N-acetylcysteine reduces cocaine-seeking behavior and anterior cingulate glutamate/glutamine levels among cocaine-dependent individuals

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Abstract

N-acetylcysteine (NAC) is a cystine prodrug shown to reduce cocaine- and cue-primed reinstatement of cocaine-seeking behavior in preclinical studies. In this inpatient study, the effects of NAC maintenance versus placebo on cocaine-seeking behavior were examined during cocaine-primed and unprimed self-administration sessions among non-treatment-seeking, cocaine-dependent individuals. Twelve participants completed this double-blind, placebo-controlled, within-subject crossover study. Each participant was maintained for 1 week (Sat–Fri) on NAC (1200-mg TID; 3600 mg/day total) and 1 week on placebo (0-mg TID); medication order was randomized. A subset of participants underwent proton magnetic resonance spectroscopy scans ($n = 8$) on the third day of medication (Mon) to assess neurochemistry in the rostral anterior cingulate (rACC; voxel = 4.5 cm^3). In four randomized sessions (Tue–Fri) each week, each participant could earn unit amounts of cocaine (10 mg, fixed) versus money ($0.50 vs. $1.50) on a choice, progressive ratio schedule after insufflating active versus placebo cocaine-priming doses (110 mg vs. 4 mg). Relative to the placebo priming dose, the active cocaine priming dose (110 mg) increased cocaine-seeking behavior ($p = .003$). NAC reduced cocaine-primed cocaine-seeking behavior compared with placebo levels ($p = .044$) but did not alter placebo-primed cocaine-seeking behavior. The larger money alternative ($1.50) suppressed cocaine-seeking behavior relative to the smaller money alternative ($0.50; $p = .011$). Compared with placebo levels, NAC significantly decreased rACC glutamate + glutamine levels ($p = .035$) and numerically decreased rACC glutamate levels ($p = .085$). These preliminary findings indicate that NAC suppresses

cocaine-seeking behavior in some, but not all, experimental scenarios. Further, our findings suggest NAC may exert its therapeutic effects by modulating excitatory tone in the rACC.

Keywords

anterior cingulate cortex; cocaine; drug-seeking behavior; glutamate; *N*-acetylcysteine; relapse

1 | INTRODUCTION

Chronic cocaine use poses serious public health, economic, and societal problems. At present, there are no Food and Drug Administration (FDA)-approved medications to treat cocaine use disorder (CUD). However, one medication that has received considerable recent interest is *N*-acetylcysteine (NAC). NAC is a cystine prodrug that binds to the cystine-glutamate antiporter located on glial cells and exchanges 1:1 with glutamate, thereby increasing extracellular glutamate levels. This mechanism aligns well with CUD neurobiological deficits. Preclinical studies indicate that chronic cocaine exposure significantly lowers basal extrasynaptic glutamate levels throughout corticostriatal brain regions, which are central to cocaine-seeking behavior.[1–5] NAC has been shown to reverse this deficit by restoring cystine-glutamate exchange, normalizing glial glutamate transporter (GLT-1) activity[6–8] and regulating presynaptic $mGluR_{2/3}$ inhibition of neurotransmission.[9,10] These glutamatergic effects are thought to underlie NAC's therapeutic effects. NAC has been reliably shown to reduce cocaine-primed *reinstatement* of cocaine-seeking behavior in animal models.[6,9,11,12] The extant literature indicates NAC does not decrease *stable*, nonprimed cocaine-seeking behavior,[13] except when paired with punishment.[14] The preclinical literature suggests NAC may be an effective therapeutic agent for prolonging abstinence, but not for abstinence initiation. That is, NAC has been shown to attenuate the reinforcing properties of cocaine but only *after* a period of cocaine abstinence, which can be as brief as 1 day.[11,12]

Clinical research has investigated NAC's effects on cocaine craving/withdrawal symptoms, cocaine and cue reactivity, and outpatient cocaine use. In an open-label, 4-week outpatient study, NAC was well tolerated at doses up to 3600 mg/day.[15] Subject retention was better for higher doses (2400 and 3600 mg/day) compared with 1200 mg/day.[15] Among patients who completed the study, cocaine use and craving scores significantly decreased with NAC dosing; however, these encouraging findings must be tempered as placebo control and experimental blinding were not implemented.[15] In two double-blind, placebo-controlled crossover studies among cocaine-dependent individuals, 2400 mg of NAC did not significantly reduce cocaine withdrawal symptoms and craving[16] but did significantly decrease cocaine cue-induced craving, interest in cocaine, and cue viewing time.[17] Results from a small pilot trial comparing the effect of 4-day NAC dosing (400-mg TID [1200 mg] or 800-mg TID [2400 mg]) on cocaine versus neutral cue reactivity and physiological and subjective responses to 20 mg/70 kg intravenous cocaine showed that NAC did not alter cocaine's subjective or physiological effects but did significantly reduce cocaine priming-induced craving, compared with an earlier phase when NAC was not administered.[11] A randomized clinical trial compared the effects of placebo, NAC 1200 mg/day, and

NAC 2400 mg/day on outpatient cocaine use, cocaine craving, dropout, and medication adherence.[18] NAC was not more effective than placebo for reducing cocaine use, craving, dropout or improving medication adherence.[18] However, among patients who were cocaine abstinent in the week prior to starting NAC, the 2400 mg/day NAC dose significantly increased time-to-lapse compared with placebo, providing clinical evidence of NAC's therapeutic efficacy for relapse prevention.[18] Finally, Bolin et al.[19] conducted a double-blind, placebo-controlled, within-subject crossover study to examine whether short-term maintenance on NAC 2400 mg/day versus placebo altered cocaine-cue attentional bias, and reinforcing, subjective, and physiological effects of intranasal cocaine (0, 30, and 60 mg).[19] NAC reduced cocaine-cue attentional bias and subjective responses to intranasal cocaine and reduced cocaine choice but only among participants who received NAC first before placebo.[19] In summary, clinical evidence indicates NAC can reduce cocaine cue salience, attenuate subjective responses to cocaine administration and cocaine cues, and prolongs abstinence among individuals who were cocaine abstinent prior to NAC dosing.

