covariance structure with rho=0.9[80] (previous studied range 0.80-0.95). The number of participants per group required to achieve this power is therefore estimated to be n=62; however, this number needs to be adjusted for dropout between randomization and end of study. While the R21 dropout rate was 19.4%, this study will include a longer compensated abstinence period and longer follow-up period, which may increase dropout to 25%. Therefore, **83 participants** will be randomized per group after adjusting for dropout[81]. Secondary Hypothesis 1b: Studies[82,83] have shown that smokers with larger reductions in CPD may have increased odds of cessation compared to those with smaller reductions. Preliminary analysis from the R21 showed that 52% of participants in Group R-E had achieved a ≥60% reduction in CPD during the 2 weeks leading up to the 1 month follow-up visit relative to the 2 weeks prior to study treatment while only 26% of Group N-RE achieved the same milestone. The randomized sample size of 83 participants per group (62 completers) will provide greater than 80% power (α < .05) to detect a similar difference between group R-E relative to each control group. Hypothesis 3: In the R21, cue-elicited craving was measured during post-treatment test sessions at 24-hrs, 2-wks and 1-mo. In the 1-mo test session, differences between R-E and NR-E groups emerged, showing a decrease in cue-elicited craving in Group R-E as compared to Group NR-E (d=0.44). In the proposed study, data will be collected at each of the 7 test sessions (over 6-months) and we assume an attenuated effect size (for the same reason noted above for CPD) relative to the R21 findings (d=0.40). Given a moderate Rho (=0.5), the sample will provide greater than 80% power to detect an effect size of d=0.4 between Group R-E and controls. Thus, the sample size of **83 participants per treatment group (n=166 total)** will provide sufficient power (≥80%; and α < .05) to detect clinically meaningful smoking efficacy and craving differences for group R-E compared to controls, after accounting for up to 25% dropout.

fMRI sample size estimation. Though we have not assessed the effects of RET on fMRI BOLD response, the four human fear conditioning studies of RET described above reported significant BOLD and connectivity changes with n's ranging from 19 to 23[39,40,45,48]. Additionally, some of our previous work provides strong support that we will observe significant effects with group n's in this range. In two prior fMRI CR studies[58,59] of smoking/smoking abstinence (n's=17-19), effects sizes for CR-BOLD response ranged between d=.65 to 1.5. In two prior rsFC studies in cigarette smokers (n's 16-18), we found smoking abstinence, compared to satiety, strengthened rsFC in an amygdala-striatal circuit (rZ's between .56 and .91); whereas in Froeliger et al 2015[71] the strength of rsFC in the frontostriatal pathway was associated with less craving (R^2=.48) and smoking (CO confirmed:R^2=.50). In the proposed study, we will examine the effects of RET on drug CR-BOLD response (Hypothesis 2a) and rsFC (Hypothesis 2b). If we assume, based on our prior work and that of others, that these effects are of medium to large size (e.g. D≈.8), then with a **full fMRI sample of N=68** (34 per group) and α =.05 (1-tailed), a final sample of ≈23 participants in each group (accounting for fMRI ineligibility and 30% attrition) will result in power >.80 for detecting effects on CR-BOLD response and rsFC.

iv. Cue Reactivity Measures (CR lab-based & fMRI): All of the following measures have strong psychometric properties and have been routinely employed in laboratory studies performed by our research group, including the R21 on which this study is based. They are brief and/or unobtrusive enough to be administered between multiple cue administrations. They include: Craving Questionnaire (CQ) is a brief, 4-item self-report questionnaire[84] that assesses urge to smoke. It has the benefits of multi-item craving assessment without being intrusive or time consuming; Mood Form (MF) is a 9-item form[85] that will provide an immediate, corroborative assessment of current positive and negative mood states. Our modified MF contains an item to assess subjective stress; Heart rate (HR), skin conductance (SC) and blood pressure (BP) will be measured repeatedly over the course of the laboratory sessions. HR will be collected via two electrodes affixed to the participant's ribcage (minimizes movement artifact) while 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/SC signals will be amplified via Biopac MP100 data acquisition system). BP will be measured via a non-invasive arm cuff.

v. Experimental Stimuli: The baseline CR assessment will involve presentation of the smoking video described below. The three intervention sessions will have two components: retrieval and extinction training. Retrieval in Group R-E will involve the viewing of a smoking video whereas retrieval in Group NR-E will involve the viewing of a non-smoking 'neutral content' video. Ten minutes after retrieval, extinction training will commence and will consist of exposure to four sequences of cues, with each sequence consisting of video, in vivo and picture cue formats. Within a stimulus sequence, each of the three formats will be presented only once and the order of presentation of the first two formats will be randomly determined. For Groups R-E and NR-E, extinction training will consist of cue sequences with smoking content. Following intervention, all groups will undergo follow-up test sessions that will involve presentation of smoking video and novel picture cues only. All stimuli for the proposed study (described below) have been used in other studies by our group, including the R21.

