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Ceftriaxone attenuates acquisition and facilitates extinction of cocaine-induced suppression of saccharin intake in C57BL/6J mice

Christopher S. Freet^a and Antoneal L. Lawrence^b

^aThe Pennsylvania State University College of Medicine, Department of Neural and Behavioral Sciences, 500 University Drive, Hershey, PA 17033, USA

^bThe Lincoln University, 1570 Baltimore Pike, Lincoln University, PA 19352, USA

Abstract

Growing evidence implicates glutamate homeostasis in a number of behaviors observed in addiction such as acquisition of drug taking, motivation, and reinstatement. To date, however, the role of glutamate homeostasis in the avoidance of natural rewards due to exposure to drugs of abuse has received little attention. The aim of the current study was to evaluate the beta-lactam antibiotic, ceftriaxone, which has been shown to normalize disrupted glutamate homeostasis associated with exposure to drugs of abuse, in cocaine-induced suppression of saccharin intake in C57BL/6J mice. Briefly, C57BL/6J mice received daily injections of either 200 mg/kg ceftriaxone or saline. Mice were then given access to 0.15% saccharin for 1 hour and immediately injected intraperitoneally with either saline or 30 mg/kg cocaine; taste-drug pairings occurred every 24 hours for 5 trials followed by a final CS only trial. One week following taste-drug pairings, extinction was evaluated in a series of one- and two-bottle saccharin intake tests. Individual differences in cocaine-induced suppression were observed (i.e., low and high suppressors) with differential effects of ceftriaxone. Ceftriaxone delayed suppression of saccharin intake in high suppressors but prevented suppression in low suppressors. In addition, ceftriaxone history facilitated extinction in the high suppressors. These data suggest that changes in glutamate homeostasis may be involved in the formation and expression of cocaine-induced suppression of saccharin intake in mice.

Keywords

addiction; glutamate; glia; natural rewards; reward comparison

Corresponding Author: Christopher S. Freet, Department of Neural and Behavioral Sciences, The Pennsylvania State University College of Medicine, 500 University Drive, H181, Hershey, PA 17033 USA, Phone: (717) 531-3758, FAX: (717) 531-6916, csf5@psu.edu.

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1. INTRODUCTION

Disruption of glutamate homeostasis has been associated with a number of neurological diseases including Alzheimer's disease, Parkinson's disease, and schizophrenia and it is now recognized that glutamate release and clearance are altered during the development of addiction as well (for review, see Bridges *et al.*, 2012). In the nucleus accumbens (NAc), cocaine-induced synaptic plasticity results in elevated presynaptic glutamate release in response to cocaine and increased postsynaptic sensitivity to glutamate (Luscher, 2013, Smith *et al.*, 1995). Cocaine also decreases function of EAAT-2 (GLT-1), a major glutamate transporter responsible for synaptic glutamate clearance (Knackstedt *et al.*, 2010, Rothstein *et al.*, 1996). In the ventral tegmental area (VTA), even a single injection of cocaine produces a persistent (~1 week) increase in the AMPA- to NMDA-mediated synaptic current ratio (Ungless *et al.*, 2001).

While glutamate is elevated in the synaptic space in response to cocaine, evidence indicates that glutamatergic tone in the perisynaptic space is decreased. The loss of glutamatergic tone appears to be, at least in part, due to disruption in the function of the astrocytic cystine-glutamate exchanger. Under drug-naïve conditions, the exchanger serves as an antiporter, importing extrasynaptic cystine and exporting intracellular glutamate at a 1:1 ratio (Bannai, 1986, Bannai and Kitamura, 1980). This process contributes up to 60% of the 1–3 μM glutamate in the perisynaptic space (Baker *et al.*, 2002). Repeated cocaine exposure decreases exchanger function as demonstrated by the increased K_m of cystine uptake in NAc tissue slices (Baker *et al.*, 2003, Bridges *et al.*, 2012) and blockade of the cystine-glutamate exchanger decreases extracellular glutamate levels in drug-naïve, but not cocaine-withdrawn, rats (Baker *et al.*, 2003).

Interestingly, the beta-lactam antibiotic ceftriaxone has been shown to normalize deficits in cystine-glutamate exchanger (and GLT-1) function associated with exposure to cocaine (Knackstedt *et al.*, 2010, Trantham-Davidson *et al.*, 2012). Ceftriaxone also attenuates a number of cocaine-related behaviors in rodent models including drug-taking behavior (self-administration, Ward *et al.*, 2011), motivation for cocaine (progressive ratio, Ward *et al.*, 2011), and relapse (cue- and drug-primed reinstatement, Knackstedt *et al.*, 2010, Sari *et al.*, 2009, Sondheimer and Knackstedt, 2011). Based on these data, we hypothesized that ceftriaxone also will attenuate the drug-induced avoidance of natural rewards as modeled by cocaine-induced suppression of saccharin intake in mice (Grigson, 1997, Grigson and Twining, 2002, Grigson *et al.*, 2009). Mice and rats avoid intake of a palatable natural reward (such as saccharin) when paired with a drug of abuse such as cocaine (Cappell *et al.*, 1973, Carey and Goodall, 1974, Goudie *et al.*, 1978). Data demonstrate greater drug-induced suppression of saccharin intake is correlated with greater cocaine self-administration (Grigson and Twining, 2002) and elevated corticosterone levels (Gomez *et al.*, 2000) and a single saccharin-morphine pairing blunts accumbens dopamine release in response to saccharin (Grigson and Hajnal, 2007). While dopamine and drug-induced suppression has received a fair amount of attention, the present study represents an initial examination into the relatively unexplored role of glutamate signaling and homeostasis in this model.