To our knowledge, only one clinical study has investigated NAC's putative neurochemical mechanism of action, that is, modulation of glutamatergic tone, among cocaine-dependent individuals. In a randomized crossover, open-label proton magnetic resonance spectroscopy (^{1}H MRS) study, Schmaal et al.[20] reported that basal dorsal anterior cingulate (dACC) glutamate to total creatine ratio levels (GLU/tCr) among abstinent cocaine-dependent individuals ($n = 8$) were higher than healthy volunteers ($n = 14$). A single unblinded dose of 2400-mg NAC 1 h before ^{1}H MRS significantly reduced dACC GLU/tCr levels among cocaine-dependent individuals compared with basal levels but did not alter GLU/tCr levels among controls. After NAC dosing, dACC GLU/tCr levels among cocaine-dependent individuals were not different from controls. The authors concluded that NAC "normalized" dACC GLU levels among cocaine-dependent individuals.[20] Although that study provided the first clinical evidence of NAC's effects on neurochemistry, only a single dose of NAC was administered; thus, effects of repeated NAC dosing remain unclear. Finally, Schmaal et al did not measure cocaine-seeking/self-administration behavior; thus, NAC's effects on neurochemistry and cocaine self-administration have yet to be reported in the same individuals.

The present study investigated four gaps in the literature. First, this study extended the Schmaal et al. study by investigating rACC neurochemistry after repeated NAC dosing under double-blind and placebo-controlled conditions. Second, this is the first study in humans to measure NAC's effects on neurochemistry and cocaine-seeking behavior in the same participants. Third, we evaluated NAC's effects on cocaine-seeking behavior under cocaine-primed and unprimed conditions, that is, translation of robust preclinical findings. Fourth, we evaluated NAC's effects on cocaine-seeking behavior in combination with a human laboratory analogue of contingency management, that is, by modifying the alternative choice money amount in a cocaine versus money choice progressive ratio paradigm.[21] Contingency management is well established as an effective behavioral strategy for promoting cocaine abstinence[22–24] and may augment NAC's efficacy. Using a double-blind, placebo-controlled, randomized-crossover design, we evaluated whether maintenance on a high oral dose of NAC (3600 mg/day) decreases cocaine-seeking behavior using a cocaine versus money choice progressive ratio paradigm (e.g., Greenwald et al[25]) among cocaine-dependent

individuals during a 16-day continuous, monitored inpatient stay. Specifically, we tested whether NAC alters cocaine seeking under cocaine-primed and unprimed conditions and whether NAC's effects on cocaine seeking interact with alternative money amount. Finally, using single-voxel ^{1}H MRS, we measured rostral ACC glutamate (GLU) and GLX (glutamate + glutamine) levels after 3 days of NAC versus placebo during recent cocaine abstinence. The rostral ACC was selected because previous research indicates cocaine users exhibit structural, functional, and neurochemical impairments in the rACC.[26–28] Further, the rACC is implicated in response inhibition: a cognitive process shown to be impaired among cocaine users[29] and one that may be rescued by NAC dosing.[30] We hypothesized the following: (1) cocaine priming would increase cocaine-seeking behavior, (2) NAC would reduce cocaine-primed cocaine seeking, (3) the higher money amount alternative would reduce cocaine seeking, (4) NAC would augment the effect of the higher money amount alternative to further reduce cocaine seeking, and (5) NAC would decrease GLU and GLX levels in the rACC.

2 | METHODS

2.1 | Participant recruiting and selection

The local Institutional Review Board approved this study, which was conducted according to the Declaration of Helsinki. Volunteers aged 18–55 years, regardless of racial/ethnic background, were recruited by newspaper advertisements and word-of-mouth referral and were not seeking substance use disorder treatment. All volunteers provided informed consent for screening and separate consent to participate in the study.

All candidates received routine medical (history and physical) exam with standard laboratory tests (complete blood chemistry, urinalysis, urine pregnancy test for females, tuberculin screening), 12-lead ECG, and psychiatric interview (SCID-IV[31]). Table S1 presents all inclusion and exclusion criteria. Fifty-three candidates were screened, and 36 were excluded. Of the 17 individuals who enrolled, three were lost to contact prior to the cocaine reinforcement qualifying session. Experimental cocaine did not function as a reinforcer for two participants during a cocaine reinforcement screen, and thus, they were excluded (defined below).

The Cocaine Purchasing and Use Patterns (CPUP) semistructured interview was used to characterize past-month sources and amounts of income, drug purchasing and consumption, and price- and availability-related factors (e.g., number of dealers, time, and distance costs) related to these behaviors.[32]

A single-session cocaine reinforcement screen served as the final selection criterion.[21] Participants insufflated *Drug A* (4 or 110 mg) at 9:00 a.m. and *Drug B* (110 or 4 mg) at 11:00 a.m., in counterbalanced order. Subjective and physiological effects were measured at −0.5 h (baseline), and 0.25-, 0.5-, 0.75-, 1.0-, and 1.5-h postdrug administration. An 11-trial *Drug A* versus *Drug B* choice progressive ratio (PR) task was conducted from 1:00 to 4:00 p.m. during which participants could earn 10 mg and/or 0 mg units (max earned dose = 110 mg), with response requirements for each option increasing independently and exponentially across the 11 choice trials (100, 250, 505, 915, 1530, 2400, 3575, 5105, 7040, 9430, and

12325). Cocaine reinforcement was defined as occurring when cocaine (10-mg unit dose) maintained global preference (earned ≥6 of 11 trials) and the participant earned cocaine ≥2 more trials than placebo. Two subjects did not meet these criteria and were excluded.

2.2 | Experimental design

For cocaine-seeking behavior, there were three factors: Medication Dose (NAC 3600 mg vs. 0 mg) × Priming Dose (Cocaine 110 mg vs. 4 mg [placebo]) × Money Alternative ($1.50 vs. $0.50). For ^{1}H MRS metabolites, Medication Dose was the sole factor. For subjective and physiological response measures, there were three factors: Medication Dose (NAC 3600 mg vs. 0 mg) × Priming Dose (Cocaine 110 mg vs. 4 mg) × Session Time (prepriming vs. postpriming dose).

