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Blunted Cystine–Glutamate Antiporter Function in the Nucleus Accumbens Promotes Cocaine-induced Drug Seeking

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Abstract: Repeated cocaine alters glutamate neurotransmission, in part, by reducing cystine-glutamate exchange via system x_c-, which maintains glutamate levels and receptor stimulation in the extrasynaptic compartment. In the present study, we undertook two approaches to determine the significance of plasticity involving system x_c-. First, we examined whether the cysteine prodrug N-acetylcysteine attenuates cocaine-primed reinstatement by targeting system x_c-. Rats were trained to self-administer cocaine (1 mg/kg/200 µl, IV) under extended access conditions (6 hr/day). After extinction training, cocaine (10 mg/kg, IP) primed reinstatement was assessed in rats pretreated with N-acetylcysteine (0–60 mg/kg, IP) in the presence or absence of the system x_c- inhibitor (S)-4-carboxyphenylglycine (CPG; 0.5 µM; infused into the nucleus accumbens). N-acetylcysteine attenuated cocaine-primed reinstatement, and this effect was reversed by co-administration of CPG. Secondly, we examined whether reduced system x_c- activity is necessary for cocaine-primed reinstatement. To do this, we administered N-acetylcysteine (0 or 90 mg/kg, IP) prior to twelve daily self-administration sessions (1 mg/kg/200 µl, IV; 6 hr/day) since this procedure has previously been shown to prevent reduced activity of system x_c-. On the reinstatement test day, we then acutely impaired system x_c- in some of the rats by infusing CPG (0.5 µM) into the nucleus accumbens. Rats that had received N-acetylcysteine prior to daily self-administration sessions exhibited diminished cocaine-primed reinstatement; this effect was reversed by infusing the cystine-glutamate exchange inhibitor CPG into the nucleus accumbens. Collectively these data establish system x_c- in the nucleus accumbens as a key mechanism contributing to cocaine-primed reinstatement.

Keywords: extrasynaptic, nonvesicular, glutamate, microdialysis, cystine-glutamate antiporter, reinstatement

Long-term plasticity resulting in altered excitatory neurotransmission within corticostriatal pathways has been implicated in addiction. Human cocaine abusers exposed to craving-inducing stimuli exhibit increased activation of excitatory circuits originating in cortical regions, including orbital or prefrontal cortex, and projecting to the ventral striatum (Breiter et al., 1997; Dackis and O'Brien, 2005; Volkow et al., 2005). Preclinical data indicate that an injection of cocaine increases Fos protein expression throughout the corticostriatal pathway in rats engaged in cocaine seeking (Neisewander et al., 2000) and that transient inactivation of the prefrontal cortex or nucleus accumbens core blocks cocaine-primed reinstatement in rats (McFarland and Kalivas, 2001). Inhibition of excitatory neurotransmission in the nucleus accumbens by preventing activation

of corticostriatal pathways, stimulating group II metabotropic glutamate autoreceptors, or blocking AMPA receptors also blocks cocaine-primed reinstatement in rats (Cornish and Kalivas, 2000; Park et al., 2002; Baker et al., 2003; McFarland et al., 2003; Schmidt et al., 2005; Peters and Kalivas, 2006). These data indicate that regulation of synaptic glutamate represents a novel approach in the treatment of cocaine addiction (Volkow and Fowler, 2000; Dackis, 2004).

System x_c- may be a key mechanism underlying cocaine-induced changes in glutamate signaling within corticostriatal pathways that contribute to pathological cocaine seeking. First, cystine-glutamate exchange via system x_c- supplies nonvesicular glutamate in the extrasynaptic compartment that stimulates extrasynaptic group II metabotropic glutamate receptors (mGluR) in the nucleus accumbens and prefrontal cortex (Baker et al., 2002; Xi et al., 2002a; Moran et al., 2005). This is important because stimulation of group II mGluRs inhibits synaptic release of glutamate (Baskys and Malenka, 1991; Cochilla and Alford, 1998; Schoepp, 2001; Moran et al., 2005). Repeated cocaine blunts cystine-glutamate exchange (Baker et al., 2003; Madayag et al., 2007) which results in reduced basal glutamate levels in the nucleus accumbens (Pierce et al., 1996; Reid and Berger, 1996; Baker et al., 2003), and reduced autoregulation of synaptic glutamate by group II mGluRs (Xi et al., 2002b). Likely as a result, a cocaine challenge increases synaptic release of glutamate in cocaine-withdrawn rats (Pierce et al., 1996; Reid and Berger, 1996; Baker et al., 2003) that occurs as a result of activation of corticostriatal pathways (McFarland et al., 2003).

Preclinical studies have shown N-acetylcysteine to be effective in blocking compulsive drug-seeking in rodents (Baker et al., 2003; Madayag et al., 2007; Zhou and Kalivas, 2007) and data from open-label trials have shown reduced cocaine use and craving in human cocaine abusers (Larowe et al., 2006; Mardikian et al., 2007). Although the effects of N-acetylcysteine were attributed to increased cystine-glutamate exchange by system x_c-, data indicate that N-acetylcysteine may alter glutamate signaling through cellular mechanisms distinct from system x_c-. Specifically, N-acetylcysteine or cysteine resulting from deacetylation of N-acetylcysteine has been shown to influence the activity of sodium-dependent glutamate

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transporters and glutamate receptors including NMDA and AMPA receptors (Janaky et al., 2000; Aoyama et al., 2006; Chase et al., 2007). As a result, the mechanism underlying the effects of N-acetylcysteine needs to be identified.

