Ceftriaxone normalizes nucleus accumbens synaptic transmission, glutamate transport and export following cocaine self-administration and extinction training

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Abstract

Decreased basal glutamate levels are observed in the rat nucleus accumbens (NA) core following cocaine self-administration. This disruption of glutamate homeostasis arises from a reduction in the export of glutamate via system x_{C}- and is accompanied by a decrease in expression of xCT, the catalytic subunit of system x_{C}-. A second hallmark of disrupted homeostasis is a decrease in expression and function of the major glutamate transporter, GLT-1. We have previously shown that chronic treatment with the antibiotic ceftriaxone restores xCT and GLT-1 expression following cocaine self-administration and attenuates both cue- and cocaine-primed reinstatement. Here we used a ^{3}H-glutamate uptake assay and microdialysis to test the hypothesis that ceftriaxone restores the function of both GLT-1 and xCT (glutamate reuptake and export, respectively) in the NA core following cocaine self-administration. We also used electrophysiology to investigate the ability of ceftriaxone to normalize measures of synaptic plasticity following cocaine. We found that 5 days of ceftriaxone treatment following cocaine self-administration restores basal glutamate levels in the accumbens core, likely through an upregulation of system x_{C}- function. We also found that ceftriaxone restores glutamate re-uptake and attenuates the increase in synaptically-released glutamate that accompanies cocaine-primed reinstatement. Ceftriaxone also reversed the cocaine-induced synaptic potentiation in the accumbens core, evidenced by normalized spontaneous EPSC amplitude and frequency and evoked EPSC amplitude. These data indicate that ceftriaxone normalizes multiple aspects of glutamate homeostasis following cocaine self-administration and thus holds the potential to reduce relapse in human cocaine addicts.

Disrupted glutamate homeostasis is observed in the nucleus accumbens (NA) core following cocaine administration. The hallmarks of this homeostatic dysregulation are decreased basal levels of glutamate (Baker et al., 2003; Moussawi et al., 2011), enhanced synaptically-released glutamate during cocaine-primed reinstatement (McFarland et al., 2003) and decreased sodium-dependent glutamate uptake accompanied by decreased expression of GLT-1 (Knackstedt et al., 2010). GLT-1 is the major glutamate transporter, accounting for

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approximately 94% of total CNS glutamate uptake (Tanaka et al., 1997). System x_C-, which exchanges extracellular cystine for intracellular glutamate in a sodium-independent manner, accounts for the majority of basal extracellular glutamate in the NA core (Baker et al., 2002). Cocaine produces deficits in NA core system x_C- function, as well as decreased expression of its catalytic subunit, xCT (Knackstedt et al., 2010).

The beta-lactam family of antibiotics increases the expression and function of GLT-1 in vitro and in vivo, with ceftriaxone possessing the greatest potency (Rothstein et al., 2005). GLT-1 upregulation is attributable to an increase in gene transcription through the nuclear factor-$\kappa\beta$ signaling cascade (Lee et al., 2008). Ceftriaxone is also a transcriptional regulator of xCT, Nrf2, and thereby increases system x_C- activity (Lewerenz et al., 2009). Accordingly, we have shown that ceftriaxone normalizes the expression of xCT and GLT-1 in the NA core following cocaine self-administration and extinction (Knackstedt et al., 2010). Furthermore, ceftriaxone attenuates cue- (Sari et al., 2009; Knackstedt et al., 2010) and cocaine-primed reinstatement (Knackstedt et al., 2010), an effect that persists weeks after cessation of ceftriaxone treatment (Sondheimer and Knackstedt, 2011).

Here we investigated whether ceftriaxone can restore compromised glutamate transport and basal glutamate levels following cocaine self-administration. We also investigated the ability of ceftriaxone to reverse cocaine-induced alterations in synaptic plasticity by measuring spontaneous and evoked EPSCs in the NA core of animals with a history of cocaine self-administration.

METHODS

Subjects

Male Sprague-Dawley rats (Charles River Laboratories, Indianapolis, IN) weighing 250–275g were housed individually in a temperature controlled vivarium on a 12h/12h reverse light cycle. All experiments were conducted during the dark phase. Animals were provided with ad libitum water and 20g of food/day. All experiments were conducted in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. A total of 86 rats were used for this study.

Drugs

Cocaine hydrochloride was generously donated by the National Institute on Drug Abuse (Bethesda, MD) and was dissolved in 0.9% physiological saline. For the purposes of self-administration, a 4 mg/ml solution was prepared and subjects received 0.25 mg/infusion. Cocaine-primed reinstatement testing was conducted using the dose of 10 mg/kg cocaine (IP). Ceftriaxone (Nova Plus, Irving, TX) was dissolved in saline and administered in a dose of 200 mg/kg.

