# Ceftriaxone Restores Glutamate Homeostasis and Prevents Relapse to Cocaine Seeking

Lori A. Knackstedt, Roberto I. Melendez, and Peter W. Kalivas

**Background:** The cystine-glutamate exchanger is downregulated after chronic cocaine, resulting in reduced extracellular levels of nucleus accumbens glutamate. The importance of cocaine-induced loss of glutamate homeostasis is revealed by N-acetylcysteine restoring cystine-glutamate exchange and attenuating reinstatement to cocaine seeking. Another regulator of extracellular glutamate is the glial glutamate transporter GLT-1. We hypothesized that cocaine self-administration reduces GLT-1 and that GLT-1 upregulation inhibits cocaine seeking.

**Methods:** We measured [$^3$H] glutamate uptake and protein expression of GLT-1 and xCT, the catalytic subunit of the cystine-glutamate exchanger, following cocaine self-administration and 3 weeks of extinction training. We also examined the affect of ceftriaxone (previously shown to increase GLT-1) and N-acetylcysteine treatment on the expression of GLT-1 and xCT. Ceftriaxone was also tested for the capacity to inhibit cue- and cocaine-induced relapse.

**Results:** Cocaine self-administration reduced glutamate uptake and the expression of both GLT-1 and xCT. Ceftriaxone restored GLT-1 and xCT levels and prevented cue- and cocaine-induced reinstatement of drug seeking. N-acetylcysteine also restored GLT-1 and xCT levels.

**Conclusions:** These results indicate that glutamate transport and cystine-glutamate exchange may be coregulated and provide further evidence that targeting glutamate homeostasis is a potential method for treating cocaine relapse.

**Key Words:** Cocaine, accumbens, cystine-glutamate exchange, glutamate uptake, GLT-1, self-administration

Relapse to cocaine seeking in the animal model of reinstatement is strongly associated with changes in the extracellular levels of glutamate in the nucleus accumbens (NAcc) core, namely decreased basal levels of glutamate in the NAcc core as well as enhanced extracellular glutamate levels in response to a cocaine challenge (1). Glutamate is released into the extracellular space from synaptic and nonsynaptic sources and is eliminated through a family of glutamate uptake transporters (2). The balance between synaptic and nonsynaptic glutamate release and elimination is termed "glutamate homeostasis," which modulates synaptic activity and plasticity by controlling the stimulation of iontotropic and metabotropic glutamate receptors (3,4). The cystine-glutamate exchanger, which exchanges one extracellular cystine molecule for one intracellular glutamate molecule (5), accounts for most nonsynaptic extracellular glutamate in the nucleus accumbens (6), and its activity is downregulated after chronic cocaine administration (7,8). The nutritional supplement N-acetylcysteine restores the function of the cystine-glutamate exchanger, increases the basal levels of extracellular glutamate in the accumbens after withdrawal from cocaine, and thereby attenuates reinstatement to cocaine seeking in animals (3,7,8) and cocaine cue reactivity in humans (9).

In addition to the cystine-glutamate exchanger, sodium-dependent glutamate transport into glia is a critical regulator of extracellular glutamate concentrations; this type of transport is maintained primarily by the major glial glutamate transporter, GLT-1 (EAAT2) (2). GLT-1 is responsible for 90% of total brain glutamate uptake (10). Given the apparent imbalance in glutamate homeostasis associated with withdrawal from cocaine self-administration, we postulated that cocaine also induces changes in glutamate transport. Recently, this hypothesis was supported by mathematical modeling of glutamate homeostasis at glutamatergic synapses in the nucleus accumbens that predicted both cystine-glutamate exchange and sodium-dependent uptake would be reduced by cocaine self-administration (11). Thus, it is possible that these two mechanisms of glutamate transport, glutamate uptake and cystine-glutamate exchange, are coregulated. Supporting this possibility, Bannai (12) reported that cystine-glutamate exchange is stimulated by increased activity of sodium-dependent glutamate transport. Additionally, downregulated transporter activity leaves excess glutamate in the extrasynaptic space, which has been found to inhibit the cystine-glutamate exchanger from exporting glutamate (13). The catalytic subunit of the cystine-glutamate exchanger is the protein xCT (5), and nicotine self-administration was recently shown to reduce the levels of both xCT and GLT-1 in the nucleus accumbens (14).