The present study examines the therapeutic potential of targeting system x_c- to reduce drug-seeking behavior. First, we examined whether the cysteine prodrug N-acetylcysteine attenuates cocaine-primed reinstatement by targeting system x_c-. To do this, we examined the capacity of N-acetylcysteine to block cocaine-primed reinstatement in the presence or absence of the system x_c- inhibitor (S)-4-carboxyphenylglycine (Ye et al., 1999; Patel et al., 2004) (CPG; 0.5 μM; infused into the nucleus accumbens). N-acetylcysteine attenuated cocaine-primed reinstatement and this effect was reversed by co-administration of CPG. Secondly, we examined whether reduced system x_c- activity is necessary for cocaine-primed reinstatement. To do this, we administered N-acetylcysteine (0 or 90 mg/kg, IP) prior to twelve daily self-administration sessions (1 mg/kg, IV; 6 hr/day). This procedure has previously been shown to prevent reduced activity of system x_c- and cocaine-primed reinstatement (Madayag et al., 2007).

Experimental Procedures

Subjects

These experiments utilized male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 275–325 grams upon arrival. Rats were individually housed in a temperature-controlled colony room with a 12-h reversed light/dark cycle. Housing conditions and experimental protocols were approved by the Marquette University Institutional Animal Care and Use Committee and carried out according to the NIH Guide for the Care and Use of Laboratory Animals (revised 1996).

Surgeries

Rats included in self-administration studies were implanted with indwelling catheters under ketamine HCl (100 mg/kg, IP, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (2 mg/kg, IP, Lloyd Laboratories, Shenandoah, IA, USA) anesthesia. A silicon tubing

catheter (Dow Corning Co., Midland, MI; 0.64 mm ID; 1.19 mm OD) was implanted such that it entered the jugular vein through the right posterior facial vein and terminated at the right atrium. The catheter was sutured to the vein at the entry point. The distal aspect of the catheter, which consisted of a 22-gauge guide cannula (Plastics One Inc., Roanoke, VA) attached with dental acrylic to a piece of polypropylene monofilament surgical mesh (Atrium Medical, Co., Hudson, NH), exited 2 cm posterior to the scapulae. Throughout the experiment, catheters were filled daily with a heparin solution (83 i.u./ml; Elkins-Sinn, Inc., Cherry Hill, NJ) and capped when disconnected from the leash/delivery line assembly.

Rats included in microdialysis studies were also implanted with indwelling bilateral guide cannulae (20 gauge, 14 mm; Plastics One, Roanoke VA) using the following coordinates derived from Paxinos and Watson (1986): + 0.9 mm anterior, ± 2.5 mm mediolateral to Bregma, and −4.4 mm from the surface of the skull at a 6° angle from vertical. The placement of the active region of the microdialysis probe, which began 2 mm beyond the ventral tip of the guide cannulae, was primarily in the nucleus accumbens core. Following surgery, rats were given at least five days to recover prior to testing. During this time, rats were provided acetaminophen (480 mg/L) in their drinking water and injected daily with a sterile cefazolin antibiotic solution (15 mg, IV; West-Ward Pharmaceutical Co., Eatontown, NJ).

Cocaine Self-Administration Training

Self-administration occurred in operant chambers (ENV-008CT, MED-Associates Inc., St Albans, VT, USA) housed in sound attenuating cubicles (ENV-016M, MED-Associates Inc.) and equipped with two retractable levers, two stimulus lights, and a water bottle. At least five days after surgery, rats were food restricted for 18 hr with water available ad libitum. Rats were then placed into the operant chambers overnight and responses on the lever designated as active resulted in the delivery of food pellets under a fixed ratio 1 schedule of reinforcement. Daily food training continued until subjects received at least 150 food rewards in a session, which typically occurred following the first session. Rats then underwent drug self-administration training during daily 2-hr sessions in which operant responses on the active

lever were reinforced with an infusion of cocaine (0.5 mg/kg/200 µl IV, National Institute on Drug Abuse, Bethesda, MD, USA) under a fixed ratio 1 schedule of reinforcement. Each reinforced lever response resulted in the illumination of the stimulus light located above the active lever and was followed by a 25-s time-out period. Responding on a second, inactive lever located on the back wall was recorded but had no programmed consequences. Acquisition of cocaine self-administration was operationally defined as < 10% variation in daily responding over at least three consecutive sessions. Once rats met the acquisition criteria, they were advanced to maintenance sessions in which saline or cocaine was self-administered under extended-access conditions (1.0 mg/kg/200 µl IV; 6-hr/day for 12 days).

