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Glutamatergic Mechanisms of Comorbidity Between Acute Stress and Cocaine Self-administration

Constanza Garcia-Keller[1,2], **Yonatan Kupchik**[3], **Cassandra D Gipson**[1], **Robyn M Brown**[4], **Sade Spencer**[1], **Flavia Bollati**[2], **Maria A Esparza**[2], **Doug Roberts-Wolfe**[1], **Jasper Heinsbroek**[1], **Ana-Clara Bobadilla**[1], **Liliana M Cancela**[2], and **Peter W Kalivas**[1,*]

[1]Department of Neuroscience, Medical University of South Carolina, Charleston, SC, USA

[2]IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

[3]Department of Medical Neurobiology, Institute for Medical Research Israel-Canada, Faculty of Medicine, The Hebrew University, Jerusalem, Israel 9112102

[4]Florey Institute of Neuroscience & Mental Health, University of Melbourne, Parkville, Australia

Abstract

There is substantial comorbidity between stress disorders and substance use disorders (SUDs), and acute stress augments the locomotor stimulant effect of cocaine in animal models. Here we endeavor to understand the neural underpinnings of comorbid stress disorders and drug use by determining if the glutamatergic neuroadaptations that characterize cocaine self-administration are induced by acute stress. Rats were exposed to acute (2 h) immobilization stress and 3 weeks later the nucleus accumbens core was examined for changes in glutamate transport, glutamate mediated synaptic currents, and dendritic spine morphology. We also determined if acute stress potentiated the acquisition of cocaine self-administration. Acute stress produced an enduring reduction in glutamate transport, and potentiated excitatory synapses on medium spiny neurons. Acute stress also augmented the acquisition of cocaine self-administration. Importantly, by restoring glutamate transport in the accumbens core with ceftriaxone the capacity of acute stress to augment the acquisition of cocaine self-administration was abolished. Similarly, ceftriaxone treatment prevented stress-induced potentiation of cocaine-induced locomotor activity. However, ceftriaxone did not reverse stress-induced synaptic potentiation, indicating that this effect of stress exposure did not underpin the increased acquisition of cocaine self-administration. Reversing acute stress-induced vulnerability to self-administer cocaine by normalizing glutamate transport poses a novel treatment possibility for reducing comorbid SUDs in stress disorders.

Individuals suffering from stress disorders are vulnerable to developing substance use disorders (SUDs). This is well documented in United States Veterans returning from combat

*Corresponding Author. Peter Kalivas, Ph.D., kalivasp@musc.edu, Phone: 843-876-2340, FAX: 843-792-4423.

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in Vietnam, Iraq and Afghanistan who have ~20% incidence of post-traumatic stress disorder (PTSD) and a 30–50% comorbidity of PTSD and SUDs[1–4]. Using rodent models of stress and substance use, most studies reveal that previous exposure to stress predisposes animals to the behavioral effects of psychostimulants and opioids, including the development of behavioral sensitization and drug self-administration[5–10]. While the face validity of these animal models relative to stress disorders and addiction can be argued[11–13], stress exposure potentiates both the rewarding and psychomotor stimulant effects of addictive drugs, and understanding the neurobiological underpinnings of this interaction could provide avenues for developing treatments for comorbid stress disorders and SUDs.

Previous studies have largely focused on stress-induced release of corticotropin releasing hormone and its effects on dopamine cells in the ventral tegmental area as mechanisms of comorbidity between stress disorders and SUDs[14–17]. Accordingly, pre-exposure to stress promotes the capacity of psychostimulants to release dopamine in the nucleus accumbens, and this is associated with stress facilitating the locomotor response to acute psychostimulant administration[6, 7, 9]. Recently, it was proposed that allocortical regions sending glutamatergic projections to the nucleus accumbens, such as the amygdala and hippocampus, may contribute to the effects of stress on substance use[18, 19].

Here we modeled how exposure to a single acute stressful event can create an enduring vulnerability to developing SUDs and quantified nucleus accumbens biomarkers of glutamate transmission previously shown to be relevant to cocaine use[20–24], including glutamate receptor-mediated currents, dendritic spine morphology and glutamate transport, at 3 weeks following exposure to a single immobilization stress. Following acute stress all three parameters of glutamate synapses showed enduring changes akin to what is seen after withdrawal from cocaine, and animals pre-exposed to acute stress showed augmented acquisition of cocaine self-administration. We then validated the importance of stress-induced reductions in glutamate transport in the enhanced responsiveness to cocaine by showing that restoring glutamate uptake with ceftriaxone abolished stress-induced increases in cocaine self-administration and locomotor activity.

METHODS

Animal Housing and Stress

Male Sprague-Dawley rats (250 g; Charles River Laboratories) were double housed with a 12:12 hr dark/light cycle. The animals were approximately 2 month old (± 1 week). All experimentation occurred in the light cycle. Rats received food and water ad libitum and were allowed at least 1 week to acclimate to the vivarium before any treatment. The acute stress group was restrained for 2 hours (anytime between 10:00 and 14:00 h) in restraining devices, while sham animals were left undisturbed in their home cages. The Plexiglas cylinders were designed so that the rats' tails emerged from the rear. The animals appeared healthy as shown by their coat texture and no difference in body weight was detected between sham and stress exposed rats at the time animals were used for behavior or sacrificed for the different measurements. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Assessment and Accreditation of Laboratory Animal Care.

