

**Chronic N-acetylcysteine during abstinence or extinction following cocaine
self-administration produces enduring reductions in drug-seeking**

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Abstract

The cysteine prodrug N-acetylcysteine (NAC) has been shown to reduce reinstatement of cocaine-seeking by normalization of glutamatergic tone. However, enduring inhibition of cocaine-seeking produced by NAC has not been explored under different withdrawal conditions. Thus, the present study determined whether chronic NAC administered during daily extinction training or daily abstinence following withdrawal from cocaine self-administration would reduce cocaine-seeking. Rats self-administered intravenous cocaine during daily 2-h sessions for 12 days, followed by daily extinction or abstinence sessions. During this period, rats received daily injections of saline, 60, or 100 mg/kg NAC. Subsequently, rats were tested for cocaine-seeking via conditioned cue, cue+cocaine-primed, and context induced relapse. Chronic NAC administration blunted cocaine-seeking under multiple experimental protocols. Specifically, NAC attenuated responding during cue and cue+cocaine-primed reinstatement tests following extinction, and context, cue, and cue+cocaine relapse tests following abstinence. Protection from relapse by NAC persisted well after treatment was discontinued, particularly when the high dose was combined with extinction trials. The finding that NAC reduced cocaine-seeking after drug treatment was discontinued has important implications for the development of effective anti-relapse medications. These results support recent preclinical and clinical findings that NAC may serve as an effective treatment for inhibiting relapse in cocaine addicts.

Introduction

A key feature of drug addiction is the inability of addicts to inhibit relapse. Thus, the development of effective anti-relapse medication is a critical goal of pharmacotherapy for addiction. While a large number of pharmacological agents have been assessed in animal models of addiction and clinical studies (Yahyavi-Firouz-Abadi and See, 2009), there are no currently available drugs with established efficacy for the treatment of psychostimulant addiction and relapse.

Drug-induced plasticity in central glutamate function has been increasingly implicated in the process of cocaine addiction and relapse (Kalivas, 2009). For example, chronic cocaine self-administration reduces basal concentrations of extracellular glutamate, but potentiates nucleus accumbens glutamate release during cocaine-primed reinstatement of extinguished drug-seeking (McFarland et al., 2003; Madayag et al., 2007). Basal glutamate concentrations are regulated by the glutamate/cystine antiporter (Baker et al., 2002), which are down regulated during withdrawal (Baker et al., 2003a; Madayag et al., 2007). *N*-acetylcysteine (NAC) is a cystine prodrug that restores basal glutamate levels and prevents increased glutamate during reinstatement by reversing glutamate dysregulation after cocaine self-administration in rats (Baker et al., 2002; Madayag et al., 2007; Kalivas, 2009; Moussawi et al., 2009). Acute NAC administration prevents cocaine-induced increases in extinguished drug-seeking (Baker et al., 2003b; Madayag et al., 2007; Kau et al., 2008). Chronic NAC given during cocaine self-administration does not impact cocaine intake with daily 2-h access conditions (Madayag et al., 2007; Amen et al., 2011), but does reduce and block escalation of intake in extended 6-h access conditions (Madayag et al., 2007; Kau et al., 2008). Consequently,

NAC does not change the reinforcing mechanisms associated with cocaine, but instead prevents drug-seeking by a reduction or reversal of the development of neuroplasticity required for cocaine-induced reinstatement (Madayag et al., 2007; Amen et al., 2011; Moussawi et al., 2011).

Recently, we demonstrated that repeated NAC administration during daily extinction trials reduces reinstatement triggered by either cocaine associated conditioned cues, or the combination of drug-associated cues and a priming injection of cocaine (Moussawi et al., 2011). Similarly, chronic NAC during extinction produced lasting reductions in heroin-seeking in rats (Zhou and Kalivas, 2008), and repeated NAC decreased cocaine-primed reinstatement of drug-seeking when tested 24 h after the last injection (Amen et al., 2011). Finally, initial clinical assessments indicate promising results for reduced craving in cocaine addicts following repeated NAC treatment (Amen et al., 2011; LaRowe et al., 2007). These studies suggest that NAC may serve as an effective anti-relapse medication.

Extinction occurs when a Pavlovian signal or an instrumental action is repeatedly presented without its associated reinforcer. Ample evidence shows extinction does not result in the “unlearning” of the original association, but instead involves new learning of the altered contingency (Bouton, 2002; Quirk and Mueller, 2008). The extinction-reinstatement model involves a period of maintained drug self-administration for intravenous infusions, followed by extinction training, whereby operant responding decreases in the drug associated context when lever pressing no longer results in reinforcement. Drug-seeking is reinstated by exposure to a priming injection of drug, stress, or previously drug associated cues. While most animal models of relapse have

implemented extinction-reinstatement procedures (Shaham et al., 2003), most “treatment-as-usual” protocols for human drug addicts do not explicitly extinguish drug related cues. An alternative approach in animal models has been to assess drug-seeking after a period of forced abstinence with no daily extinction trials (Reichel and Bevins, 2009). Rather than extinguishing the operant behavior (e.g., lever pressing), subjects are kept away from the self-administration environment for a prescribed amount of time. At the time of relapse testing, the drug-reinforced associations (i.e., lever presses, environmental context, and conditioned cues) are fully intact, having never been experienced in the absence of the drug. It is important to study both extinction-reinstatement and abstinence-relapse models because the incorporation of extinction training into an abstinence period results in different behavioral profiles (Neisewander et al., 2000; Zavala et al., 2007) and neuroadaptations related to relapse (Schmidt et al., 2001; Sutton et al., 2003; Self, 2004; Fuchs et al., 2006; Knackstedt et al., 2010).