Extinction training

After completing twelve maintenance self-administration sessions, rats remained in their home cages for seven days prior to extinction training. A seven day delay was used to ensure an adequate abstinent period prior to reinstatement, even in rats that quickly extinguished responding. Extinction training involved placing rats into the operant chambers for 2-hr/day as described above in the self-administration section except each active lever press now resulted in an infusion of saline. This continued until the mean number of lever presses was ≤ 10 responses across at least three sessions, at which point rats were tested for drug-primed reinstatement. Because the average number of extinction sessions needed to meet criteria (± SEM) was 15.4 ± 4.3, reinstatement testing occurred on average 22 days after the last self-administration session.

Histology

Rats included in the microdialysis studies were given an overdose of pentobarbital (60 mg/kg, IP), and the brains fixed by intracardiac infusion of 0.9% saline followed by 2.5% formalin solution. Brains were then removed and stored in 2.5 % formalin for at least seven days prior to sectioning. The tissue was then blocked and coronal sections (100 µM) were cut and stained with cresyl violet to verify probe placements.

Statistical analyses

The SPSS statistics package (version 16) was used to perform the statistical analyses. Data was analyzed using analysis of variance (ANOVA) with drug treatment (e.g., dose of N-acetylcysteine, cocaine history) as between subject factors and self-administration session or phase of experiment (extinction, reinstatement) as repeated factors. Tukeys HSD test were used to analyze significant ($p \leq .05$) interactions or main effects. Student t-tests were used in instances where main effects or simple main effects involving only two groups were further analyzed as a result of a significant interaction.

Experiment 1

N-acetylcysteine has been shown to block cocaine-primed reinstatement in rats that had self-administered under short access conditions (Baker et al., 2003). In the present study, we examined whether N-acetylcysteine targets cystine-glutamate exchange by system x_c-to produce this effect. Rats were trained to self-administer cocaine as described above under extended access conditions. Following extinction training, rats were tested for cocaine-primed reinstatement. On the night before the reinstatement test, microdialysis probes, constructed as previously described (Baker et al., 2003), were inserted into indwelling guide cannula. Rats were then housed overnight in the self-administration chambers. The next day, dialysis buffer (5 mM glucose, 140 mM NaCl, 1.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.15% phosphate buffer saline, pH 7.4) was pumped through the probes at a rate of 1 µl/min for at least 3 hr. After this, vehicle or N-acetylcysteine (30–60 mg/kg, IP) was administered at the same time that vehicle or (S)-4 carboxyphenylglycine (CPG; 0.5 µM) was added to the dialysis buffer. Sixty minutes later, rats received a systemic injection of cocaine (10 mg/kg, IP). The levers were then extended into the chambers and responding was monitored for 120 min.

Experiment 2

Repeated cocaine produces a reduction in cystine-glutamate exchange from system x_c-. In the present study, we explored the

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contribution of diminished system x_c- activity to cocaine-induced reinstatement of drug seeking. Rats were trained to self-administer cocaine as described above with the exception that rats received saline or N-acetylcysteine (90 mg/kg, IP) injections thirty minutes prior to each session of cocaine self-administration during both the acquisition and maintenance phases. Note, administration of N-acetylcysteine prior to daily cocaine self-administration has been shown to prevent reduced cystine-glutamate exchange by system x_c- (Madayag et al., 2007). Once self-administration was complete, rats underwent a seven day forced abstinence period, which was followed by extinction training as described above. On the night before the reinstatement test, microdialysis probes, constructed as previously described (Baker et al., 2003), were inserted into indwelling guide cannula. Rats were then housed overnight in the self-administration chambers. The next day, dialysis buffer (5 mM glucose, 140 mM NaCl, 1.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.15% phosphate buffer saline, pH 7.4) was pumped through the probes at a rate of 1 µl/min for at least 3 hr. After this, vehicle or CPG (0.5 µM) was added to the dialysis buffer for the duration of the experiment. Sixty minutes later, rats received a systemic injection of cocaine (10 mg/kg, IP). The levers were then extended into the chambers and responding was monitored for 120 min.

Results

N-acetylcysteine Blocks Cocaine-Primed Reinstatement by Targeting System x_c-

In the present study, we tested the hypothesis that N-acetylcysteine blocks cocaine-induced reinstatement of drug seeking by targeting system x_c-. Figure 1 illustrates the impact of acute administration of N-acetylcysteine in the presence or absence of the cystine-glutamate exchange inhibitor CPG on cocaine-primed reinstatement of drug seeking. A comparison of lever pressing on the last day of extinction or the reinstatement test day produced an interaction between day and drug treatment (ANOVA: $F_{4,35}=18.949$, $p<.001$). Post hoc analyses indicated that administration of the high dose of N-acetylcysteine in the absence of cocaine failed to reinstate cocaine seeking since there was no increase in lever pressing on the

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reinstatement test relative to behavior expressed on the last day of extinction (Students paired T-test, $p<.05$). In contrast, a cocaine prime reinstated cocaine seeking in all groups, although the magnitude of reinstatement was significantly lower in rats treated with either dose of N-acetylcysteine when administered in the absence of CPG infusions into the nucleus accumbens (Tukey HSD, $p<.05$). Interestingly, infusion of CPG into the nucleus accumbens reversed the effects of acute N-acetylcysteine administration such that these rats exhibited significantly more lever presses relative to rats pretreated with N-acetylcysteine in the absence of CPG (Tukey HSD, $p<.05$).