Surgery and Cocaine Self-Administration

Some rats were anesthetized and implanted with indwelling jugular catheters at two weeks after acute stress or sham. Seven days after surgery, rats began daily 2 hr cocaine self-administration on a fixed radio 1 (FR1), in which one response on the active lever yielded one intravenous cocaine infusion (0.2 mg/infusion, followed by a 20 s timeout period), paired with a white cue light above the active lever and a discrete tone cue. An inactive lever was also available throughout each session. Rats were trained for seven days and the time to reach cocaine self-administration criterion, defined as the first day animals obtain ≥10 infusions, was recorded. In some experiments, animals were treated with ceftriaxone (200 mg/kg IP after each operant session) or vehicle (saline) for 3 days prior and 7 days during the acquisition of cocaine self-administration. The exclusion criteria used was if the animals the first days of the self-administration start randomly pressing the both levers (> 30 inactive lever press). To further investigate the effect of stress on the acquisition of cocaine self-administration, infusions of cocaine were converted to brain concentrations of cocaine using the following equation:

$$c = \frac{\mathrm{d}k}{\nu(\alpha - \beta)}(e^{-\beta t} - e^{-\alpha t})$$

Where d is the dose of cocaine per infusion (0.2 mg; 0.659 μmol), k the rate constant of transfer between blood and brain (0.223), and v the apparent volume of distribution in the brain (0.044). Constants α (0.642) and β (0.097) represent the rate of distribution between blood and brain and the rate of clearance from the body, respectively.[25]

Locomotor Activity

Three weeks after acute stress or sham, rats were tested in an apparatus consisting of rectangular cages equipped with parallel infrared photocell beams located 3 cm above the floor. Beam interruptions were monitored over 10-min intervals. At two weeks following acute immobilization stress, rats were pretreated with 5 daily injections of saline, ip, or ceftriaxone (200 mg/kg, ip), and one hr after the 5th injection were given saline or cocaine injection (15 mg/kg, ip).

^{3}H-Glutamate Uptake Assay

Glutamate uptake was measured using an *in vitro* slice preparation as described previously[26]. Briefly, two weeks after stress or sham animals received daily ceftriaxone (200 mg/kg, ip) or vehicle (saline) for 5 days prior to bilateral dissection of the NAcore or dorsolateral striatum. The tissue was sliced into 250 × 250 μm sections, and each hemisphere incubated at 37°C in either oxygenated Kreb's-Ringer's solution phosphate buffer (KRP) (in mM: 140 NaCl, 1.2 $CaCl_2$, KH_2PO_4, 5 HEPES, 10 glucose, and 1 $MgCl_2$) for Na^+-dependent glutamate uptake, or in KRP buffer with NaCl replaced by 140 mM choline chloride for Na^+-independent glutamate uptake. Glutamate uptake measurements were initiated by adding ^{3}H-glutamate (40 nM, 50 Ci/mmol; PerkinElmer) in the presence of unlabeled glutamate (10 μM) in a final volume of 250 μl of KRP buffer. The reaction was conducted at 37°C for 15 min, and was terminated by washing the slices with ice-cold

sodium-free buffer. The tissue was then solubilized using 1% SDS, and radioactivity and protein content quantified. Counts per minute were converted to uptake/mg protein/15 min.

Tissue Fractionation and Western Blotting

Crude membrane and gliosome fractions were prepared for assessing GLT-1 protein expression. Briefly, two weeks after stress or sham, animals were administered ceftriaxone (200 mg/kg, ip) or vehicle (saline) for 5 days prior to being decapitated. The NAcore was dissected and the bilateral slices were pooled and homogenization in 0.2 mL ice-cold buffer containing Na^+ Hepes and sucrose (pH: 7.4) or in 0.32 M sucrose, 1 mM EDTA, 5 mM Tris, pH 7.4. All buffers were supplemented with 1:100 protease/phosphatase inhibitors. Homogenates for the membrane subfraction were centrifuged at 1,000×g for 10 min at 4 °C, and the pellet homogenized with additional 0.2 ml homogenization buffer and recentrifuged. Supernatants were centrifuged at 12,000×g for 20 min, and the pellet resuspended in 30 μl radioimmunoprecipitation assay (RIPA) buffer (Thermo Scientific, Waltham, USA) supplemented with 1% SDS. A final centrifugation step at 10,000×g for 5 min was performed to remove insoluble material.[2626 26 26 26 26 26 26 26 2626] For gliosome fractionation the homogenates were centrifuged at 1,000×g for 10 min at 4°C. Percoll gradients were placed in tubes from higher to lower concentration and seeded with the supernatant[27]. The gradients were centrifuged at 31,000×g for 5 min at 4°C, and the layer between 2% and 6% Percoll was removed[28]. The gliosome fraction was resuspended in 2.0 ml buffer (in mM: 125 NaCl, 3.5 KCl, 1.25 KH_2PO_4, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.45 $CaCl_2$, 10 HEPES and 10 glucose, pH 7.4) and centrifuged at 20,000×g for 30 min. The resulting pellet was resuspended in 30μL RIPA buffer, and 20 μg of protein/sample was probed using GLT-1 glutamate transporter antibody (1:1000, ab 3838, Cell Signaling, Beverly, USA) using PVDF membranes. Data were normalized to β-actin (1:500, sc 1616, polyclonal actin, Santa Cruz Biotecnology, Santa Cruz, CA, USA) a loading control and the average of sham controls.

