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Effect of Selective Inhibition of Reactivated Nicotine-Associated Memories With Propranolol on Nicotine Craving

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Abstract

IMPORTANCE—A relapse into nicotine addiction during abstinence often occurs after the reactivation of nicotine reward memories, either by acute exposure to nicotine (a smoking episode) or by smoking-associated conditioned stimuli (CS). Preclinical studies suggest that drug reward memories can undergo memory reconsolidation after being reactivated, during which they can be weakened or erased by pharmacological or behavioral manipulations. However, translational clinical studies using CS-induced memory retrieval-reconsolidation procedures to decrease drug craving reported inconsistent results.

OBJECTIVE—To develop and test an unconditioned stimulus (UCS)-induced retrieval-reconsolidation procedure to decrease nicotine craving among people who smoke.

DESIGN, SETTING, AND PARTICIPANTS—A translational rat study and human study in an academic outpatient medical center among 96 male smokers (aged 18–45 years) to determine the association of propranolol administration within the time window of memory reconsolidation (after retrieval of the nicotine-associated memories by nicotine UCS exposure) with relapse to

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nicotine-conditioned place preference (CPP) and operant nicotine seeking in rats, and measures of preference to nicotine-associated CS and nicotine craving among people who smoke.

INTERVENTION—The study rats were injected noncontingently with the UCS (nicotine 0.15 mg/kg, subcutaneous) in their home cage, and the human study participants administered a dose of propranolol (40 mg, per os; Zhongnuo Pharma).

MAIN OUTCOMES AND MEASURES—Nicotine CPP and operant nicotine seeking in rats, and preference and craving ratings for newly learned and preexisting real-life nicotine-associated CS among people who smoke.

RESULTS—Sixty-nine male smokers completed the experiment and were included for statistical analysis: 24 in the group that received placebo plus 1 hour plus UCS, 23 who received propranolol plus 1 hour plus UCS, and 22 who received UCS plus 6 hours plus propranolol. In rat relapse models, propranolol injections administered immediately after nicotine UCS-induced memory retrieval inhibited subsequent nicotine CPP and operant nicotine seeking after short (CPP, $d = 1.72$, 95% CI, 0.63–2.77; operant seeking, $d = 1.61$, 95% CI, 0.59–2.60) or prolonged abstinence (CPP, $d = 1.46$, 95% CI, 0.42–2.47; operant seeking: $d = 1.69$, 95% CI, 0.66–2.69), as well as nicotine priming-induced reinstatement of nicotine CPP ($d = 1.28$, 95% CI, 0.27–2.26) and operant nicotine seeking ($d = 1.61$, 95% CI, 0.59–2.60) after extinction. Among the smokers, oral propranolol administered prior to nicotine UCS-induced memory retrieval decreased subsequent nicotine preference induced by newly learned nicotine CS (CS1, Cohen $d = 0.61$, 95% CI, 0.02–1.19 and CS2, $d = 0.69$, 95% CI, 0.10–1.28, respectively), preexisting nicotine CS ($d = 0.57$, 95% CI, –0.02 to 1.15), and nicotine priming (CS1, $d = 0.82$, 95% CI, 0.22–1.41 and CS2, $d = 0.78$, 95% CI, 0.18–1.37, respectively; preexisting nicotine CS, $d = 0.92$, 95% CI, 0.31–1.52), as well as nicotine craving induced by the preexisting nicotine CS ($d = 0.64$, 95% CI, 0.05–1.22), and nicotine priming ($d = 1.15$, 95% CI, 0.52–1.76).

CONCLUSIONS AND RELEVANCE—In rat-to-human translational study, a novel UCS-induced memory retrieval-reconsolidation interference procedure inhibited nicotine craving induced by exposure to diverse nicotine-associated CS and nicotine itself. This procedure should be studied further in clinical trials.

A core feature of nicotine addiction is high relapse rates during abstinence.^{1–3} In human studies⁴ and in animal models,^{5,6} key triggers of craving and relapse during abstinence are acute re-exposure to nicotine (the unconditioned stimulus, [UCS], a smoking episode for people or noncontingent nicotine priming injections in laboratory animals) or nicotine-associated conditioned stimuli (CS), presumably because of reactivation of nicotine-associated memories.^{7,8}

Nicotine-associated memories may undergo reconsolidation, a time-dependent process in which consolidated memories become unstable for several hours after their reactivation.^{9–11} Preclinical studies suggest that reconsolidation requires activation of the noradrenergic system.^{12–14} Human studies have shown that the administration of the β -adrenoceptor blocker propranolol before or after memory reactivation decreases conditioned fear among healthy individuals, fear of spiders among arachnophobic individuals,¹⁵ or psychophysiological responses induced by traumatic imagery scripts among individuals with posttraumatic stress disorder.^{16,17} Propranolol administration after memory retrieval also

impairs reconsolidation of drug-related memories in rat models,^{14,18–23} and among people addicted to heroin.²⁴ However, other studies failed to demonstrate an effect of propranolol on drug craving among people who use drugs.^{25–27}

One potential reason for the limited “translational” utility of propranolol’s effect on drug craving is that previous human studies exposed participants to drug-associated CS, not (UCS), to induce memory retrieval and reconsolidation. Under these experimental conditions, propranolol or other neuropharmacological manipulations interfere with the reconsolidation of memories selectively associated with the reactivated CS.^{28–31} In the context of nicotine addiction, smoking is associated with multiple CS that vary across individuals.³² It is not feasible to reactivate all of a smoker’s nicotine-associated CS in a clinical or laboratory setting.