2.3 | Protocol timeline

Each participant lived on a residential unit for 16 consecutive nights. The participant was admitted Thursday afternoon (procedural training) and, after passing the cocaine reinforcement qualifying session (Friday), maintained on a medication (NAC or placebo) for the first experimental week (Saturday–Friday), then crossed over to the other medication condition for the second week (Saturday–Friday), and was discharged Saturday morning after the final Friday session. Importantly, NAC dosing was initiated during experimental cocaine abstinence (Saturday–Monday), consistent with the preclinical literature.[11,12] ^{1}H MRS scans were acquired on Monday of each week. Thus, for participants who received NAC first, there was a 3-day washout period (Friday–Monday) before ^{1}H MRS, and a 4-day washout period (Friday–Tuesday) before behavioral testing, to prevent possible carry-over effects (e.g., Bolin et al[19]). Participants were transported to/from the residential unit and laboratory under staff observation. Daily urinalysis ensured abstinence from unsanctioned drug use. Participants earned $40 per night on the residential unit.

2.4 | Drug administration

Cocaine HCl powder (Research Triangle Institute, NC) was prepared in 110-mg constant-volume doses. Placebo contained 106-mg lactose and 4-mg cocaine. Response-contingent doses contained the earned cocaine dose with the remainder being lactose. The cocaine/lactose mixture was placed in a small plastic cup, and the participant insufflated the powder through a short plastic straw, while the research assistant observed.

NAC 600-mg capsules (Swanson Health Products, Fargo, ND) were placed inside opaque size-00 capsules with lactose filler. Each placebo dose (0 mg) consisted of lactose placed inside these same-colored opaque size-00 capsules. NAC or placebo doses were self-administered orally three times daily at 7:00 a.m., 3:00 p.m., and 11:00 p.m. under staff supervision.

2.5 | Experimental sessions

2.5.1 | Cocaine/money choice—Participants completed eight choice sessions (four per week), in randomized order, involving all medication-dose, cocaine priming-dose, and money-alternative combinations. Cigarette smoking was prohibited starting 1-h prior to each session. At 9:20 a.m., 10 min prior to the 3-h choice task (9:30 a.m. to 12:30 p.m.), the

participant insufflated 110-mg powder that contained a placebo (4 mg) or active (110 mg) cocaine dose. The drug code referring to the active cocaine dose from the reinforcement-screening session (*Drug A* or *Drug B*, based on each participant's random assignment) was used to label the active dose for choice sessions, and participants were told they could work for unit amounts of the same drug they preferred in the qualifying session.

During the choice PR task, the participant could earn fixed amounts of cocaine (10-mg unit dose) or money ($1.50 or $0.50, varied across sessions); the participant was also informed she/he was not obligated to respond at all. Cocaine-priming doses and money amounts were orthogonal factors, nested within medication dose (NAC or placebo) maintenance week. Response requirements on cocaine and money options were identical to the reinforcement-qualifying session. Maximum earned cocaine dose was 110 mg (10-mg units × 11 possible choices), and maximum earned money amount per condition was $16.50 ($1.50 per choice) or $5.50 ($0.50 per choice). The participant was told there was no advantage to completing the choice task before the 3-h time limit, as she/he could not receive the earned cocaine dose before the 3-h time limit and money earnings were deferred until end of participation.

2.5.2 | Subjective and physiological effects—Vital signs (respiration rate, oxygen saturation, heart rate, and blood pressure) and subjective effects (see below) were assessed 15 min before and 10 min after the cocaine/placebo priming dose (just before the choice task), 3 h later just after the choice task (before earned cocaine self-administration), and, for safety, monitored for 2.5 h after earned cocaine self-administration.

Craving was measured with the 45-item, 7-point Likert format, Cocaine Craving Questionnaire (CCQ).[33] CCQ subscales (*Desire to Use Cocaine*, *Intention and Planning to Use Cocaine*, *Anticipation of Positive Outcome*, *Anticipation of Relief from Withdrawal or Dysphoria*, and *Lack of Control Over Use*) were analyzed separately.

Eight visual analog scale (VAS) drug-effect ratings were obtained: Any Effect, High, Liking, Good Drug Effect, Bad Drug Effect, Stimulated, Sedated, and Want to Take Drug Again. Each VAS rating was on a scale from 0 (*not at all*) to 100 (*extremely*).

Psychostimulant effects were assessed with the 21-item, 5-point Likert format, Stimulant-Sensitive Adjective Rating Scale.[34]

2.5.3 | Brain chemistry—Eight participants (one excluded for MRI contraindications; three excluded due to scanner unavailability) were scanned each Monday (third day of medication maintenance dose and cocaine abstinence) on a 3T Tesla Siemens Verio system. Cigarette smoking was prohibited starting 1 h prior to each scan. Anatomical T_1-weighted images were acquired using the magnetization prepared rapid gradient echo sequence (TR = 2.2 s, TE = 3 ms, TI = 799 ms, flip angle = 13°, field-of-view [FOV] = 256 × 256 × 160 mm, 256 × 1 mm thick axial slices, matrix = 176 × 256, 1 mm^3 pixel resolution). A voxel (2.0 × 1.5 × 1.5 cm; 4.5 cm^3) was positioned in the rACC (Figure 3A). Anatomical landmarks and each participant's prior scan guided voxel placement during the second scan. Water suppressed single-voxel ^{1}H MRS spectra were acquired (eight measurements; 16 averages/measurement) using point resolved spectroscopy sequence (TE = 22 ms, TR = 4.0 s, data

points = 2048, bandwidth = 2 kHz, no apodization) from each voxel location, followed by unsuppressed water spectra acquisition (one measurement of two averages; TR = 10 s).

Postprocessing and quantification steps were 100% automated. For each ^{1}H MRS scan, the eight spectra were phase-, shift-, and eddy current corrected before averaging and quantification using LCModel version 6.3 with a simulated basis set.[35] T_1-weighted images were B_1-field bias-corrected, brain extracted, and segmented into partial volume maps of gray and white matter and cerebrospinal fluid using FreeSurfer and FSL tools.[36,37] Voxel tissue composition and appropriate relaxation values, along with other correction factors, were used to quantify absolute metabolite values expressed as institutional units.[38] Voxel replacement accuracy was confirmed by nonsignificant differences in gray/white matter and cerebrospinal fluid tissue fractions between scans 1 and 2. One participant exhibited poor data quality (possibly due to motion) and was excluded from analyses.

2.6 | Data analyses

All variable distributions were evaluated for normality (skewness and kurtosis statistics) and corrected as needed (e.g., winsorization and log-10 transformations) prior to outcome analyses.

