Supplemental Information

Supplemental Methods

Recruitment, Inclusion and Exclusion Criteria

There were two study sites (University of Chicago, Chicago, IL, and the National Institute on Drug Abuse Intramural Research Program (NIDA IRP), Baltimore, MD). At both sites, participants were recruited with newspaper and internet advertisements and word-of-mouth. Initial inclusion criteria were: 1) ≥18 years old, and 2) ≥10 cigarettes daily. Candidates underwent structured clinical screening interviews and were excluded based on: 1) medical conditions contraindicating participation, 2) lifetime diagnosis or treatment for psychosis or mania, 3) other psychiatric diagnosis or treatment in the past year, 4) current use of psychiatric medication, 5) current Substance Dependence except nicotine (1), 6) pregnancy, and 7) inability to understand study procedures. Participants were given detailed study descriptions, provided written informed consent, and were debriefed at study completion, as approved by the University of Chicago and NIDA IRP Institutional Review Boards.

Biochemical Verification of Abstinence

During abstinence, participants attended the laboratory daily to provide urine and breath samples for biochemical abstinence verification. Expired air carbon monoxide (CO, Vitalograph BreathCO Carbon Monoxide Monitor, Vitalograph Inc., Lenexa, KS) and urinary cotinine (NicAlert Urine Screen, Nymox Pharmaceutical Corp., Hasbrouck Heights, NJ) were monitored daily. Abstinence criteria were a combination of breath CO and urine cotinine concentration. For CO, participants were required to have CO ≤10 ppm (2) beginning on Day 2. For urinary cotinine, decreasing levels were expected in the first 10 days, and levels of ≤500ng/ml (3) were

required after Day 10. The CO cutoff was set liberally at ≤10 ppm to allow some exposure to second-hand smoke. The cotinine requirements were also somewhat liberal (2), but allowed us to detect active smoking (3). Immunoassays of cotinine (e.g. NicAlert) show 12-40% cross reactivity with the cotinine metabolite 3-OH-cotinine (2, 4), also supporting the use of a liberal threshold. Participants were instructed not to use illegal drugs; compliance was verified with random urine assays (QuickTox Urine Drug Test, Branan Medical Corporation, Irvine, CA).

Cue Sessions

Cue sessions lasted for 2 hours. Participants first provided breath and urine samples for CO and cotinine testing and to ensure that they were free of drugs and alcohol (Alco-sensor III, Intoximeters, St. Louis, MO). Baseline cardiovascular (CV) and subjective measurements and a saliva sample for cortisol assessment were collected. To make the smoking and neutral cues more distinctive, we used separate rooms for each cue type. After baseline measures, participants were escorted to Cue Room 1 and exposed to the first cues. Immediately after exposure, CV and subjective measures were collected, after which participants returned to the first room ("Home Room") for 20 minutes of free/reading time. A saliva sample and CV and subjective measurements were again collected just before participants moved to Cue Room 2 for the second cue set, which was followed by CV and subjective measurements. They returned to the Home Room for 20 minutes of free/reading time and a final saliva sample. Cues included visual, olfactory, and tactile stimuli. Visual smoking cues consisted of 30 photographs of cigarettes and people smoking, each presented for 7-seconds in a slide show. Olfactory and tactile cues came from a lit cigarette of the participant's preferred brand that the participant held, without smoking, while viewing photographs. The cigarette, smoke, and ashtray also provided visual cues. Neutral

cues consisted of 30 neutral pictures without explicit smoking cues. Participants held a pencil cut to cigarette length, and a scented candle provided an olfactory cue. No photographs were repeated within or across cue sessions.

Assessment Measures

General intelligence, depressive symptomatology, and nicotine dependence were assessed during initial screening. Participants completed the Shipley Institute of Living Scale (SILS) (5), which estimates verbal and performance intelligence, the Beck Depression Inventory (BDI) (6) to measure depression symptoms, and the Fagerström Test for Nicotine Dependence (FTND) (7) which assesses severity of nicotine dependence.

At each daily visit during abstinence, participants completed a rating of nicotine withdrawal (Minnesota Nicotine Withdrawal Scale; MNWS). The MNWS (8), which is based on DSM-IV withdrawal criteria (1), assesses daily withdrawal symptoms rated from 0 (“not at all”) to 4 (“extreme”).

