

Original Investigation

Association Between Nicotine Withdrawal and Reward Responsiveness in Humans and Rats

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IMPORTANCE Reward-related disturbances after withdrawal from nicotine are hypothesized to contribute to relapse to tobacco smoking but mechanisms underlying and linking such processes remain largely unknown.

OBJECTIVE To determine whether withdrawal from nicotine affects reward responsiveness (ie, the propensity to modulate behavior as a function of prior reinforcement experience) across species using translational behavioral assessments in humans and rats.

DESIGN, SETTING, PARTICIPANTS Experimental studies used analogous reward responsiveness tasks in both humans and rats to examine whether reward responsiveness varied in (1) an ad libitum smoking condition compared with a 24-hour acute nicotine abstinence condition in 31 human smokers with (n = 17) or without (n = 14) a history of depression; (2) rats 24 hours after withdrawal from chronic nicotine (n = 19) or saline (n = 20); and (3) rats following acute nicotine exposure after withdrawal from either chronic nicotine or saline administration.

MAIN OUTCOMES AND MEASURES Performance on a reward responsiveness task under nicotine and nonnicotine conditions.

RESULTS In both human smokers and nicotine-treated rats, reward responsiveness was significantly reduced after 24-hour withdrawal from nicotine ($P < .05$). In humans, withdrawal-induced deficits in reward responsiveness were greater in those with a history of depression. In rats previously exposed to chronic nicotine, acute nicotine reexposure long after withdrawal potentiated reward responsiveness ($P < .05$).

CONCLUSIONS AND RELEVANCE These findings across species converge in suggesting that organisms have diminished ability to modulate behavior as a function of reward during withdrawal of nicotine. This blunting may contribute to relapse to tobacco smoking, particularly in depression-vulnerable individuals, to reinstate responsiveness to natural rewards and to experience potentiated nicotine-induced reward responsiveness. Moreover, demonstration of behavioral homology across humans and rodents provides a strong translational framework for the investigation and development of clinical treatments targeting reward responsiveness deficits during early withdrawal of nicotine.

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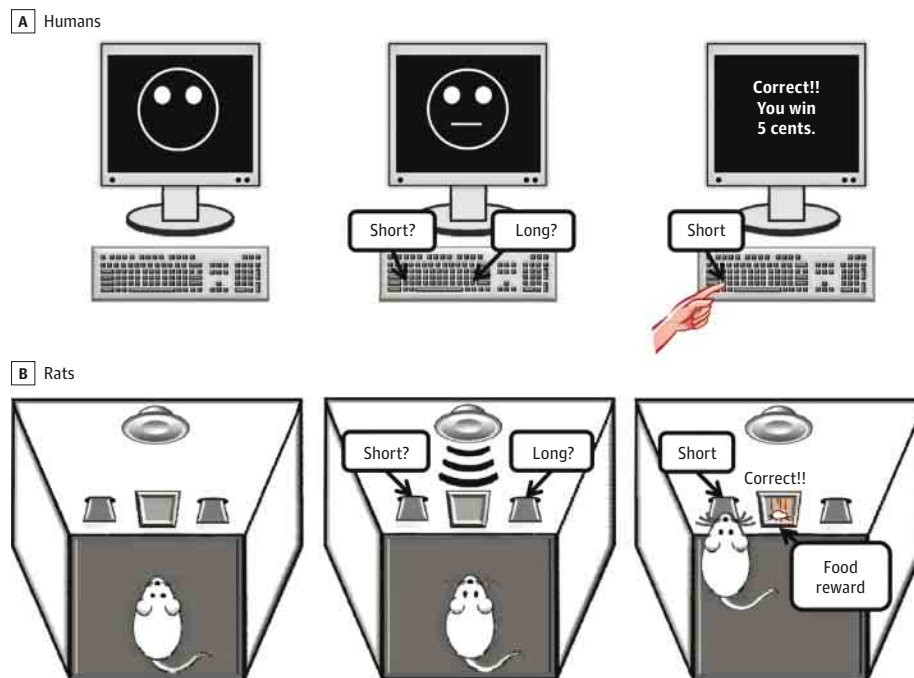
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Smoking is a leading cause of disease and mortality worldwide,^{1,2} and many smokers experience difficulty with quitting and with nicotine withdrawal.³⁻⁸ While exposure to nicotine is associated with increased responsiveness to rewards⁹⁻¹⁸ in rodents and humans, less is known about the role of different reward-related processes during nicotine withdrawal. Studies in rodents using the intracranial self-stimulation procedure¹⁹⁻²¹ have consistently shown decrements in brain reward function during nicotine withdrawal but assessments of motivation and effort for natural rewards in rodents²²⁻²⁴ and humans^{14,16,25,26} have produced less-consistent results likely owing to the heterogeneity of tests measuring motivation and reward responsiveness between humans and rodents. Thus, it remains unclear which reward-related processes are compromised after withdrawal from nicotine, hindering development of cessation treatments.