2. METHODS

2.1 Subjects

Subjects were 60 naïve male C57BL/6J mice obtained from Jackson Laboratories. Mice weighed between 17.3 and 22.4 grams (mean: 20.2 grams) at the beginning of the experiment. All subjects were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 am) and experimental manipulations begun 1.5 hours into the light phase of the cycle. Mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water except where otherwise noted.

2.2 Apparatus

All experimental manipulations were conducted in the home cages. Modified serological pipettes (Fisher Scientific; 07-200-574) were used to provide purified H₂O and saccharin access. Pipettes were converted to plastic cylinders by removing the tapered ends. A stainless steel spout (Girton Manufacturing Co., Inc.; ST300C) was then placed in the bottom of the cylinder while a silicon stopper (Fisher Scientific; 09-704-1D) sealed the top of the cylinder. Intake of H₂O and saccharin was recorded in 1/10 milliliters.

2.3 Procedure

Procedural organization and time course of all phases is illustrated in Figure 1. *Baseline intake.* All subjects were weighed once a day throughout the study. For baseline measurements, all mice were placed on a water restriction schedule which consisted of access to purified H₂O for 1 hour in the morning and tap water (for rehydration) 2 hours in the afternoon. Baseline intake and body weight were recorded for 8 days (Trials B1–B8). Daily intraperitoneal (i.p.) administration of 200 mg/kg ceftriaxone (n=30) or saline (n=30) began six days into baseline intake (Trial B6) and continued up to and including the fifth taste-drug pairing (Trial 5) for a total of eight daily injections. Ceftriaxone or saline injections were administered six hours into the light cycle (1:00 pm), at least 1 hr after morning intake testing and 1 hr before afternoon rehydration. *Saccharin-cocaine pairings.* Mice remained on the water restriction schedule as described above during taste-drug pairings. For each pairing (which occurred every 24 hours), all mice received 1 hour access to 0.15% saccharin followed by an i.p. injection of either saline (n=15/cell) or 30 mg/kg cocaine (n=15/cell). The 30 mg/kg cocaine dose was used in the current study as published data (Freet *et al.*, 2013) indicates that, in C57BL/6J mice at least, it is the minimum dose required for significant suppression of saccharin intake (i.e., 10 and 20 mg/kg are ineffective in this strain). Taste-drug pairings occurred in the morning for 5 trials (Trials 1–5). A sixth trial was conducted with CS only access (no injection, Trial 6). *Extinction testing.* Seven days following taste-drug pairings, extinction testing began. All mice received the same 3-day cycle, repeated five times (total of 15 extinction trials, E1–E15). Each 3-day extinction cycle consisted of two 1-bottle test days followed by a 2-bottle test day. On 1-bottle test days, all mice received 1 hr access to 0.15% saccharin; for 2-bottle test days all mice received 1 hr access to 0.15% saccharin and purified H₂O simultaneously. The location of saccharin and H₂O were counterbalanced within groups and across test days. In all phases of

testing, saccharin and H₂O were presented at room temperature; cocaine HCl was provided by the National Institute on Drug Abuse.

2.4 Statistical Analysis

Repeated measures analysis of variance (ANOVA) varying appropriate factors were conducted using Statistica 7.1 software, Stat Soft, Inc., Tulsa, OK. Newman-Keuls post hoc analyses of significant interactions were conducted where appropriate.

3. RESULTS

3.1 Saccharin Intake in Saccharin-Cocaine Pairings

Saccharin intake was analyzed using $2 \times 2 \times 6$ repeated measures ANOVA varying Treatment (saline, ceftriaxone), Drug (saline, cocaine), and Trials (1–6) and the data are presented in Figure 2. Although there was a significant main effect of Treatment [$F(1,56)=35.63$, $p<.001$], Drug [$F(1,56)=33.79$, $p<.001$], and Trials [$F(5,280)=22.55$, $p<.001$], the Treatment $\times$ Drug $\times$ Trials interaction was not significant [$F(5,280)=0.16$, $p=.97$]. Given the significant main effect of Treatment, separate 2×6 repeated measures ANOVAs varying Drug (saline, cocaine) and Trials (1–6) for each Treatment (saline, ceftriaxone) were conducted. In the saline treatment, Newman-Keuls post hoc analysis of the significant 2-way interaction [$F(5,140)=9.07$, $p<.001$] indicates that, compared with saline controls, the cocaine group demonstrated significant suppression on all trials [$p<.01$]. In the ceftriaxone treatment, Newman-Keuls post hoc analysis of the significant 2-way interaction [$F(5,140)=6.88$, $p<.001$] indicated that the cocaine group demonstrated significant suppression on trials 3–6 [$p<.01$].