In the current study, we compared the impact of chronic NAC treatment during abstinence vs. chronic NAC during daily extinction trials on subsequent cocaine-seeking at both early and late withdrawal points. Although extinction and abstinence may involve differing neuroadaptations, the ability of NAC to prevent cocaine-seeking relies on the restoration of glutamate homeostasis and the reversal of cocaine-induced synaptic potentiation (Baker et al., 2003a; Kalivas, 2009; Moussawi et al., 2011). Therefore, we hypothesized that cocaine-seeking should be reduced by chronic NAC regardless of whether or not explicit extinction training occurred during treatment.

Methods

Subjects

Ninety-three male Sprague-Dawley rats (Charles-River) weighing 250-300 g at the time of delivery were maintained on a reversed 12:12 light-dark cycle in a temperature- and humidity-controlled vivarium. Rats were individually housed and received *ad libitum* water throughout the study and 25 g of standard rat chow (Harlan, Indianapolis, IN, USA) daily until self-administration stabilized, at which time animals were maintained *ad libitum*. Procedures were conducted in accordance with the “Guide for the Care and Use of Laboratory Rats” (Institute of Laboratory Animal Resources on Life Sciences, National Research Council) and approved by the IACUC of the Medical University of South Carolina.

Apparatus

Self-administration chambers (30×20×20 cm, Med Associates) were housed inside sound-attenuating cubicles fitted with a fan for airflow. Each chamber contained two retractable levers, two stimulus lights, a speaker for tone delivery, and a house light to provide general illumination. Additionally, each chamber was equipped with a balanced metal arm and spring leash attached to a swivel (Instech). Tygon® tubing extended through the leash and was connected to a 10 ml syringe mounted on an infusion pump located outside the sound-attenuating cubicle.

Drugs

Cocaine hydrochloride dissolved in 0.9% sterile saline (generously provided by the National Institute on Drug Abuse, Research Triangle Park, NC) was administered IV at a volume of 50 µg/50 µl infusion. N-acetylcysteine (Sigma, St. Louis, MO, USA) was dissolved in saline, adjusted with NaOH to 7.2 pH, and injected at a volume of 1 ml/kg, IP. Drugs used for anesthesia were ketamine (Vedco Inc, St Joseph, MO, USA), xylazine

(Lloyd Laboratories, Shenandoah, IA, USA), equithesin (sodium pentobarbital 4 mg/kg, chloral hydrate 17 mg/kg, and 21.3 mg/kg magnesium sulfate heptahydrate dissolved in 44% propylene glycol, 10 % ethanol solution), and ketorolac (Sigma, St. Louis, MO, USA). Catheters were flushed with heparin (Elkins-Sinn, Cherry Hill, NJ, USA) and cephazolin (Schein Pharmaceuticals, Florham Park, NJ, USA). Catheter patency was verified with methohexital sodium (Eli Lilly, Indianapolis, IN, USA).

Surgery

Anesthesia consisted of IP injections of ketamine (66 mg/kg), xylazine (1.3 mg/kg), and equithesin (0.5 ml/kg). Ketorolac (2.0 mg/kg, IP) was given just prior to surgery as an analgesic. One end of a silastic catheter was inserted 33 mm into the right jugular and secured with 4.0 silk sutures. The other end ran subcutaneously and exited from a small incision just below the scapula. This end attached to an infusion harness (Instech Solomon, Plymouth Meeting, PA, USA) that provided access to an external port for IV drug delivery. An antibiotic solution of cefazolin (10 mg/0.1 ml) was given post surgery and during recovery along with 0.1 ml 70 U/ml heparinized saline. During self-administration, rats received an IV infusion (0.1 ml) of 10 U/ml heparinized saline before each session. After each session, catheters were flushed with cefazolin and 0.1 ml 70 U/ml heparinized saline. Catheter patency was periodically verified with IV methohexital sodium (10 mg/ml dissolved in 0.9% physiological saline), a short-acting barbiturate that produces a rapid loss of muscle tone.

Cocaine self-administration

Following at least 5 days of recovery, rats were given daily 2 h sessions to self-administer cocaine on a fixed ratio 1 schedule of reinforcement. The house light always

signaled the beginning of a session and remained on throughout the session. During the sessions, a response on the active lever resulted in activation of the pump for a 2-s infusion (50 μ g/50 μ l bolus infusion) and presentation of a stimulus complex consisting of a 5-s tone (78 dB, 4.5 kHz) and a white stimulus light over the active lever, followed by a 20-s time out. Responses that occurred during the time out and on the inactive lever were recorded, but had no scheduled consequences. All sessions took place during the dark cycle and were conducted 6 days/week until each rat reached a criterion of >10 infusions during a session for 12 cumulative days. All ensuing experiments utilized the same self-administration procedures.