During cue sessions, we employed two validated measures to capture different aspects of the multidimensional construct of cigarette craving (9-10). The Tobacco Craving Questionnaire – Short Form (TCQ-SF) (9) includes 12 statements such as “I could control things better right now if I could smoke,” rated on a 7-point scale from “strongly disagree” to “strongly agree.” Although this scale can be divided into four subscales, we employed the Total score to reduce the number of analyses undertaken. The Brief Questionnaire of Smoking Urges (QSU-B) (10) consists of 10 statements (e.g. “I have a desire for a cigarette right now”) rated on a 7-point scale anchored with “strongly disagree” and “strongly agree.” The QSU-B contains two subscales;

Factor 1 reflects desire to smoke for pleasure, whereas Factor 2 reflects urge to smoke for relief from negative emotions.

We also employed the Positive and Negative Affect Schedule (PANAS) (11) to measure changes in affect after cues. The PANAS is a well-validated measure including 20 mood adjectives (e.g. "excited"). Participants rate each word as a descriptor of their current affect on a 5-point scale, from "very slightly or not at all" to "extremely." The PANAS is scored into Positive and Negative Affect subscales.

Statistical Analyses

Between Group Analyses:

Demographics. To assess possible confounds, we examined differences between Groups 1, 2, and 3 in demographics and drug use with ANOVAs and post-hoc *t*-tests, or with chi-square tests.

Cue Reactivity – Smoking versus Neutral Cues. To assess whether the smoking cues were effective overall, we combined data from Groups 1, 2, and 3, and used paired *t*-tests comparing within-session change scores (post-cue minus pre-cue) for smoking versus neutral cues.

Cue Reactivity and Baseline Craving as a Function of Length of Abstinence. Only outcome measures that were sensitive to smoking cues overall were included. Group differences were assessed with one-way ANOVAs, using polynomial contrasts testing for linear trends (i.e., Group 1>2>3 or Group 1<2<3). Planned comparisons were made between Group 1 and Group 2, and Group 1 and Group 3. The dependent variables were within-session change scores (post-cue minus pre-cue), with smoking and neutral cues analyzed separately. We used a similar approach to test group differences in baseline craving reports at cue sessions.

The two demographic variables that differed between groups (BDI and number of cigarettes smoked) were considered for inclusion as covariates, but neither variable correlated significantly with outcome measures; therefore, neither was included. There was no effect of cue order in between-group analyses.

Within-Group Analyses:

Cue Reactivity and Baseline Craving as a Function of Length of Abstinence. In Group 4, we assessed cue-reactivity measures and cue session baseline scores in repeated-measures regression models (Proc Mixed in SAS 9.0) using day (7, 14, and 35) as the predictor; Tukey-Kramer pairwise comparisons were then made between days. Repeated-measures regression produces output similar to that of repeated-measures ANOVA, without requiring imputation of missing data points.

Time Course of Daily Withdrawal Symptoms:

To examine group differences in the time course of daily withdrawal symptoms, we used repeated-measures regression (Proc Mixed in SAS 9.0) with MNWS data as the dependent variable. In one model, Groups 1, 2, and 3 were compared across the first 7 days of abstinence. In a second model, Groups 2 and 3 were compared across the first 14 days of abstinence. We did not compare Groups 3 and 4 across the full 35 days of abstinence because Group 4 was enrolled and run as a separate cohort, precluding direct comparisons. To assess effects of time on withdrawal symptoms within Groups 1, 2 and 3, we conducted repeated-measures regression (Proc Mixed) across abstinence days, from Day 2 (participants would not be expected to be in full withdrawal on Day 1) until the final abstinence day, using polynomial contrasts to test for linear decreases over time.

Supplemental Results

Participants

Of 181 participants enrolled, 93 completed the assigned abstinence period. Seven participants' data were removed from analyses; four due to positive drug screens (two for cannabis and two for cocaine), and three due to intermittent cotinine and/or carbon monoxide readings indicating possible smoking during the assigned abstinence period. Enrollment details are presented in Figure S1 below. There was no difference between the four groups in numbers of participants who failed to complete the study (χ^2 (3) = 0.2, p = 0.98).