Here, we examined the effects of withdrawal of nicotine on *reward responsiveness*, defined as the propensity to modulate behavior as a function of prior reinforcement experience,²⁷⁻²⁹ using a Response Bias Probabilistic Reward Task (RB-PRT) developed to objectively quantify reward responsiveness in humans²⁷ and rats.³⁰ During this task, both rats and humans must distinguish between 2 ambiguous stimuli, whereby correct identification of either stimulus is partially reinforced (Figure 1). Unbeknownst to them, throughout the test session, correct identification of 1 stimulus (rich) is rewarded 3 times more frequently than correct identification of the other stimulus (lean). Because of the differential reinforcement schedule, healthy rats and humans develop a response bias in favor of the more frequently rewarded (rich) stimulus. In a placebo-controlled study, acute nicotine administration in current nonsmokers was associated with

Figure 1. Schematic Representation of the Human and Rat Response Bias Probabilistic Reward Task



A, In each trial, human participants were asked to choose whether a short (11.5 mm) or long (13 mm) mouth (briefly flashed for 100 milliseconds) had been presented on a mouthless schematic face by pressing a key (eg, z for short and / for long). In each of the 3 blocks (100 trials/block), the mouth stimuli were pseudorandomly presented in an equal number. For some of the correct trials, the participant received monetary reinforcement (5 cents). Unbeknownst to the participants, the reinforcement schedule was designed to favor 1 mouth length (ie, rich) over the other (ie, lean) in a 3:1 ratio. Only 40 correct trials were rewarded in each block (30 rich and 10 lean). Participants were instructed that the goal of the task was to win as much money as possible and that not all correct responses would receive a reward feedback. Response bias, our main variable of interest, was calculated as $\log b = \frac{1}{2} \log \left[\frac{\text{rich}_{\text{correct}} * \text{lean}_{\text{incorrect}}}{(\text{rich}_{\text{incorrect}} * \text{lean}_{\text{correct}})} \right]$. As evident from the formula, a high response bias emerges when participants tend to correctly identify the rich stimulus and misclassify the lean stimulus. Discriminability, which is the degree to which the participant can distinguish the 2 target stimuli and is a measure of task difficulty, was used as a control variable and was calculated as $\log d = \frac{1}{2} \log \left[\frac{(\text{rich}_{\text{correct}} * \text{lean}_{\text{correct}})}{(\text{rich}_{\text{incorrect}} * \text{lean}_{\text{incorrect}})} \right]$. These formulae include the addition of 0.5 to each cell to allow for estimation in cases with a zero cell. Accuracy (percentage hit rate) and reaction time in response to the rich and lean

stimuli represented additional secondary behavioral variables. B, Rats were food restricted and trained to discriminate between 2 tones varying in duration (5 kHz or 60 dB and 0.5 seconds or 2 seconds) by pressing 1 of the 2 levers associated with each tone. Tone durations and lever sides were counterbalanced across subjects and tones were presented in a random order over 100 trials. Each trial was initiated with presentation of a tone, after which levers were extended and rats had a 5-second limited hold period to respond. In each trial, correct identification of tones resulted in a single 45-mg food pellet (Test Diet 5TUM; Richmond, Indiana). Both levers retracted after a correct, incorrect, or omitted response, followed by a variable intertrial interval (5-8 seconds). Rats were trained daily until achieving at least 70% accuracy for 5 consecutive days. Rats that were successful in discriminating the tones were then trained with tone durations of 0.7 and 1.8 seconds for 2 days and tone durations of 0.9 and 1.6 seconds for 2 days. During a subsequent test session, the ambiguous tone durations (ie, 0.9 and 1.6 seconds) were reinforced for 60% and 20% of correct responses (counterbalanced across subjects) over 100 trials, which is identical to the 3:1 reinforcement ratio used in the human Response Bias Probabilistic Reward Task.³⁰ Response bias, the primary variable, as well as the 3 secondary behavioral variables (discriminability, accuracy, and reaction time), were computed using identical formulae as for the human experimental data.

potentiated reward responsiveness.¹⁸ However, the study included individuals with prior smoking history, which may differentially mediate reward responsiveness during acute nicotine reexposure relative to those without prior smoking experience. Notably, human participants with elevated depression-related symptoms^{27,28,31} show blunted reward responsiveness (ie, reduced response bias) in this task.

Given that nicotine withdrawal is characterized by depression-like symptoms,³² these previous findings for depression^{27,28,31} may suggest that withdrawal of nicotine is also associated with blunted reward responsiveness. Moreover, many smokers have a history of major depression^{33,34}; such individuals are more likely to experience nicotine-withdrawal symptoms and continue smoking,^{4,33,35-38} and trait anhedonia is associated with relapse to smoking.^{39,40} Such findings promoted the hypothesis that many smokers are self-medicating an underlying depressive vulnerability,^{41,42} which has received varying degrees of support. In smokers with trait anhedonia or history of depression, nicotine use is related to increased positive mood,^{43,44} while abstinence is associated with reduced attentional bias toward positive stimuli.⁴⁵ Similarly, smokers with a history of depression ascribe greater value to cigarettes relative to natural rewards,⁴⁶ which may hinder substitution of healthy rewards for cigarettes during cessation. However, to our knowledge, there has been limited consideration of depression history in regards to the effects of withdrawal of nicotine on reward processes. Moreover, the high rate of relapse to smoking during withdrawal from nicotine⁴⁷⁻⁴⁹ may potentially arise from reward responsiveness deficits, with the resumption of nicotine use reversing such deficits.

In light of prior independent lines of evidence, we hypothesized that (1) withdrawal from chronic nicotine exposure would be associated with blunted reward responsiveness (ie, reduced response bias) in human smokers and rats; (2) withdrawal-related changes in reward responsiveness would be exacerbated in human smokers with a history of major depressive disorder; and (3) acute nicotine reexposure after nicotine withdrawal would enhance reward responsiveness in rats.

Methods

Humans

Participants

Heavy smokers (smoking ≥ 15 cigarettes per day and smoking for ≥ 5 years) not planning to quit permanently over the next month participated. Exclusion criteria included age younger than 18 years, current use of smoking-cessation aids, and current or planned pregnancy. Ninety-three percent of ineligible candidates ($n = 314$) did not meet cigarette use criteria or planned to quit cigarettes permanently over the next month. Eligible candidates were scheduled for a screening interview and study overview, read and signed an informed consent, and verified smoking status using an ecolyzer to measure expired carbon monoxide. All procedures were approved by the Human Research Protection Office at Washington University.