Abstinence, extinction, and reinstatement

The term “reinstatement” connotes responding subsequent to extinction of the lever press response (Bouton, 2002). Adhering to the traditional extinction-reinstatement model (Shaham et al., 2003), tests conducted after extinction are termed “reinstatement tests”, while tests that occur following abstinence in the absence of daily extinction are referred to as “relapse”. Following cocaine self-administration, rats were placed into abstinence and/or extinction before testing. During abstinence, rats were transported, handled, and injected daily with saline, 60, or 100 mg/kg NAC, but were not placed in the self-administration chamber. Recent clinical studies report dose ranges from 1200-3600 mg in a 24-hr period (LaRowe et al., 2007; Mardikian et al., 2007; Amen et al., 2010). Using an average body weight of 70 kg, this range converts to 17.14-51.43 mg/kg. Keeping in mind species differences and administration routes, our NAC doses can be considered to be in the higher range. After abstinence, relapse tests consisted of placing rats back into the self-administration context for a 2 h test. Responding was recorded, but

had no consequences except during cue-induced reinstatement, whereby each response resulted in presentation of the stimulus complex. During extinction, rats were placed in the self-administration chamber for daily 2-h sessions for 12 days, and responding on either lever had no scheduled consequences. To have met extinction criterion, responses on the active (i.e., drug-seeking) lever needed to be ≤ 25 presses for 2 consecutive days. When this criterion was met, rats were tested for cue-induced, cocaine-primed, and/or cue+cocaine-primed reinstatement. For cue-induced reinstatement tests, active lever presses resulted in presentation of the conditioned light+tone in the same manner as during self-administration. For cocaine-primed reinstatement tests, rats received a cocaine injection (10 mg/kg) prior to testing and responses on both levers were recorded, but had no consequences. The cue+cocaine condition consisted of the cocaine priming injection and presentation of cues. Daily extinction sessions occurred between reinstatement tests until the criterion of ≤ 25 presses for 2 consecutive days was met.

Experiments 1 and 2: Effects of chronic NAC (60 and 100 mg/kg) during extinction on subsequent reinstatement

Figures 1A and 2A depict the time line for experiments 1 and 2, respectively. Rats experienced 12 days of cocaine self-administration, followed by at least 12 days of extinction. During extinction, rats received saline, 60 mg/kg NAC (Experiment 1), or 100 mg/kg NAC (Experiment 2) 2 h before each extinction trial. At the end of extinction, rats were tested for cue and cue+cocaine-primed reinstatement. Between tests, rats were re-extinguished to criterion and NAC or saline treatment continued. After 2 weeks of abstinence, rats were retested in the absence of NAC. A subset of rats used in experiment 2 has been previously included in a published data set (Moussawi et al., 2011).

Experiments 3 and 4: Effects of chronic NAC (60 and 100 mg/kg) during abstinence on subsequent relapse and reinstatement

Figures 3A and 4A depict the time line for experiments 3 and 4, respectively. Rats underwent 12 days of cocaine self-administration, followed by two weeks of abstinence. During abstinence, rats were transported, handled, and injected daily with saline, 60 mg/kg NAC (Experiment 3), or 100 mg/kg NAC (Experiment 4), but were not placed back into the self-administration environment. After 2 weeks, rats were tested for relapse to cocaine-seeking by placement into the chambers. Responding was subsequently extinguished in daily trials and rats were tested for cue and cocaine-primed reinstatement.

Experiment 5: Effects of chronic NAC (100 mg/kg) during abstinence on cue and cue+cocaine-primed relapse

Figure 5A depicts the time line for experiment 5. Rats experienced 12 days of cocaine self-administration followed by two weeks of abstinence. During abstinence, rats were transported, handled, and injected daily with saline or 100 mg/kg NAC, but were not placed back into the self-administration environment. After 12-14 days, rats were tested for cue and cue+cocaine-primed relapse while receiving NAC. Two weeks after NAC was discontinued, rats were again tested for cue and cue+cocaine-primed relapse.

Data analysis

The number of active and inactive lever presses, as well as cocaine intake served as the dependent measures. To determine that groups did not differ before NAC treatment, data from the last 3 days of self-administration were averaged for each variable and analyzed with t-tests. The effects of NAC on extinction responding used a mixed analysis of variance (ANOVA), with drug as the between subjects factor and session as

the within-subjects factor. Based on *a priori* assumptions, planned comparisons (t-tests, one-tail) were limited to assessment of reductions in reinstatement responding produced by NAC treatment relative to saline-treated rats. Data points that were two standard deviations from the mean were excluded (Hill and Lewicki, 2007). Based on very low responses in all groups, data for inactive lever presses are not presented. All data are presented as mean \pm SEM and the alpha was set at $p < 0.05$.

Results

Experiments 1 and 2: NAC attenuates reinstatement following extinction

Experiment 1: 60 mg/kg NAC during extinction.

Rats given saline or 60 mg/kg NAC during extinction did not differ on cocaine intake (saline=17.96 \pm 1.47; NAC=18.60 \pm 0.87) or lever responding during SA (Fig. 1B). During extinction (Fig. 1C), lever pressing decreased across the 12 sessions for both groups on the active lever [session main effect, $F(11,154)=32.96$, $p < 0.05$]. However, saline and NAC treated rats did not differ, nor did treatment interact with session. Data from the first cue-induced and cue+cocaine-primed reinstatement tests are shown in Fig. 1D, while rats were still receiving NAC. NAC-treated rats had lower responding relative to saline-treated rats on the cue test [$t(14)=2.7$, $p < 0.05$]; and marginally lower responding on the cue+cocaine test [$t(14)=1.7$, $p=0.06$]. During the second series of reinstatement tests at 2 weeks after saline or NAC injections (Fig. 1E), the groups did not differ.