Measurement of AMPA and NMDA Currents

As previously reported[29, 30], rats were anesthetized with ketamine HCl (1 mg/kg Ketaset) and decapitated. The brain was removed from the skull and coronal accumbens brain slices (220 μm) were collected into a vial containing artificial cerebrospinal fluid (aCSF) (in mM: 126 NaCl, 1.4 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 2.5 KCl, 2.0 NaPyruvate, 0.4 ascorbic acid, bubbled with 95% O_2 and 5% CO_2) and a mixture of 5 mM kynurenic acid and 50 μM D-(−)-2-Amino-5-phosphonopentanoic acid (D-AP5). All recordings were collected at 32°C in the dorsomedial NAcore. Inhibitory synaptic transmission was blocked with picrotoxin (50 μM), and excitatory postsynaptic currents (EPSCs) were recorded in whole cell patch-clamp configuration using glass microelectrodes (1–2 MΩ) filled with cesium-based internal solution (in mM: 124 cesium methanesulfonate, 10 HEPES potassium, 1 EGTA, 1 $MgCl_2$, 10 NaCl, 2.0 MgATP, and 0.3 NaGTP, 1 QX-314, pH 7.2–7.3, 275 mOsm). Data were acquired at 10 kHz, filtered at 2 kHz, and EPSCs evoked using a bipolar stimulating electrode placed ~300 μm dorsomedial of the recorded cell to maximize stimulating prelimbic afferents. Stimulation intensity was adjusted to evoke an EPSC of 200–500 pA. Recordings were collected every 20 sec. Series resistance (Rs) and holding current were monitored online, and recordings with unstable Rs, or Rs >10 MΩ

were aborted. Recordings began 10 min after the cell membrane was ruptured, and AMPA currents were first measured at -80 mV to ensure stability followed by recordings at +40 mV. After EPSCs containing AMPA and NMDA currents were obtained, AP5 was bath-applied (50 μM) and AMPA currents recorded at +40 mV. NMDA currents were obtained by subtracting the AMPA currents from the total current at +40 mV. In a different set of cells NMDA currents were recorded at +40mV in the presence of CNQX (10 μM) to block AMPA currents[29]. EPSCs were evoked by a train of one, two, four, or eight pulses given at 100 Hz with 20 s interval between trains. The time-constant of NMDA current decay was determined from eight traces, averaged and fit with a single exponential.

Statistics

All statistical analyses were performed using Graphpad Prism 6.0. Two-tailed unpaired t-tests and 1- or 2-way ANOVAs were used as all data were normally distributed according to a D'Agostino-Pearson omnibus normality test. However, when survival analysis was conducted for reaching the self-administration criterion, data were analyzed using a log-rank Mantel-Cox test. Electrophysiology experiments were performed with experimenter blinded to condition, while glutamate uptake, behavioral studies and Western blotting were not blinded. No randomization was used in any experiments. Statistical tests are indicated in the text or figure legend. All experiments were replicated at least twice. Total number of animals per group was determined by an a priori power analysis conducted to engender 95% power assuming a moderate effect size (0.5).This resulted in N=8–12 for the majority of experiments[31].

RESULTS

Stress facilitates the acquisition of cocaine self-administration

Three weeks after acute immobilization stress the acquisition of cocaine self-administration was facilitated (figure 1). Stress-induced facilitation was not significant when quantified by number of active lever presses or cocaine infusions (figure 1a,b). However, transforming the data according to a criterion of the first day animals self-administered at least 10 cocaine infusions, stress animals acquired cocaine self-administration more rapidly than sham animals over the 7 day test period (figure 1c).

Inspecting lever presses of individual rats over time during self-administration revealed faster learning and stabilization of responding for cocaine in stress-exposed animals. Either raster ploting of lever presses or converting infusions to brain cocaine concentration[25] shows that the best performing stress animal reached stable brain cocaine levels by day 4, whereas the best performing sham animal did not yet stabilize responding until day 7 (figure 1d). When the brain cocaine concentration across self-administration sessions was averaged within treatment groups, a heat map reveals that stressed rats show a more rapid acquisition and stabilization of self-administration (figure 1e).

Acute stress enhanced glutamate currents and dendritic spine density

Withdrawal from cocaine is associated with measures of potentiated excitatory synapses in NAcore MSNs, including elevated AMPA currents (normalized to NMDA current

amplitude) and increases in dendritic spine density and head diameter (d_h)[32, 33]. Both AMPA/NMDA (figure 2) and spine density (supplemental figure 1) were elevated in NAcore at 3 weeks after an acute immobilization stress, but no change in d_h was measured. The increase in AMPA/NMDA may result from elevated AMPA currents since chronic immobilization stress increases surface expression of GluA1 in accumbens[6]. There was no stress-induced change in spontaneous EPSC amplitude (sham- 23.97 ± 1.36 pA, n= 15; stress- 26.90 ± 1.92, n=14, Student's $t_{(27)}$= 1.26, p= 0.220) or frequency (sham- 1.71 ± 0.32 Hz, n= 15; stress- 2.75 ± 0.78 Hz, n=14, Student's $t_{(27)}$= 1.27, p= 0.216).

Reduced GLT-1 and glutamate uptake after acute stress and restoration with ceftriaxone

A feature of rats trained to self-administer cocaine, heroin, alcohol or nicotine is an enduring reduction in the glial glutamate transporter GLT-1 in the NAcore[26, 29, 34, 35]. Using ^{3}H-glutamate uptake in NAcore or striatal tissue slices, we measured a marked reduction in glutamate uptake 3 weeks after acute stress (figure 3a,b). Acute stress did not affect Na^+-independent ^{3}H-glutamate uptake, which estimates cystine-glutamate exchanger activity[26], but significantly reduced Na^+-dependent uptake. The glial glutamate transporter GLT-1 contributes to >90% of extracellular glutamate removal from the extrasynaptic space[36], and GLT-1 expression was significantly reduced in the NAcore of pre-stressed animals compared with sham controls (figure 3c,d). The reduction in glutamate uptake was verified using whole cell patch of NAcore MSNs by showing that the spillover of synaptic glutamate (evoked by 100 Hz trains and estimated by the decay time constant in NMDA currents) was greater in stress than in sham animals, especially when the highest number of stimulations in a train (8) was applied (figure 3e,f). All these measures of stress-induced reduction in glutamate transport in the NAcore were successfully normalized by 5 days of ceftriaxone administration (figure 3).