Surgery

Rats were anesthetized with ketamine HCl (87.5 mg/kg, IM) and xylazine (5 mg/kg, IM). Ketorolac (3 mg/kg, IP) was administered for analgesia. For catheter implantation, SILASTIC tubing (Dow Corning, Midland, MI) was implanted into the jugular vein. The tubing exited the vein and traveled subcutaneously to exit on the back. The tubing was attached to a stainless-steel cannulae which was held within a harness (Instech, Plymouth Meeting, PA). For microdialysis, cannulas (20 gauge; Plastics One) were implanted bilaterally, aimed at the NAcore [anteroposterior (AP), + 1.4 mm; mediolateral (ML), ± 2.5 mm at 6° angle; dorsoventral (DV), −5.0 mm; all coordinates from Paxinos and Watson (2005)]. Catheters were flushed with heparin (100 units/mL) and timentin (25 mg/day) for 10 d after surgery and then heparin alone throughout the remainder of self-administration.

Self-administration and extinction procedures

Animals self-administered cocaine in a two-lever operant chamber (Med Associates, St. Albans, VT). Yoked-saline (SAL) controls received saline infusions when their cocaine counterpart received cocaine. Cocaine was available on an FR-1 schedule of reinforcement in which each press on the active lever (always the right lever) resulted in the delivery of 0.25 mL cocaine, the illumination of the stimulus light above the active lever and the delivery of a tone (2900 Hz). The light signified the length of the time-out (TO) period (20 s) during which presses on the active lever were recorded but did not result in delivery of drug. To be included in the study, rats were required to earn at least 10 infusions/2 hr session for 12 days. Animals which failed to meet this criterion were eliminated from the study. Subsequent to the completion of the cocaine self-administration phase of the experiment, animals underwent daily extinction training during which time responses on the previously active lever no longer produced cocaine or drug-paired cues. Animals underwent extinction training for 2–3 weeks prior to being used in either microdialysis, uptake, or electrophysiological studies as described below.

Microdialysis and HPLC for quantification of glutamate

Hollow fiber microdialysis tubing was affixed to a 24 gauge cannula (Plastics One). The end of the tubing was sealed with epoxy leaving 2 mm of active membrane between the glued membrane and the end of the cannula. The other end of the cannulae was threaded with the inlet and outlet tubing made of fused silica.

For microdialysis procedures during reinstatement testing, subjects were implanted with a microdialysis probe and placed into the self-administration chamber the night prior to testing. Subjects were perfused overnight with artificial cerebral spinal fluid (aCSF) composed of the following (in mM): 5 d-glucose, 2.7 KCl, 140 NaCl, 1.2 MgCl$_2$, 1.4 CaCl$_2$, pH 7.2) at a rate of 0.2 μL/min. Food and water were available overnight. The next morning, the flow rate was increased to 2 μL/min for 2 hrs followed by collection of twelve 10-min baseline samples. Animals received an injection of cocaine (10 mg/kg IP) and the levers were extended for the reinstatement trial. Samples were collected at 10-min intervals during the 2 hr reinstatement test, yielding twelve samples.

For no-net flux microdialysis, animals were probed and placed into a plexiglass chamber overnight, and dialysis was performed as described above. After collection of baseline samples for 2 hr, the no-net flux procedure began by changing the perfusate to dialysis buffer containing 2.5, 5, or 10 μM glutamate. Liquid switches were used to minimize the pressure fluctuations while dialysis buffers containing varying concentrations of glutamate were perfused. Four samples were collected at 20 min intervals for each concentration.

Glutamate levels were analyzed using a high-pressure liquid chromatography (HPLC) system with electrochemical detection. The mobile phase consisted of MeOH (15% v/v), acetylnitrile (2.5% v/v), 100 mM sodium dihydrogen phosphate monobasic, pH 6.0. Precolumn derivatization with o-phthalaldehyde was performed using an ESA Model 540 autosampler (ESA Inc, Bedford, MA). Separation was done with a Shiseido column and glutamate was detected by a spectrophotometer (ESA Inc.). The glutamate content in each sample was analyzed by area under the curve and compared with an external standard curve for quantification.

^{3}H Glutamate Uptake

Glutamate uptake was measured using an in-vitro slice preparation as described previously (Knackstedt et al., 2010). Briefly, rats were decapitated and bilateral punches of the NA core were dissected and sliced into 250×250 μm sections using a McIllwan tissue chopper (St.

Louis, MO). Slices were incubated at 37°C in either oxygenated Krebs-Ringer's solution phosphate buffer (in mM: 140 NaCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 5 HEPES, 10 glucose and 1 $MgCl_2$, pH 7.4) to quantify sodium-dependent uptake, or an identical buffer in which NaCl was replaced with 140 mM choline chloride to measure sodium-independent uptake. Uptake was stimulated by the addition of L-[^{3}H] glutamate (40 nM, 51 Ci/mM; Perkin-Elmer, Boston MA) to slices in the presence of 3 μM L-glutamate. The reaction was conducted at 37°C for 15 minutes and terminated by ice-cold, sodium-free buffer. Slices were solubilized with 1 % sodium dodecyl sulfate and radioactivity determined using a liquid scintillation counter. Protein content was measured using a bicinchoninic acid assay protein assay (Pierce) and cpm/mg protein calculated.