Based on these considerations and findings that interference with reconsolidation after retrieval by UCS (aversive shock) prevented subsequent fear expression induced by multiple CS,^{28,29,33} we recently developed a UCS-induced memory retrieval-reconsolidation interference procedure and demonstrated its efficacy in decreasing relapse to cocaine seeking and craving in rat models.³¹ In the present study, we introduced a memory retrieval-reconsolidation interference procedure that is based on our rat study³¹ in which we reactivated nicotine reward memories in both rat models and among people who smoke by acute exposure to nicotine (the UCS) and then interfered with memory reconsolidation using propranolol. In the rat study, we assessed the efficacy of our procedure using animal models of drug relapse^{6,34} based on the CPP³⁵ and intravenous drug self-administration³⁶ procedures. In the human study, we assessed the efficacy of our new procedure using a laboratory method in which drug craving during abstinence was assessed after exposing the participants to drug-associated cues^{27,37–39} or drug priming.⁴⁰

Methods

Rat Study

The timelines of the experiments are provided in eFigure 1 and 2 in the Supplement.

We housed male 156 Sprague-Dawley rats (260–280 g; Vitalriver Company) in individual cages in a temperature-(23±2°C) and humidity- (50 ±5%) controlled animal facility with unlimited access to food and water. We performed the experimental procedures in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the procedures were approved by the Biomedical Ethics Committee for animal use and protection of Peking University. The procedures for training and testing for nicotine CPP are similar to those of our previous studies.^{41–43} The training and extinction procedures are also similar to those of previous studies by our group and others.^{44–48} We injected the rats noncontingently with the previously self-administered drug (nicotine 0.15 mg/kg, subcutaneous, the UCS) in their home cage. We based the nicotine UCS retrieval dose on previous studies using the reinstatement procedure,^{46,48,49} and these studies also guided our choice for the nicotine priming dose during the reinstatement tests described later. We based the propranolol dose (10 mg/kg, intraperitoneal) and the timing of the injections on previous studies of reconsolidation of drug memories in rats.^{18–20} The UCS and CS memory retrieval

manipulations were based on those used in our previous studies.^{31,45} This information is described in the eMethods in the Supplement.

Human Study

The timeline of the study is provided in eFigure 3 in the Supplement and a description of the experimental phases is provided in the eMethods in the Supplement.

Participants

We recruited 96 healthy young men through posters and online advertisements. Because of the multisession aspect of the study, we enrolled only men to avoid the influence of the menstrual cycle phase on the response to nicotine stimulant effects and raving induced by nicotine-associated cues.^{50,51} The inclusion criteria were that participants had to be men between the ages of 18 and 45 years who had been smoking 10 or more cigarettes per day continuously for at least 12 months prior to the screening, were willing to refrain from smoking for at least a night, had an afternoon end-exhaled carbon monoxide concentration less than or equal to 10 ppm (Bedfont Mini2 Smokerlyzer; Bedfont Scientific Ltd), and were in general good health as determined by a physician. Participants were excluded if they had used addictive drugs other than nicotine based on self-reporting or had a positive urine test result during either the recruitment screening or testing, had a current or past history of DSM-IV Axis I disorders, were currently using antidepressants or had depression diagnosed, had clinically evident cognitive impairment, or had self-reported treatment with any prescription drugs during the previous 2 weeks or any over-the-counter drugs during the 3 days prior to the experimental sessions.

Eligible candidates were scheduled for a screening interview during which they read the study protocol and signed the informed consent form. The study was approved by the institutional review boards of Peking University Health Center, and all participants were paid for their participation. During the base-line session, we collected demographic data (age, sex, and education) and self-reports of the number of cigarettes per day and the Fagerström Test of Nicotine Dependence Score, and we also assessed depression and anxiety using standard measures.

