

Supplementary Online Content

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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* x *Block* x *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* x *Stimulus Type* [$F(1,36) = 4.43$; $p = 0.04$; $\eta_p^2 = 0.11$] and *Block* x *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 ($p < 0.001$).

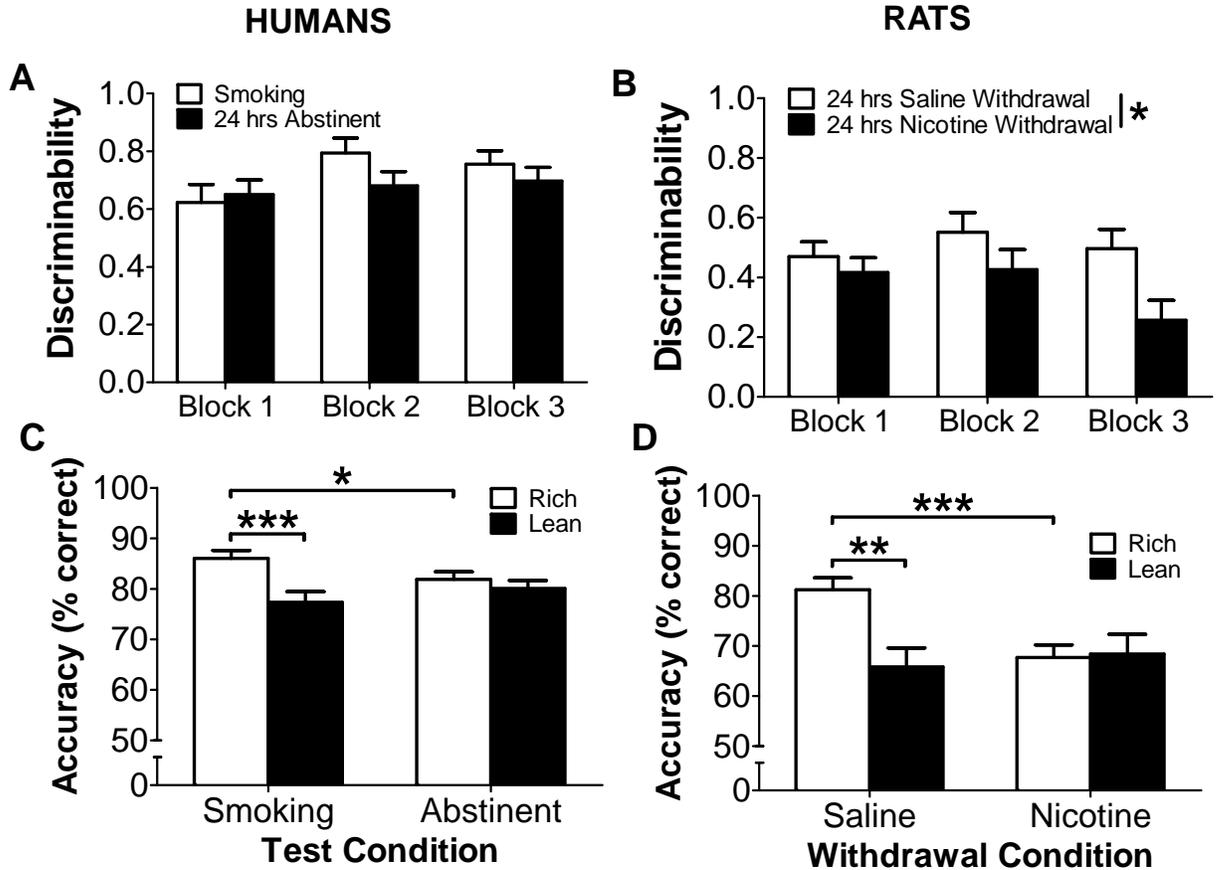
Reaction Time (humans). The *Nicotine Status* x *Block* x *History of Depression* x *Stimulus Type* ANOVA revealed a significant main effect of *Stimulus Type* [$F(1,29) = 7.58$; $p = 0.01$; $\eta_p^2 = 0.21$], due to faster reaction times for the rich relative to the lean stimulus. The only other significant effect was the *Block* x *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* x *Block* x *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* x *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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