2.6.1 | Cocaine seeking—Three-factor (2 Medication Doses [NAC vs. Placebo] × 2 Priming Doses [110 mg vs. 4 mg Cocaine] × 2 Money Alternative [$1.50 vs. $0.50]) repeated measures analyses of variance (rmANOVAs) were used to analyze total cocaine choices and cocaine breakpoint (separately).

2.6.2 | Cocaine priming—Three-factor rmANOVAs (Medication Dose × Priming Dose × Session Time) were used to evaluate subjective and physiological responses to placebo versus cocaine priming doses.

2.6.3 | Brain chemistry—The effect of NAC (vs. Placebo) on glutamate (GLU), glutamate plus glutamine (GLX), and other metabolite levels was analyzed via rmANOVAs.

The threshold for statistical significance for rmANOVA main effects was p .05 and partial η^2 effect sizes were interpreted as follows: "small" 0.09, 0.10 "moderate" 0.24, 0.25 "large" 0.49, and "very large" 0.50. Bonferroni-corrected thresholds were adopted within each set of analyses to avoid false positives from multiple comparisons. Descriptive statistics are presented as mean ± 1 standard deviation (SD) in the text and mean ± 1 standard error of the mean in figures, unless otherwise noted.

3 | RESULTS

3.1 | Participant characteristics

The 12 participants who completed the behavioral procedures were 48.1 ± 4.5 years old and reported lifetime cocaine-use duration of 20.7 ± 6.7 years (Table 1). Eleven smoked cocaine, whereas one injected. All reported smoking tobacco cigarettes, averaging 5.3 ± 2.5 daily.

Using median values from the CPUP, participants reported past-month total income of $1057, spending a total of $390 on cocaine. Participants had easy access to cocaine from their suppliers: eight walked and three drove to get cocaine, and one had it delivered. Participants reported a median of 3.5 weekly cocaine purchases, subjective cocaine purity of 50%, round-trip purchase time of 20 min, and purchase amount per episode of $40.

3.2 | Cocaine-seeking behavior

3.2.1 | Cocaine breakpoints—Three-factor rmANOVA indicated significant cocaine breakpoint effects for Cocaine-Priming, Money Amount Alternative, and Medication Dose by Cocaine-Priming interaction ($ps < .05$), but a nonsignificant Medication Dose effect, $F(1,11) = 2.84$, $p = .12$, partial $\eta^2 = 0.21$, moderate-to-large effect (Figure 1; Table S2. Relative to placebo-priming, cocaine-priming significantly increased cocaine breakpoint, $F(1,11) = 14.11$, $p = .003$, partial $\eta^2 = 0.56$, very large effect. Relative to $0.50, the $1.50 money alternative significantly decreased cocaine breakpoint, $F(1,11) = 9.35$, $p = .011$, partial $\eta^2 = 0.46$, large effect. Finally, relative to the placebo dose, NAC attenuated the effect of cocaine-priming on cocaine breakpoint, $F(1,11) = 5.19$, $p = .044$, partial $\eta^2 = 0.32$, large effect.

3.2.2 | Cocaine choices—Consistent with the cocaine breakpoint findings, three-factor rmANOVA exhibited significant effects for Cocaine-Priming, Money Amount Alternative, and Medication Dose by Cocaine-Priming interaction, but a non-significant Medication Dose effect, $F(1,11) = 2.60$, $p = .14$, partial $\eta^2 = 0.19$, moderate-to-large effect (Figure S1). Relative to the placebo-priming dose, the cocaine-priming dose significantly increased total number of cocaine choices, $F(1,11) = 10.53$, $p = .008$, partial $\eta^2 = 0.49$, large effect (not shown). Relative to $0.50, the $1.50 money alternative significantly decreased total cocaine choices, $F(1,11) = 13.20$, $p = .004$, partial $\eta^2 = 0.55$, very large effect. Finally, relative to the placebo dose, NAC attenuated the effect of cocaine-priming on total number of cocaine choices, $F(1,11) = 4.71$, $p = .05$, partial $\eta^2 = 0.30$, large effect.

3.2.3 | Naturalistic and experimental cocaine-seeking behavior are correlated—Pearson correlations indicated that CPUP cocaine purchasing time was significantly positively correlated with cocaine breakpoint and cocaine choices in seven of the eight experimental conditions ($rs = 0.58–0.94$, $ps < .05$; all except for the placebo-medication, cocaine-primed, and $0.50 money alternative condition). Three of eight experimental conditions remained significant at the Bonferroni-corrected threshold ($p < .0063$) for both cocaine breakpoint and cocaine choices ($rs = 0.74–0.94$; $ps < .0063$; Figure 2) and accounted for 55.4%–87.4% of the variance. The extent to which individuals "worked" to buy cocaine outside of the laboratory (i.e., purchase travel time) correlated with the extent to which individuals "worked" to earn cocaine in this laboratory paradigm (i.e., cocaine breakpoint) and thus support the external validity of our cocaine-seeking findings.

3.3 | Brain chemistry

3.3.1 | rACC ¹H MRS—Spectra quality and LCModel fit was very good (SNR = 18, CRLB% GLU = 4.5%, and CRLB% GLN = 14.6%) and did not differ by medication (NAC vs. placebo; Table S3). Relative to placebo levels, rACC GLX levels were significantly

lower by 11.5%, $F(1,6) = 7.36$, $p = .035$ (partial $\eta^2 = 0.55$; very large effect; GLX: 15.86 ± 0.81 vs. 13.94 ± 0.60), and rACC GLU levels were lower by 8.2%, during NAC dosing, $F(1,6) = 4.23$, $p = .085$ (partial $\eta^2 = 0.41$; large effect; GLU: 11.77 ± 0.61 vs. 10.70 ± 0.45; Figure 3). No other metabolites approached significance (ps > .30).

3.4 | Subjective and physiological effects

3.4.1 | Vital signs—Relative to session baseline, cocaine-priming significantly increased heart rate and systolic and diastolic blood pressure more than placebo-priming (Priming × Time interactions; all ps < .001; Bonferroni-corrected threshold: $p < .01$; Figure 4) but did not affect oxygen saturation. NAC decreased oxygen saturation to a statistically (but not clinically) significant degree relative to placebo levels, $F(1,11) = 9.52$, $p < .01$ (Figure 4). NAC did not alter cocaine-priming effects on any physiological measures.