Intervention

We chose the dose of propranolol (40 mg, oral; Zhongnuo Pharma) based on previous studies on memory reconsolidation among people with spider phobias¹⁵ and people addicted to heroin.²⁴ We administered propranolol 1 hour before the memory retrieval manipulation rather than 1 hour after retrieval as in the rat study, because plasma concentrations of propranolol peak approximately 1 hour after oral administration,⁵² and we were concerned that postretrieval oral administration might delay effective brain concentrations of propranolol until after the typical 1 to 2 hour “reconsolidation window.”^{53,54} The delayed 6-hour propranolol administration condition (a standard control condition in rodent and human memory studies) is based on previous human studies on propranolol’s effect on reconsolidation of fear memories.^{53,54}

Overall Design

In a double-blind experimental design, we recruited 96 participants and randomly assigned them to 1 of 3 groups, but in the statistical analyses, we excluded 27 participants because they did not complete the experiment. The 3 groups of participants included: placebo plus 1 hour plus UCS, propranolol plus 1 hour plus UCS, and UCS plus 6 hours plus propranolol, all in a 1:1:1 ratio. During the randomization period, an in-dependent (blind) team member, who was only involved in the rat experiments, generated a table of randomization codes using Microsoft Excel software. Other team members who were responsible for the random allocation of the patients, drug administration, and the subsequent memory tests during the study were blind to the drug condition. We gave the participants the placebo and propranolol in identical capsules. We used methods from previous studies with some modifications.^{55,56} There were 6 sessions: screening and baseline tests of preference (liking) and craving for “pre-existing” nicotine CS (day 1), conditioning for 3 different CS to be paired or not paired (CS-) with smoking (day 2–4), postconditioning test of preference and craving for the CS (day 5), UCS memory reactivation and propranolol or placebo administration (day 6), a posttreatment test (day 7), and a priming test (day 8) (eFigure 3 in the Supplement). We asked the participants to abstain from smoking for at least 8 hours (overnight) before the daily experimental sessions, and verified short-term abstinence by examining their carbon monoxide level (less than 8 ppm) before the sessions.⁵⁷ We instructed the participants to continue their usual daily smoking habits and not to attempt to quit smoking while in the study.

Assessment of Nicotine Craving and Preference (Liking)

Based on previous studies,^{45,55} we measured subjective ratings of “liking” and craving. For ratings of “liking,” we marked the scale at the left and right ends with –5 (“dislike”) and 5 (“like very much”) and with 0 (“neutral”) at the midpoint. For ratings of craving, we marked the scale at the left and right ends with 0 (“extremely low”) and 10 (“extremely high”). The participants had to rate their current craving level to smoke or their preference for the different CS after exposure to the different experimental manipulations (see earlier).

Statistical Analysis

We reported the results as mean (standard error of the mean) and analyzed the data by analyses of variance (ANOVAs) with the appropriate between- and within-subject factors for each experiment (eTable in the Supplement). We excluded 22 participants because they did not meet the smoking criteria and 5 participants because they decided to quit the study during the conditioning phase and included 69 participants (24 for placebo plus 1 hour plus UCS, 23 for propranolol plus 1 hour plus UCS, and 22 for UCS plus 6 hours plus propranolol) for data analysis. No statistical methods were used to predetermine sample sizes, but they are similar to those reported in our previous studies of rats and humans.^{14,21,45} We performed post hoc analyses of significant effects in ANOVAs using the Tukey test. We also performed post hoc analyses and report the Cohen d estimate and its 95% CIs using R software (3.3.1 version). Values of P less than or equal to .05, 2-tailed, were considered statistically significant.

Results

Effect of Propranolol on Reconsolidation of Multiple Nicotine Reward Memories After UCS Retrieval in Rats

In the first experiment, we found that propranolol administration (10 mg/kg, intraperitoneal) within the reconsolidation time window after UCS exposure decreased nicotine preference in the CPP procedure and relapse to nicotine seeking in the drug self-administration procedure (eFigures 4 and 5 in the Supplement).

In the second experiment, we developed a modified training procedure in which we trained rats for both nicotine self-administration (14 days) and then nicotine CPP (8 days), resulting in the formation of both Pavlovian and operant reward memories in the same rat (eFigures 1 and 2 in the Supplement). For the nicotine UCS retrieval procedure, the analysis showed a significant main effect of the propranolol dose (0, 10 mg/kg) on both the CPP test (mixed ANOVA, $F_{1,15} = 6.59$, $P = .02$, Figure 1A) and the nicotine-seeking test ($F_{1,15} = 13.65$, $P = .002$, Figure 1B). By contrast, for the operant CS retrieval procedure, the analysis showed a significant main effect of the propranolol dose on the nicotine-seeking test (mixed ANOVA, $F_{1,13} = 13.94$, $P = .003$, Figure 2) but not the CPP test ($F_{1,13} = 0.02$, $P = .88$, Figure 2).

These results indicate that propranolol injections after UCS retrieval disrupted both nicotine CPP and self-administration memories, leading to the inhibition of nicotine CPP and operant nicotine seeking. By contrast, propranolol injections administered after operant CS memory retrieval selectively disrupted nicotine self-administration memories, leading to the inhibition of operant nicotine seeking but not nicotine CPP.

We also examined whether propranolol injections administered after nicotine UCS retrieval affect reward memories for sucrose. We found that propranolol prevented nicotine CPP 1 day later but had no effect on sucrose seeking in the extinction test (eFigure 3 in the Supplement).