Experiment 2: 100 mg/kg NAC during extinction.

Rats given saline or 100 mg/kg NAC during extinction did not differ on cocaine intake (saline=18.85 \pm 1.25 and NAC=18.08 \pm 0.90) or lever responding during SA (Fig. 2B). During extinction (Fig. 2C), lever pressing decreased across the 12 sessions for both

groups on the active lever [session main effect, $F(11,209)=27.08$, $p<0.05$]. Rats treated with 100 mg/kg NAC pressed the lever significantly less than saline-treated rats throughout the extinction period [group main effect, $F(1,19)=7.57$, $p<0.05$]. However, group did not interact with session. Data from the first cue-induced reinstatement and cue+cocaine-primed reinstatement tests are represented in Fig. 2D, while rats were still receiving NAC. NAC-treated rats had lower responding relative to saline-treated rats on the cue test [$t(19)=1.8$, $p<0.05$] and cue+cocaine test [$t(19)=1.7$, $p<0.05$]. Similarly, during the second series of reinstatement tests 2 weeks after saline or NAC injections (Fig. 2E), NAC-treated rats had lower responding relative to saline-treated rats on the cue test [$t(19)=2.6$, $p<0.05$] and cue+cocaine test [$t(19)=2.2$, $p<0.05$].

Experiments 3 and 4: N-acetylcysteine (60 and 100 mg/kg) during abstinence has dose dependent effects on context-induced relapse

Experiment 3: 60 mg/kg NAC during abstinence.

Rats given saline or 60 mg/kg NAC during abstinence did not differ on cocaine intake (saline= 18.37 ± 1.2 and NAC= 20.04 ± 1.6) or lever responding during SA (Fig. 3B). On the relapse test (Fig. 3C), NAC and saline rats also showed no significant differences. During extinction (Fig. 3D), lever pressing decreased across the 10 sessions for both groups on the active lever [session main effect, $F(9,126)=9.39$, $p<0.05$]. NAC-treated rats maintained higher levels of responding on days 2 and 3 of extinction [session x group, $F(9,126)=2.93$, $p<0.05$, and Tukey HSD, $p<0.05$]. During the cued-induced reinstatement and cocaine-primed reinstatement tests (Fig. 3E), there were no group differences.

Experiment 4: 100 mg/kg NAC during abstinence.

Rats given saline or 100 mg/kg NAC during abstinence did not differ on cocaine intake (saline=21.48±1.63 and NAC=19.63±1.47) or lever responding during SA (Fig. 4B). On the context relapse test (Fig. 4C), NAC-treated rats showed lower responding relative to saline-treated rats on the test day [$F(1,26)=7.8$, $p<0.05$]. During extinction (Fig. 4D), lever pressing decreased across the 10 sessions for both groups [session main effect, $F(9,264)=27.6$, $p<0.05$]. During the cued-induced reinstatement and cocaine-primed reinstatement tests (Fig. 4E), there were no group differences.

Experiment 5: N-acetylcysteine (100 mg/kg) during abstinence attenuates cue and cue+cocaine-induced relapse

Rats given saline or 100 mg/kg NAC during abstinence did not differ on cocaine intake (saline=16.83±1.66 and NAC=17.58±1.69) or lever responding during SA (saline=33.62 ± 2.54 and NAC=37.95 ± 4.16). Data from the first cue-induced reinstatement and cue+cocaine-primed reinstatement tests are represented in Fig. 5B, while rats were still receiving NAC. NAC-treated rats showed lower responding relative to saline-treated rats on the cue test [$t(12)=2.7$, $p<0.05$] and the cue+cocaine test [$t(12)=2.3$, $p<0.05$]. During the second series of reinstatement tests at 2 weeks after saline or NAC injections (Fig. 5C), saline and NAC-treated rats responded the same on the cue test; however, NAC-treated rats had lower responding on the cue+cocaine test [$t(12)=3.2$, $p<0.05$].

Discussion

In the current study, chronic NAC administration blunted cocaine-seeking under multiple experimental protocols of extinction and abstinence following cocaine self-administration. Specifically, NAC decreased responding during cue-induced and

cue+cocaine-primed reinstatement tests following extinction, and context, cue, and cue+cocaine relapse tests following abstinence. The reduction of cocaine-seeking by NAC persisted well after treatment was discontinued, particularly when the high dose (100 mg/kg) was administered during the extinction trials. These results support and extend earlier findings in animal models suggesting that chronic NAC may act as an effective anti-relapse medication (Madayag et al., 2007; Zhou and Kalivas, 2008; Amen et al., 2011).