Electrophysiology

Rats were anesthetized with isoflurane and decapitated. Brains were removed and immersed in cold (4°C) oxygenated artificial CSF (aCSF) composed of the following (in mM): 200 sucrose, 1.9 KCl, 33 $Na2HCO_3$, 6 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose, and 0.4 ascorbic acid. 300 μm coronal slices containing the accumbens were obtained with a vibratome (Leica VT1000S) and incubated for 1 h at room temperature in aCSF containing the following (in mM): 126 NaCl, 2.5 KCl, 25 $NaHCO_3$, 10 glucose, 4 $MgCl_2$, and $1CaCl_2$, 3 kynurenic acid. Slices were immersed in a recording solution composed of the following (in mM): 126 NaCl, 3 KCl, 26 $NaHCO_3$, 1 $MgCl_2$, 1.3 $CaCl_2$, and 10 glucose, maintained at 28–32°C at a perfusion rate of 1.5–2 ml/min and viewed by differential interference contrast (DIC) optics. The core compartment of the nucleus accumbens was localized using the anterior commissure as a landmark.

Borosilicate pipettes were filled with the following (in mM): 135 CsCl, 2 $MgCl_2$, 10 HEPES, 1 EGTA, 4 NaCl, 2 Na-ATP, and 0.3 Tris-GTP. QX-314 Cl- (2%) was added to pipettes during the evoked experiment to block voltage-sensitive sodium channels from generating action potentials. Pipettes were connected to the headstage of a Multiclamp 700B amplifier (Molecular Devices), with Ag/AgCl wire. An Ag/AgCl reference wire or pellet was placed in the bath and, by using offset, Vm shifts were corrected. Amplifier was controlled by Multiclamp Commander (Molecular Devices). Signals were digitized and recorded on a Windows-based PC running Axograph X software. Voltage-clamp recordings were obtained in continuous voltage-clamp mode and filtered at 2.9 kHz. Series resistance and capacitance were compensated automatically by Multiclamp Commander and optimized manually. Access resistance was monitored continuously, and a 15% change was deemed acceptable. Spontaneous EPSCs (sEPSCs) and evoked EPSCs (eEPSCs) were collected in the presence of picrotoxin (100 μM). Data was analyzed using Minianalysis software (Synaptosoft, Decatur, GA). A bipolar stimulating electrode (World Precision Instruments, Sarasota, FL) was placed medial to the lateral ventricle in the prelimbic cortex, and responses were recorded in the accumbens. Approximately 20 min after break-in, eEPSC data was collected with 10 sweeps at 60 s intervals. The pulse was 0.1 ms and its intensity was set to yield a stable response equal to approximately 75% of maximum amplitude (derived from the input-output function). For paired-pulse analysis, a set of two pulses was delivered with an inter-pulse interval of 50 ms, and the pairs of pulses were applied every 60 s.

RESULTS

Cocaine self-administration

Animals self-administered cocaine for 12 days and subsequently underwent extinction training. During extinction training, animals were treated with either vehicle (0.3 mL i.p.) or ceftriaxone (200 mg/kg i.p.) for five days prior to microdialysis procedures or sacrifice for

uptake assays or electrophysiology. Vehicle and ceftriaxone-treated animals displayed similar levels of cocaine intake (Figure 1A) and extinction responding (Figure 1B).

Ceftriaxone reversed cocaine-induced deficits in both glutamate uptake and export

We have previously shown that sodium-dependent glutamate uptake is reduced following cocaine self-administration and extinction training, accompanied by a decrease in Vmax but not Km (Knackstedt et al., 2010). Here, we incubated NA core slices in a concentration of glutamate in which uptake was previously found to be reduced (3 μM). In agreement with our previous results, we found a significant decrease in glutamate uptake in tissue taken from animals with a history of cocaine self-administration; ceftriaxone prevented this decrease (Figure 2A; $F_{(1,27)}=10.066$, $p<0.01$). When conducted in sodium-free conditions, a [3H]-glutamate uptake assay can accurately assess the activity of system x_C- (Melendez et al., 2005). This assay revealed that ceftriaxone also reversed a deficit in NA core system x_C- activity produced by cocaine (Figure 2B; $F_{(1,3)}= 7.534$, $p <0.05$).