Effect of UCS-Induced Memory Interference Procedure on Multiple Nicotine-Associated Memories and Nicotine Craving Among Smokers

In the third experiment, we tested whether the findings from the rat studies would generalize in relation to people who smoke. We used a variation of a cue preference procedure, designed as a human version of the CPP procedure.^{55,56} We assigned the 96 smokers (69 of whom completed the experiment) to 3 experimental groups: placebo plus 1 hour plus UCS retrieval, propranolol plus 1 hour plus UCS retrieval, or UCS retrieval plus 6-hours plus propranolol (eFigure 3 in the Supplement). The participants' demographic data are shown in the Table.

Nicotine Preference

The analysis of preference (liking) for the newly learned CS on days 5 and 7 showed a significant group $\times$ test interaction (mixed ANOVA, $F_{2,198} = 7.75$, $P = .001$) and cue type $\times$ test interaction ($F_{2,198} = 4.69$, $P = .01$) (Figure 3A). The analysis of preference (liking) for the newly learned CS during the priming test showed significant main effects of group (2-

way ANOVA, $F_{2,198}=10.19$, $P<.001$) and cue type ($F_{2,198}=6.75$, $P=.001$) (Figure 3A). The analysis of preference (liking) for the preexisting real-life CS indicated a significant group $\times$ test interaction (mixed ANOVA, $F_{2,66}=3.74$, $P=.03$) (Figure 3B) in the posttreatment test and a significant main effect of group (1-way ANOVA, $F_{2,66}=11.14$, $P<.001$) in the nicotine priming test (Figure 3B). These results demonstrate that propranolol administration within the time window of memory reconsolidation after UCS retrieval decreased the participants' preference (liking) to both newly learned and preexisting nicotine-associated CS.

Nicotine Craving

We also examined the effect of propranolol plus UCS retrieval on nicotine craving induced by newly learned and preexisting CS and nicotine priming. For newly learned CS, there were no significant group differences (mixed-measures ANOVA, $P>.1$) in the posttreatment test (Figure 4A). By contrast, for preexisting CSs, there was a significant group $\times$ test interaction (mixed ANOVA, $F_{2,66}=5.82$, $P=.005$) in the post-treatment test and a significant main effect of group (1-way ANOVA, $F_{2,66}=7.91$, $P=.001$) in the priming test (Figure 4B). These results showed that nicotine craving in our procedure was induced by preexisting but not newly learned nicotine-associated cues, but more importantly, that propranolol plus UCS retrieval decreased the nicotine craving induced by preexisting CS and nicotine priming.

Discussion

In this rat-to-human translational study, we introduced a UCS-induced memory retrieval-reconsolidation interference procedure in which we reactivated nicotine reward memories by acute exposure to nicotine (the UCS) and then pharmacologically interfered with the putative process of memory reconsolidation. In rat relapse models, systemic injections of the β -adrenoceptor blocker propranolol immediately after UCS-induced, but not CS-induced, memory retrieval inhibited sub-sequent nicotine CPP and relapse to operant nicotine seeking after short or prolonged abstinence. Among people who smoke, propranolol administration decreased subsequent nicotine preference induced by both newly learned and preexisting (ie, real-life) nicotine CS, and nicotine craving induced by the preexisting nicotine CS and nicotine priming. In both rats and humans, the pharmacological manipulations had no effect on any of these behavioral measures when administered 6 hours after UCS retrieval (outside the temporal window within which reconsolidation is thought to occur⁵⁸), supporting a memory reconsolidation account of the present results.

UCS Memory Retrieval vs CS Memory Retrieval

Since 2005,^{59,60} many studies using rats and mice showed that consolidated drug-associated memories undergo reconsolidation when reactivated by drug-associated CS, and that neuropharmacological or behavioral interference with reconsolidation can inhibit subsequent drug CPP and relapse into operant drug seeking.^{14,21,61,62} However, with few exceptions,^{45,63} the "translation" of these preclinical studies to people addicted to drugs has not been successful.²⁵⁻²⁷ This situation, and the realization that a major limitation of CS-induced retrieval and reconsolidation procedures is their specificity to the reactivated CS,^{28,29,33}

inspired us to develop an alternative procedure in which the drug-reward memories are reactivated by exposure to the drug itself (the UCS).

In a recent study,³¹ we introduced a UCS memory retrieval-extinction procedure and showed that, unlike the more established CS-induced procedure,^{45,64} the UCS-induced procedure can prevent relapse into cocaine seeking induced by multiple cocaine-associated CS under a wide range of experimental conditions. However, from a clinical perspective, the UCS memory retrieval-extinction procedure can be difficult to implement, because it requires multiple sessions of UCS retrieval plus extinction training. By contrast, the UCS memory retrieval-reconsolidation procedure described here was effective among rats and humans after a single UCS session. Additionally, in the human translational study, the procedure's effects generalized to multiple nicotine CS during the early abstinence period. Furthermore, in rats trained for both nicotine CPP and operant nicotine self-administration, reconsolidation interference after nicotine UCS retrieval inhibited both nicotine CPP and operant nicotine seeking. By contrast, reconsolidation interference after CPP-CS or operant CS memory retrieval selectively inhibited subsequent nicotine CPP, but not operant nicotine seeking and vice versa, respectively.