Video cues. The content for the video cues were acquired from Dr. Joel Erblich[86] and consisted of 12, 30-sec high-resolution video segments of individuals of both sexes engaging in smoking-related behaviors (e.g., lighting up a cigarette and smoking) or non-smoking behaviors (e.g., person reading a book). The smoking and non-smoking (neutral) video segments were staged and filmed by a professional cinematographer with the goal of maximizing inter-video similarity on elements of lighting and visual complexity. The craving and cue reactivity potency of the smoking videos has been demonstrated in smokers[86]. A selection of 10 video segments has been assembled into a continuous 5-min smoking video or non-smoking (neutral) video for use in the proposed study (also used in the R21). The smoking video will be used in the baseline cue reactivity assessment, will serve as the 'retrieval' cue for Group R-E, and will serve as the 'familiar' smoking cues in all test sessions. The neutral video will serve as the non-smoking neutral 'retrieval' cue for participants assigned to Group NR-E.

In vivo cues. The smoking in vivo cues in the intervention sessions will be based on those used in our previous cue reactivity research with smokers[87,88], including the R21. Briefly, a pack of the participant's preferred brand of cigarettes and a lighter will be placed in a covered box. The participant will be signaled to lift the cover off the box and look at/handle the cigarette pack and lighter for approximately 60-90 sec. Then, participants will take one cigarette from the pack and hold it between his/her fingers as they normally do while smoking (approx. 60 sec). Participants will be instructed to handle the cigarette and then to smell the cigarette. The participants will then be instructed to pick up the lighter and flick the lighter without actually lighting the cigarette. Next, the participants will receive instruction to light the cigarette without smoking. Participants will be instructed to deposit the lit cigarette in a container filled with water. Duration of this experience will be 5 min.

Picture cues. The smoking and neutral picture cues in the intervention sessions will be a set of 30 images. This includes images acquired from Dr. Stephen Tiffany's research group (SUNY, University at Buffalo). These images have been used extensively by Dr. Tiffany to study craving and cue reactivity in smokers and their potency has been documented in several publications[89-91]. As in the R21, the images will be displayed on a computer monitor located directly in front of the participant. Each image will be displayed for 8-sec, with a two second delay between images (total approximate duration will be 5-min). The smoking images will be part of the stimulus sequence used in the intervention extinction training of Groups R-E and NR-E.

Picture cues for test sessions: To assess whether the effects of treatment generalize to novel smoking stimuli, participants will be administered a brief "novel" smoking picture cue exposure sequence in each test session. The picture sequence will occur approximately 30 min. after the video cues and will consist of 12 novel smoking pictures, presented for 8-sec. each (two sec. delay between each). The pictures will be obtained from standardized stimulus sets we have used in our previous work[92,93] and from the work of others[94-96].

fMRI cues: A novel set of smoking images[96] will be presented in each of two fMRI CR task (see below).

vi. General Neuroimaging Methods: Blood-oxygenation-level-dependent (BOLD) imaging. Imaging will be performed on a 3T Siemens scanner. During each task, whole brain blood-oxygenation-level-dependent (BOLD) contrast sensitive images will be acquired using an EPI

sequence (36 slices, TR = 2200 ms, TE = 35 ms, FOV =192, matrix = 64x64, 3mm^3 voxels). The participant's head will be immobilized. Prior to the acquisition of images, the anterior (AC) and posterior commissures (PC) will be identified in the mid-sagittal slice of a localizer series. High-resolution anatomical imaging. A high-resolution 3D MPRAGE anatomical sequence will be acquired (matrix = 256, flip angle = 9°, 166 slices, slice thickness = 1 mm). Following preprocessing procedure and 1st level modeling as described in each respective specific aim, 2nd level modeling will be conducted within the confines of an ROI mask acquired from the Wake Forest University Pickatlas[97] and drawn on the MNI template. This ROI approach reduces Type I error by restricting analyses to a smaller set of voxels[98]. Follow-up voxel-wise exploratory analyses will be conducted (p>.05, FWE).