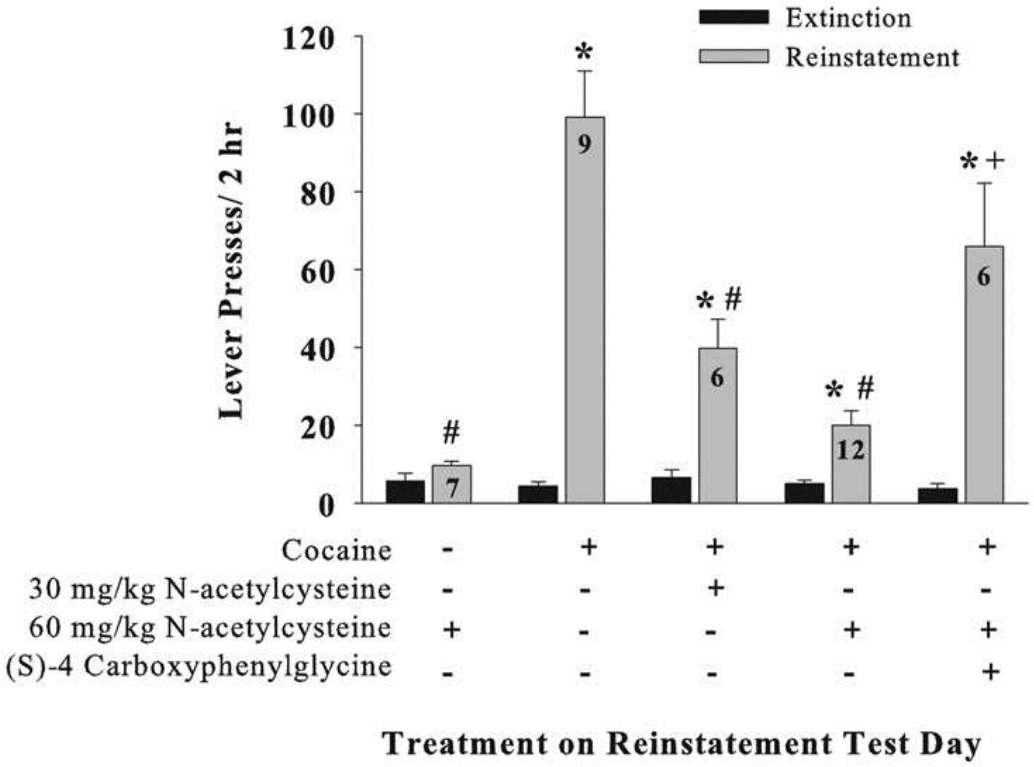

Figure 1 N-acetylcysteine blocks cocaine-primed reinstatement by targeting system xc-. The data depict the mean (+ SEM) number of lever presses on the last extinction session or on the reinstatement test day. Drug assignments indicate treatment on only the reinstatement test day (N=6–12/group as indicated in the bar graphs). N-acetylcysteine (0–60 mg/kg, IP) was injected at the time that (S)-4 carboxyphenylglycine (CPG; 0–0.5 μM) was added to the dialysis buffer; rats then received a cocaine injection (10 mg/kg, IP) sixty min later. The operant levers were then extended into the chamber and responding was monitored for 120 min. * indicates a significant difference from extinction responding (t-test, $p<.05$); # indicates a significant difference from rats treated with cocaine only (Tukey, $p<.05$); +

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indicates a significant increase relative to rats treated with cocaine + N-acetylcysteine (60 mg/kg; Tukey, $p<.05$).

Decreased cystine-glutamate exchange contributes to cocaine-primed reinstatement

The present study tested the hypothesis that reduced cystine-glutamate exchange by system x_c- is necessary for cocaine-primed reinstatement. To do this, N-acetylcysteine was administered prior to each daily session of cocaine self-administration, which has previously been shown to prevent cocaine-induced plasticity involving system x_c- (Madayag et al., 2007). In figure 2, we present the impact of daily N-acetylcysteine on cocaine intake during twelve maintenance sessions of cocaine self-administration under extended-access conditions. A comparison of the number of cocaine infusions with drug treatment as a between subjects variable and time or daily session as a repeated measure failed to produce an interaction between the terms (ANOVA: $F_{11,264}=0.683$, $p=.754$), but produced a significant main effect of time (ANOVA: $F_{11,264}=8.54$, $p<.001$) and a strong trend toward a main effect of drug treatment (ANOVA: $F_{1,24}=4.16$, $p=.053$).