Ceftriaxone treatment normalizes both synaptically-released glutamate and non-synaptic glutamate

A two-way RM ANOVA conducted on the no-net-flux dialysis data revealed a significant Group × Concentration interaction ($F_{(3,30)}=4.614$, $p<0.001$), a significant effect of Group ($F_{(3,30)} = 8.737$, $p<0.001$), and a significant effect of Concentration ($F_{(3,90)} = 11.871$, $p<0.001$). Using the point of y=0 to determine basal glutamate, ceftriaxone increased basal glutamate relative to vehicle-treated animals (Figure 3B; $F_{(1,3)} = 5.207$, $p<0.05$). A 2-way ANOVA determined that the slope of the regression line was significantly different between the ceftriaxone- and vehicle-treated groups (Figure 3C; $F_{(1,3)}=15.865$, $p<0.001$). A 2-way ANOVA revealed that vehicle-treated animals displayed a significant increase in glutamate during a cocaine-primed reinstatement test while ceftriaxone-treated animals did not (Figure 3D). This test revealed a significant Time X Group interaction ($F_{(8,88)}=3.118$, $p<0.01$), effect of Group ($F_{(1,11)} = 4.72$, $p=0.05$), and Time ($F_{(8,88)}= 2.530$, $p<0.05$). There was a significant reduction in active lever presses during the reinstatement test in ceftriaxone-treated animals (Cef=35.43, Veh= 81.6; $t_{(11)}=4.158$, $p=0.002$). Figure 3E indicates the placement of microdialysis probes for animals used in the microdialysis studies. Accurate placement was achieved when 2/3 of the active dialysis membrane was within the NA core. Data from animals with misplaced cannulae are not shown and were excluded from analysis.

Ceftriaxone normalized NA core excitatory transmission following cocaine self-administration

Two-way ANOVAs revealed that both sEPSC frequency and amplitude were increased following cocaine self-administration, and ceftriaxone reversed this effect (Figure 4). These tests revealed a significant Ceftriaxone X Cocaine interaction ($F_{(1,4)} = 15.423$, $p <0.001$) for sEPSC frequency (Figure 4A) as well as sEPSC amplitude (Figure 4B; $F_{(1,4)} = 4.452$, $p <0.05$). Cocaine self-administration also increased the amplitude of synaptic evoked EPSC's, an effect that was normalized by ceftriaxone (Figure 4C; $F_{(1,4)} = 8.1$, $p <0.01$). Thus we observed identical effects under stimulated (evoked) and basal (spontaneous) conditions. We found no significant effect on paired-pulse facilitation (data not shown; $F_{(1,4)} = 0.348$, n.s.).

DISCUSSION

We have previously shown that ceftriaxone restores GLT-1 expression following cocaine self-administration while attenuating cue- and cocaine-primed reinstatement (Knackstedt et al., 2010). Ceftriaxone may exert protection against reinstatement by increasing GLT-1 expression and function, thus increasing glutamate uptake and preventing the overflow of synaptically-released glutamate during reinstatement. In support of this hypothesis,

ceftriaxone restored sodium-dependent glutamate uptake in the NA core following cocaine self-administration (Figure 2A). Furthermore, ceftriaxone attenuated cocaine-induced glutamate overflow during a reinstatement test (Figure 3C), an effect that may be the result of increased uptake.

The therapeutic effects of ceftriaxone may also derive from its ability to increase system x_C- activity (Knackstedt et al, 2010; Lewerenz et al., 2009). Accordingly, here we found that ceftriaxone increased basal glutamate in the NA core of cocaine self-administering animals (Figure 3A,B). However, reports exist which indicate that ceftriaxone decreases glutamate in the striatum (Miller et al., 2008; Rasmussen et al., 2011), a discrepancy that may arise from differences in species and technique. Miller and colleagues sampled from a larger striatal region in the mouse, whereas in the rat, our probes were localized to the NA core where system x_C- strongly contributes to basal glutamate levels (Baker et al., 2002). Rasmussen and colleagues used qualitative microdialysis but did not take into account probe recovery; estimates of basal neurotransmitter levels obtained via this method can be misleading [see Chefer et al., (2009) for review].

In light of the reports of reduced basal glutamate following ceftriaxone, we conducted a [3H]-glutamate uptake assay to measure the activity of system x_C- following cocaine and ceftriaxone. When conducted in sodium-free conditions, this assay characterizes the activity of system x_C- (Melendez et al., 2005). We found that ceftriaxone reverses cocaine-induced deficits in system x_C- activity (Figure 2B). Furthermore, the pattern of ceftriaxone and cocaine-induced changes in basal glutamate (Figure 3B) and system x_C- activity (Figure 2B) are similar, providing support for the idea that an increase in system x_C- activity is the mechanism by which ceftriaxone restores basal glutamate. Furthermore, as a ceftriaxone-induced increase in NA core GLT-1 activity (Figure 2A) is not accompanied by reduced basal glutamate levels (Figure 3), it can be surmised that upregulated GLT-1 is not sufficient to decrease basal glutamate levels.