3.4.2 | Subjective drug effects—Relative to placebo-priming, cocaine-priming significantly increased VAS ratings of "good drug effect," "want drug again," "liking," "high," and "stimulated" (all ps < .01; Bonferroni-corrected threshold: $p < .012$; Figure 4). Stimulant-Sensitive Adjective Rating Scale scores also significantly increased after cocaine-priming ($p = .001$). The only significant interaction of NAC × Priming indicated that NAC attenuated the cocaine-priming effect on CCQ "Lack of Control Over Use" scores, $F(1,11) = 10.30$, $p < .01$; Bonferroni-corrected threshold: $p < .01$. A complete description of these data is provided in Table S4.

4 | DISCUSSION

This well-controlled inpatient human laboratory study evaluated the effects of NAC versus placebo on cocaine-seeking behavior, rACC neurochemistry, and subjective and physiological effects of cocaine self-administration. There were four primary significant findings: (1) cocaine-priming increased cocaine-seeking behavior, (2) NAC attenuated cocaine-primed increases in cocaine seeking, (3) the higher money amount alternative suppressed cocaine seeking, and (4) NAC reduced rACC GLX levels. Here, we report the first clinical evidence that NAC can suppress cocaine-primed cocaine-seeking behavior and replicate prior research indicating NAC modulates rACC neurochemistry in cocaine users. Finally, we report the first evidence of NAC's therapeutic effects on cocaine-seeking behavior in parallel with its neurochemical effects, that is, modulation of rACC excitatory tone, which is theorized to underlie NAC's therapeutic efficacy.

Animal research indicates that NAC reliably attenuates *reinstatement* of cocaine-seeking behavior after cue- and cocaine-priming challenges.[6,9,11,12] The present study translated robust preclinical findings by evaluating NAC effects on cocaine seeking under cocaine-primed and unprimed conditions in cocaine-dependent individuals. A critical feature of the study design was the onset of NAC maintenance during a 3-day period of experimental cocaine abstinence, similar to preclinical reinstatement models. In the absence of NAC, cocaine-priming significantly increased cocaine seeking by 47% and 48% for the $0.50 and $1.50 money amounts, respectively, compared with placebo-priming. This finding is analogous to cocaine-induced reinstatement in preclinical models and replicates prior human laboratory research.[39] NAC significantly attenuated cocaine-primed cocaine seeking on

both behavioral metrics (cocaine breakpoint and total number of cocaine choices) with a large effect size. Relative to placebo levels, NAC reduced the total number of cocaine choices after cocaine-priming by 30%, on average. This is additional evidence that NAC may be an effective therapeutic agent for suppressing cocaine-seeking behavior, building on preclinical data.[6,9,11,12] Interestingly, NAC did not attenuate physiological responses to the cocaine-priming dose (i.e., blood pressure and heart rate), suggesting that NAC's behavioral effects may be driven by neurochemical/cognitive changes. Our data indicate NAC did not alter placebo-primed cocaine seeking at either money alternative amount. It is important not to overinterpret a null finding, especially in a pilot study. However, this is consistent with the extant literature that indicates that NAC does not alter *stable*, cocaine-reinforced responding in preclinical research,[13] nor outpatient cocaine use among actively using patients,[18] suggesting NAC may not be effective for abstinence initiation. Finally, NAC attenuated cocaine-primed increases in subjective "Loss of Control Over Use." NAC may reduce cocaine's acute disinhibiting effects, which may account for NAC's effective attenuation of cocaine-primed cocaine seeking. In summary, we provide the first clinical evidence that NAC suppresses cocaine-primed cocaine-seeking behavior. Our findings contribute to the growing literature that NAC has greater therapeutic promise for prolonging cocaine abstinence, that is, relapse prevention, than abstinence initiation.

One prior study has reported NAC's effect on neurochemistry among cocaine-dependent individuals. Using single-voxel ^{1}H MRS, Schmaal and colleagues found a single oral dose of 2400 mg NAC "normalized" dACC GLU/tCr ratio levels among abstinent cocaine users, compared with controls.[20] We extended that initial study in three important ways: (1) rather than a single NAC acute dose, NAC's effect on glutamate neurochemistry was evaluated after 3 days of repeated NAC dosing, to simulate the onset of a therapeutic regimen, (2) placebo control was implemented, and (3) cocaine-seeking behavior was measured in parallel. Our findings indicate that repeated NAC dosing significantly reduced rACC GLX levels by 12% and numerically reduced GLU levels by 8% (both large effect sizes). While the brain region and calculated endpoint are different than Schmaal et al., the consistency of NAC's effect on neurochemistry is noteworthy. Taken together with Schmaal et al., NAC can reduce ACC glutamate + glutamine levels among cocaine users and may restore glutamatergic homeostasis.[20] ^{1}H MRS does not distinguish glutamate pools (neuronal vs. glial vs. extracellular), but the predominant source of the ^{1}H MRS glutamate signal is intracellular.[40] Based on preclinical findings,[9,10] we speculate NAC's neurochemical effect is driven by increased inhibitory tone via mGluR$_{2/3}$ signaling. Prior research guides interpretation of these neurochemical findings. rACC glutamate levels have been linked to impulsivity,[20,41] cocaine cue-reactivity,[42–44] and years of cocaine use.[28] We found that NAC attenuated cocaine's subjective disinhibiting effect, whereas prior research indicated that NAC improved inhibitory control[30] and reduced cocaine cue-induced craving[16,17] and cocaine attentional bias.[19] NAC's effect on cocaine-seeking behavior may be mediated by changes in any (or all) of these processes via modulation of rACC excitatory tone.

Contingency management is a robust behavioral intervention for initiating cocaine abstinence.[22–24] In this study, we implemented a contingency management analogue adapted for the money versus cocaine choice progressive ratio paradigm. Specifically, on half of the experimental testing days, the alternative choice money amount was three times greater

than other days ($1.50 vs. $0.50). Results indicated the higher alternative money amount significantly suppressed cocaine seeking by ~32% across all experimental conditions (large effect sizes), consistent with prior research.[39] However, NAC did not augment the suppressing effect of the higher money amount alternative on cocaine-seeking behavior. We stress that these findings should be strictly interpreted within the context of money versus drug choice paradigms and *should not* be extended to contingency management interventions in general.