Procedures: Items viii thru xi below describe major procedural elements in the study (cf., **Fig. 6** above).

vii. Clinical Assessment and Randomization: A brief phone screening will assess participant suitability via inclusion/exclusion criteria. Qualifying individuals will be scheduled to undergo (i) informed consent, (ii) demographic information collection, and (iii) comprehensive clinical assessment of smoking behavior/nicotine dependence, general psychiatric functioning, and substance use. The instruments employed in this assessment are the same as those used in the R21; they are widely cited and have strong psychometric properties. Assessments include the: a) Smoking History Form[99], a 20-item interview that solicits detailed information about smoking history, including initiation and duration; b) Fagerström Test for Nicotine Dependence (FTND[100]), a brief measure of nicotine dependence; c) Wisconsin Inventory of Smoking Dependence Motives (WISDM-68[101]), a multidimensional assessment of nicotine dependence; d) Contemplation Ladder[102] to measure readiness to quit; e) Wisconsin Predicting Patients' Relapse questionnaire (WPPR[103]) which assesses relapse potential and may serve as covariate in the analysis; f) Michigan Nicotine Reinforcement Questionnaire (MNRQ[104]) which assesses both positive and negative reinforcement from smoking; g) Beck Depression Inventory (BDI[105]); and h) the Mini-International Neuropsychiatric Interview or MINI[106] which assesses general psychiatric functioning and other alcohol/substance dependence (per inclusion/exclusion criteria). Quantity and frequency assessment of smoking and other substance use in the three months prior to study involvement will be assessed using the Timeline Follow-Back (TLFB[107]). At the conclusion of the assessment, a saliva sample will also be collected to establish baseline cotinine levels; next, participants will be scheduled for their baseline cue reactivity assessment and fMRI session. They will be informed that they will be (1) required to be abstinent from alcohol and other drugs on the day of the session, and (2) compensated to remain abstinent from smoking starting at bedtime the night before their appointment. Overnight smoking/substance abstinence prior to baseline session is necessary so that responses are collected under the same conditions of compensated abstinence as those that will be in force during the intervention and initial 24-hr. post-treatment cue reactivity/fMRI test session.

Lastly, study staff will randomize participants to treatment group and fMRI condition. Participants will be randomized to the Group R-E or NR-E in a 1:1 allocation. Randomization will be done using a stratified random block design; randomization blocks will vary in size (3, 6 and 9) and randomization will be stratified across gender and baseline nicotine dependency (FTND score ≤ 5 vs. > 5). Relevant data from the clinical assessment will be used to assign participants to the corresponding randomization strata; then randomized within the current block of the strata. Following treatment randomization, participants will be randomized to receive fMRI or no fMRI in a 1:1 allocation (within each treatment strata) until 34 participants in each treatment assignment (68 total) have been successfully randomized to the fMRI protocol. This approach to randomization should mitigate accidental bias and produce comparable groups across possible confounds.

viii. Baseline Cue Reactivity/fMRI Assessment: The baseline CR session will be administered at one of our two identical lab locations (ABRL or TRAIN). Upon arrival, participants will undergo a smoking abstinence assessment involving self-report verification followed by breath CO assessment. Per recent study guidelines[108,109], participants will be considered overnight abstinent if their breath CO level is ≤ 10 ppm. Abstinence from alcohol/other drug use will be

assessed via breathalyzer and urine drug screen (UDS). Failure to meet abstinence requirements will result in rescheduling. Following the abstinence assessment, participants will be seated comfortably in front of a computer monitor and have the HR and SC sensors and BP cuff affixed as described above. They will remain seated comfortably for 10 min, after which 50-sec of continuous HR and SC data will be collected; next the participant will complete the CQ, MF and have their BP assessed. Having completed pre-cue assessments, the participant will remain seated for 5-min; they will then view the smoking-related video described above. Continuous HR and SC will be collected during the first 50-sec of the video whereas the CQ, MF and BP will be collected immediately after viewing the video. Following a break, participants randomized to the fMRI condition will be escorted to the fMRI suite. Participants will practice the experimental task while inside a mock scanner in order to reduce scan-related anxiety and enhance data integrity. Next, participants will be administered the fMRI procedures. In brief, we will collect a hi-res MPRAGE structural image, followed by 6-minute eyes-closed resting-state sequence and then participants will be scanned while performing a smoking CR task (8 ½ min duration) that involves viewing alternating blocks of neutral cues (e.g. pencil: 40 sec), fixation and craving ratings (30 sec) and smoking cues (e.g. cigarette: 40 sec). Participants not randomized to the fMRI condition will remain in the CR laboratory and receive the same CR regimen as administered in the fMRI condition. This ensures that all participants have equivalent stimulus exposure.