Of the 99 individuals enrolled, 60 completed baseline and 2 test sessions (described further on). The RB-PRT was added

halfway through data collection for 37 completed participants (details provided in eAppendix 1 in the Supplement). This sample of 37 had the following characteristics: mean (SD) cigarettes smoked per day, 22.3 (6.0); mean (SD) years smoking, 23.3 (13.5); mean (SD) age, 41.1 (14.2) years; 54% women; 57% with a lifetime history of major depression; and 89% with a high school education or higher.

Procedures and Assessments

Baseline Visit | Candidates meeting preliminary inclusion criteria were administered self-report questionnaires and a diagnostic interview, a modified Semi-Structured Assessment for the Genetics of Alcoholism,⁵⁰ with the smoking section modified from the Composite International Diagnostic Interview,⁵¹ which included lifetime assessments of nicotine withdrawal and major depression.³²

Approximately 90-Minute Test Sessions | During sessions separated by a median of 7 days and counterbalanced across participants, participants completed self-report questionnaires and were tested under (1) ad libitum smoking and (2) 24-hour nicotine-abstinence conditions. Smoking and abstinence were verified by self-report and a noninvasive breath test ecolyzer measurement of exhaled carbon monoxide (eAppendix 1 in the Supplement). The RB-PRT was administered to quantify reward responsiveness. Response bias (the main variable of interest; see Figure 1 for calculation details), discriminability (control variable), accuracy (ie, correct responses/[correct + incorrect responses]) and reaction time for each stimulus type (ie, rich/lean) were calculated. Reaction time shorter than 150 milliseconds or longer than 2500 milliseconds were removed; participants with more than 10% of trials with outlying reaction times were removed entirely ($n = 6$), leaving 31 participants with valid data from both test sessions. The 6 individuals removed were similar in sample characteristics from the remaining 31 participants (all $P > .05$). Of these 31 smokers, 55% ($n = 17$) had a history of lifetime major depression. The sample was sufficiently remitted at baseline, reflected by the average Profile of Mood States Total Mood Disturbance Scale⁵² score of participants without (mean [SD], 9.9 [23.1]) and with (mean [SD], 17.8 [31.3]) a history of depression being lower and within range of the average score published for normative nonpsychiatric participants (range, 17-19),⁵³ respectively.

Statistical Analyses

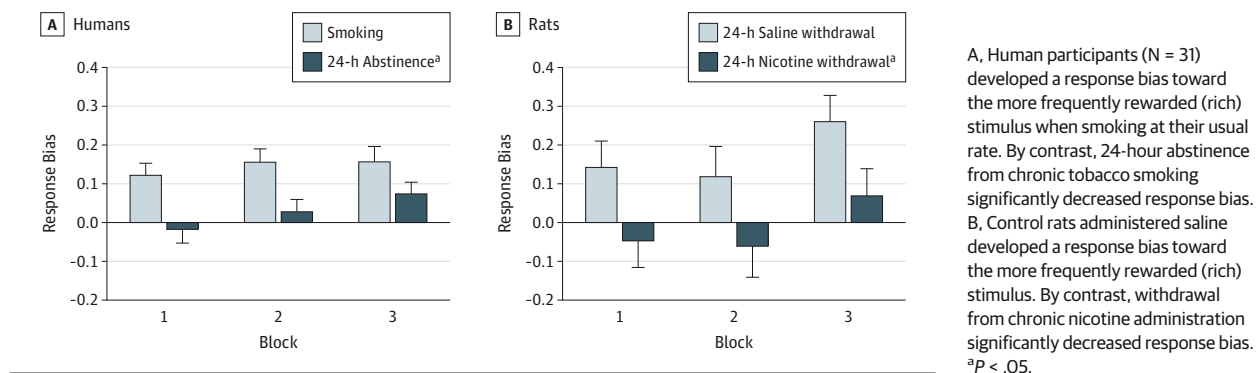
For response bias, mixed analysis of variance with nicotine status (smoking or 24-hour abstinence) and block (1, 2, or 3; 100 trials/block) as repeated measures and history of depression (present or absent) as the between-subjects factor were performed. Greenhouse-Geisser corrected estimates are reported.

Rats

Subjects

Forty-six adult male Wistar rats (Charles River Laboratories, Raleigh, North Carolina) were pair housed with food and water available ad libitum prior to behavioral training. All procedures were conducted in accordance with guidelines from the National Institutes of Health and the Association for the

Figure 2. Withdrawal of Nicotine and Blunted Reward Responsiveness in Humans and Rats



Assessment and Accreditation of Laboratory Animal Care and were approved by the University of California San Diego's institutional animal care and use committee.

Apparatus

Training and testing were conducted in operant chambers (Med Associates; St. Albans, Vermont) consisting of 2 metal retractable levers, a food receptacle located between the levers, and a speaker located above the food receptacle. Tones were generated using a multipurpose sound generator. All programs and data collection were controlled by a computer running MED-PC IV software.³⁰

Procedure

Rats were trained on the RB-PRT and tested under baseline conditions (see Figure 1 and the study by Der-Avakian et al³⁰ for details). Rats were then surgically prepared with subcutaneous osmotic minipumps (Alzet Osmotic Pumps; Cupertino, California) delivering either a 6.32-mg/kg/d (base) (-)-nicotine hydrogen tartrate solution (Sigma, St Louis, Missouri) or vehicle (sterile 0.9% saline) for 28 days.

Rats continued to train during drug administration with the parameters described in Figure 1. Before minipump removal, rats received increasingly ambiguous tones as stimuli while being equally reinforced for all correct responses. Twenty-four hours after minipump removal, rats were tested with the same tone and reinforcement parameters as during the baseline test session.