Together, the findings across species suggest that the UCS-induced memory retrieval-reconsolidation interference procedures are more promising for relapse prevention than the CS-induced procedures.

Methodological and Translational Considerations and Limitations

Before we reach the goal of clinical implementation, several issues should be considered. First, the results of our human study were limited to the measurement of craving and preference during an early abstinence period within a laboratory setting. Craving often precedes and predicts drug relapse, but is neither necessary nor sufficient for it to occur.^{65,66} Additionally, the response to drug-associated CS in rat models⁶⁷ and among people⁶⁸ is often context-specific. Even after prolonged extinction of the response to drug CS within a non-drug context, the same CS can provoke relapse in the drug environment. Thus, we still need to test whether the UCS-induced memory retrieval-reconsolidation interference procedure will decrease craving and relapse in the smokers' home environments. In the absence of data from a larger clinical study with additional outcome measures, we cannot draw strong conclusions about the clinical utility of the procedure.

Another issue to consider from a translational perspective is that the timing of propranolol administration differed between our rat and human studies: we administered propranolol after UCS retrieval in rats but prior to UCS retrieval in the human study because of the pharmacokinetics of oral administration in humans. This was a procedural necessity, but it complicates our data interpretation. Specifically, it cannot be ruled out that propranolol's effect on nicotine preference and craving among people was because of interference with memory retrieval rather than memory reconsolidation. Another issue arising in our human study is that under our experimental conditions, nicotine craving were only observed after exposure to preexisting CS, not newly learned CS. This observation agrees with results from a previous study using a similar procedure⁶⁹ and is likely because of insufficient pairings between the new CS and cigarette smoking.

Finally, the generalizability of our results is limited by our having used only males of each species. We recognize that this approach is no longer tenable⁷⁰ because of sex differences in nicotine's effects and smoking cessation,^{50,51,71} and we plan to address it in the future.

Conclusions

We introduced a UCS-induced memory retrieval-reconsolidation interference procedure and demonstrated its efficacy in simultaneously preventing nicotine CPP and operant nicotine seeking during prolonged abstinence periods in a rat model. Our results among people who smoke suggest that a UCS-induced procedure may be a promising method for decreasing nicotine craving. Additionally, to the degree that results from rat models generalize to drug addiction among people,⁷² the potential value of the procedure should be tested for the prevention of relapse to smoking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Hunt WA, Barnett LW, Branch LG. Relapse rates in addiction programs. *J Clin Psychol.* 1971;27(4): 455–456. [PubMed: 5115648]
2. Zhou X, Nonnemaker J, Sherrill B, Gilsenan AW, Coste F, West R. Attempts to quit smoking and relapse: factors associated with success or failure from the ATTEMPT cohort study. *Addict Behav.* 2009;34(4):365–373. [PubMed: 19097706]
3. 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. [PubMed: 19922574]
4. Shiffman S. Dynamic influences on smoking relapse process. *J Pers.* 2005;73(6):1715–1748. [PubMed: 16274451]
5. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. *Trends Neurosci.* 2011;34(8):411–420. [PubMed: 21764143]
6. Shaham Y, Shalev U, Lu L, De Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl).* 2003;168(1–2):3–20. [PubMed: 12402102]
7. Jasinska AJ, Stein EA, Kaiser J, Naumer MJ, Yalachkov Y. Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. *Neurosci Biobehav Rev.* 2014;38:1–16. [PubMed: 24211373]
8. Stoker AK, Markou A. Neurobiological bases of cue- and nicotine-induced reinstatement of nicotine seeking: implications for the development of smoking cessation medications. *Curr Top Behav Neurosci.* 2015;24:125–154. [PubMed: 25638336]