At the completion of the baseline CR/fMRI assessment, participants will schedule their next visit with study staff, and they will be reminded that their quit attempt will begin the night before and that it will be the first of four consecutive daily visits for which they will receive abstinence-based compensation. The rationale for implementing a reinforced abstinence procedure was to ensure that extra-study smoking (e.g., at home) does not interfere with any effects of treatment (RET or otherwise) or the treatment effects assessed in the initial 24-hr test session. Lastly, participants will receive compensation.

ix. Intervention: Three intervention sessions will be performed on consecutive days. Participants will arrive at the ABRL/TRAIN lab and compliance with abstinence (breath CO, UDS and breathalyzer) will be assessed. If the participant fails the abstinence assessment, they will be rescheduled; however, if the participant fails the abstinence assessment on the second/third day, they will be permitted to continue in the protocol. This is a departure from the R21, where participants violating the abstinence assessment were discharged from the study. Importantly, the compensation schedule we used to reinforce abstinence resulted in only 10% of the sample being discontinued during treatment. In the present study, we have elected to retain these individuals in the interest of being able to conduct a full intent-to-treat analysis, which we believe is a strength. To discourage multiple violations of abstinence, participants will lose their compensation on the day of the violation but will have the ability to re-earn it by remaining abstinent for the remaining treatment session(s) and the 24-hour test session. Following the abstinence assessment, use of any nicotine products (e.g., e-cigarettes, dip, gum, patch) during the prior 24-hr. period will be assessed. Next, pre-cue measures of craving and physiological reactivity will be obtained as described above for the baseline assessment. After pre-cue measures, individuals in Group R-E will watch the 5-min. video with smoking-related content (same as viewed during baseline cue reactivity) whereas individuals in Group NR-E will view the 5-min. control video with neutral, smoking-irrelevant content. Continuous HR and SC will be collected during the video and the remaining assessments (CQ, MF and BP) will be obtained after. Participants in all three groups will remain seated for 10-min and then Groups R-E and NR-E will be administered a 1-hr of extinction training. As noted above, this 1-hr. training will consist of four sequences of video, picture and in vivo cue exposure. Continuous HR and SC will be collected during the first cue format presented in each sequence and the CQ, MF and BP will be obtained immediately following the last cue format of each sequence (i.e., during a 2-min period where no stimuli are presented). At the completion of exposure training, participants will be asked to sit comfortably for 5-min while the study coordinator removes the HR/SC sensors and the BP cuff. Participants will be reminded that they are being compensated to remain abstinent from smoking and other substance use during the remaining intervention session(s) and/or the 24-hr. test session.