Demographics

Tables S1 and S2 (below) presents demographic and drug-use characteristics for Groups 1, 2, 3, and 4 and comparisons between the three single-cue groups, which were well matched on most demographic and drug-use variables. Group 2 endorsed more depressive symptoms than Groups 1 and 3, but mean scores were well below 10, the threshold score for mild depression (6). Group 1 also smoked more cigarettes per day than Group 3.

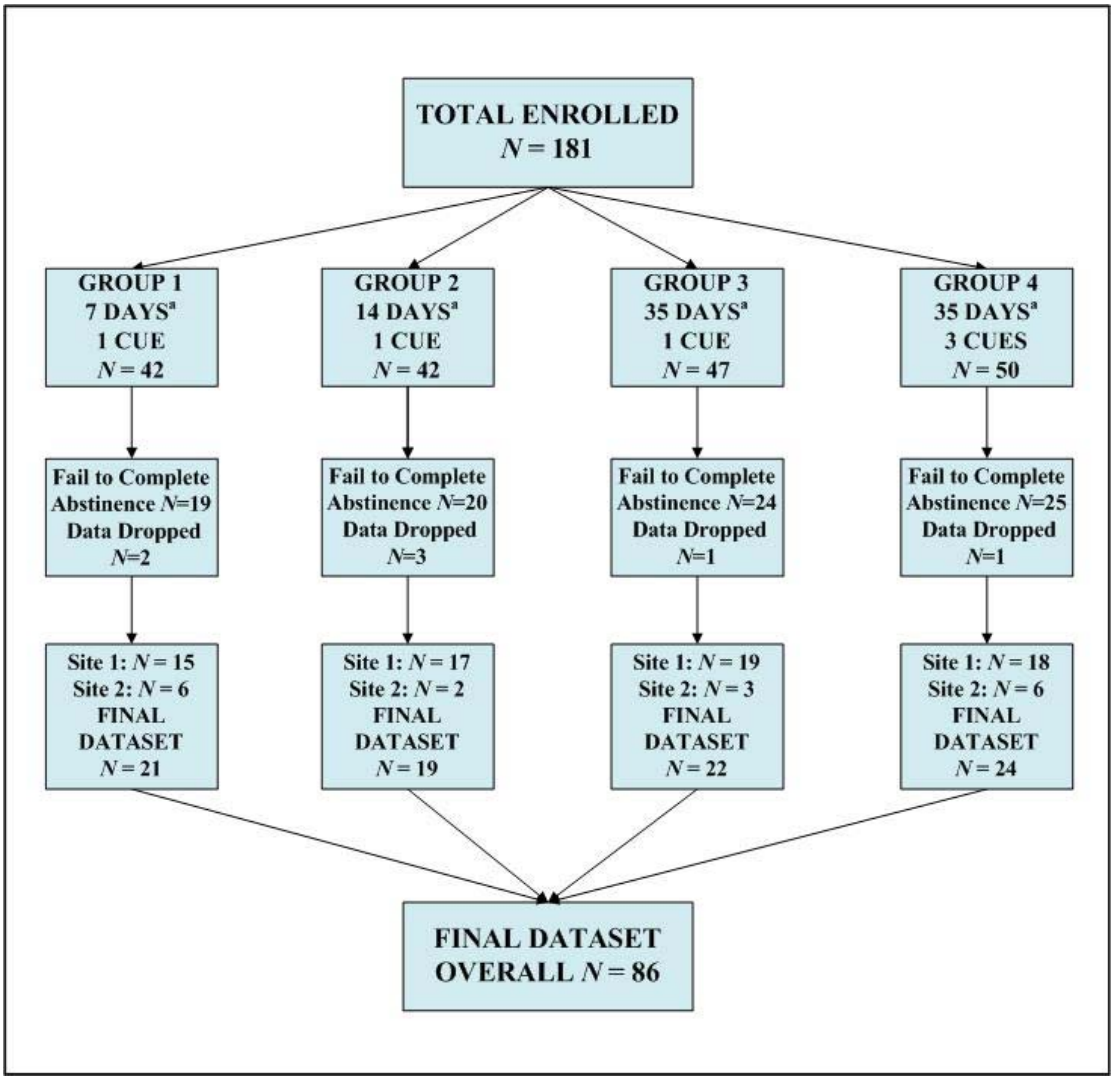

Figure S1. Flowchart of participant enrollment and completion by group. [a] Length of required abstinence period. Site 1 = Human Behavioral Pharmacology Laboratory, University of Chicago. Site 2 = NIDA Intramural Research Program, Baltimore.