The slope of the line of regression for no-net-flux dialysis plots reflects the extraction fraction (E_d), which is potentially an estimate of neurotransmitter clearance (Bungay et al., 2003). The slope has been empirically shown to be a measure of clearance for the neurotransmitters dopamine (Smith and Justice, 1994) and acetylcholine (Vinson and Justice, 1997) following infusion of inhibitors of uptake, such that a lower E_d indicates less clearance. A study of this sort has not yet been conducted for the neurotransmitter glutamate. Here we found a reduction in slope following ceftriaxone treatment (Figure 3A,C), indicating that glutamate uptake may be reduced by ceftriaxone. Obviously, this interpretation does not fit with our results from the sodium-dependent glutamate uptake assay which indicated that ceftriaxone restored uptake. Chen et al., (2006) reported that while recovery may be altered due to uptake inhibition, recovery is also dependent on the ratio of neurotransmitter release to uptake. In the case of an increase in basal glutamate arising from a decrease in glutamate uptake, slope may be an accurate indicator of clearance. However, ceftriaxone increases *both* glutamate uptake (Figure 2A) and export (Figure 2B), resulting in a more complicated relationship between glutamate uptake and the point of no-net-flux of glutamate, and thereby preventing slope from serving as an accurate estimate of uptake [see Chen (2006); Pendyam et al., (2009) for further discussion].

N-acetylcysteine (NAC) serves as an artificial source of cystine which drives the exchanger to export glutamate, thus increasing basal glutamate levels (Baker et al., 2003; Moussawi et al., 2011). Because mGluR2/3 antagonism prevents N-acetylcysteine from attenuating reinstatement (Moran et al., 2005) it has been proposed that an increase in basal glutamate restores tone to release-regulating mGluR2/3 autoreceptors, thereby dampening the synaptically-released glutamate that drives reinstatement. The data presented here (Figure 3)

supports a similar mechanism of action for ceftriaxone to attenuate reinstatement by increasing basal glutamate levels and restoring tone on mGluR2/3 autoreceptors, reducing glutamate release during reinstatement (Figure 3D). N-acetylcysteine also up-regulates GLT-1 expression (Knackstedt et al., 2010), an adaptation which may also serve to dampen glutamate release during reinstatement. At this time, the relative importance of increased basal glutamate and increased GLT-1 function is not known.

Cocaine self-administration produces enduring synaptic potentiation in the nucleus accumbens, evidenced by increased AMPA/NMDA ratio (Conrad et al., 2008; Moussawi et al., 2011). Additionally, cocaine self-administration and extinction training results in an inability to induce LTP by stimulating PFC afferents to the NA core, indicating that the accumbens neurons are already in an LTP-like state (Moussawi et al., 2009). Taken together, these results suggest that cocaine self-administration potentiates glutamatergic transmission from the PFC to the NA core, a neural pathway that has been shown to underlie the behavior of cocaine reinstatement (McFarland et al., 2003). Here we confirmed this potentiation in cocaine self-administering animals and found that it was reversed by ceftriaxone (Figure 4). The observed cocaine-induced increase in both sEPSC amplitude and frequency supports the hypothesis that there are both pre- and postsynaptic changes in glutamate transmission caused by cocaine self-administration. As discussed above, ceftriaxone may normalize pre-synaptic modulation of glutamate release by increasing basal glutamate thereby restoring tone to mGluR2/3 receptors. The restoration of basal glutamate by ceftriaxone may also normalize post-synaptic determinants of excitability, such as AMPA receptor subunit composition. The failure of either cocaine or ceftriaxone to alter paired-pulse facilitation supports our conclusion that there are both pre- and post-synaptic alterations induced by these treatments. In agreement with the ability of ceftriaxone to normalize synaptic physiology after cocaine, N-acetylcysteine has been demonstrated to normalize the AMPA/NMDA ratio and restore the ability to induce LTP following cocaine self-administration and extinction (Moussawi et al., 2009; Moussawi et al., 2011).

In conclusion, we have confirmed previous studies which have shown that cocaine self-administration and extinction training results in a reduction of basal glutamate levels, diminished glutamate transport and export, and enduring synaptic potentiation in the nucleus accumbens core. We show that ceftriaxone normalizes these pathologies. At this time it is not known whether all of these neuroadaptations must be reversed in order to attenuate the reinstatement of cocaine seeking. Further studies will be done which assess the relative importance of glutamate export by system xc- and glutamate uptake by GLT-1 in mediating the therapeutic effects of ceftriaxone.