Interestingly, the same daily pretreatment with ceftriaxone did not alter the enduring increase in AMPA/NMDA produced by acute stress pretreatment (figure 2a). Thus, the down-regulation of GLT-1 by stress does not appear to underlie the capacity of stress to elevate AMPA/NMDA in NAcore MSNs.

Ceftriaxone prevented stress-induced potentiation of cocaine behaviors

In animal models of SUDs, pharmacologically restoring GLT-1 in NAcore inhibits drug-seeking[22, 29, 34, 37]. Similarly, we found that stress-induced increases in cocaine-induced locomotor activity were abolished by pretreatment with ceftriaxone for 5 days prior to acute cocaine injection (figure 4). Also, pretreating sham and stress animals for 3 days prior to self-administration and for 7 days during acquisition of cocaine self-administration with ceftriaxone (200 mg/kg) prevented stress-potentiated self-administration when the data were analyzed as active lever presses (figure 5).

DISCUSSION

The possibility that stress and addictive drugs may elicit shared neuroadaptive changes in brain is supported by clinical co-morbidity between stress disorders and SUDs, as well as animal research showing that pre-exposure to stress potentiates the motor stimulant effects

of psychostimulants. Our study identifies enduring neuroadaptive mechanisms in common between acute restraint stress and daily cocaine self-administration, and identified one mechanism, down-regulated GLT-1, as pathogenic in acute stress facilitating the acquisition of cocaine self-administration.

We probed aspects of glutamate transmission in the NAcore known to be altered by addictive drugs, and found that akin to cocaine, at three weeks following a single exposure to stress the AMPA/NMDA ratio and dendritic spine density were increased, while glutamate uptake and GLT-1 content were reduced. Consistent with reduced glutamate elimination by GLT-1, no net flux microdialysis revealed that the basal levels of extracellular glutamate were increased for weeks after acute immobilization stress[7]. In contrast to elevated AMPA/NMDA and spine density, which occurs after withdrawal from cocaine but not heroin, reduced GLT-1 in the NAcore is observed following withdrawal from all drugs of abuse examined to date, and pharmacological restoration of GLT-1 inhibits drug seeking for all drugs tested to date[29, 34, 38, 39]. Accordingly, we used ceftriaxone to restore GLT-1 function in NAcore[37, 40], and prevented acute stress-induced increases in cocaine-mediated locomotion and acquisition of cocaine self-administration. These data provide a mechanistic linkage between acute stress-induced down-regulation of glutamate transport in NAcore and the vulnerability to use cocaine.

Glutamate uptake via GLT-1 is strategically positioned on glial processes adjacent to the synaptic cleft, which maximizes the rapid elimination of synaptically released glutamate[41, 42]. Accordingly, enduring downregulation of GLT-1 in rats pre-exposed to acute stress would be predicted to reduce the elimination of synaptically released glutamate; thereby allowing glutamate to more freely diffuse outside the synaptic cleft. Here we showed that electrically released synaptic glutamate induces NMDA currents with a longer decay time, indicative of synaptic glutamate having access to NMDA receptors at a greater distance from the synapse in previously stressed animals[29]. Moreover, when GLT-1 was restored by ceftriaxone treatment glutamate spillover in stressed animals was normalized; thereby directly implicating reduced glutamate uptake. While these data indicate that the spillover in stress animals results from reduced clearance by GLT-1, it remains possible that increased synaptic release also contributes.

Interestingly, restoring GLT-1 normalized the behavioral response to cocaine did not affect stress-induced elevation in AMPA/NMDA in NAcore MSNs. While down-regulated GLT-1 is a shared adaptation between different classes of addictive drug, the increase in AMPA/NMDA is not and differs between cocaine and heroin[21, 30, 43]. Since stress can potentiate both cocaine and heroin behaviors[5–10], it is consistent that a shared neuroadaptation, such as down-regulated GLT-1, would mediate stress-induced vulnerability to drug self-administration and that a neuroadaptation not in common between cocaine and heroin would not. A number of other studies have used chronic restraint or social stress animal models to evaluate changes in excitatory transmission in the nucleus accumbens, and there is a consensus that chronic stress elicits adaptations in AMPA receptors and currents. For example, resilience to social defeat stress is associated with increased in excitability of accumbens neurons, as indicated by an induction GluA2 subunit of AMPA receptors[44]. Also, resilience to chronic social stress is supported by stimulating prefrontal cortical or

amygdala inputs and is associated with a reduction in activity of hippocampal glutamatergic afferents[19], while mice most susceptible to social stress exhibit increased synaptic strength a glutamatergic afferents from the intralaminar thalamus[45]. Finally, using a chronic immobilization stress protocol, it was shown that stressed mice have a reduction in AMPA/NMDA selectively in D1-expressing accumbens neurons[46]. In comparing these studies, it is clear that further work is necessary to understand how different stress protocols (chronic versus acute or immobilization versus social defeat) may differentially affect distinct accumbens glutamatergic afferents in terms of potentiating and depotentiating glutamatergic synapses. Regardless, the present findings show that the stress-induced changes in the excitability of synapses may not be the pathogenic in increased susceptibility to acquire cocaine use since restoring GLT-1 prevented augmented cocaine acquisition, but did not alter stress-induced increases in AMPA/NMDA.

Although we showed a role for stress-induced down-regulation of GLT-1, stress-induced increases in glucocorticoid hormones, through actions on mesocorticolimbic dopamine neurons, also contribute to the increased vulnerability to drug use[47, 48]. In addition, selective deletion of Nr3c1 (the glucocorticoid receptor gene) in mouse dopaminoceptive neurons expressing D1 dopamine receptors decreases the motivation of mice to self-administer cocaine[49]. Moreover, neurons containing corticotropin releasing factor (CRF) synapse within the ventral tegmental area (VTA)[50], and microinjecting CRF antagonists into the VTA inhibits stress-induced reinstatement of cocaine-seeking[16, 51, 52]. CRF antagonists also prevent stress cross-sensitization to psychostimulants[53, 54], dopamine sensitization and escalated cocaine consumption[54]. Studies of the role for corticosterone, CRF and dopamine generally involve multiple stress exposures, while we used only a single stressor in an effort to more closely mimic the human situation where a single stressful experience can precipitate stress disorders.