After the withdrawal test, rats were exposed to the training parameters for 2 weeks and tested in response to acute nicotine administration. Two days prior to the initial acute nicotine test, all rats received 0.125 mg/kg of nicotine (base) subcutaneously after the training session to habituate to the subjective experience of acute nicotine exposure. Rats then received either 0, 0.125, 0.25, or 0.5 mg/kg of nicotine (base; 15-minute pretreatment) in a within-subjects Latin-square design, and reward responsiveness was assessed 2, 4, 6, and 8 weeks after the withdrawal test.

Statistical Analyses

Data were cumulated and analyzed across blocks 1 (trials 1-33), 2 (trials 34-67), and 3 (trials 68-100). Rats were excluded owing to insufficient accuracy during discrimination training (ie,

<70%; n = 5) and complications with minipumps (1 nicotine and 1 saline). Thus, data from 39 rats were available for the withdrawal test. Rats with less than 30% accuracy for either stimulus during testing were excluded because insufficient responding prevents the differential (ie, 3:1) reward distribution, as in the human task. Five chronic saline-treated rats and 2 chronic nicotine-treated rats were excluded from the acute nicotine test. Response bias was calculated as described here for humans. For the withdrawal test, response bias was analyzed with a 2-way mixed analysis of covariance with chronic drug treatment (between subjects) and block (within subjects) as factors. For the acute nicotine tests, acute nicotine dose was included as a within-subjects factor. Inherent side biases unrelated to the differential reinforcement schedule during testing were controlled as a covariate, defined as the change in response bias from blocks 1 to 3 during the pretest training session.

For human and rat data analyses, significant main and interaction effects involving analysis of variance factors (eg, nicotine status, block, and depression in smokers and acute nicotine dose in rats) were clarified using post hoc *t* tests. The significance level was 0.05. Additional detail on samples and procedures for humans and rats are available in eAppendix 1 in the Supplement.

Results

Response Bias in Humans

Among adult heavy-smoking humans, a 3-way analysis of variance with nicotine status (ad libitum smoking or 24-hour abstinence), block (1, 2, or 3), and history of depression (present or absent) as factors revealed that 24-hour nicotine abstinence was associated with a significant reduction in response bias (nicotine status: $F_{1,29} = 6.61$, $P = .02$; partial eta squared [η_p^2] = 0.19) (Figure 2A). No other effects emerged. Although the nicotine status by history of depression interaction reached only a statistical trend ($P = .10$; $\eta_p^2 = 0.09$), a priori subsidiary analyses found that smokers without depression history exhibited significant increases in response bias (ie, reward learning) across blocks during abstinence ($P = .03$, $\eta_p^2 = 0.25$; ad libitum: $P = .94$, $\eta_p^2 = 0.01$). Smokers with a history of depression failed to show changes in response bias across blocks (abstinence: $P = .46$, $\eta_p^2 = 0.05$; ad libitum:

Figure 3. Nicotine Abstinence and Reward Responsiveness in Humans Without (n=14) and With (n=17) a History of Depression

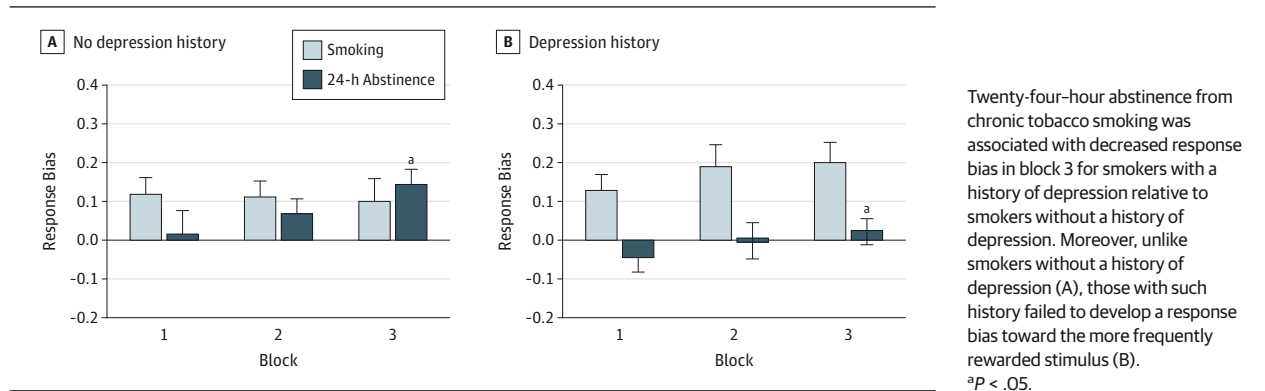
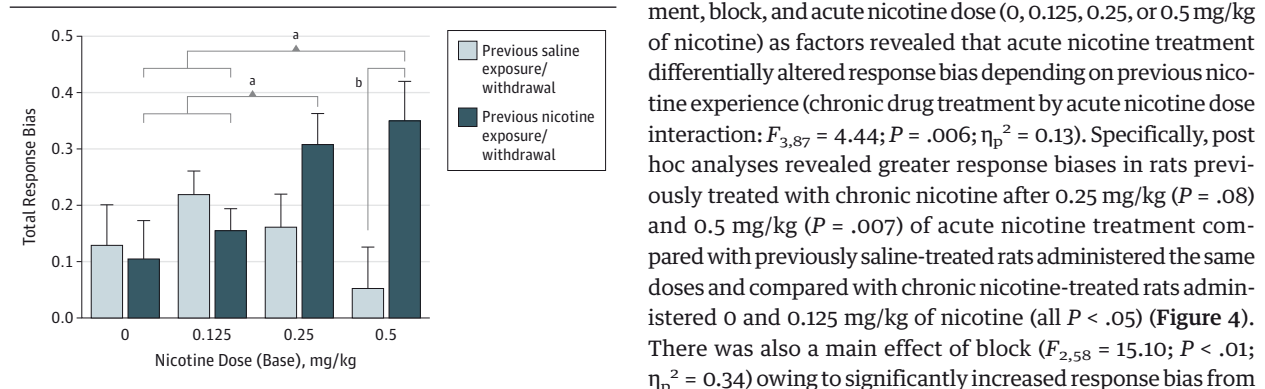