Table S1. Demographic Features of Participants in Groups 1, 2, 3, and 4

	Group 1 – 7 days *N* = 21	**Group 2 – 14 days *N* = 19**	**Group 3 – 35 days *N* = 22**	**Group 4 – 35 days Repeat cues *N* = 24**	**Overall differences – Groups 1, 2, & 3[e]**	**1 vs 2**	**1 vs 3**	**2 vs 3**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	***F* (*df*)**	***t* (*df*)**	***t* (*df*)**	***t* (*df*)**
Age	33.0 (11.3)	36.3 (12.2)	28.8 (8.9)	35.3 (12.2)	2.4 (2, 59)	-	-	-
Shipley Score	104.5 (8.6)	104.9[a] (11.6)	106.8[b] (8.6)	99.8 [c] (13.7)	0.3 (2, 57)	-	-	-
BDI Score	1.7 (2.2)	5.1 (4.3)	2.1[b] (3.7)	3.8 (3.4)	5.3* (2, 58)	3.0* (26.4)	0.5 (40)	2.3* (38)
FTND Score	4.6 (1.9)	4.3 (1.9)	4.4 (1.8)	4.1 [d] (1.9)	0.1 (2, 59)	-	-	-
					Group Differences – 1, 2 & 3[e]			
	***N* (%)**	***N* (%)**	***N* (%)**	***N* (%)**	**χ^2 (*df*)**			
Sex, female	10 (48)	6 (32)	9 (41)	9 (38)	1.1 (2)			
Race, Caucasian	10 (48)	10 (53)	12 (55)	10 (43)[d]				
Race, African American	9 (43)	8 (42)	6 (27)	11 (48)[d]	2.6 (4)			
Race, Other	2 (9)	1 (5)	4 (18)	2 (9)[d]				

[a] $N = 18$ due to missing data.
[b] $N = 21$ due to missing data.
[c] $N = 22$ due to missing data.
[d] $N = 23$ due to missing data, where applicable valid percentages are presented.
[e]Only single cue groups (Groups 1, 2, and 3) are included in the between-group analyses.
* $p < .05$.
BDI, Beck Depression Inventory; FTND, Fagerstrom Test for Nicotine Dependence.

Table S2. Drug Use of Participants in Groups 1, 2, 3, and 4

	Group 1 – 7 days ***N* = 21**	**Group 2 – 14 days** ***N* = 19**	**Group 3 – 35 days** ***N* = 22**	**Group 4 – 35 days Repeat cues** ***N* = 24**	**Overall differences – Groups 1, 2, & 3[i]**	**1 vs 2**	**1 vs 3**	**2 vs 3**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	***F* (*df*)**	***t* (*df*)**	***t* (*df*)**	***t* (*df*)**
Cigarettes Per Day[a]	16.1 (5.3)	13.6 (4.5)	12.5 (3.3)	17.1 (8.5)	3.8* (2, 59)	1.6 (38)	2.7* (41)	0.9 (39)
Caffeine Beverages Per Week[a]	16.5[b] (12.5)	17.1 (12.5)	15.2 (18.8)	16.6[c] (14.2)	0.1 (2, 57)	-	-	-
Alcohol Drinks Per Week[a]	7.2 (7.1)	7.2 (6.0)	8.0 (8.5)	7.3 (7.6)	0.1 (2, 59)	-	-	-
MJ Occasions of Use[a]	0.6 (1.4)	1.2 (3.6)	1.4 (2.7)	1.8 (5.0)	0.5 (2, 59)	-	-	-
	Group 1 – 7 days ***N* = 21**	**Group 2 – 14 days** ***N* = 19**	**Group 3 – 35 days** ***N* = 22**	**Group 4 – 35 days Repeat cues** ***N* = 24**	**Group Differences – Groups 1, 2 & 3[i]**			
	***N* (%)**	***N* (%)**	***N* (%)**	***N* (%)**	χ^2 ***(df = 2)***			
MJ Use Lifetime, Never	1 (5)	1 (5)	3 (14)	2 (8)	1.7 (4)			
MJ Use Lifetime, 1-100 Times	12 (57)	11 (58)	10 (46)	10 (42)				
MJ Use Lifetime, 100+ Times	8 (38)	7 (37)	9 (41)	12 (50)				
Stimulant[e] Use Lifetime	10 (48)	8 (42)	8 (36)	12 (50)	0.6 (2)			
Opiate[f] Use Lifetime	5 (24)	3 (16)	5 (23)	7 (29)	0.5 (2)			
Hallucinogen[g] Use Lifetime	7 (33)	6 (33)[d]	8 (36)	12 (50)	0.1 (2)			
Tranquilizer[h] Use Lifetime	3 (14)	4 (21)	2 (9)	3 (13)	1.2 (2)			