The fact that a single stressor produced such long-lasting alterations at glutamatergic synapses in the NAcore, and facilitated the acquisition of cocaine self-administration poses possible shared pathological substrates that may predispose individuals suffering from stress disorders to develop comorbid SUDs. In future studies, it will be important to understand the interaction between corticosterone, CRF and dopamine in contributing to these glutamatergic adaptations. Moreover, the similarity between acute stress-induced glutamatergic neuroadaptations in NAcore and those produced by the self-administration of addictive drugs, poses common points of pharmacological intervention that may be particularly useful in treating stress disorder and SUDs comorbidity. Indeed, here we identify normalizing GLT-1 with ceftriaxone as a potential pharmacological intervention. This possibility is consistent with an emerging clinical literature with another compound known to normalize GLT-1 in animal models of addiction, N-acetylcysteine, that has been generally successful at reducing drug craving and other forms of intrusive thinking associated with other neuropsychiatric diseases, such as obsessive compulsive disorder, PTSD and major depression[55, 56].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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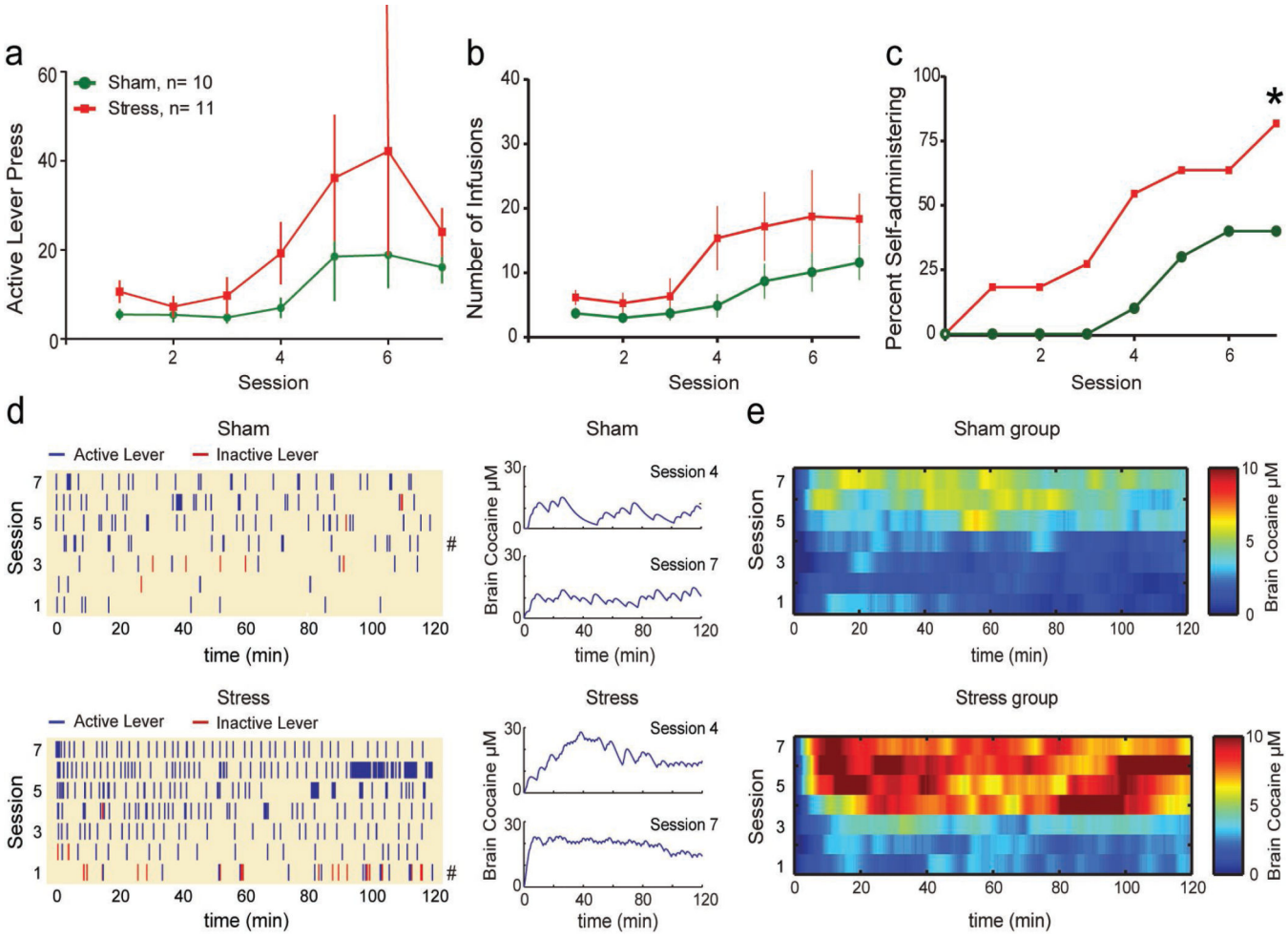

Figure 1.