Figure 4. Acute Nicotine-Induced Changes in Reward Responsiveness in Rats Previously Exposed to Chronic Nicotine (n=17) or Saline (n=15)



Acute nicotine reexposure in rats previously treated with chronic nicotine significantly potentiated response bias compared with acute saline exposure and compared with acute nicotine exposure in rats previously treated with chronic saline. Moreover, acute nicotine treatment did not affect reward responsiveness in previously nicotine-naïve rats.
^aDifferent from chronic nicotine-treated rats administered 0 and 0.125 mg/kg of acute nicotine ($P < .05$).
^bDifferent from chronic saline-treated rats administered the same acute nicotine dose ($P < .01$).

$P = .45$, $\eta_p^2 = 0.05$). This group effect was detectable by block 3, whereby smokers with a history of depression had a smaller response bias during 24-hour abstinence than smokers without such history ($t = 2.06$, $P = .048$, $\eta_p^2 = 0.13$; ad libitum: $t = -1.30$, $P = .21$, $\eta_p^2 = 0.06$) (Figure 3).

Response Bias in Rats

A 2-way analysis of covariance with chronic drug treatment (nicotine or saline) and block (1, 2, or 3) as factors and inherent bias as a covariate revealed that withdrawal from chronic nicotine administration significantly reduced response bias relative to saline treatment (chronic drug treatment: $F_{1,36} = 4.18$; $P = .048$; $\eta_p^2 = 0.10$) (Figure 2B). There was also a main effect of block ($F_{2,72} = 6.05$; $P = .004$; $\eta_p^2 = 0.14$) due to significantly higher response bias in block 3 relative to block 1 ($t_{38} = -2.49$; $P = .02$) and block 2 ($t_{38} = -3.64$; $P < .001$), indicating that the differential reinforcement schedule was effective.

After withdrawal from chronic nicotine or saline administration, a 3-way analysis of covariance with chronic drug treatment, block, and acute nicotine dose (0, 0.125, 0.25, or 0.5 mg/kg of nicotine) as factors revealed that acute nicotine treatment differentially altered response bias depending on previous nicotine experience (chronic drug treatment by acute nicotine dose interaction: $F_{3,87} = 4.44$; $P = .006$; $\eta_p^2 = 0.13$). Specifically, post hoc analyses revealed greater response biases in rats previously treated with chronic nicotine after 0.25 mg/kg ($P = .08$) and 0.5 mg/kg ($P = .007$) of acute nicotine treatment compared with previously saline-treated rats administered the same doses and compared with chronic nicotine-treated rats administered 0 and 0.125 mg/kg of nicotine (all $P < .05$) (Figure 4). There was also a main effect of block ($F_{2,58} = 15.10$; $P < .01$; $\eta_p^2 = 0.34$) owing to significantly increased response bias from block 1 to block 2 to block 3 (all $P < .05$).

Secondary analyses of discriminability, accuracy, and reaction time for humans and rats are detailed in eAppendix 2 and the eFigure in the Supplement.

Discussion

Capitalizing on a task rooted in signal detection theory previously shown to be sensitive to detecting reward responsiveness deficits in depression and other mood disorders,^{27-29,31,54} the current results provide converging evidence across human smokers and rats chronically administered nicotine that withdrawal from nicotine is associated with reduced reward responsiveness. This compromised ability to modulate behavior as a function of rewarding experiences after withdrawal from chronic nicotine exposure, an effect that was exacerbated in humans with a history of major depression, was reversed with acute nicotine reexposure in rats. The results suggest that restoring or potentiating responsiveness to natural rewards through nicotine reexposure may contribute to relapse to tobacco smoking. Furthermore, these findings may help rectify previous inconsistent findings across species,^{14,16,19-26} which used heterogeneous measures to assess reward processing during withdrawal from nicotine, generated mixed results, and thus yielded limited translational opportunities. Our findings highlight the

value of using a conceptually identical reward task across species to objectively measure withdrawal-related decrements in reward responsiveness and provide a strong translational framework for identifying novel treatment strategies for smoking cessation.

Increased depressive symptoms³² and subjective stress levels⁵⁵ during withdrawal from chronic nicotine may accompany reward responsiveness deficits, and resuming nicotine use may act to reverse these deficits. Fitting this hypothesis, in the current study, acute nicotine exposure potentiated reward responsiveness in rats previously treated with chronic nicotine without affecting reward responsiveness in nicotine-naïve rats. These acute nicotine effects were observed 2 to 8 weeks after initiation of withdrawal from chronic nicotine. Moreover, human participants not currently smoking, but some with a history of smoking, showed similar acute nicotine-induced enhancement of reward responsiveness in a previous study.¹⁸ However, it is unclear whether individuals without a history of smoking, who were included in that overall analysis,¹⁸ displayed similar increases in reward responsiveness. By contrast, somatic signs of withdrawal in rats peaked within the first 24 hours of and dissipated 3 days after termination of chronic nicotine exposure.¹⁹ These results raise the possibility that enhanced reward responsiveness that is produced by acute nicotine reexposure long after initiation of abstinence, when other symptoms of withdrawal have dissipated, may contribute to relapse that occurs during protracted abstinence. Subsequent studies should consider the extent to which these results relate to putative therapeutic effects of smoking cessation treatment.