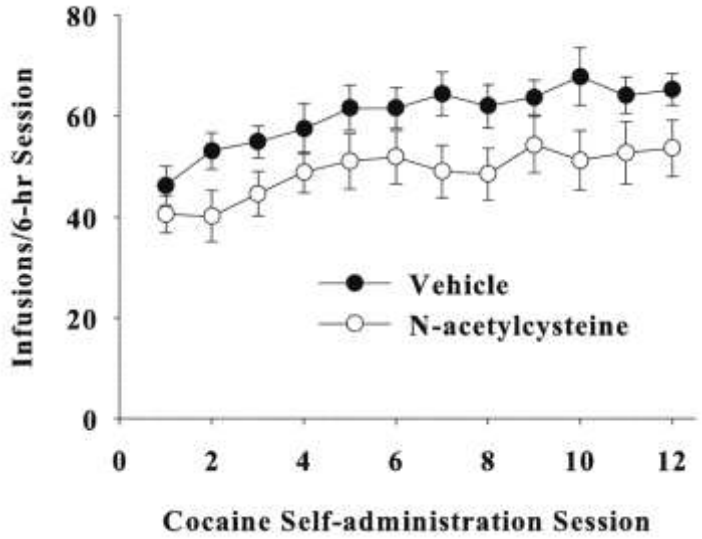

Figure 2 N-acetylcysteine reduces cocaine intake across daily sessions of cocaine self-administration. Data depict the mean (± SEM) number of cocaine infusions (1.0 mg/kg/200 µl, IV) obtained during twelve daily maintenance self-administration sessions (6-hr/day). Thirty min prior to each daily session, rats received either vehicle (1 ml/kg; N=14) or N-acetylcysteine (90 mg/kg, IP; N=12).

Figure 3 illustrates the impact of N-acetylcysteine administered prior to daily sessions of cocaine self-administration on extinction responding following a seven day forced abstinence period. A comparison of the number of the number of lever presses with pretreatment history as a between subjects variable and day of the experiment as a repeated measure failed to produce an interaction between the terms (ANOVA: $F_{4,96}$=0.364, p=.833), but produced a significant main effect of time (ANOVA: $F_{4,96}$=11.95, p<.001) and pretreatment history (ANOVA: $F_{1,24}$=6.165, p=.02). Note, the above analyses were conducted on just the first five extinction sessions since some rats met the acquisition criteria within five sessions.

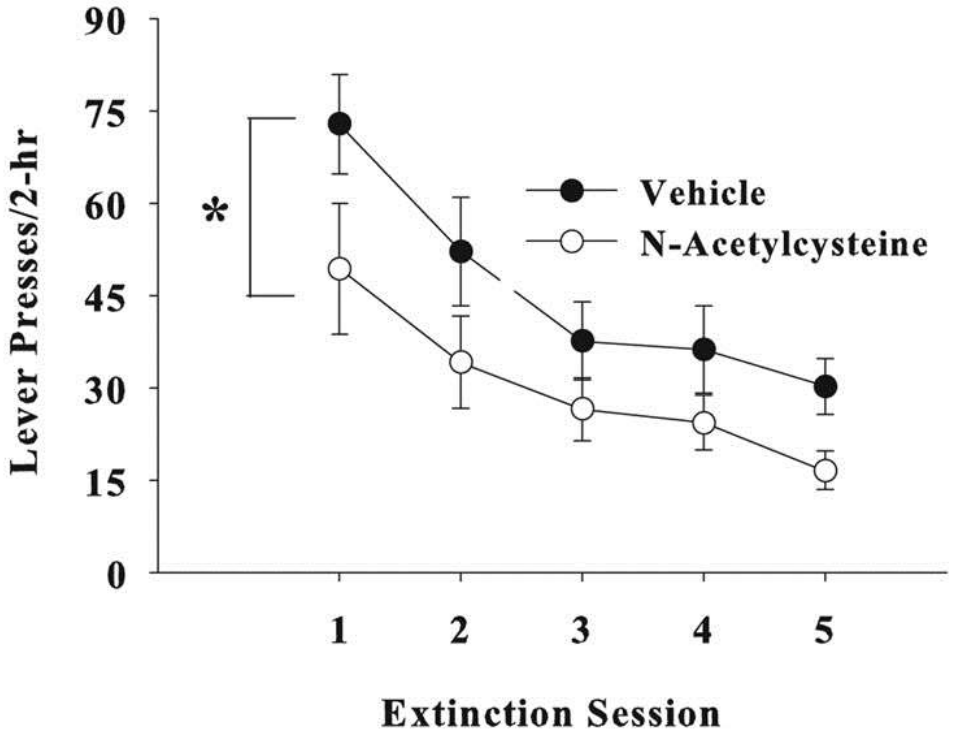

Figure 3 N-acetylcysteine pretreatment during self-administration training results in lower levels of extinction responding. Following self-administration training, rats remained in the home cage for 7 days, and then underwent extinction training in which lever presses resulted in a saline infusion. Data depict the mean (± SEM) number of lever presses during the first five extinction sessions (2 hr/day). The first five sessions are depicted because some rats met the extinction criteria prior to the sixth session. Drug assignments indicate treatment during self-administration training (N=12–14/group). * indicates a main effect of drug treatment (ANOVA, p<.05).