Stress pre-exposure potentiated the acquisition of cocaine self-administration. **a)** Number of active lever presses made over the 7-day test for acquiring cocaine self-administration. N is shown in the graph legend. 2-way ANOVA revealed no effect of treatment group $F_{(1,19)}$= 2.41, p=0.137 or interaction $F_{(6,114)}$= 0.61, p=0.724, but a significant effect of time $F_{(6,114)}$= 4.04, p=0.001. **b)** Number of cocaine infusions taken over the 7-day test for acquiring cocaine self-administration. 2-way ANOVA revealed no effect of treatment group or interaction. 2-way ANOVA revealed no effect of treatment group $F_{(1,19)}$= 1.47, p=0.240 or interaction $F_{(6,114)}$= 1.02, p=0.417, but a significant effect of time $F_{(6,114)}$= 7.80, p<0.001. **c)** Using a criterion of the first day a rat self-administered 10 or more cocaine infusions, stress exposure potentiated the acquisition of cocaine self-administration. Log-rank Mantel-Cox test Chi^2= 4.33, * p= 0.038, comparing stress to sham. **d)** Example raster plots of the best performing sham (top) and stress exposed animal (bottom). Presses on the active (blue) and inactive (red) lever. #= day achieving criterion of 10 infusions. Modeled brain cocaine concentrations in these same animals reveal differences in brain cocaine levels on days 4 and 7 of self-administration. **e)** Heat maps of average modeled brain cocaine concentration in sham and stress exposed animals. Data are shown as mean (± SEM).

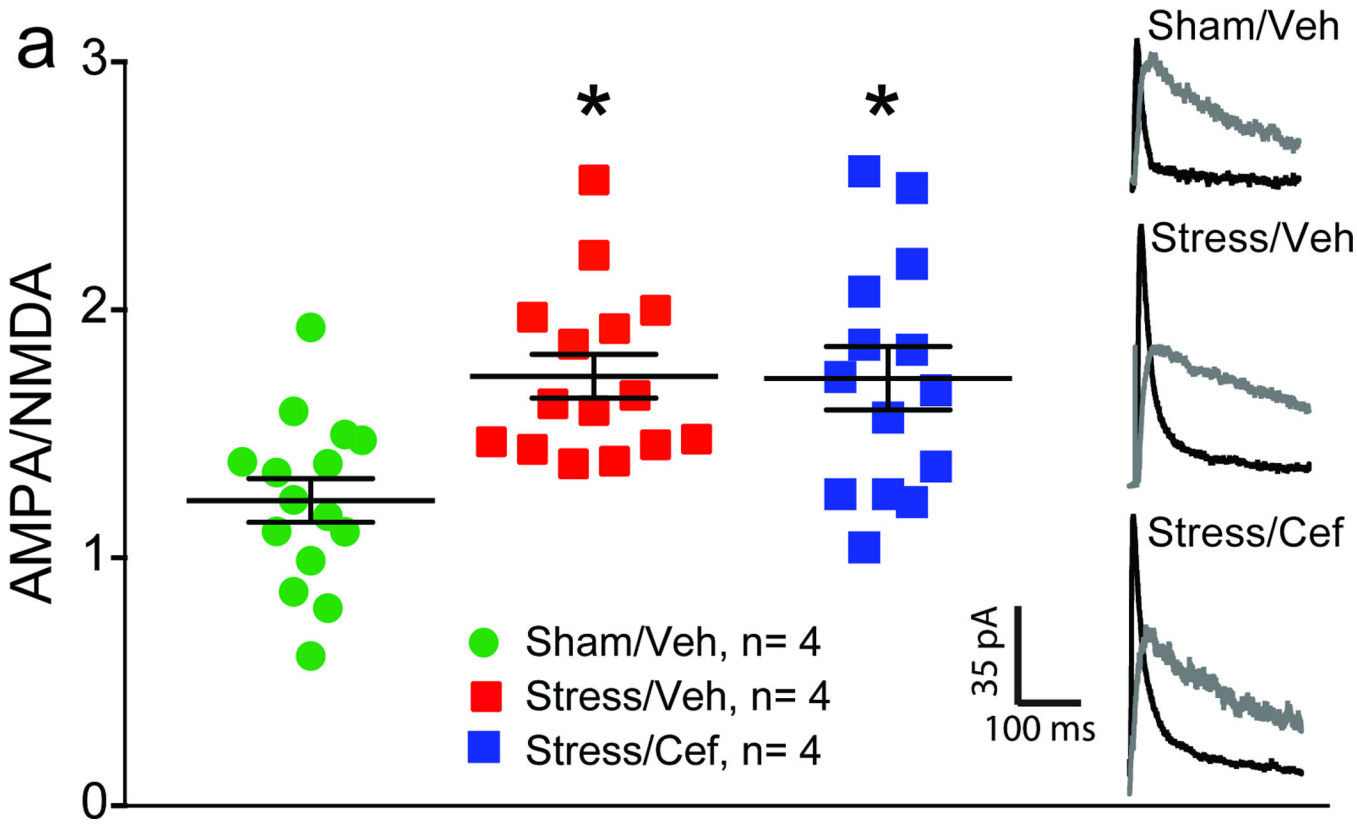

Figure 2.

Acute stress-induced increase in the AMPA/NMDA ratio in the NAcore. Increase in AMPA/NMDA by stress, one-way ANOVA $F_{(2,43)}$= 8.14, p<0.001, which was not reversed by ceftriaxone (Cef). Also are shown are representative AMPA (black) and NMDA (gray) traces. Veh= vehicle. N is shown in the graph legend.

Data are shown as mean (± SEM). *p< 0.05 compared to sham using a Bonferoni post hoc in panel.