Limitations of this study deserve note. First, sample size was relatively small, suggesting caution in generalizing these findings. Nonetheless, we observed significant NAC-induced reduction of cocaine-primed cocaine seeking, consistent with preclinical data. Second, ^{1}H MRS data of acceptable quality were only available for seven of 12 participants who completed behavioral choice procedures. Brain chemistry measures were considered secondary so as not to rate-limit enrollment and evaluation of the primary behavioral outcomes. Although ^{1}H MRS data are consistent with prior findings[20] and exhibited large effect sizes, future studies are needed to confirm these findings and investigate brain–behavior relationships, which were underpowered herein. Third, we note NAC may exhibit other (possibly related) neurobiological mechanisms of action not examined in this study, for example, modulation of dopamine release in the striatum.[13]

In conclusion, this study offers translational experimental evidence regarding the effect of NAC on cocaine-seeking/self-administration behavior in the human laboratory setting. Our findings indicate NAC reduced cocaine seeking following an active cocaine priming dose and reduced rACC glutamate + glutamine levels. This is the first report of NAC's putative neurochemical mechanism of action, that is, modulation of rACC excitatory tone, measured in parallel with its therapeutic effect on cocaine-seeking behavior. Finally, we report that a higher alternative choice money amount suppressed cocaine-seeking behavior, analogous to continency management, but did not exhibit additive effects when combined with NAC dosing in our human laboratory paradigm. Taken together with prior research, NAC may be an effective intervention for "short-circuiting" lapses and prolonging cocaine abstinence but may not be effective for abstinence initiation. CUD is a complex problem that will likely require multiple strategies. Future research is needed to investigate the therapeutic efficacy of NAC (or NAC-amide, which has better bioavailability than NAC) in combination with an effective intervention for initiating abstinence, such as contingency management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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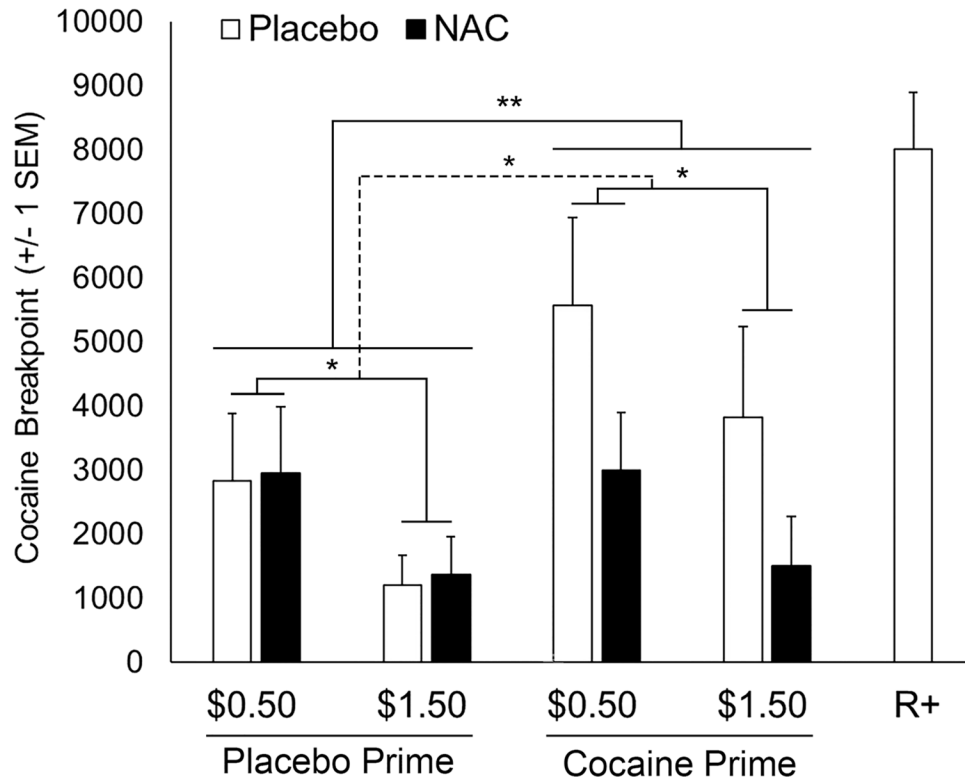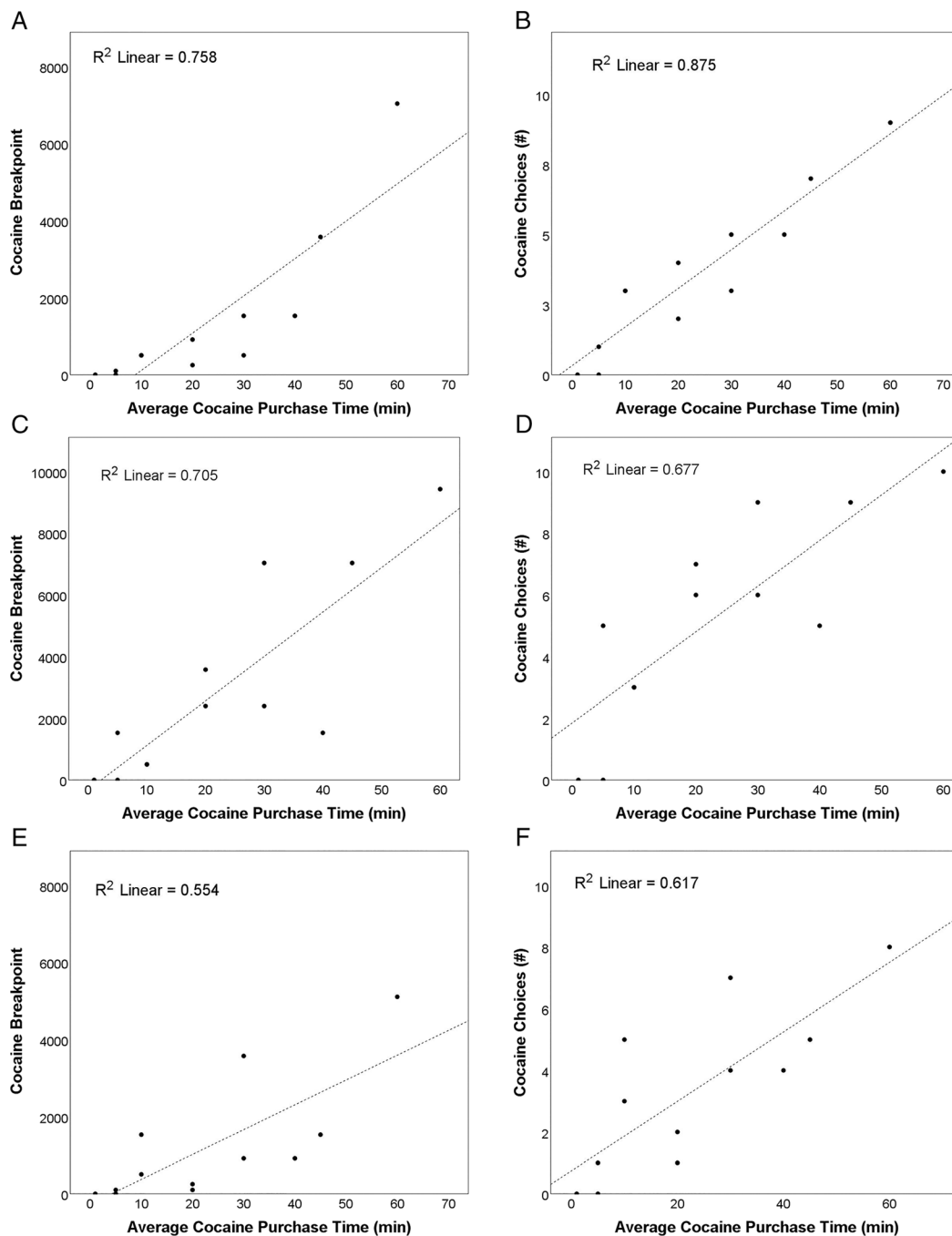
FIGURE 1.