Evidence suggests that NAC reduces cocaine-seeking by restoration of corticostriatal glutamate homeostasis and the reversal of cocaine-induced synaptic potentiation (Baker et al., 2003a; Kalivas, 2009; Moussawi et al., 2011). Thus, it may be expected that NAC should reduce cocaine-seeking, regardless of experience during the withdrawal phase (i.e., extinction or no extinction). The current data generally support this contention in several ways. First, chronic NAC (60 and 100 mg/kg) during extinction reduced both cue-induced and cue+cocaine-primed reinstatement, and the reduced responding was still maintained at two weeks after final NAC treatment for the high dose group. Second, specific for the high dose, NAC significantly attenuated cocaine-seeking on a context-relapse test. Importantly, in this condition, rats never received NAC in the self-administration chamber. Thus, NAC never influenced the learning of a new contingency between lever responding and a lack of reinforcement. The mere exposure to chronic NAC treatment was sufficient to reduce relapse behavior when rats were placed drug free into the cocaine-associated environment. Finally, chronic NAC (100 mg/kg) administered during abstinence blunted both cue-induced and cue+cocaine relapse

(experiment 5), with persistently reduced cocaine-seeking during the cue+cocaine test at two weeks after final NAC treatment.

Importantly, the ability of NAC to blunt cocaine-seeking under multiple experimental conditions is unlikely to result from non-specific motor alterations. Inactive lever responding remained low and relatively unchanged across all saline and NAC treated rats. In our laboratory, we have confirmed that NAC (100 mg/kg) has no impact on activity in a novel environment in cocaine-exposed rats with a previous history of NAC treatment relative to saline-treated rats (unpublished data). Furthermore, NAC has been consistently found to produce neither increases nor decreases in locomotor activity (Ferreira et al., 2008; Prakash and Kumar, 2009). Thus, non-specific changes in activity are unlikely to contribute to the ameliorative effects of chronic NAC treatment on motivated cocaine-seeking.

The NAC-induced reductions in cocaine-seeking under conditions of abstinence and extinction are modest relative to some direct pharmacological challenges. For example, systemic administration of metabotropic glutamate receptor (mGluR) receptor antagonists can completely block conditioned cue-induced and cocaine-primed reinstatement (Peters and Kalivas, 2006; Kumaresan et al., 2009). These drugs are administered via one-time injections and act directly on the specific receptor subtypes that mediate reinstatement. Further, intracranial infusions of mGluR antagonists into the nucleus accumbens can completely block cocaine-primed reinstatement (Peters and Kalivas, 2006; Kumaresan et al., 2009). Mechanistically, these studies differ from the current results, since chronic NAC treatment provides an enduring increase in overall glutamatergic tone, resulting in restoration of glutamate homeostasis, rather than directly blocking receptor activity.

Additionally, clinical studies cannot take advantage of intracranial infusions for drug administration protocols. As such, the chronic dosing paradigm we used here is more akin to clinical treatment protocols, thus enhancing the translational relevance of our findings.

Although NAC effectively reduced reinstatement and relapse following extinction and abstinence, our findings do suggest that repeated NAC exerts more profound effects in reducing relapse when NAC modifies the neuroadaptations resulting from explicit extinction training. For example, NAC at 60 mg/kg was effective in combination with extinction training, but not when the contextual associations remained intact following abstinence. Additionally, only two treatment circumstances provided enduring protection from relapse: First, 100 mg/kg NAC administered during extinction (Experiment 2); and second, 100 mg/kg NAC given during abstinence and reinstatement (Experiment 5). In both experiments, animals had systemically available NAC while in the self-administration chamber. In experiment 1, NAC was biologically available during the extinction session, while in experiment 5, NAC was present during both the cue and cue+cocaine reinstatement tests (see Fig 5A). Thus, for abstinent rats in Experiment 5, these test sessions would have served as extinction sessions due to the lack of primary reinforcement with cocaine. As such, enduring reductions in cocaine-seeking were evident in both conditions when concurrent NAC treatment occurred in combination with extinction training.

Reduced cocaine-seeking after discontinuation of pharmacotherapy is a desirable milestone for the successful development of effective anti-relapse medications. Although it is difficult to directly extrapolate these findings to the treatment of cocaine dependent

patients, our data suggest that NAC may reduce the probability of relapse in these patients, particularly when combined with extinction. It is conceivable that NAC improves extinction learning of the new contingency between lever pressing and lack of drug reinforcement. Support for this possibility comes from studies on the cognitive enhancing effects of NAC in rodents. Acute NAC improved memory retention in the Morris water maze in rats exposed to chronic aluminium (Prakash and Kumar, 2009) and improved cognition in aged mice on a T-maze footshock avoidance procedure (Farr et al., 2003; Prakash and Kumar, 2009). Acute NAC also improved memory consolidation in a conditioned place preference procedure (Achat-Mendes et al., 2007). Future comparisons are warranted to test whether NAC enhances extinction learning and differentially modifies the neural adaptations that occur with or without explicit extinction. For example, extinction-induced upregulation of Homer 1b/c enhanced internalization and sequestering of mGluR5, a mechanism that likely accounted for a loss of mGluR5-dependent LTD in extinguished, but not abstinent rats (Knackstedt et al., 2010). Cystine-glutamate exchange is down regulated after chronic cocaine, thereby reducing glutamatergic tone on mGluR5 in the accumbens (Madayag et al., 2007; Moussawi et al., 2009). Restoration of this tone with NAC during extinction training may restore the capacity to induce LTD in the nucleus accumbens core, and potentiates cocaine-induced reinstatement in the presence of an mGluR5 positive allosteric modulator (Moussawi et al., 2009). Taken together, extinction specific changes combined with restoration of glutamatergic tone by NAC administration during extinction could enhance protection from relapse.