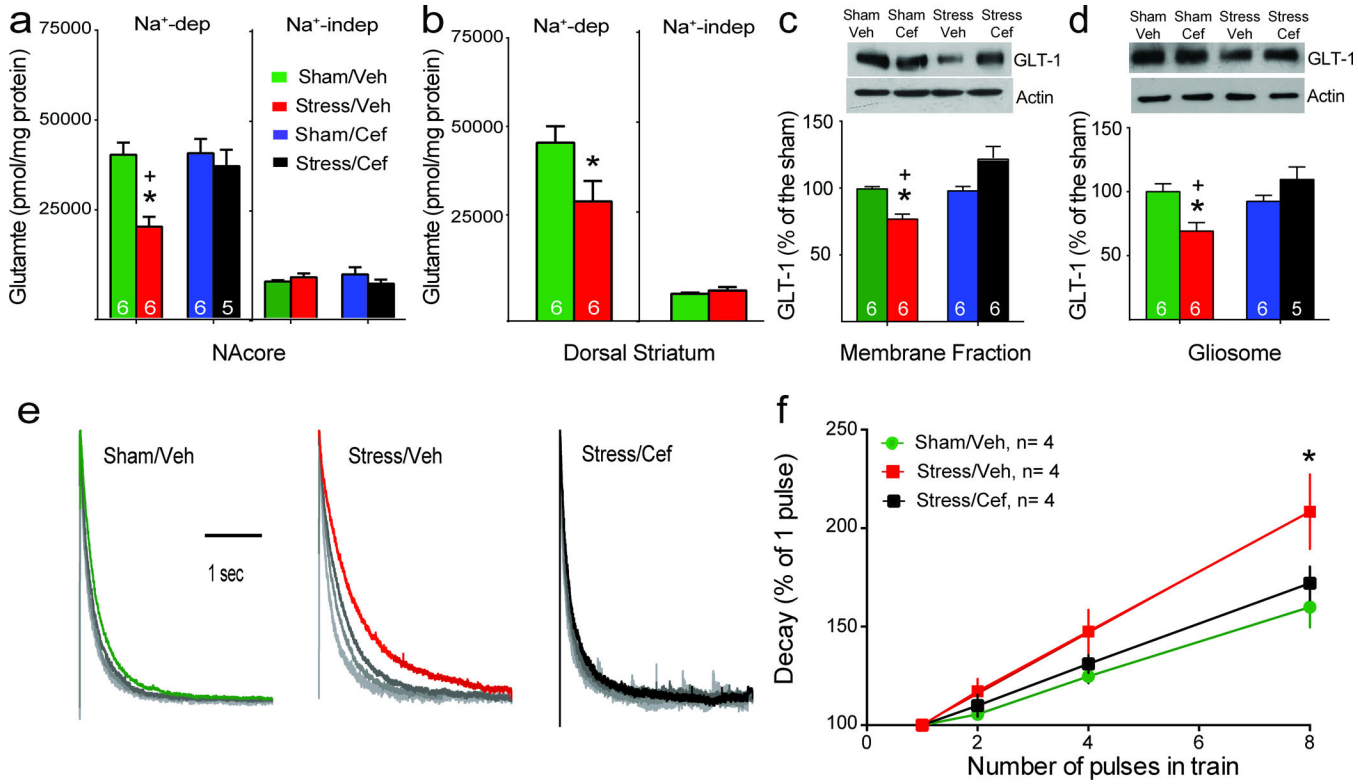

Figure 3.

Ceftriaxone (cef) restored stress-induced decrease in glutamate uptake and synaptic glutamate overflow. **a)** Acute stress reduced Na^+-dependent, but not Na^+-independent uptake of ^3H-glutamate into slices of the NAcore compared to sham animals. Na^+-dependent uptake, 2-way ANOVA stress vs sham $F_{(1,19)}= 8.98$, $p< 0.01$; Veh vs Cef $F_{(1,19)}= 11.12$, $p< 0.001$; interaction $F_{(1,19)}= 10.20$, $p< 0.001$. N is shown in bars. **b)** Acute stress reduced Na^+-dependent, but not Na^+-independent glutamate uptake into slices of the dorsal striatum. Student's t-test $t_{(10)}= 2.25$, $p< 0.05$. N is shown in bars. **c–d)** GLT-1 protein content was reduced in NAcore by acute stress in both the whole cell lysates (2-way ANOVA Veh vs Cef $F_{(1,20)}= 12.98$, $p< 0.01$; interaction $F_{(1,20)}= 19.78$, $p< 0.001$) and gliosome subfraction (2-way ANOVA Veh vs Cef $F_{(1,19)}= 4.86$, $p< 0.05$; interaction $F_{(1,19)}= 10.57$, $p< 0.01$). N is shown in bars. **e)** Representative examples of increasing NMDA decay time with increasing number of 100 Hz trains (1,2,4,8). Colored trace shows 8 trains for each treatment group. **f)** Stress-induced increase in NMDA decay time in the NAcore with an 8-pulse 100 Hz train (reflecting increased synaptic glutamate spillover) was normalized by ceftriaxone treatment; 2-way ANOVA treatment $F_{(2,64)}= 6.14$, $p= 0.005$; Veh vs Cef $F_{(3,64)}= 50.49$, $p< 0.001$. N is shown in the graph legend.

Data are shown as mean (± SEM). *$p< 0.05$, compared to sham using a Bonferroni's post hoc, $^+p< 0.05$, compared to stress/Cef using a Bonferroni's post hoc.