We also found suggestive evidence that nicotine abstinence resulted in an exacerbated decrease in reward responsiveness for smokers with a history of depression relative to smokers without such history. This finding extends prior reports that trait anhedonia is associated with reduced attentional bias toward positive stimuli during nicotine abstinence⁴⁵ and increased risk for relapse to smoking.^{39,40} While there is debate regarding the impact of negative affect on relapse, deficits in reward responsiveness observed here appear to be unrelated to negative affect. Consistent with the literature,⁶ our human sample exhibited increased negative affect after 24 hours of withdrawal from nicotine, as measured by increases in the Profile of Mood States Total Mood Disturbance Scale⁵² score ($F_{1,28} = 26.2; P < .001$). However, changes in Total Mood Disturbance Scale score were not correlated with changes in reward responsiveness ($r = -0.09$), suggesting that reward responsiveness deficits observed during withdrawal of nicotine may be distinct from the nicotine withdrawal syndrome characterized by negative mood symptoms.^{6,32}

Blunted reward responsiveness is likely not associated with decrements in discriminability (observed in rats) or cognitive processes, such as attention, as accuracy for the lean stimulus was similar during withdrawal and smoking/control conditions in humans and rats, respectively. Furthermore, only accuracy for the rich stimulus was disrupted during withdrawal in both species, suggesting that deficits in responding during the task were selective for the rich stimulus rather than globally for both stimuli, reflecting decreased reward respon-

siveness and unimpaired cognitive processing (eAppendix 2 in the Supplement). Although the average response bias during abstinence/withdrawal was lower than levels observed during smoking/saline treatment, response bias slightly increased across blocks. This pattern of results may suggest that reinforcement learning was occurring during withdrawal of nicotine but at a slower rate than smoking/saline conditions. Indeed, reinforcement learning (ie, changing behavior based on prior reinforcement) is a key component of reward responsiveness. Future work may further examine how blunted reward responsiveness interrelates with additional cognitive processes across species.

Because of the nature of human and rodent research, it remains challenging to implement completely homologous cross-species procedures. One strength of the RB-PRT used here is its complete objectivity that allows for assessment across species and comparable statistical analyses and data interpretation. The experimental manipulations, while analogous, have some noted dissimilarities. For example, humans intermittently smoke cigarettes throughout the day while ingesting numerous chemicals in addition to nicotine, whereas rats were administered only nicotine continuously via osmotic pumps. While not identical, continuous nicotine infusion is preferred over repeated, intermittent nicotine administration because it more effectively upregulates neuronal nicotinic receptors,⁵⁶ as observed in human heavy smokers.⁵⁷ Moreover, strictly controlling for administration of nicotine in the present rat study suggests that nicotine, and not necessarily other components of cigarette smoke, contribute to deficits in reward responsiveness observed in humans during withdrawal. Lastly, spontaneous withdrawal signs have not been observed after chronic exposure to tobacco smoke vapor in rats,⁵⁸ whereas signs of withdrawal have been well characterized using the same continuous nicotine exposure procedure as presented here.⁵⁹ Thus, the continuous nicotine infusion procedure used in rats is the most appropriate method for replicating the effects of spontaneous withdrawal of chronic nicotine in heavy-smoking humans. The extent to which our findings generalize to lighter smokers should be examined in future investigations.

Conclusions

Using an analogous reward responsiveness task in humans and rats, we found that reward responsiveness was significantly reduced after withdrawal from nicotine. Our strong phenotypic alignment is directed at circumventing the typical translational bottleneck, which continues to impede progress in psychiatric treatments.^{60,61} The fact that humans and rats showed similar deficits in reward responsiveness using conceptually and procedurally identical versions of the RB-PRT reflects the strong convergent validity of this objective measure. Importantly, our cross-species behavioral paradigm developed and validated in this study may facilitate the identification of novel neurobiological substrates mediating nicotine withdrawal and the testing of new smoking-cessation treatments.

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Conflict of Interest Disclosures: During the last 3 years, Dr Shiffman has received consulting fees from GlaxoSmithKline and has been a partner in a venture to develop nicotine medications. During the last 3 years, Dr Markou has received consulting fees from AbbVie and contract research support from Bristol-Myers Squibb, Forest Laboratories, and AstraZeneca for studies unrelated to this work. Dr Markou has a patent on the use of metabotropic glutamate receptor compounds for the treatment of drug dependence. Over the last 3 years, Dr Pizzagalli has received consulting fees from Advanced Neuro Technology, AstraZeneca, Ono Pharma USA, Pfizer, Servier, and Shire Pharmaceuticals for studies unrelated to this work. No other disclosures were reported.

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Supplementary Online Content

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eAppendix 1. Additional Methodological Details Regarding the Human and Rat Studies

eAppendix 2. Additional Results From the Human and Rat Studies

eFigure. Discriminability and Accuracy During Withdrawal of Nicotine in Humans and Rats

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Additional Methodological Details Regarding the Human and Rat Studies

Humans

Participants

Smokers were recruited through advertisements and from the service provided by the Volunteers for Health at Washington University School of Medicine (a database that assists researchers of clinical studies to find participants meeting qualifications) for a study examining the validity and reliability of self-reported and behavioral responses associated with nicotine withdrawal in heavy smokers¹. Project staff described the protocol to 521 candidates and performed a brief telephone screen to assess whether eligibility criteria were met. Of the 99 individuals who met criteria and enrolled in the study, 60 completed the baseline and two test sessions (smoking *ad libitum* and nicotine abstinence- randomly counterbalanced across subjects, with abstinence verified by self-report and an ecolyzer to measure expired carbon monoxide (CO < 9ppm)), as well as 1-week and 1-month follow-up sessions. Fifty percent of the participants self-reported themselves to be African American, 48.3% as White and 1.7% as Other (data were collected by self-report as per NIH policy²). The two test days included self-reported withdrawal-related symptoms³⁻⁶ and behavioral tasks (adapted from⁷⁻¹⁵), including the Response Bias Probabilistic Reward Task (RB-PRT)¹⁶ (included half-way through the study). The current translational study focuses on this reward task, which has been validated for use in humans¹⁶ and rats¹⁷, as inclusion of other measures not yet validated within and/or across species would be premature for this purpose.