In summary, the current data suggest that repeated NAC treatment results in enduring attenuation of cocaine-seeking under different withdrawal regimens of abstinence and extinction, but that the persisting anti-relapse features of NAC are enhanced in conjunction with explicit extinction training. Extinction techniques are integral to intervention approaches, such as guided imagery and cue-exposure therapy (Conklin, 2006; Lee et al., 2007). However, these techniques to date have shown only moderate clinical success (Childress et al., 1993; Marissen et al., 2007). Newer applications of cue-exposure show promise in enhancing cue-exposure therapies, including the use of computer-based virtual environments (Lee et al., 2007; Culbertson et al., 2010). Additionally, medication-facilitated cue exposure therapies have begun to emerge in several fields of psychiatry, with the most notable success seen in the area of phobia treatment (Ressler et al., 2004; Davis et al., 2006). Such approaches have also been suggested for addiction treatment (Otto et al., 2004), and NAC may offer a useful approach for restoration of glutamatergic tone during extinction-based therapy in the treatment of cocaine and other drug addictions.

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Authorship Contributions

Participated in research design: Reichel, See, Moussawi, Kalivas

Conducted experiments: Do, Reichel, Moussawi

Performed data analysis: Reichel, See

Wrote or contributed to the writing of the manuscript: Reichel, See, Kalivas

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Footnotes

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Legends for Figures

Figure 1. Experiment 1: 60 mg/kg NAC during extinction. (A) Time course for experiment 1. NAC (60 mg/kg) treatment occurred during daily extinction sessions and on cue-induced and cue+cocaine-primed reinstatement test days. Rats were retested following 2 weeks of abstinence without NAC. (B) Active lever presses during the last 3 days of self-administration. (C) Lever responding over the 12 days of extinction. (D) Reinstatement of cocaine-seeking by cues or cues+cocaine (*Reduced responding by NAC treatment relative to saline treatment; $p<0.05$). (E) Cocaine-seeking at two weeks after discontinuation of NAC treatment.

Figure 2. Experiment 2: 100 mg/kg NAC during extinction. (A) Time course for experiment 2. NAC (100 mg/kg) treatment occurred during daily extinction sessions and on cue and cue+cocaine primed reinstatement testing. Rats were retested following 2 weeks of abstinence without NAC. (B) Active lever presses during the last 3 days of self-administration. (C) Lever responding over the 12 days of extinction (main effect of group, $p<0.05$). (D) Reinstatement of cocaine-seeking by cues or cues+cocaine (*Reduced responding by NAC treatment relative to saline treatment; $p<0.05$). (E) Cocaine-seeking at two weeks after discontinuation of NAC treatment (*Reduced responding by NAC treatment relative to saline treatment; $p<0.05$).

Figure 3. Experiment 3: 60 mg/kg NAC during abstinence. (A) Time course for experiment 3. NAC (60 mg/kg) treatment occurred daily during abstinence but not on

tests or during extinction. Rats were tested for context relapse and went through subsequent extinction and reinstatement testing. (B) Active lever presses during the last 3 days of self-administration. (C) Relapse to the cocaine context after abstinence. (D) Lever responding over the 11 days of extinction. (E) Reinstatement of cocaine-seeking by cues or cocaine.

Figure 4. Experiment 4: 100 mg/kg NAC during abstinence. (A) Time course for experiment 4. NAC (100 mg/kg) treatment occurred daily during abstinence, but not on tests or during extinction. Rats were tested for context relapse and went through subsequent extinction and reinstatement testing. (B) Active lever presses during the last 3 days of self-administration. (C) Relapse to the cocaine context after abstinence (*Reduced responding by NAC treatment relative to saline treatment; $p < 0.05$). (D) Lever responding over the 11 days of extinction. (E) Reinstatement of cocaine-seeking by cues or cocaine.

Figure 5. Experiment 5: 100 mg/kg NAC during abstinence. (A) Time course for experiment 5. NAC (100 mg/kg) treatment occurred daily during abstinence and on cue and cue+cocaine primed reinstatement testing. Rats were retested following 2 weeks of abstinence without NAC. (B) Relapse of cocaine-seeking by cues or cues+cocaine (*Reduced responding by NAC treatment relative to saline treatment; $p < 0.05$). (C) Cocaine-seeking at two weeks after discontinuation of NAC treatment (*Reduced responding by NAC treatment relative to saline treatment; $p < 0.05$).