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Figure 4 illustrates the impact of the cystine-glutamate exchange inhibitor CPG infused into the nucleus accumbens on cocaine-primed reinstatement by rats that had received N-acetylcysteine prior to daily sessions of cocaine self-administration. A comparison of lever pressing on the last day of extinction or the reinstatement test day produced an interaction between day and drug treatment history (ANOVA: $F_{2,22}$=5.45, p=.012). Analysis of the simple main effect comparing response rates on the reinstatement test session only yielded a significant effect of drug treatment history (ANOVA: $F_{2,22}$=5.40, p=.012). Post hoc analyses indicated that rats that had received N-acetylcysteine prior to daily sessions of cocaine self-administration, a procedure previously shown to prevent reduced cystine-glutamate exchange (Madayag et al., 2007), exhibited blunted cocaine-primed reinstatement (Tukey HSD, p<.05), even though testing occurred at least 12 days after the last administration of N-acetylcysteine (figure 4). Further, acute disruption of cystine-glutamate exchange following intra-accumbens infusion of CPG in rats that had received N-acetylcysteine pretreatments during cocaine self-administration training restored cocaine-primed reinstatement to levels observed in controls that had no history of N-acetylcysteine pretreatments (Tukey HSD, p<.05). Although the nucleus accumbens was the region primarily infused with CPG, the microdialysis probe for some subjects also extended into the striatum dorsal to the nucleus accumbens or the olfactory tubercles (figure 5).

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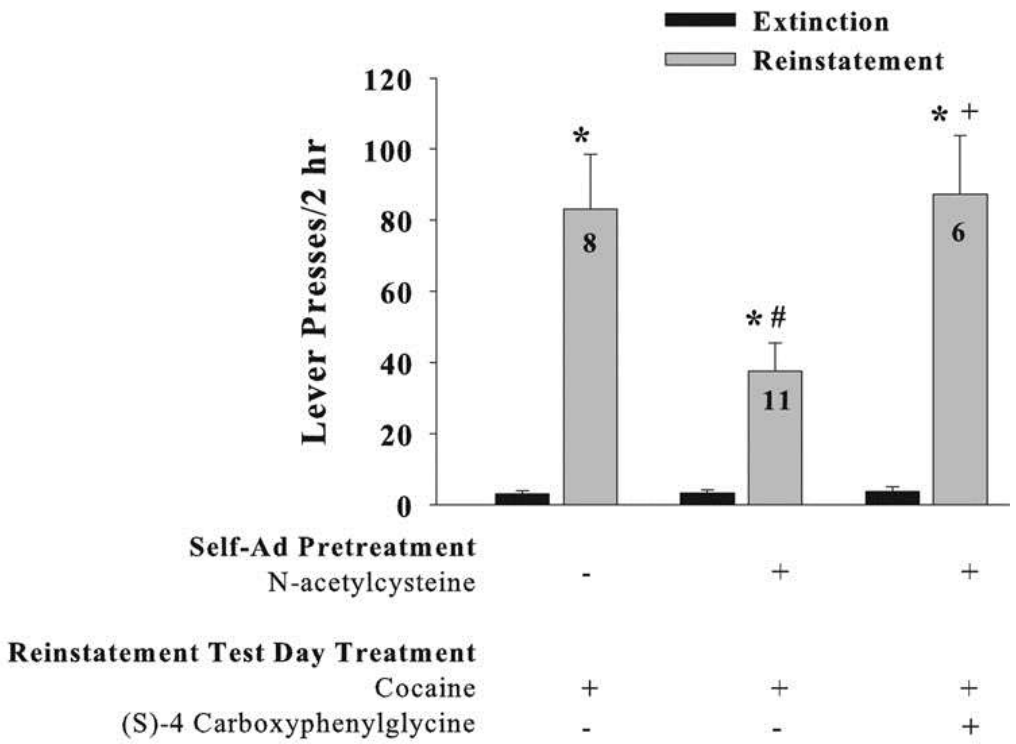

Figure 4 Acute disruption of cystine-glutamate exchange reverses the impact of daily N-acetylcysteine pretreatments on cocaine-primed reinstatement. Data depict the mean number of lever presses (± SEM) on the last extinction session or the reinstatement test day. Rats had a history of vehicle or N-acetylcysteine pretreatments prior to each daily session of cocaine self-administration. On the reinstatement test day, rats received intra-accumbens infusions of vehicle or CPG sixty min prior to an injection of cocaine (10 mg/kg, IP). The operant levers were then extended into the chamber and responding was monitored for 120 min.

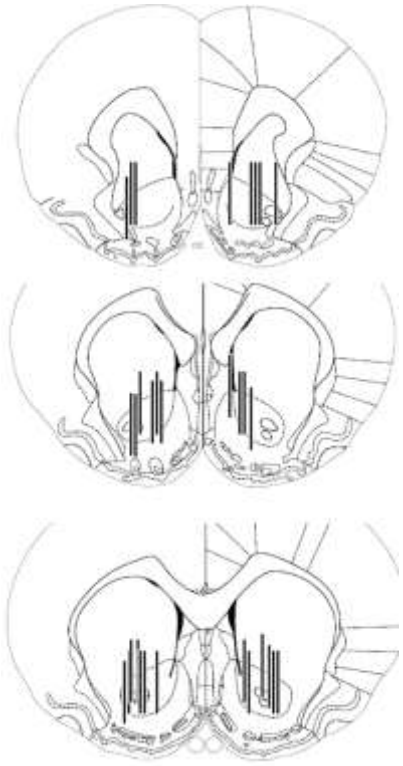

Figure 5 A schematic illustrating the placement of the 2 mm active membrane portion of the microdialysis probe for the rats included in the microdialysis study. The active regions of the microdialysis probes were primarily located in the nucleus accumbens core, although aspects of nucleus accumbens shell, the striatum dorsal to the nucleus accumbens, and the olfactory tubercles were likely sampled as well.