Heavy smokers (smoking ≥ 15 cigarettes per day and smoking for ≥ 5 years) not planning to quit permanently over the next month participated in the experiment. This level and length of smoking was chosen because previous withdrawal-related research¹⁸ using this threshold found reliable within-subject increases in withdrawal symptomology during acute nicotine abstinence.

The sample characteristics of the N=60 who completed all study sessions included: 22.1 ± 6.1 (SD) cigarettes smoked/day, 22.8 ± 12.6 years smoked, 41.7 ± 12.9 years old, 55% women, 55% with a lifetime history of major depression, and 90% with a high school education or higher. These characteristics did not differ significantly from the remaining N=39 who enrolled in the study but did not complete the study, except that this latter group included proportionally more men (74%) than women (26%). The RB-PRT was added halfway through data collection; thus, we collected complete reward responsiveness data from 37 subjects. This sample had the following characteristics: 22.3 ± 6.0 cigarettes smoked/day, 23.3 ± 13.5 years smoked, 41.1 ± 14.2 years old, 54% women, 57% with a lifetime history of major depression and 89% with a high school education or higher. These characteristics did not differ significantly from the remaining N=62 who enrolled in the study. Smoking rates (number of cigarettes over the last 24-hours, cpd) and CO at the *ad libitum* session (cpd=20.8, CO=20.9) and at the baseline session (cpd=21.2, CO=21.1) did not significantly differ, suggesting that smokers were continuing to smoke at their normal rates, which could then effectively be compared within subject to a 24-hour withdrawal condition, where cpd=0 and CO=4.3.

Rats

Subjects

Forty-six adult male Wistar rats (Charles River Laboratories, Raleigh, NC, USA) were housed in pairs in standard rat Plexiglas cages with food and water available *ad libitum* prior to initiation of behavioral training. Rats were maintained in a climate-controlled colony room at 21°C on a 12-hour reverse light/dark cycle (lights off at 06:00); all experiments were conducted during the dark (i.e., active) phase in rooms illuminated by red light.

Procedure

Rats were anesthetized with isoflurane and prepared for surgery using aseptic procedures. A 2 cm lateral incision was made in either the right or left flank, and each minipump was placed in the subcutaneous space caudal to the incision and parallel to the spine. The incision was then closed using 9-mm stainless steel wound clips (Becton Dickinson Primary Care Diagnostics, Sparks, MD, USA) and treated with topical antibiotic (bacitracin) ointment.

eAppendix 2. Additional Results From the Human and Rat Studies

Secondary analyses of the nicotine withdrawal tests in humans and rats focusing on discriminability, accuracy and reaction time were conducted to fully characterize the findings:

Discriminability (humans). The only effect emerging from the *Nicotine Status x Block x History of Depression* ANOVA was the main effect of *Block* [$F(2,57)=5.28$; $p=0.01$; $\eta_p^2 = 0.15$] (eFigure 1A). No other effects, including the *Nicotine Status* main effect, were significant.

Discriminability (rats). The *Chronic Drug Treatment x Block* ANCOVA revealed that nicotine withdrawal reduced discriminability compared to saline treatment [*Chronic Drug Treatment*: $F(1,36)=5.52$; $p=0.02$; $\eta_p^2=0.13$] (eFigure 1B). No other statistically significant effects emerged.

Accuracy (humans). The *Nicotine Status x Block x History of Depression x Stimulus Type* (rich, lean) ANOVA revealed significant main effects of *Block* [$F(2,53)=6.80$; $p=0.003$; $\eta_p^2 = 0.19$] and *Stimulus Type* [$F(1,29)=35.21$; $p<0.001$; $\eta_p^2 = 0.55$], which were qualified by significant interactions of *Nicotine Status x Stimulus Type* [$F(1,29)=6.54$; $p=0.02$; $\eta_p^2 = 0.19$] and *Block x Stimulus Type* [$F(2,52)=3.72$; $p=0.04$; $\eta_p^2 = 0.11$] interaction effects (eFigure 1C). *Post*

hoc analyses revealed that accuracy for the rich stimuli was significantly greater than for the lean stimuli during satiety ($p < 0.001$); moreover, relative to satiety, nicotine withdrawal was associated with reduced accuracy for the rich stimuli ($p = 0.01$), but no change in accuracy for the lean stimuli ($p = 0.17$). Accuracy for the rich stimuli also increased from block 1 to 2 ($p = 0.001$), whereas accuracy for the lean stimuli did not change across blocks (all p 's > 0.05).

Accuracy (rats). The *Chronic Drug Treatment* \times *Block* \times *Stimulus Type* ANCOVA revealed a significant main effect of *Stimulus Type* [$F(1,36) = 6.86$; $p = 0.01$; $\eta_p^2 = 0.16$]. As in humans, this effect was qualified by significant *Chronic Drug Treatment* \times *Stimulus Type* [$F(1,36) = 4.43$; $p = 0.04$; $\eta_p^2 = 0.11$] and *Block* \times *Stimulus Type* [$F(2,72) = 6.72$; $p = 0.002$; $\eta_p^2 = 0.16$] interactions (eFigure 1D). *Post hoc* analyses revealed that saline-treated rats were significantly more accurate for rich vs. lean stimuli ($p = 0.005$), while nicotine withdrawing rats had similar accuracy for rich and lean stimuli. Nicotine withdrawing rats were also less accurate than saline-treated rats for the rich stimulus ($p < 0.001$).