The effects of cocaine- versus placebo-priming, money-alternative amount ($0.50 vs. $1.50 per choice), and NAC versus placebo on mean (±1 SEM) cocaine breakpoint values (10 mg unit dose) are depicted. Relative to placebo-priming, cocaine-priming significantly increased cocaine breakpoint, $F(1,11) = 14.11$, **$p = .003$, partial $\eta^2 = 0.56$, very large effect (**solid line). Relative to the $0.50 money alternative, the $1.50 money alternative significantly decreased cocaine breakpoint for placebo- and cocaine-priming, $F(1,11) = 9.35$, *$p = .011$, partial $\eta^2 = 0.46$, large effect (*solid lines). Relative to the placebo dose, NAC (3600 mg/day) attenuated the effect of cocaine-priming on cocaine breakpoint, $F(1,11) = 5.19$, *$p = .044$, partial $\eta^2 = 0.32$, large effect (*dashed line). For comparison, the rightmost bar indicates cocaine (10-mg unit dose vs. placebo [no money] choice) breakpoint during the reinforcement screening session (R+) prior to medication exposure

FIGURE 2.

Significant Pearson correlations (Bonferroni-corrected threshold; $p < .0063$) between average cocaine purchase time (minute) and cocaine-seeking behavior (cocaine breakpoint depicted in the left column; total cocaine choices depicted in the right column) under different experimental conditions are denoted: (A) and (B) NAC dosing, placebo-prime, and $1.50 money alternative ($r = 0.87$, $p < .001$ and $r = 0.94$, $p < .001$; respectively); (C) and (D) NAC dosing, cocaine-prime, and $0.50 money alternative ($r = 0.82$, $p = .001$ and $r = 0.84$,

$p = .001$, respectively); and (E) and (F) placebo dosing, placebo-prime, and \$1.50 money alternative ($r = 0.79$, $p = .002$ and $r = 0.74$, $p = .006$, respectively)
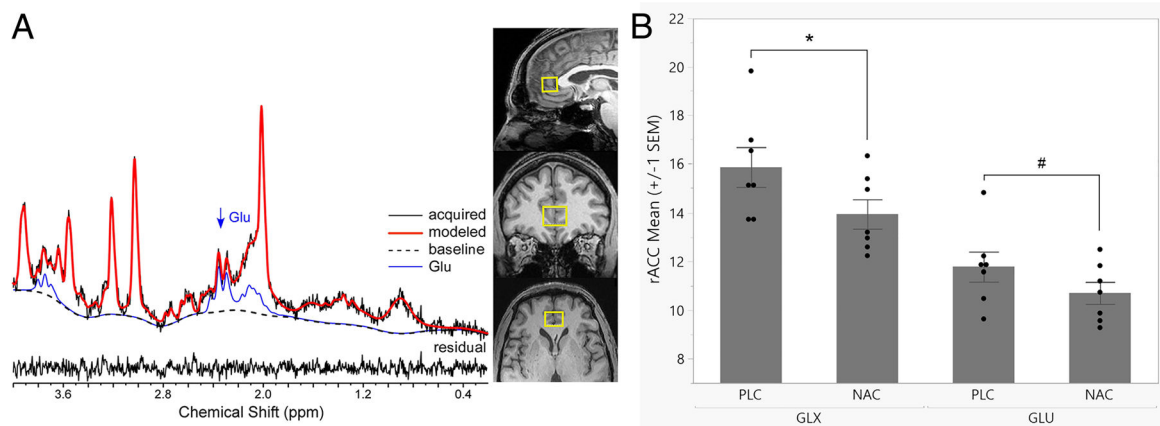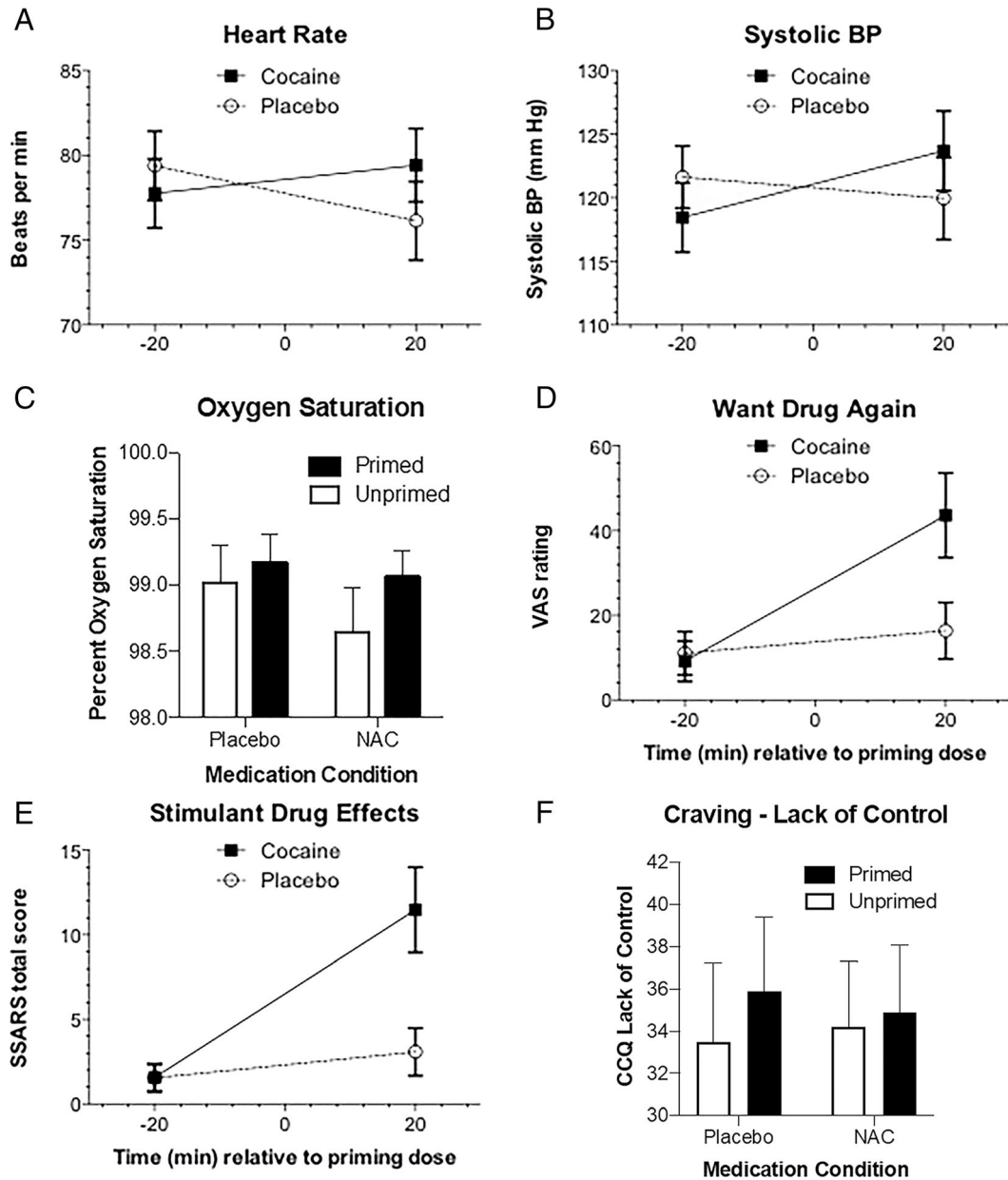
FIGURE 3.