Discussion

Plasticity resulting in abnormal activation of corticostrial pathways appears to contribute to craving in human drug abusers (Breiter et al., 1997; Dackis and O'Brien, 2005; Volkow et al., 2005). Previous reports have provided indirect evidence that reduced nonvesicular glutamate release by system x_c^- may contribute to cocaine-primed reinstatement (Madayag et al., 2007), and that it represents a novel target in the treatment of cocaine addiction (Baker et al., 2003; Larowe et al., 2006; Mardikian et al., 2007). In the present report, we undertook two approaches to directly examine the contribution of system x_c^- to cocaine-induced reinstatement. First, we demonstrate that the cysteine prodrug N-acetylcysteine attenuates cocaine-primed reinstatement by targeting system x_c^-. Second, we found that reduced system x_c^- activity is necessary for robust cocaine-

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primed reinstatement. Collectively, these data establish system x_c- in the nucleus accumbens as a key mechanism contributing to cocaine-primed reinstatement and further establish it as a novel target for the treatment of cocaine addiction.

N-acetylcysteine Blunts Cocaine-Induced Reinstatement by Targeting System x_c-

Acute administration of N-acetylcysteine blocks cocaine-induced reinstatement (Baker et al., 2003), and this was thought to be due to increased cystine-glutamate exchange. However, recent data indicate that N-acetylcysteine may block reinstatement by targeting sites other than cystine-glutamate exchange. Specifically, N-acetylcysteine or cysteine resulting from deacetylation of N-acetylcysteine has been shown to regulate the activity of sodium-dependent glutamate transporters, NMDA receptors, and AMPA receptors (Janaky et al., 2000; Aoyama et al., 2006; Chase et al., 2007). In the present study, we replicate finding that acute administration of N-acetylcysteine attenuates cocaine-primed reinstatement when administered prior to a cocaine injection. Moreover, we found that this effect is blocked by infusion of the cystine-glutamate exchange inhibitor CPG into the nucleus accumbens. Because CPG also acts at metabotropic glutamate receptors, we selected a concentration (500 nM) that is significantly lower than the EC50s of CPG to block group I mGluRs (40–65 μM) or stimulate group II mGluRs (570–970 μM) (Cavanni et al., 1994; Thomsen et al., 1994; Sekiyama et al., 1996). As a result, the present findings indicate that N-acetylcysteine blocks cocaine-induced reinstatement of drug seeking by targeting cystine-glutamate exchange by system x_c- in the nucleus accumbens.

It is noteworthy that blockade of reinstatement by N-acetylcysteine in the present study occurred in rats trained to self-administer cocaine under extended-access conditions, which has been suggested to represent a more stringent test of the therapeutic potential (Ahmed and Koob, 1998; Mantsch et al., 2004; Kippin et al., 2006). Further, these data are consistent with the findings of recent open-label trials demonstrating reduced cocaine craving and use in human cocaine abusers receiving N-acetylcysteine (Larowe et al., 2006; Mardikian et al., 2007). Although clinical trials are needed to

more stringently determine the anticraving efficacy of N-acetylcysteine, these data establish, to at least some degree, predictive validity for the reinstatement paradigm as a screen for novel pharmacotherapies for addiction.

Administration of N-acetylcysteine prior to daily sessions of cocaine self-administration under extended access conditions has previously been shown to prevent escalation of drug intake (Madayag et al., 2007). In the present report, we obtained a significant increase in drug intake regardless of N-acetylcysteine pretreatment. Differences between the two studies include dose of N-acetylcysteine, number of self-administration sessions, and subjects per group. The latter may be particularly important because the present study obtained a strong, but nonsignificant trend towards N-acetylcysteine-induced blockade of escalation ($p=.053$). Further, the magnitude of escalation observed in the control subjects of the present study was larger than we previously obtained. Collectively, additional data may be needed to determine the role of cystine-glutamate exchange in the emergence of escalation of drug intake.

It may seem counterintuitive that increased glutamate release from system x_c^- reduces cocaine-induced behaviors in rodents and drug-craving and use in humans that are thought to be dependent, at least in part, on glutamate signaling (Cornish and Kalivas, 2000; Baker et al., 2003; Di Ciano and Everitt, 2003; McFarland et al., 2003; Larowe et al., 2006). However, this may reflect the existence of multiple, functionally-distinct pools of glutamate. Cocaine increases glutamate in the synaptic cleft following corticostriatal activation thereby generating behaviors dependent upon postsynaptic receptor stimulation (Cornish and Kalivas, 2000; McFarland et al., 2003; Di Ciano and Everitt, 2004; Suto et al., 2004). System x_c^- releases glutamate into the extrasynaptic compartment resulting in the stimulation of group II mGluRs (Baker et al., 2002; Baker et al., 2003; Moran et al., 2005). By stimulating extrasynaptic group II mGluRs without exerting postsynaptic effects, extrasynaptic glutamate appears to inhibit synaptic release (Baker et al., 2002; Moran et al., 2005). Sodium-dependent glutamate transporters may partition the two pools by limiting glutamate overflow from the synapse into the extrasynaptic compartment (Danbolt, 2001), and restricting entry of nonvesicular glutamate into the synapse (Jabaudon et al., 1999). To the extent that

NR2B receptors are located outside the synapse, this hypothesis is supported by the recent observation that glutamate release from astrocytes appears to stimulate extrasynaptic, but not synaptic NMDA receptors (D'Ascenzo et al., 2007).