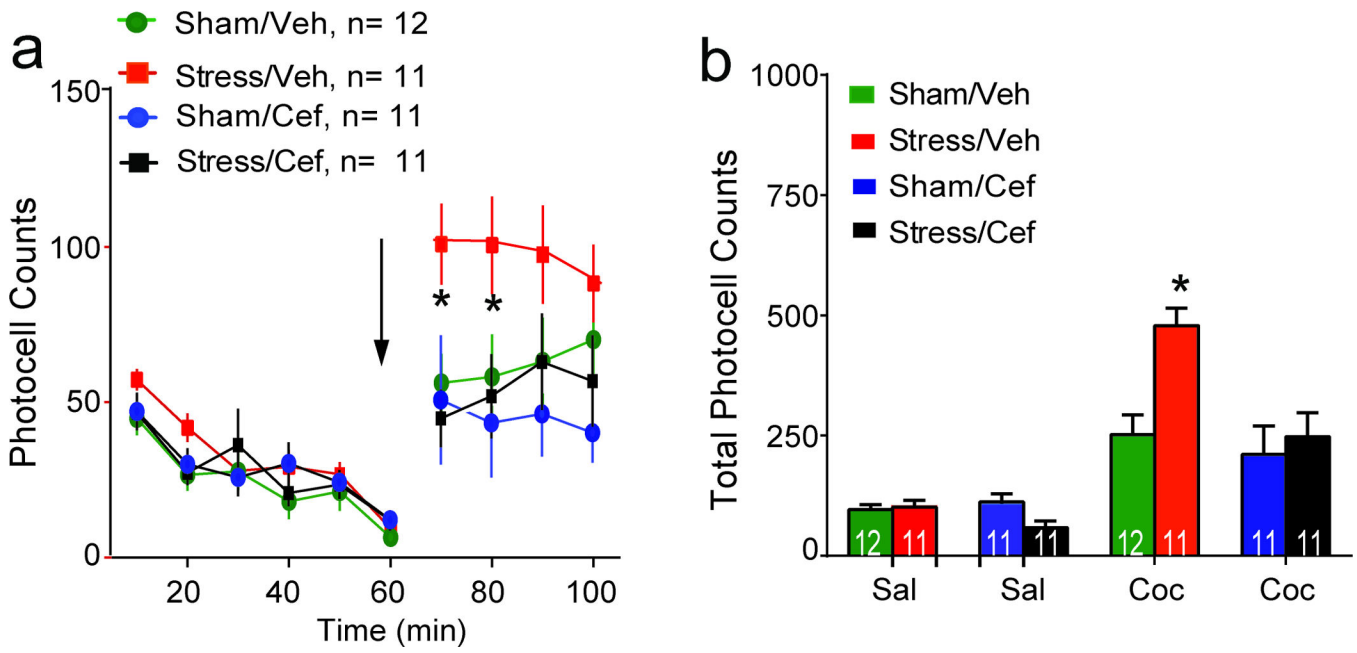

Figure 4.

Ceftriaxone reversed stress-induced potentiation in locomotor response to acute cocaine administration. **a)** Time course of stress-induced potentiation of the motor stimulant effect of cocaine. 2-way ANOVA stress v sham $F_{(1,21)}= 5.68$, $p< 0.05$; time $F_{(9,189)}= 25.27$, $p< 0.001$; interaction $F_{(9,189)}= 2.03$, $p< 0.05$. N is shown in the graph legend **b)** Total photocell counts over 40 min show stress-induced sensitization to motor stimulant effect of cocaine compared to sham and ceftriaxone pre-treatment abolished the sensitization. 2-way ANOVA stress vs sham $F_{(1,80)}= 4.83$, $p< 0.05$; saline v cocaine $F_{(3,80)}= 27.81$, $p< 0.001$; interaction $F_{(3,80)}= 5.74$, $p< 0.01$. N is shown in bars.

Data are shown as mean (± SEM). *$p< 0.05$, comparing Stress/Veh to all other groups using a Bonferroni's post hoc.

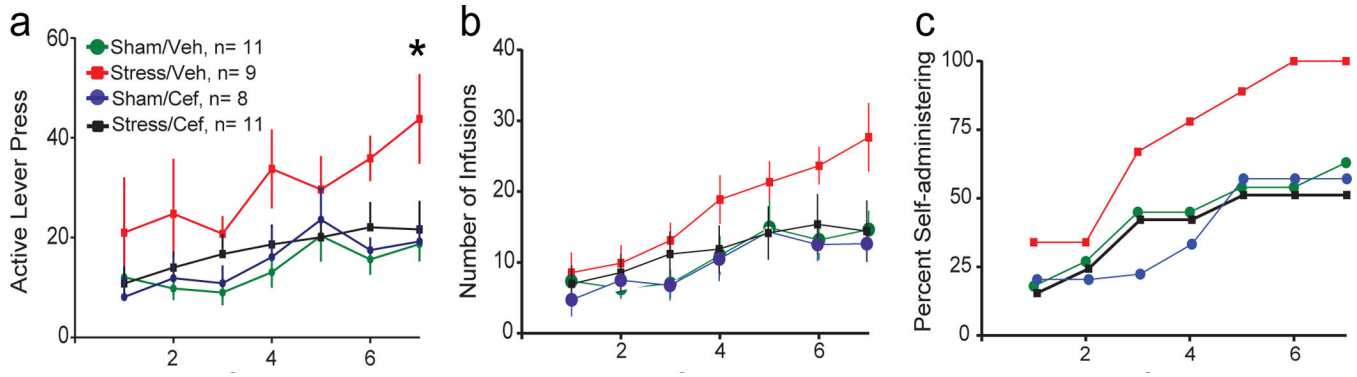

Figure 5.

Ceftriaxone reversed stress-induce potentiation in acquisition of cocaine self-administration. **a)** Number of active lever presses over the 10-day test for acquiring cocaine self-administration vehicle (Veh) vs ceftriaxone (Cef) pre-treatment. 2-way ANOVA treatment $F_{(3,35)}= 3.16$, *p= 0.037, comparing Sham/Veh to all other groups. N is shown in the graph legend. Data are shown as mean (± SEM). **b)** Number of cocaine infusions over the 7-day test for acquiring cocaine self-administration were not statistically different between treatment groups. **c)** Using a criterion of the first day a rat self-administered 10 or more cocaine infusions, the acquisition of cocaine self-administration trended higher in Stress/Veh compared with other treatment groups. and ceftriaxone pre-treatment reduced the percentage of animals achieving criterion. $Chi^2_{(3)}= 5.51$, p= 0.138.