x. Test Sessions: Participants will complete seven follow-up test sessions: 24-hours, 2-weeks and 1-month, 6-weeks, 2-, 3- and 6-months. We use monetary compensation to minimize test session attrition (only 17.2% of participants did not complete the R21). Consistent with the R21 and Xue et al., 2012, each test sessions will involve a CR assessment identical to the baseline CR assessment described above except that a brief novel smoking picture cue sequence will also be presented. Importantly, some procedural elements will not occur in all test sessions. First, only the 24-hour follow-up test session will be performed under conditions of reinforced abstinence. The goal will be to assess the acute effects of the interventions under the same conditions of abstinence that occurred during the intervention. Participants will be encouraged to maintain their smoking abstinence at the conclusion of this session but will not be compensated for it (participants will be given a referral to the SC state Quitline, a free, anonymous service that supports abstinence). Second, for individuals randomized to the fMRI condition, the 24-hr test session is the only one in which an fMRI session (identical to the one in the baseline assessment) will be performed immediately after the initial CR assessment (cf., **Fig. 6**); those not assigned to the fMRI condition will remain in the CR laboratory and receive the same CR regimen as administered in the fMRI condition. Third, at the end of all but the 6-mo. test session, participants will receive a smoking diary in which to record the occurrence of both daily smoking and craving. A TLFB assessment of smoking behavior will be performed whenever diary data is not available; all but one of the R21 participants used their diary to collect smoking data as instructed. For those follow-up periods where more than 2-weeks elapse between visits (i.e., period between the 2 and 3 mo. follow-up visit and the period between the 3 and 6 mo. visit), study staff will phone contact participants every 2 weeks to collect diary data. Fourth, in all but the 24-hr. follow-up test session, expired CO and a saliva sample will be collected in order to biochemically corroborate, via CO and cotinine levels, self-report smoking/abstinence (salivary cotinine abstinence cutoff ≤15 ng/ml per SRNT Guidelines[110] and CO abstinence cutoff ≤ 5 ppm[108,109]). Assessment of the use of any nicotine products (e.g., e-cigarettes, dip, gum, patch) will be assessed at all follow-up visits. At study completion, debriefing will occur in which participant questions will be addressed and recommendations for additional cessation treatment will be provided.

xii. Participant Compensation: Study retention will be promoted by the following compensation schedule: Clinical assessment = $30.00; Baseline cue reactivity/fMRI = $60.00; Intervention sessions 1, 2 & 3 = $75.00, $100.00 and $125.00, respectively; 24-hr. follow-up test session 1 = $150.00; Test sessions at 2-wks, 1-mo., 6-wks, 2-mo., 3-mo., & 6-mo. = $100.00. Maximum compensation for participation is $1,140.00. Retention will also be fostered by frequent phone/text contacts between study staff and participants.

12.0 Data Management

Baseline demographic and clinical measures will be compared across groups using standard statistical methods (e.g., Chi square test of independence for categorical variables; Univariate ANOVA for continuous variables). Characteristics that (i) display imbalance at baseline, (ii) are associated with a dependent measure, or (iii) are known to be predictive of study outcomes (e.g., craving, smoking) will be assessed for inclusion as covariates in the final models. **Primary Aims Analysis:** The primary hypothesis of Aim 1 is that Group R-E will evidence decreased smoking behavior during follow relative to both control groups. Smoking behavior will be measured via smoking diary/TLFB recovered at each visit or by phone, from which, the mean CPD will be calculated between each visit for the period until the last attended visit. Additionally, the smoking diary/TLFB data will be used to compute an indicator of those that achieved a ≥60% reduction in CPD relative to baseline smoking at each visit. Generalized linear mixed effects models will be used to analyze the longitudinal smoking behavior data during follow up (using appropriate

distributions). Overall statistical significance for the effects of treatment group, time, and their interaction will be assessed using a likelihood ratio test that compares the final model to a model consisting of an intercept term alone. The pre-specified hypothesis will be tested using model-based estimates to construct group comparisons across all the planned time points. Normality of residuals will be tested for models where appropriate and when found in error, appropriate transformations or non-parametric methods will be employed. Although mixed effects models have been proven highly efficient in cases where follow up data is missing at random, to accommodate other types of missing data, we will perform analyses using multiple imputation methods (Bayesian approach using both MCMC and FCS). The primary hypothesis associated with Aim 3 is that Group R-E will evidence decreased cue-elicited craving during follow-up testing. General linear mixed effects models will be constructed to assess group differences in craving over time. **Secondary Analysis:** Smoking relapse[111] (i.e., days to 3 consecutive days of smoking ≥5 cigarettes) will be tested using a Cox Proportional Hazards regression model. Log-Log plots of the survival function and inclusion of a time variable treatment term will assess the proportionality assumptions and if models fail this assumption, a stratified Cox Regression model[112] will be used. Also, we will measure end of study 7-day point prevalence abstinence as well as continuous abstinence using smoking diary/TLFB data. The effect of R-E training on abstinence outcomes will be tested using logistic regression models. Biological Variables: Exploratory analysis of sex effects on study outcomes will be assessed through main effects and the interaction between sex and study treatment assignment. Where significant interactions are found, sex stratified analyses will be conducted to assess the role of sex in the effect of RET on study outcomes. Other secondary analyses will assess i) differences between the control groups on main outcomes, ii) craving and cue reactivity differences during the three intervention sessions, and iii) the effects of treatment on ambient or background craving and cue reactivity[113,114] (i.e., non-cue-elicited outcomes obtained early in test sessions). All analysis will be conducted using SAS/STAT v. 9.4 and Stata v. 14.