Targeting system x_c- to prevent plasticity involving extracellular glutamate

Cocaine-induced seeking in rodents and use in humans arises, at least in part, as a result of corticostriatal circuits that are rendered overactive as a result of drug-induced plasticity (Breiter et al., 1997; Volkow et al., 1999; Park et al., 2002; McFarland et al., 2003; Schmidt et al., 2005; Volkow et al., 2005). Reduced cystine-glutamate exchange may contribute to this process since it provides endogenous tone to group II mGluRs that function to inhibit synaptic release (Baskys and Malenka, 1991; Cochilla and Alford, 1998; Schoepp, 2001; Moran et al., 2005). Further, repeated cocaine produces a persistent decrease in cystine-glutamate exchange by system x_c- in the nucleus accumbens (Baker et al., 2003; Madayag et al., 2007), and these changes may be necessary for cocaine-induced behavioral plasticity used to model aspects of addiction. In support, we have previously shown that the cysteine prodrug N-acetylcysteine administered prior to daily sessions of cocaine self-administration or experimenter-delivered cocaine prevents plasticity involving system x_c-, escalation of drug intake, behavioral sensitization, and cocaine-primed reinstatement (Madayag et al., 2007). Further, in the present study, rats pretreated with N-acetylcysteine prior to daily cocaine self-administration show a strong trend toward blunted cocaine intake, reduced extinction responding, and lower levels of cocaine seeking following a cocaine prime. These findings are similar to an earlier report that administration of daily N-acetylcysteine after heroin self-administration but before extinction training resulted in blunted heroin seeking during extinction and reinstatement testing (Zhou and Kalivas, 2007). Interestingly, acute disruption of system x_c- following infusion of the cystine-glutamate exchange inhibitor CPG into the nucleus accumbens during the test for cocaine-primed reinstatement restored cocaine seeking to the level observed in cocaine control rats that had not received N-acetylcysteine prior to daily sessions of cocaine self-administration. Collectively these data support the hypothesis that

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reduced system x_c- activity promotes the capacity of a cocaine prime to elicit cocaine seeking.

Additional work is needed to understand how N-acetylcysteine is preventing plasticity involving system x_c-. First, N-acetylcysteine may maintain normal levels of cystine-glutamate exchange, which then prevents cocaine-induced synaptic glutamate release by providing normal tone on group II mGluRs. Thus, CPG may have restored reinstatement by transiently lowering basal glutamate levels and reducing tone on group II mGluRs resulting in an enhanced glutamate response following a cocaine challenge. Alternatively, repeated N-acetylcysteine may have decreased the magnitude of cocaine reinstatement by reducing cocaine intake. In support, earlier studies have revealed a correlation between cocaine intake and magnitude of cocaine-primed reinstatement (Sutton et al., 2000; Baker et al., 2001). However, N-acetylcysteine pretreatment prior to daily sessions of cocaine self administration under short-access conditions in our earlier report did not result in lower cocaine intake, but still resulted in blunted cocaine-induced reinstatement (Madayag et al., 2007). Likewise, this approach also reduced behavioral sensitization in the absence of altered cocaine intake (Madayag et al., 2007). Interestingly, repeated N-acetylcysteine administered after heroin self-administration resulted in reduced extinction responding (Zhou and Kalivas, 2007). Lastly, the effects of daily N-acetylcysteine pretreatment were reversed by producing an acute disruption of cystine-glutamate exchange in the present study.

Conclusion

These data reveal that acute administration of N-acetylcysteine targets system x_c- to block cocaine primed reinstatement in rats withdrawn from extended-access cocaine self-administration. Further, reduced cystine-glutamate exchange appears to be necessary for cocaine primed reinstatement. Collectively, these data provide further support for cysteine prodrugs, including N-acetylcysteine, as potential treatments for cocaine addiction by demonstrating efficacy after acute and repeated administration. Further, it establishes system x_c- as a novel mechanism that likely contributes to plasticity rendering corticostriatal pathways overactive. This is of particular interest

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because it is consistent with the concept that a mechanism releasing nonvesicular glutamate from glial cells into an extrasynaptic compartment contributes to pathological glutamate signaling.

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Abbreviations Used

CPG	(S)-4-carpboxyphenylglycine
system x_c^-	cystine-glutamate antiporter
mGluR	metabotropic glutamate receptor
IP	intraperitoneal
IV	intravenous

Footnotes

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