Figure 1

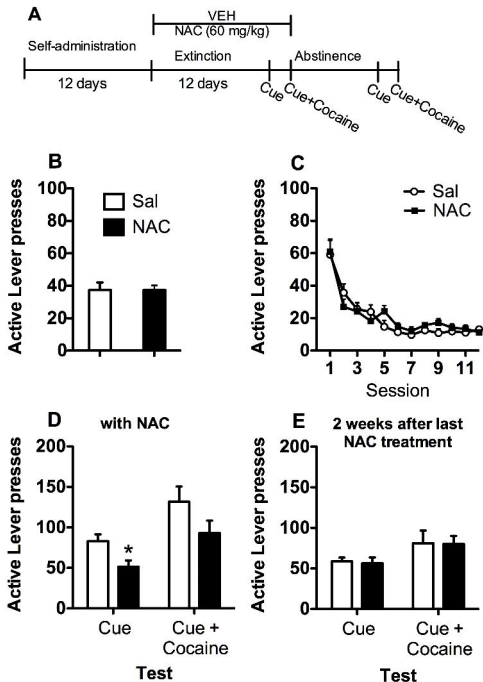
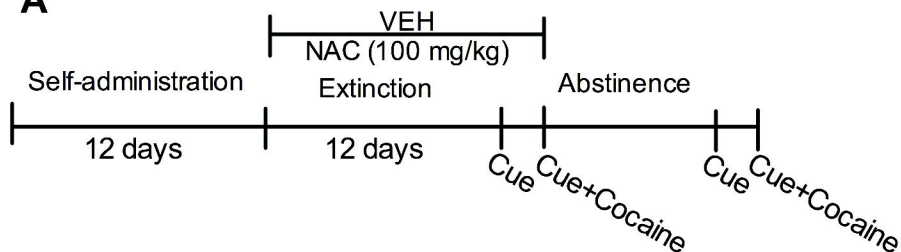
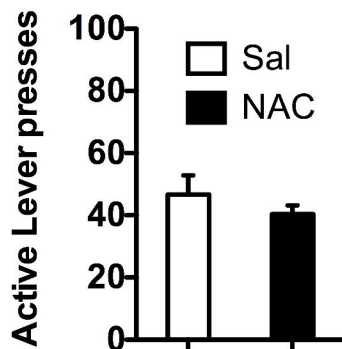


Figure 2

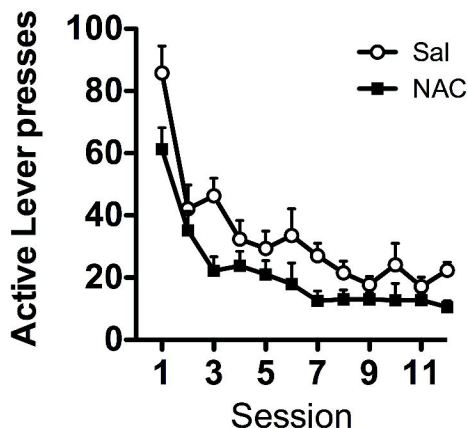
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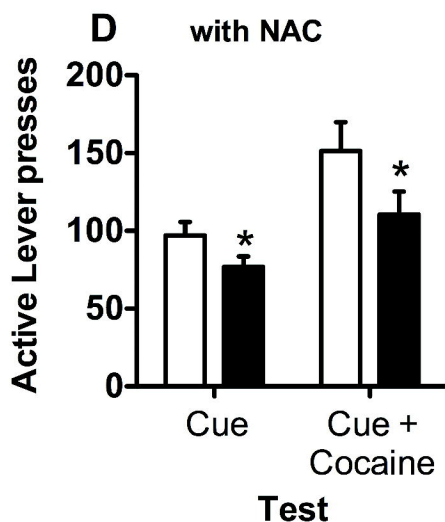
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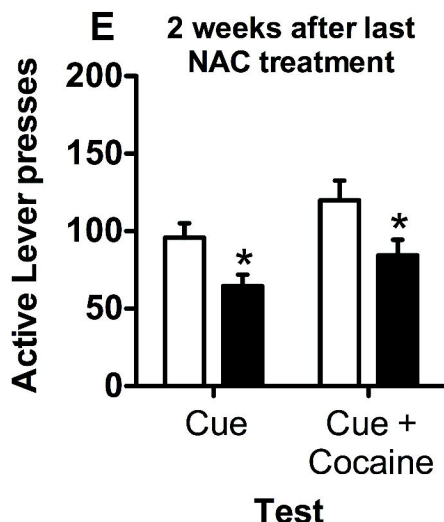