Acknowledgments

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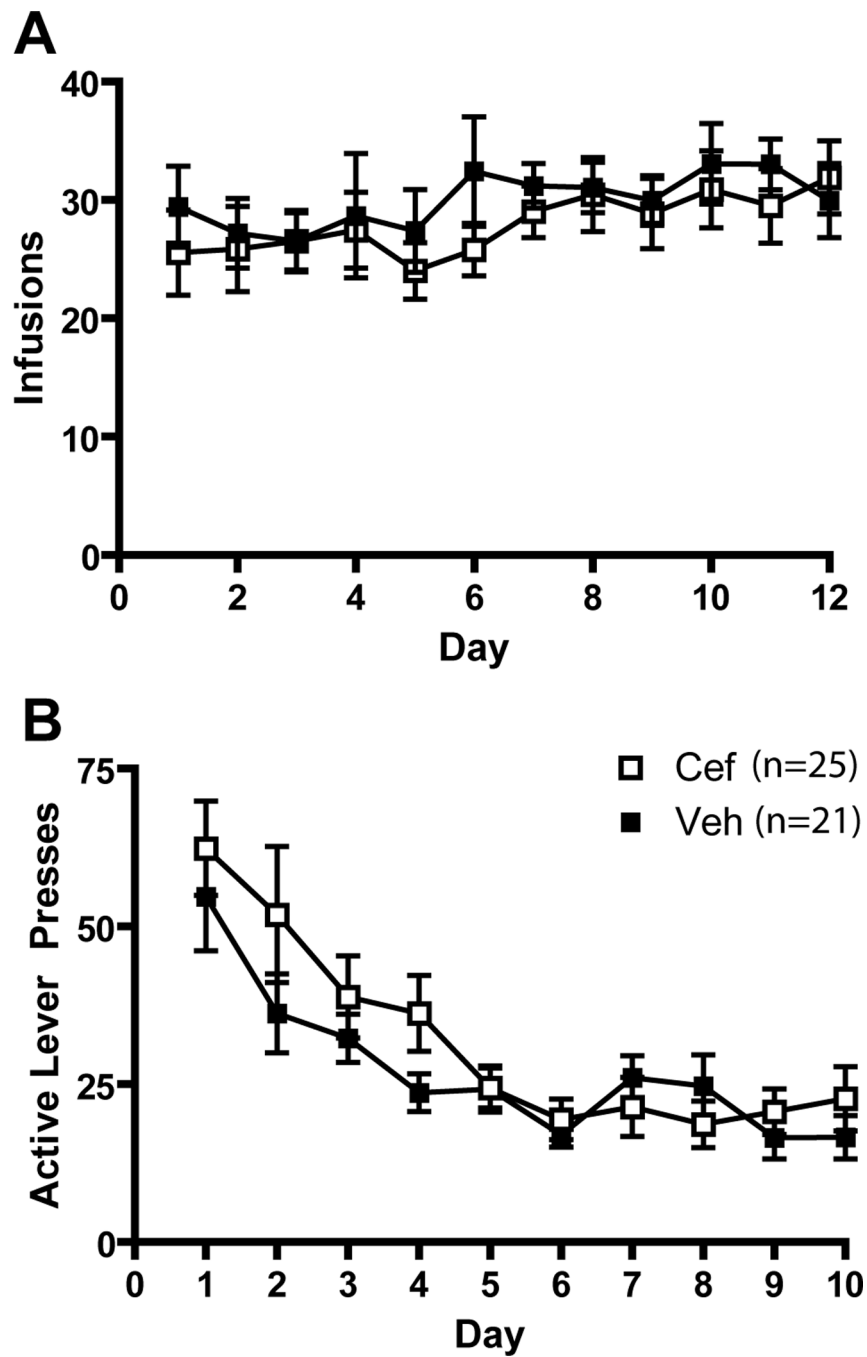
Figure 1. Cocaine self-administration and extinction training
Panel A. The mean number of infusions attained by animals later treated with ceftriaxone (Cef) and vehicle (Veh) did not differ significantly between groups. **Panel B.** There were no group differences in extinction responding. n = 21–25/group
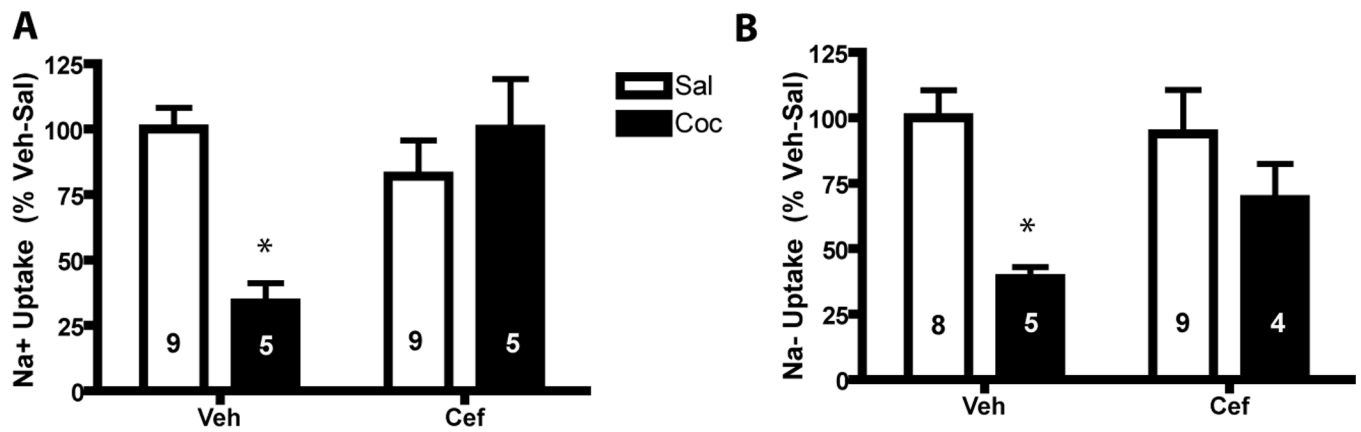
Figure 2. Ceftriaxone normalized deficits in glutamate uptake
**Panel A.** Cocaine animals displayed a significant decrease in sodium-dependent glutamate uptake that was normalized by ceftriaxone. **Panel B.** Sodium-independent uptake was reduced by cocaine. *$p< 0.05$ compared with Veh-Sal using a Bonferroni post-hoc test. n = 4–10/group
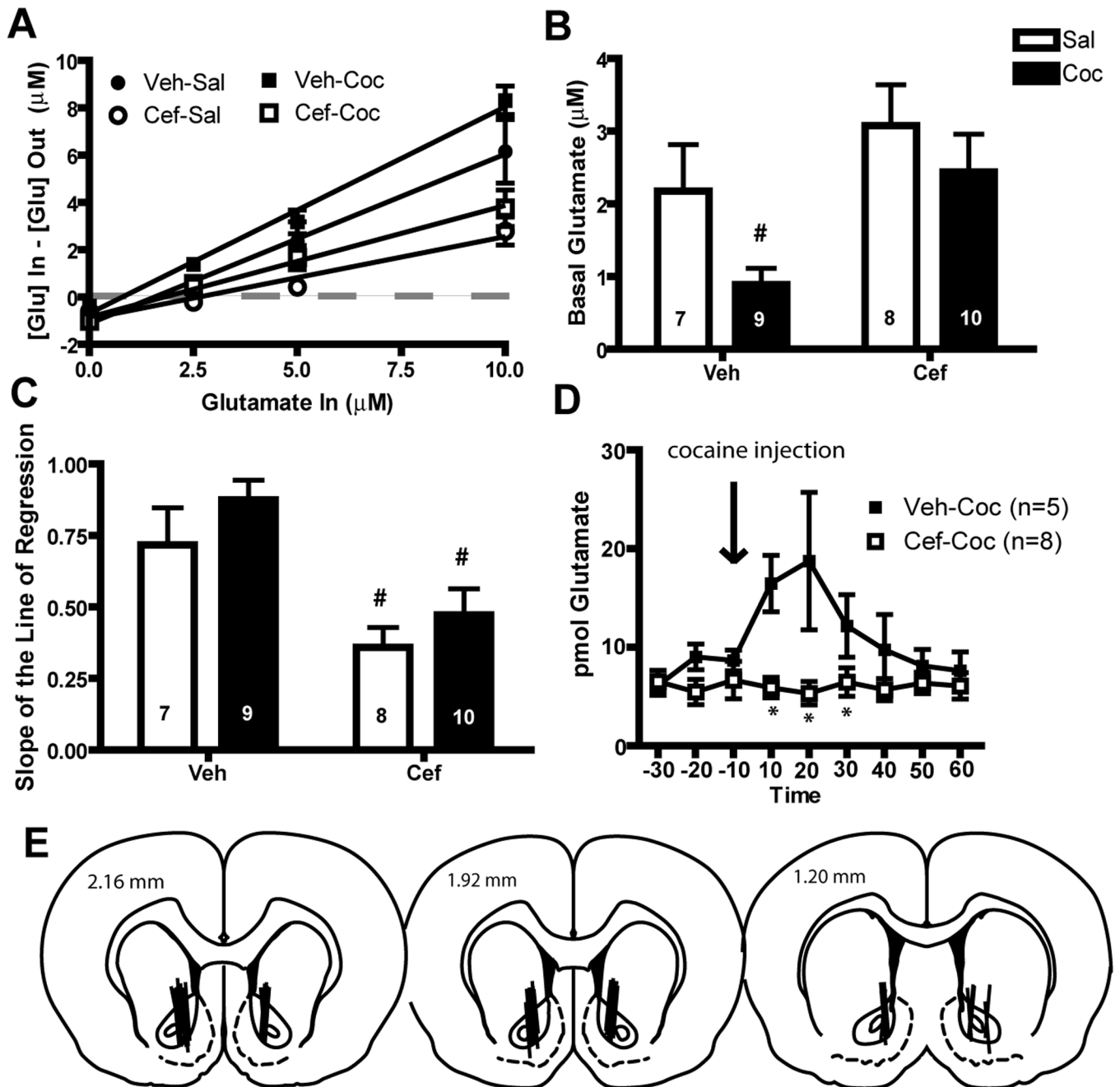