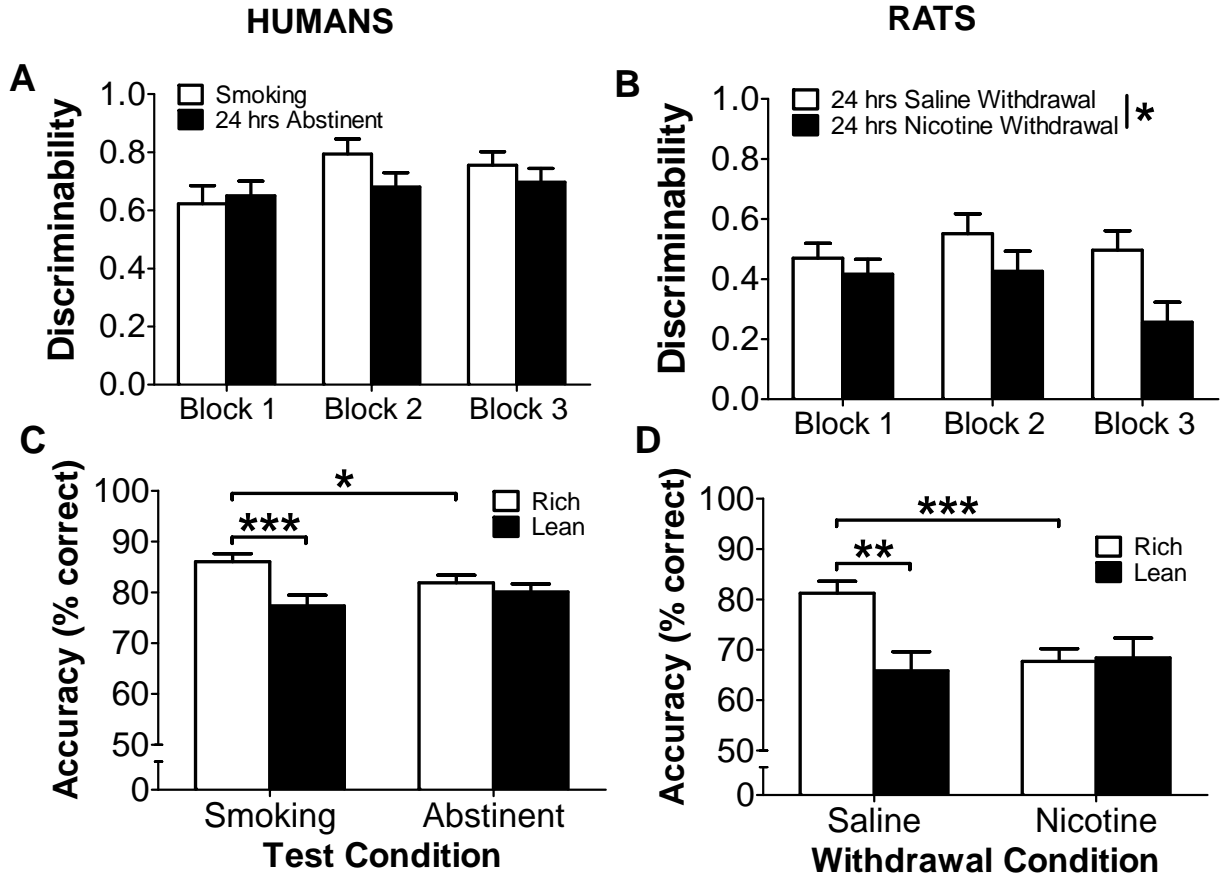
Reaction Time (humans). The *Nicotine Status* \times *Block* \times *History of Depression* \times *Stimulus Type* ANOVA revealed a significant main effect of *Stimulus Type* [$F(1,29) = 7.58$; $p = 0.01$; $\eta_p^2 = 0.21$], due to faster reaction times for the rich relative to the lean stimulus. The only other significant effect was the *Block* \times *Stimulus Type* interaction [$F(2,50) = 7.34$; $p = 0.003$; $\eta_p^2 = 0.20$]. *Post hoc* analyses revealed that, whereas reaction time for the rich stimulus decreased from blocks 1 to 2 ($p = 0.03$), reaction time did not change across blocks for the lean stimulus (all p 's > 0.05).

Reaction Time (rats). The *Chronic Drug Treatment* \times *Block* \times *Stimulus Type* ANCOVA revealed a significant main effect of *Block* [$F(2,72) = 13.03$; $p < 0.001$; $\eta_p^2 = 0.27$], which was qualified by a *Chronic Drug Treatment* \times *Block* interaction [$F(2,72) = 3.53$; $p = 0.03$; $\eta_p^2 = 0.09$].

Post hoc analyses revealed that reaction times increased from blocks 1 to 2 ($p=0.002$) and blocks 2 to 3 ($p=0.005$) in nicotine withdrawing rats, but not saline-treated rats (all p 's >0.05).

eFigure. Discriminability and Accuracy During Withdrawal of Nicotine in Humans and Rats

eFIGURE 1



(A) Nicotine abstinence did not affect discriminability in humans, and in rats (B) was associated with a decrease in discriminability. Consistent with a blunted response bias, in both human smokers (C) and rats (D), withdrawal of nicotine was associated with reduced accuracy for the rich stimuli, and no difference in accuracy for the lean stimuli. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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