[a] In last 30 days.
[b] *N* = 19 due to missing data.
[c] *N* = 22 due to missing data.
[d] *N* = 18 due to missing data, percentages are valid percentages.
[e] e.g. cocaine, methamphetamine.
[f] e.g. opium, heroin (not including medicinal opiate use).
[g] e.g. mescaline, psilocybin.
[h] e.g. diazepam, alprazolam (not including medicinal use).
[i] Only single cue groups (Groups 1, 2, and 3) are included in the between-group analyses.
* $p < .05$.
MJ, marijuana.

Time Course of Withdrawal Symptoms

The time course of nicotine-withdrawal symptoms (MNWS scores) across the abstinence periods for Groups 1, 2, and 3 is presented below in Figure S2. Symptoms followed a similar time course in each group. From Days 1 to 7, among Groups 1, 2, and 3, there was no main effect of group or Group x Day interaction. Similarly, from Days 1 to 14, between Groups 2 and 3, there was no main effect of group or Group x Day interaction. In Groups 1 and 2, there were trend-level linear decreases in withdrawal symptoms over the course of abstinence (Group 1: $F(1,94) = 3.0$, $p = 0.09$; Group 2: $F(1,212) = 3.09$, $p = 0.08$). In Group 3, the linear decrease was significant ($F(1, 679) = 5.7$, $p = 0.02$), probably due to the greater number of data points.

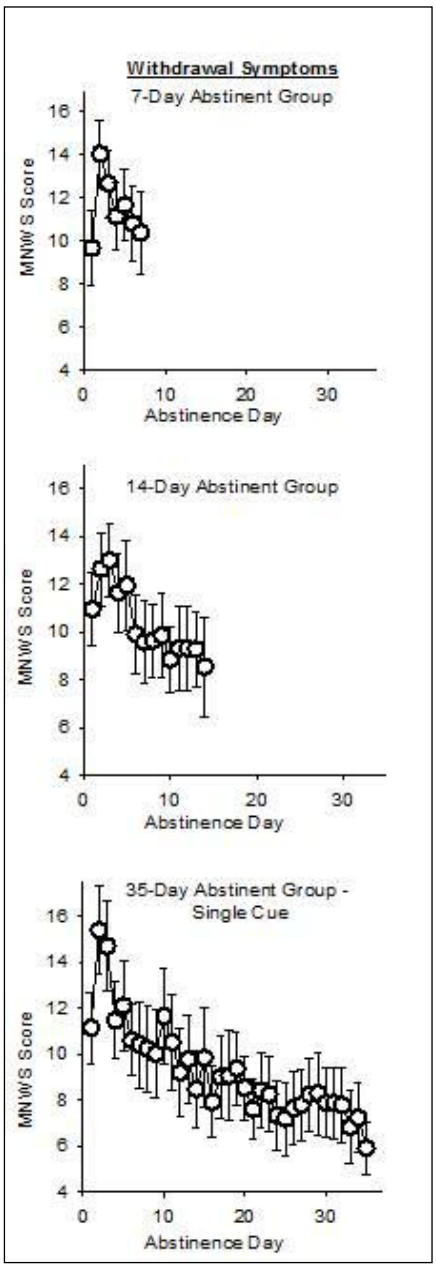

Figure S2. Time course of nicotine-withdrawal symptoms, measured with the Minnesota Nicotine Withdrawal Scale (MNWS), over the abstinence period. Top. 7-day abstinent group. Middle. 14-day abstinent group. Bottom. 35-day abstinent group (single cue). Data are group means (±S.E.M.).

Supplemental References

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