Figure 3

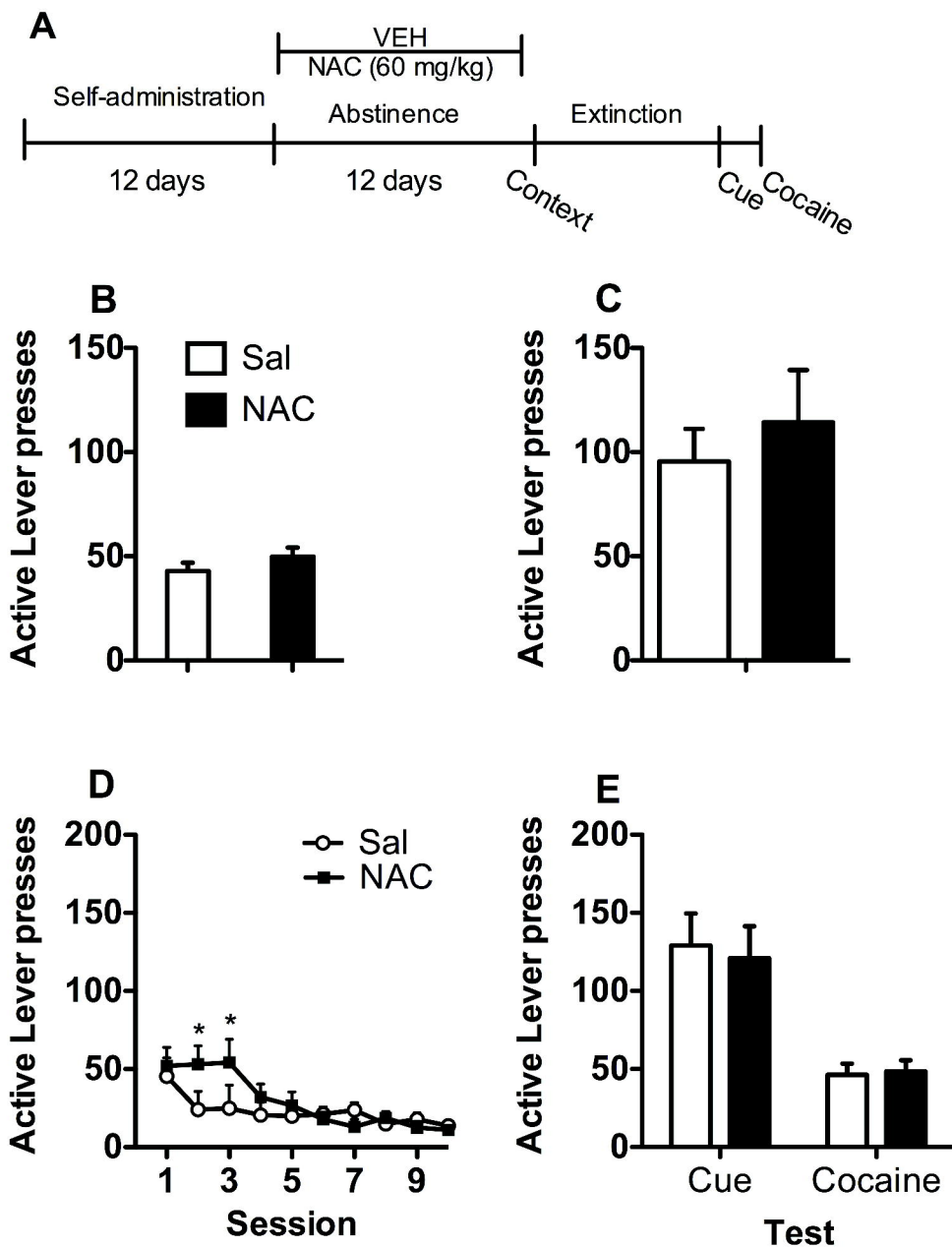


Figure 4

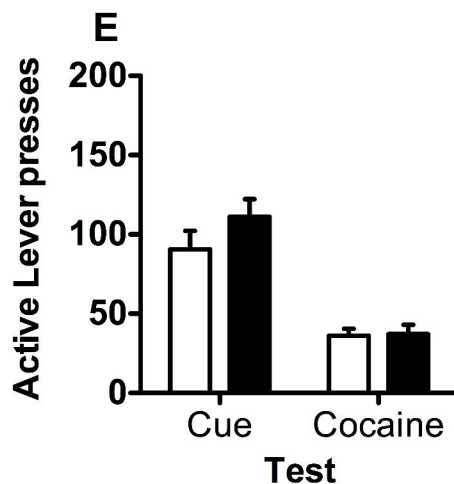
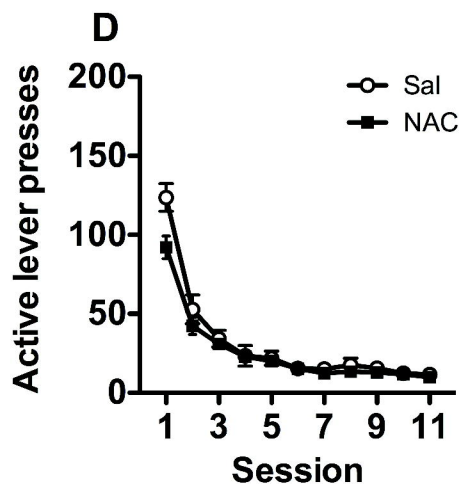
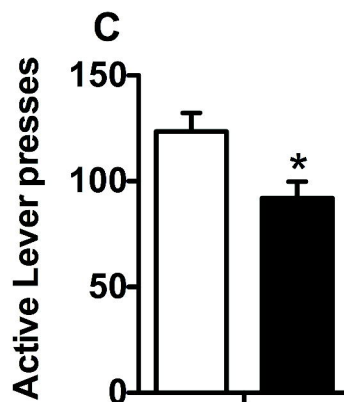
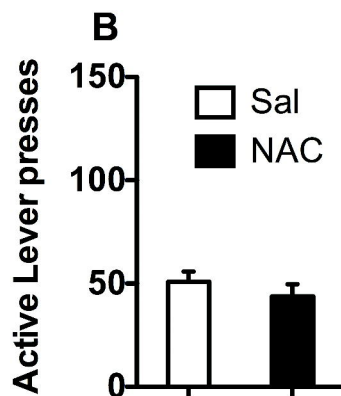
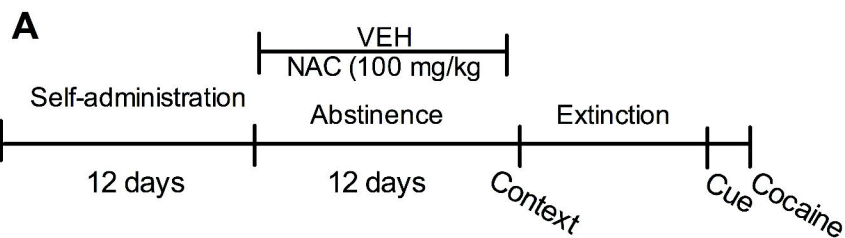


Figure 5

A