Figure 3. Ceftriaxone attenuates synaptically-released glutamate during reinstatement and increases basal glutamate in the NA core
Panel A. Ceftriaxone restores basal glutamate levels in cocaine self-administering rats.
Panel B. Ceftriaxone-treated animals displayed increased basal glutamate relative to vehicle-treated controls. **Panel C.** Ceftriaxone-treated animals display reduced slope of the line of regression relative to vehicle-treated controls. **Panel D.** Vehicle-treated animals displayed a significant increase in glutamate during a cocaine-primed reinstatement test while ceftriaxone-treated animals did not. **Panel E.** Diagrams showing dialysis probe tracks through the NA core. # = $p<0.05$ in comparison with Veh-Sal; *$p< 0.05$, comparing Cef with Veh; both using a Bonferroni post-hoc. n = 5–10/group
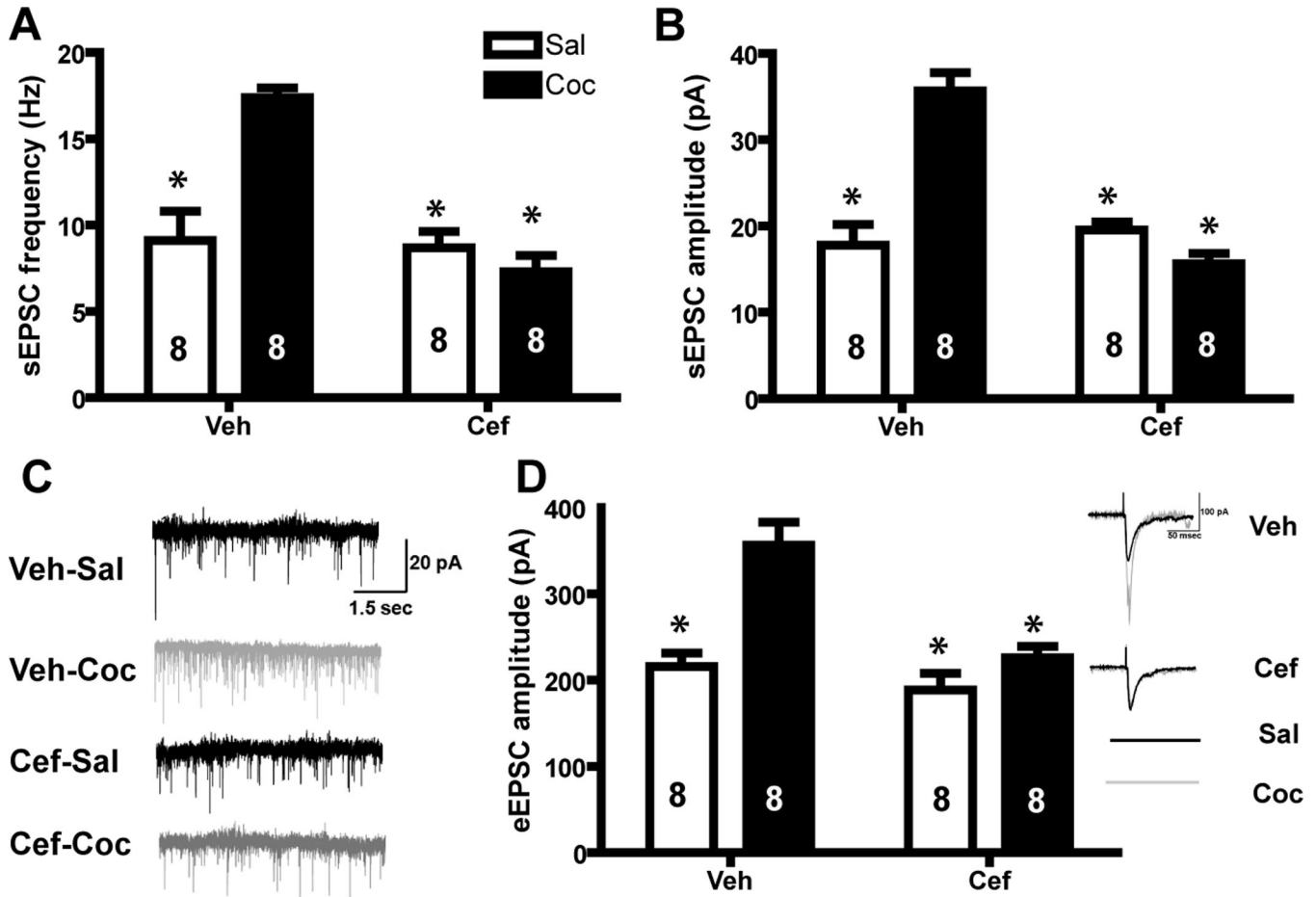
Figure 4.
Excitatory synaptic transmission is increased following cocaine self-administration and normalized by ceftriaxone. **Panel A.** sEPSC frequency is increased following cocaine self-administration, this effect is reversed by Ceftriaxone treatment. **Panel B.** sEPSC amplitude is increased following cocaine self-administration, ceftriaxone reverses this effect. **Panel C.** Sample sEPSCs: traces in black show representative samples from control animals, while gray are from cocaine-treated animals. **Panel D.** Cocaine self-administration increases synaptic evoked EPSC's, an effect that is prevented by ceftriaxone. Representative traces shown in black are from control animals, gray traces are from cocaine-treated animals. *$p < 0.05$, compared with Veh-Coc using a Bonferroni post hoc test. n = 8 cells/group