9. Alberini CM. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? *Trends Neurosci.* 2005;28(1):51–56. [PubMed: 15626497]
10. Nader K, Hardt O. A single standard for memory: the case for reconsolidation. *Nat Rev Neurosci.* 2009;10(3):224–234. [PubMed: 19229241]
11. Misanin JR, Miller RR, Lewis DJ. Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science.* 1968;160(3827):554–555. [PubMed: 5689415]
12. Debiec J, Ledoux JE. Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience.* 2004;129(2):267–272. [PubMed: 15501585]
13. Przybylski J, Roulet P, Sara SJ. Attenuation of emotional and nonemotional memories after their reactivation: role of beta adrenergic receptors. *J Neurosci.* 1999;19(15):6623–6628. [PubMed: 10414990]
14. Torregrossa MM, Taylor JR. Learning to forget: manipulating extinction and reconsolidation processes to treat addiction. *Psychopharmacology (Berl).* 2013;226(4):659–672. [PubMed: 22638814]
15. Soeter M, Kindt M. An abrupt transformation of phobic behavior after a post-retrieval amnesic agent. *Biol Psychiatry.* 2015;78(12):880–886. [PubMed: 25980916]
16. 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. [PubMed: 17588604]
17. Brunet A, Thomas É, Saumier D, et al. Trauma reactivation plus propranolol is associated with durably low physiological responding during subsequent script-driven traumatic imagery. *Can J Psychiatry.* 2014;59(4):228–232. [PubMed: 25007116]
18. Fricks-Gleason AN, Marshall JF. Post-retrieval beta-adrenergic receptor blockade: effects on extinction and reconsolidation of cocaine-cue memories. *Learn Mem.* 2008;15(9):643–648. [PubMed: 18772251]
19. Otis JM, Mueller D. Inhibition of β -adrenergic receptors induces a persistent deficit in retrieval of a cocaine-associated memory providing protection against reinstatement. *Neuropsychopharmacology.* 2011;36(9):1912–1920. [PubMed: 21544069]
20. Robinson MJ, Franklin KB. Reconsolidation of a morphine place preference: impact of the strength and age of memory on disruption by propranolol and midazolam. *Behav Brain Res.* 2010;213(2): 201–207. [PubMed: 20457186]
21. Milton AL, Everitt BJ. The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci.* 2010;31(12): 2308–2319. [PubMed: 20497475]
22. Diergaarde L, Schoffeleers AN, De Vries TJ. Pharmacological manipulation of memory reconsolidation: towards a novel treatment of pathogenic memories. *Eur J Pharmacol.* 2008;585 (2–3):453–457. [PubMed: 18410917]
23. Sorg BA. Reconsolidation of drug memories. *Neurosci Biobehav Rev.* 2012;36(5):1400–1417. [PubMed: 22342780]
24. Zhao LY, Sun LL, Shi J, Li P, Zhang Y, Lu L. Effects of β -adrenergic receptor blockade on drug-related memory reconsolidation in abstinent heroin addicts. *Drug Alcohol Depend.* 2011;118(2–3): 224–229. [PubMed: 21531091]
25. 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. [PubMed: 23460266]
26. Jobes ML, Aharonovich E, Epstein DH, et al. Effects of preactivation propranolol on cocaine craving elicited by imagery script/cue sets in opioid-dependent polydrug users: a randomized study. *J Addict Med.* 2015;9(6):491–498. [PubMed: 26501788]
27. 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. [PubMed: 25413896]

28. Debiec J, Díaz-Mataix L, Bush DE, Doyère V, Ledoux JE. The amygdala encodes specific sensory features of an aversive reinforcer. *Nat Neurosci.* 2010;13(5):536–537. [PubMed: 20348916]
29. Díaz-Mataix L, Debiec J, LeDoux JE, Doyère V. Sensory-specific associations stored in the lateral amygdala allow for selective alteration of fear memories. *J Neurosci.* 2011;31(26):9538–9543. [PubMed: 21715618]
30. Schiller D, Monfils MH, Raio CM, Johnson DC, Ledoux JE, Phelps EA. Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature.* 2010;463(7277):49–53. [PubMed: 20010606]
31. Luo YX, Xue YX, Liu JF, et al. A novel UCS memory retrieval-extinction procedure to inhibit relapse to drug seeking. *Nat Commun.* 2015;6 (7675)1–14.
32. Pericot-Valverde I, Secades-Villa R, Gutiérrez-Maldonado J, García-Rodríguez O. Effects of systematic cue exposure through virtual reality on cigarette craving. *Nicotine Tob Res.* 2014;16(11): 1470–1477. [PubMed: 24962558]
33. Liu J, Zhao L, Xue Y, et al. An unconditioned stimulus retrieval extinction procedure to prevent the return of fear memory. *Biol Psychiatry.* 2014;76 (11):895–901. [PubMed: 24813334]
34. Venniro M, Caprioli D, Shaham Y. Animal models of drug relapse and craving: from drug priming-induced reinstatement to incubation of craving after voluntary abstinence. *Prog Brain Res.* 2016;224:25–52. [PubMed: 26822352]
35. Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. *Brain Res.* 1982;243(1): 91–105. [PubMed: 6288174]
36. Schuster CR, Thompson T. Self administration of and behavioral dependence on drugs. *Annu Rev Pharmacol.* 1969;9:483–502. [PubMed: 4978013]
37. O'Brien CP, Childress AR, McLellan AT, Ehrman R. Classical conditioning in drug-dependent humans. *Ann N Y Acad Sci.* 1992;654:400–415. [PubMed: 1632593]
38. Sinha R, Fuse T, Aubin LR, O'Malley SS. Psychological stress, drug-related cues and cocaine craving. *Psychopharmacology (Berl).* 2000;152(2): 140–148. [PubMed: 11057517]
39. Janes AC, Ross RS, Farmer S, et al. Memory retrieval of smoking-related images induce greater insula activation as revealed by an fMRI-based delayed matching to sample task. *Addict Biol.* 2015; 20(2):349–356. [PubMed: 24261848]
40. Jaffe JH, Cascella NG, Kumor KM, Sherer MA. Cocaine-induced cocaine craving. *Psychopharmacology (Berl).* 1989;97(1):59–64. [PubMed: 2496428]
41. Li FQ, Xue YX, Wang JS, et al. Basolateral amygdala cdk5 activity mediates consolidation and reconsolidation of memories for cocaine cues. *J Neurosci.* 2010;30(31):10351–10359. [PubMed: 20685978]
42. Li SX, Zou Y, Liu LJ, Wu P, Lu L. Aripiprazole blocks reinstatement but not expression of morphine conditioned place preference in rats. *Pharmacol Biochem Behav.* 2009;92(2):370–375. [PubMed: 19353810]
43. Wang XY, Zhao M, Ghitza UE, Li YQ, Lu L. Stress impairs reconsolidation of drug memory via glucocorticoid receptors in the basolateral amygdala. *J Neurosci.* 2008;28(21):5602–5610. [PubMed: 18495894]
44. Guillem K, Peoples LL. Progressive and lasting amplification of accumbal nicotine-seeking neural signals. *J Neurosci.* 2010;30(1):276–286. [PubMed: 20053909]
45. Xue YX, Luo YX, Wu P, et al. A memory retrieval-extinction procedure to prevent drug craving and relapse. *Science.* 2012;336(6078): 241–245. [PubMed: 22499948]
46. Lê AD, Li Z, Funk D, Shram M, Li TK, Shaham Y. Increased vulnerability to nicotine self-administration and relapse in alcohol-naïve offspring of rats selectively bred for high alcohol intake. *J Neurosci.* 2006;26(6):1872–1879. [PubMed: 16467536]
47. Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ. Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci USA.* 2008;105(49):19480–19485.
48. Shaham Y, Adamson LK, Grocki S, Corrigall WA. Reinstatement and spontaneous recovery of nicotine seeking in rats. *Psychopharmacology (Berl).* 1997;130(4):396–403. [PubMed: 9160857]
49. Yan Y, Pushparaj A, Le Strat Y, et al. Blockade of dopamine d4 receptors attenuates reinstatement of extinguished nicotine-seeking behavior in rats. *Neuropsychopharmacology.* 2012; 37(3):685–696. [PubMed: 22030716]