Here, we measured glutamate uptake and levels of GLT-1 and xCT protein expression in nucleus accumbens tissue of rats that had self-administered cocaine and underwent 3 weeks of extinction training. The β-lactam antibiotic ceftriaxone increases the expression and activity of GLT-1 (15,16), and we tested its ability to attenuate the reinstatement of cocaine seeking and elevate GLT-1 levels following cocaine self-administration. We investigated the possibility of coregulation of the two glutamate transport systems by measuring the effects of ceftriaxone treatment on xCT expression and, conversely, the effect of N-acetylcysteine treatment on GLT-1 expression in the NAcc of rats withdrawn from cocaine self-administration.

## Methods and Materials

Rats were trained to self-administer cocaine for 2 weeks and then underwent 3 weeks of extinction training. Glutamate uptake was measured in nucleus accumbens tissue (see Supplement 1 for a detailed description of uptake methods). A separate set of animals received ceftriaxone (200 mg/kg IP), N-acetylcysteine (100 mg/kg IP), or vehicle (saline) daily for the last 7 days of

From the Department of Neurosciences, Medical University of South, Carolina, Charleston, South Carolina.

Address correspondence to Lori Knackstedt, Ph.D., Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, BSB 403, Charleston, SC 29425; E-mail: knackst@musc.edu.

Received May 6, 2009; revised Jul 1, 2009; accepted Jul 19, 2009.

0006-3223/10/$36.00
doi:10.1016/j.biopsych.2009.07.018

extinction training and were euthanized via rapid decapitation. The NAcc was dissected, and a membrane subfraction was generated. Western blotting was performed on this tissue for GLT-1 and xCT proteins (for detailed description of the membrane subfractionation and Western blotting procedures, see Supplement 1).

Animals self-administered cocaine for 2 hours/day for 2 weeks followed by extinction training for 3 weeks and until lever pressing reached 25% of self-administration levels. During extinction, animals were treated with either ceftriaxone (200 mg/kg IP) or vehicle for 5 to 12 days and then tested for both cue- and cocaine-primed reinstatement of the drug-seeking response. To ensure that ceftriaxone was not producing sedation, a separate group of animals was injected with ceftriaxone (200 mg/kg IP) for 7 days and then tested for basal locomotion and the locomotor response to a saline injection (see Supplement 1 for detailed description of the behavioral methods).

## Results

Sodium-dependent glutamate uptake in tissue slices from the accumbens was reduced in cocaine-trained compared with saline controls over a physiologic range of glutamate concentrations (1 μmol/L, 3 μmol/L, 5 μmol/L; Figure 1A). This was confirmed in a separate set of cocaine and saline subjects showing that reduced uptake resulted from decreased Vmax, without a difference in binding affinity (Kd = 2.6 μmol/L ± .9 μmol/L [cocaine], = 2.4 μmol/L ± .9 μmol/L [saline]; Figure 1B). Consistent with decreased Vmax, we observed significant downregulation of GLT-1 expression in the accumbens membrane subfraction following cocaine self-administration (Figure 1C) but not in the prefrontal cortex (Figure 1F). In the same samples used to measure GLT-1, reduced expression of xCT was also measured in the accumbens (Figure 1D) but not prefrontal cortex (although the level of xCT trended down in the cocaine animals; Figure 1F). Both N-acetylcysteine and ceftriaxone treatment alone upregulated the expression of both xCT and GLT-1 (Figure 1C–1E). Of note, this ceftriaxone treatment regimen did not upregulate GLT-1 expression in drug-naive rats (Figure 1G). Similarly, treatment with N-acetylcysteine failed to upregulate GLT-1 in drug-naive rats (Figure 1H).

There was no difference in the number of cocaine infusions attained over the course of the self-administration period for the ceftriaxone- and vehicle-treated subjects (Figure 2A; for inactive lever presses see Figure S1C in Supplement 1); likewise, self-administration levels were no different between groups used for the uptake assay, Western blots, or reinstatement tests (Figure S1A and S1B in Supplement 1). Ceftriaxone treatment had no effect on extinction levels of lever pressing (Figure 2B); however, the lever-pressing behavior was largely extinguished by Day 5, when ceftriaxone administration began. Ceftriaxone treatment significantly attenuated both cue- and cocaine-primed reinstatement (Figure 2C). Importantly, ceftriaxone potentiated sponta-