Validity and data integrity will be checked on an ongoing basis through the use of data audits. Study statisticians (Baker/Ramakrishnan) will supervise quarterly data quality assessments (i.e., examination of the outcomes database(s) for missing data, unexpected distributions or responses, and outliers) performed by the study research staff. Accuracy and completeness of the data collected will be ensured by weekly review. A 10% random sample of the primary source document will be crosschecked with the database on a quarterly basis. If inaccuracies exceed 2%, then a second 10% will be randomly chosen for audit. Should data discrepancies be discovered, they will be resolved using original source documents. The REDCap system does not accept outliers, illogical response patterns, etc. The PI will have weekly meetings with the research staff to discuss qualitative comments received during data collection and any problems in data collection or entry. The statistician will periodically examine the database to look for irregularities. Initial data analyses will examine distributions of variable scores and comparability of baseline characteristics across conditions in case analyses need to be adjusted for these.

fMRI Analysis Plan. The hypothesis for 2a (CR) is that Group R-E will evidence attenuated CR in medial prefrontal cortex (mPFC: dorsal anterior cingulate-dACC; ventromedial: vmPFC), limbic regions (amygdala, hippocampus) and ventral striatum. Greater reduction in BOLD response in regions of interest will predict decreased smoking. fMRI data will be preprocessed using recommended procedures[115] in SPM8 and as done previously[116]. At the 1st-level, data will be analyzed using the GLM[117]; events of interest (Smoking, Neutral) will be modeled as a boxcar canonically convolved hemodynamic response function, and HR and motion parameters entered as nuisance covariates. At the 2nd-level, 2 (Time: Pre, Post) x 2 (Group: R-E, NR-E) ANOVA will be used within an *a prior* ROI mask (amygdala, hippocampus, mPFC, ventral striatum; $p < .05$, pFWE). Hypothesis for 2b (rsFC) is that group R-E will evidence strengthened rsFC in frontostriatal circuitry; weakened rsFC in frontolimbic circuitry and the magnitude of rsFC will be inversely related to craving and smoking (e.g., CPD). fMRI data analysis plan. Similar to our previous work[71] along with in scanner heart-rate and respiration entered as covariates, rsFC will

be assessed using the conn13 SPM8 toolbox. Connectivity matrix for each seed (see ROI mask in 2a) will be entered into separate: 2 (Time: Pre, Post) x 2 (Group) ANOVA. Exploratory voxel-wise analyses are planned for 2a and 2b with specific focus on subdivisions of the insular cortex (cf., Addicott et. al., 2015[118]).

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

The PI (Michael E. Saladin, Ph.D.) will be responsible for monitoring the trial. Questionnaires and interviews are all non-invasive and, as such, involve minimal physical risk to participants. However, adverse events will be monitored throughout the study and all events will be followed to resolution or stabilization. AEs will be coded on a weekly basis using Medical Dictionary for Regulatory Activities (MedDRA) rules and entered into a database. For each weekly study meeting, the research staff will prepare a summary of all AEs. The PI will review this at the weekly study meeting (or before if more urgent). Serious adverse events (SAEs) are defined as events, related or unrelated, that require or prolong hospitalization or result in congenital defects, death, disability, cancer, overdose, development of drug dependency or abuse, or any other life threatening event. The Institutional Review Board (IRB) of the Medical University of South Carolina (MUSC) will be immediately informed orally of an SAE as soon as the investigator, co-investigators, or study staff are made aware of it. A written report will be filed within 72 hours to the IRB, the NIDA Project Officer through the NIDA Serious Adverse Event Tracking and Reporting System (SAETRS). As further clinical information is obtained, follow-up and final SAE reports will be filed with the IRB and NIDA. The research staff will be instructed not to reveal whether a person is a participant in the study and will report to the PI any outside requests for information about a participant or any breaches in confidentiality. All requests by participants' physicians and other medical providers will be referred directly to the PI.

Additionally, 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 tenure of the project. The board will consist of a physician, a clinical psychologist, a statistician and a Pharm.D. or Nurse practitioner. This multidisciplinary group has extensive experience with management and monitoring of clinical research involving cue reactivity/exposure. 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 federal funding agency) have been informed of the event.