50. Franklin TR, Jagannathan K, Wetherill RR, et al. Influence of menstrual cycle phase on neural and craving responses to appetitive smoking cues in naturally cycling females. *Nicotine Tob Res.* 2015;17 (4):390–397. [PubMed: 25762748]
51. Huttlin EA, Allen AM, Tosun NL, Allen SS, al'Absi M. Associations between adrenocortical activity and nicotine response in female smokers by menstrual phase. *Addict Behav.* 2015;50:135–139. [PubMed: 26135333]
52. Johnsson G, Regårdh CG. Clinical pharmacokinetics of beta-adrenoreceptor blocking drugs. *Clin Pharmacokinet.* 1976;1(4):233–263. [PubMed: 13958]
53. 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. [PubMed: 19219038]
54. Soeter M, Kindt M. Dissociating response systems: erasing fear from memory. *Neurobiol Learn Mem.* 2010;94(1):30–41. [PubMed: 20381628]
55. Childs E, de Wit H. Amphetamine-induced place preference in humans. *Biol Psychiatry.* 2009; 65(10):900–904. [PubMed: 19111278]
56. Mayo LM, Fraser D, Childs E, et al. Conditioned preference to a methamphetamine-associated contextual cue in humans. *Neuropsychopharmacology.* 2013;38(6):921–929. [PubMed: 23416857]
57. Ebbert JO, Hatsukami DK, Croghan IT, et al. Combination varenicline and bupropion SR for tobacco-dependence treatment in cigarette smokers: a randomized trial. *JAMA.* 2014;311(2): 155–163. [PubMed: 24399554]
58. 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. [PubMed: 10963596]
59. Miller CA, Marshall JF. Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron.* 2005;47(6):873–884. [PubMed: 16157281]
60. Lee JL, Di Ciano P, Thomas KL, Everitt BJ. Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron.* 2005; 47(6):795–801. [PubMed: 16157275]
61. Diergaarde L, Schoffeleer AN, De Vries TJ. Beta-adrenoceptor mediated inhibition of long-term reward-related memory reconsolidation. *Behav Brain Res.* 2006;170(2): 333–336. [PubMed: 16600394]
62. Wouda JA, Diergaarde L, Riga D, van Mourik Y, Schoffeleer AN, De Vries TJ. Disruption of long-term alcohol-related memory reconsolidation: role of β -adrenoceptors and NMDA receptors. *Front Behav Neurosci.* 2010;4(179):1–7. [PubMed: 20126432]
63. Loneragan M, Saumier D, Tremblay J, Kieffer B, Brown TG, Brunet A. Reactivating addiction-related memories under propranolol to reduce craving: A pilot randomized controlled trial. *J Behav Ther Exp Psychiatry.* 2016;50:245–249. [PubMed: 26454715]
64. Hutton-Bedbrook K, McNally GP. The promises and pitfalls of retrieval-extinction procedures in preventing relapse to drug seeking. *Front Psychiatry.* 2013;4(14):1–4. [PubMed: 23346060]
65. Tiffany ST, Carter BL. Is craving the source of compulsive drug use? *J Psychopharmacol.* 1998;12 (1):23–30. [PubMed: 9584965]
66. Epstein DH, Willner-Reid J, Vahabzadeh M, Mezghanni M, Lin JL, Preston KL. Real-time electronic diary reports of cue exposure and mood in the hours before cocaine and heroin craving and use. *Arch Gen Psychiatry.* 2009;66(1):88–94. [PubMed: 19124692]
67. Bossert JM, Marchant NJ, Calu DJ, Shaham Y. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology (Berl).* 2013;229(3):453–476. [PubMed: 23685858]
68. Carter BL, Tiffany ST. The cue-availability paradigm: the effects of cigarette availability on cue reactivity in smokers. *Exp Clin Psychopharmacol.* 2001;9(2):183–190. [PubMed: 11518094]
69. Winkler MH, Weyers P, Mucha RF, Stippekohl B, Stark R, Pauli P. Conditioned cues for smoking elicit preparatory responses in healthy smokers. *Psychopharmacology (Berl).* 2011;213(4): 781–789. [PubMed: 20953588]
70. Clayton JA. Studying both sexes: a guiding principle for biomedicine. *FASEB J.* 2016;30(2): 519–524. [PubMed: 26514164]
71. Smith PH, Bessette AJ, Weinberger AH, Sheffer CE, McKee SA. Sex/gender differences in smoking cessation: a review. *Prev Med.* 2016;92: 135–140. [PubMed: 27471021]