(A) A representative 1H MRS spectrum with LCModel fit and orthonormal slices of the rostral anterior cingulate cortex voxel (rACC; 4.5 cm^3) are depicted. (B) Mean concentrations of rACC glutamate (GLU) and GLX (glutamate + glutamine) are depicted. Relative to placebo levels, NAC significantly reduced GLX levels by 11.5%, $F(1,6) = 7.36$, $*p = .035$, partial $\eta^2 = 0.55$, very large effect; and numerically reduced GLU levels by 8.2%, $F(1,6) = 4.23$, $^\#p = .085$, partial $\eta^2 = 0.41$, large effect

FIGURE 4.

Physiological and subjective effects of placebo- versus cocaine-priming are depicted. Findings indicate significant cocaine-priming effects ($ps < .01$) on (A) heart rate; (B) systolic blood pressure (BP); (C) oxygen saturation; (D) want drug again; and (E) Stimulant-Sensitive Adjective Rating Scale (SSARS) total score. Further, a significant NAC by priming interaction, $F(1,11) = 10.30$, $p < .01$, indicated that NAC attenuated the cocaine-priming effect on (F) "Lack of Control Over Use" score

Participant characteristics

TABLE 1

| Subj. | Demographics | | | | Lifetime and recent pattern of cocaine use | | | | | | | Other past-month substance use | |
	Med First	Scan	Race	Sex	Age	Duration cocaine	Route	# quit attempts	CPUP purchase time	CPUP $ spent cocaine	# past 30d cocaine any use	# past 30d alcohol any use	# past 30d marijuana any use
1	NAC	No	AA	M	54	28	SM	10	45	500	8	0	0
2	NAC	(Yes)[a]	AA	M	51	23	SM	10	30	1200	4	25	0
3	NAC	Yes	AA	M	47	22	SM	0	10	240	12	12	12
4	NAC	Yes	C	M	47	17	SM	2	30	500	13	0	0
5	NAC	Yes	AA	M	46	27	IV	3	40	500	6	6	2
6	PLA	No	Multi	M	55	5	SM	2	5	120	9	12	0
7	PLA	Yes	AA	M	40	22	SM	2	1	2000	28	0	25
8	PLA	No	AA	F	45	20	SM	50	60	80	4	8	3
9	NAC	Yes	AA	M	45	15	SM	10	20	600	20	8	1
10	PLA	Yes	AA	M	53	25	SM	10	20	200	3	7	7
11	NAC	No	AA	M	50	28	SM	3	5	80	16	30	28
12	PLA	Yes	AA	M	44	16	SM	4	10	280	15	30	0
% or M	58%	67%	83%	92%	48.1 (4.5)	20.7 (6.7)	92%	8.8 (13.5)	23.0 (18.4)	$525.0 ($559.7)	11.1 (7.7)	11.5 (11.0)	7.1 (10.3)
(±1 SD) median	NAC	Yes	AA	M	47	22	SM	3.5	20	$390	10.5	8	1.5

Note. Patterns of cocaine use are described in terms of self-reported duration of regular use (years), current primary route of use (smoked [SM] or intravenous [IV]), and number of lifetime quit attempts.

Abbreviations: AA, African-American; C, Caucasian; Multi, multi-racial; M, Male; F, Female; NAC, N-acetylcysteine; PLA, placebo; SD, standard deviation.

[a]H MRS data quality was poor subject #2 and thus excluded from analyses of the imaging data. See text for further details on participant characteristics.