All serious adverse events will be reported to the MUSC Institutional Review Board (IRB) within 24 hrs. Follow-up of all serious adverse events will be reported as well. All adverse events are reviewed weekly by the PI and yearly by both the DSMB and the IRB. Any significant actions taken by the local IRB, including significant protocol changes, will be relayed to NIDA. We anticipate the serious adverse event rate to be extremely low. If monitoring indicates otherwise, we will convene a special meeting of the DSMB.

15.0 Risks to Subjects

Questionnaires and interviews are all non-invasive and, as such, involve minimal physical risk to the participants. Potential risks incurred by participants include:

a) Loss of confidentiality: There is a risk of loss of confidentiality regarding the information obtained during study participation. Safeguards to protect confidentiality include locked records and firewalls around password-protected electronic data, and all study data being coded, with the key linking the code with a participant's identity being kept in a separate, locked file. Similar safeguards are followed for storage and processing of MRI data. MRI data is stored on secure storage owned by the Center for Biomedical Imaging (CBI). CBI

has a longstanding policy of reviewing all scans that are transferred to the laboratory from the MRI scanners, and assuring that all participant identifiers are removed, both from the scan image itself but also the electronic headers of the scan. The MRI scans are identified only by participant code, study code, and date of acquisition.

b) Craving induction via cue reactivity/exposure: There is risk of increased craving for cigarettes or distress because of the cue-reactivity procedures. Craving may be pronounced during the intervention/24-hr test phase of the study.
c) Magnetic Resonance Imaging: Although this procedure is generally low-risk, there are some concerns. Individuals will be screened for the presence of implanted metal (including but not limited to medical devices, shrapnel, tattoos or permanent makeup); those who screen positive will be excluded from the fMRI component of the study. Some participants may feel uncomfortable or confined once positioned within the bore of the MRI system. This potential reaction is reduced by discussing the procedure prior to entry into the magnet room and by communicating with the participant regularly over the intercom. Most importantly, participants will be exposed to the scanning environment through the use of a mock scanner. During the fMRI, participants will have voice contact with a radiology technician, and may request the scan be stopped at any time. If a participant expresses discomfort in the scanner and expresses an interest in terminating the scanning procedures, the imaging procedure will be terminated and the participant will be removed from the magnet.
d) Incidental Findings: Magnetic Resonance Imaging: Another risk is the occurrence of incidental findings on MRI, such as risk of previously unrecognized stroke, hematomas, or other findings. All scans are reviewed at time of acquisition. Should any concerning findings be seen, we will obtain a consultation from a clinical neuro-radiologist who will provide an opinion on the significance of the finding and recommendations for further evaluation. Dr. Froeliger will contact the participant in question, convey these findings and recommendations, and facilitate referrals for further evaluation and treatment as needed.
e) Adverse events unrelated to study participation may occur during the course of study participation.

There are no alternative methods of obtaining this information.

Drs. Saladin and/or Carpenter will monitor all study participants for general psychological well-being/stability. The instrumentation to be used for physiological recordings meets all safety standards for non-invasive recordings, and participants will be located out of reach of any AC-powered devices in the laboratory. All laboratory sessions will be conducted under the supervision of experienced personnel at either the Addictive Behaviors Research Laboratory (ABRL) or the Translational Research of Addiction and Integrative Neuroscience (TRAIN) Laboratory. If crisis intervention is necessary, senior staff (Saladin/Carpenter) 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.

To ensure confidentiality, participant data will be number coded, and only the investigators will have access to the master list of codes and participant names. All participant records will be kept in a locked file cabinet in an office that will be locked at times when not in use. All research staff undergo rigorous training on the ethical treatment of human subjects in research and receive continuous feedback about the need to maintain confidentiality.

16.0 Potential Benefits to Subjects or Others

Benefits of study participation include detailed assessment of smoking and other substance use, potential reductions in craving to smoke and other reactions to smoking cues, reductions in smoking, abstinence from smoking and referral for additional treatment out side the context of study participation. Also, regular contact with and monitoring by research staff may serve to motivate participants in future attempts to abstain from cigarette use. Overall, given the potential benefits, it would appear that exposure to the risks associated with study participation are well justified. The risk/benefit ratio appears to favor the study participant.

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