72. Heilig M, Epstein DH, Nader MA, Shaham Y. Time to connect: bringing social context into addiction neuroscience. *Nat Rev Neurosci*. 2016;17 (9):592–599. [PubMed: 27277868]

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Key Points**Question**

How can multiple nicotine-associated memories and nicotine craving be selectively inhibited?

Findings

In rat models and a human study, propranolol administered with an unconditioned stimulus-induced memory reactivation-reconsolidation interference procedure inhibited nicotine craving induced by exposure to diverse nicotine-associated cues and nicotine itself. By contrast, the established conditioned stimulus-induced memory reactivation-reconsolidation interference procedure only inhibited nicotine craving induced by the previously reactivated nicotine-associated cues.

Meaning

These findings suggest that an unconditioned stimulus-induced procedure may be a promising method for decreasing nicotine craving and, potentially, relapse among smokers.

Figure 1.

Propranolol Disrupts Reconsolidation of Multiple Nicotine Reward Memories After Unconditional Stimulus (UCS) Memory Retrieval in Rats

Systemic injections of propranolol immediately after the UCS exposure decreased subsequent expression of nicotine conditioned place preference (CPP) (A) and operant nicotine seeking (B). Vehicle. 9 rats; propranolol. 8 rats. Data are mean (standard error of the mean).

^aDifferent from the vehicle group, mixed analysis of variance. $P < .05$.

Figure 2.

Propranolol Disrupts Reconsolidation of Specific Operant-Related Nicotine Reward Memories After Operant Conditioned Stimuli (CS) Memory Retrieval in Rats

Systemic injections of propranolol administered immediately after exposure to operant-CS retrieval decreased cue-induced operant nicotine seeking (B) but not nicotine conditioned place preference (CPP) (A). Vehicle. 7 rats; propranolol. 8 rats. Data are mean (stand error of the mean).

^aDifferent from the vehicle group, mixed analysis of variance. $P < .05$.

Figure 3.
 Propranolol Administration Before Unconditioned Stimulus (UCS) Memory Retrieval
 Decreased Subsequent Preference for Nicotine-Associated Conditioned Stimuli (CS) Among
 Smokers

Figure 4.
Propranolol Administration Before Unconditioned Stimulus (UCS) Memory Retrieval
Decreased Subsequent Craving Induced by Preexisting Cues and Nicotine Priming Among
Smokers

Table.

Participants' Demographic Information

Group	Mean(SEM)			P Value
	UCS + Placebo (n = 224 males)	UCS + Propranolol (n = 23 males)	UCS + 6 h + Propranolol (n = 22 males)	
Age, y	23.00 (0.82)	24.96 (1.24)	22.82 (0.30)	.17
Cigarettes, No.	11.79 (0.81)	12.63 (1.21)	11.64 (0.43)	.65
Education, y	14.13 (0.35)	15.17 (0.44)	14.77 (0.33)	.19
Score				
FTND	5.42 (0.20)	5.25 (0.23)	5.64 (0.14)	.42
BDI	4.92 (1.02)	4.96 (0.75)	4.73 (0.72)	.97
SAS	26.88 (0.87)	26.25 (0.93)	27.95 (1.02)	.53
SAS standard	32.13 (1.67)	32.42 (1.17)	34.64 (1.26)	.43

Abbreviations: BDI, Beck Depression Inventory; FTND, Fagerstrom Test for Nicotine Dependence; SAS, self-rating anxiety scale; UCS, unconditioned stimulus.