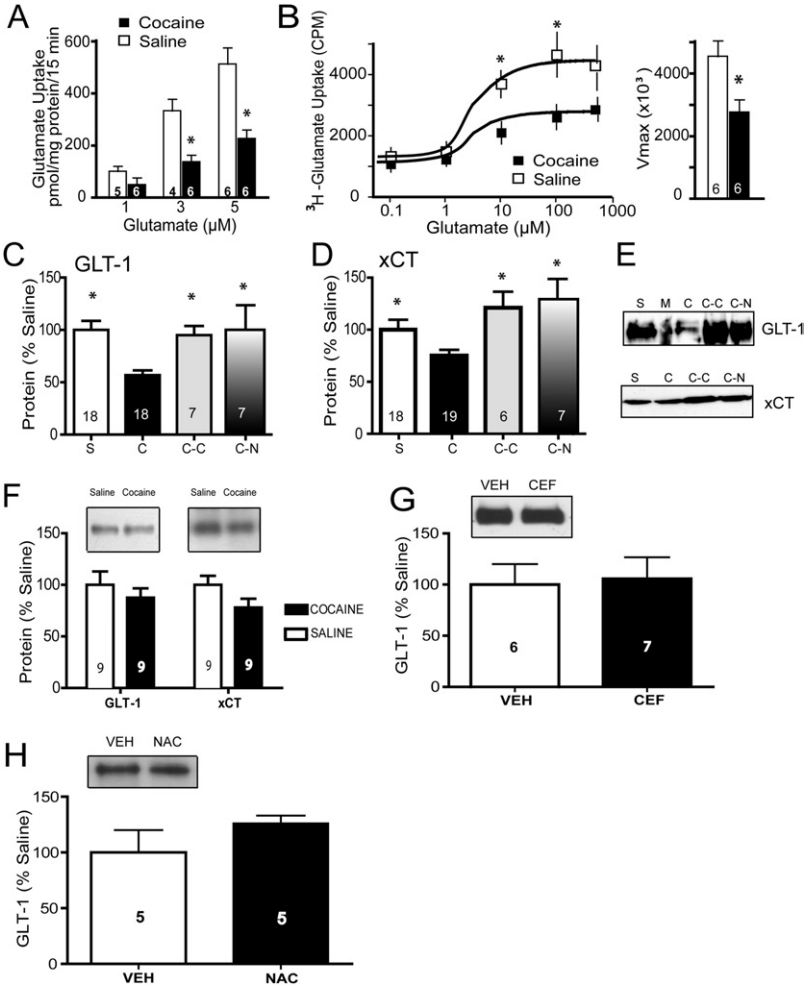


**Figure 1.** Cocaine self-administration reduces glutamate transporter expression and function. **(A)** 3H glutamate uptake was significantly decreased in accumbens tissue slices following cocaine self-administration. A two-way analysis of variance (ANOVA) revealed significant main effects of cocaine treatment [$F(1,26) = 11.36$, $p = .002$] and glutamate concentration [$F(2,26) = 14.73$, $p < .001$]; *$p < .05$). **(B)** The Vmax of glutamate uptake was reduced in cocaine subjects with no change in *Kd*. A two-way ANOVA revealed a significant effect of cocaine treatment [$F(1,49) = 11.21$, $p = .001$] and concentration of glutamate [$F(4,49) = 13.61$, $p < .001$]; *$p < .05$. **(C–E)** Cocaine self-administration followed by 3 weeks of extinction training significantly decreased GLT-1 and xCT protein expression, and chronic treatment with N-acetylcysteine or ceftriaxone restored levels of both proteins. One-way ANOVAs confirmed an effect of group for both GLT-1 [$F(3,47) = 5.34$, $p = .003$] and xCT [$F(3,45) = 4.47$, $p = .008$], *$p < .05$ compared with cocaine. S = saline; C = cocaine; C–C = cocaine + ceftriaxone; C–N = cocaine + N-acetylcysteine; M = marker ladder. **(F)** Membrane fraction levels of GLT-1 and xCT were not changed in the prefrontal cortex following cocaine self-administration. **(G)** Seven days of intraperitoneal (IP) ceftriaxone treatment (200 mg/kg) had no effect on accumbens GLT-1 levels in naive rats. **(H)** Seven days of IP N-acetylcysteine treatment (100 mg/kg) had no effect on accumbens GLT-1 levels in naive rats. VEH, vehicle; CEF, ceftriaxone; NAC, N-acetylcysteine.