3.2 Saccharin Intake in Saline Controls

A separate 2×6 repeated measures ANOVA varying Treatment (saline, ceftriaxone) and Trials (1–6) was conducted to evaluate saccharin intake by the saline groups. The main effect of Treatment was significant [$F(1,28)=12.12$, $p<.01$]; however the main effect of Trials [$F(5,140)=2.07$, $p>.05$] and the 2-way interaction [$F(5,140)=1.72$, $p=.13$] were not significant indicating that although the ceftriaxone treatment group had higher intake, they did not drink saccharin differently across trials compared with the saline treatment group.

3.3 Individual Differences

Previous literature has demonstrated individual differences in cocaine-induced suppression, i.e., with the same dose of cocaine, some rats or mice within a strain will demonstrate small suppression of intake while others show large suppression (Grigson and Twining, 2002). As individual differences were observed in the current experiment, the cocaine groups were divided post hoc into low suppressors ($n=7/\text{cell}$) and high suppressors ($n=8/\text{cell}$) for analysis using a median split of terminal intake (average of trials 5–6). Similar to the previous analysis, saccharin intake was analyzed using $2 \times 3 \times 6$ repeated measures ANOVA varying Treatment (saline, ceftriaxone), Drug (saline, cocaine [low suppressors], cocaine [high suppressors]), and Trials (1–6) and the data are presented in Figure 3. As observed previously, there was a significant main effect of Treatment [$F(1,54)=51.33$, $p<.01$], Drug [$F(2,54)=31.38$, $p<.01$], and Trials [$F(5,270)=33.05$, $p<.01$], but the Treatment $\times$ Drug $\times$

Trials interaction was not significant [$F(10,270)=0.24$, $p=.99$]. Separate 3×6 repeated measures ANOVAs varying Drug (saline, cocaine [low suppressors], cocaine [high suppressors]) and Trials (1–6) for each Treatment (saline, ceftriaxone) were conducted. In the saline treatment, Newman-Keuls post hoc analysis of the significant 2-way interaction [$F(10,135)=5.30$, $p<.01$] indicates that the high suppressor cocaine group demonstrated significant suppression on all trials [$p<.01$], while the low suppressor cocaine group demonstrated significant suppression only on trials 3–6 [$p<.02$]. Low and high suppressor groups were significantly different on trials 2, 5, and 6 [$p<.05$]. In contrast, in the ceftriaxone treatment, Newman-Keuls post hoc analysis of the significant 2-way interaction [$F(10,135)=4.85$, $p<.01$] indicated that the high suppressor cocaine group demonstrated significant suppression on trials 3–6 [$p<.01$], while the low suppressor cocaine group never demonstrated significant suppression of intake of the cocaine-paired cue [$p>.05$]. Low and high suppressor groups were significantly different on trials 2–6 [$p<.05$]. These results indicate that significant cocaine-induced suppression of saccharin intake was evident in high suppressors in all trials under the saline treatment while suppression was delayed until trial 3 in the ceftriaxone treatment. In addition, while the low suppressor group in the saline condition demonstrated significant suppression of saccharin intake on trial 3, ceftriaxone administration prevented suppression.

3.4 Baseline Water Intake

A $2 \times 2 \times 8$ repeated measures ANOVA varying Treatment (saline, ceftriaxone), Drug (saline, cocaine), and Trials (B1–B8) of baseline water intake prior to taste-drug pairings did not produce a significant 3-way interaction [$F(7,392)=.48$, $p=.85$]. The main effects of Treatment [$F(1,56)=2.83$, $p>.05$] and Drug [$F(1,56)=.84$, $p>.05$] were non-significant; the main effect of Trials [$F(7,392)=22.94$, $p<.001$] was significant. The results indicate that, although mice increased intake of water over the baseline period, there were no significant differences in water intake prior to taste-drug pairings or as a result of ceftriaxone administration on trials B6–B8 (data not shown).

3.5 Saccharin Intake in 2-Bottle Extinction

Extinction of the avoidance behavior for saccharin was evaluated using the 2-bottle intake data from trials E3, E6, E9, E12, and E15 (the 2-bottle data from Cycles 1–5) only as it was more informative and generally paralleled 1-bottle data. A $2 \times 2 \times 2 \times 5$ repeated measures ANOVA varying Treatment (saline, ceftriaxone), Drug (saline, cocaine), Bottle (saccharin, water), and Cycle (cycles 1–5) was conducted to evaluate saccharin and water intake over extinction cycles (Figure 4). All main effects were significant [Treatment, $F(1,56)=6.51$, $p=.01$, Drug, $F(1,56)=7.08$, $p=.01$, Bottle, $F(1,56)=152.76$, $p<.001$, and Cycle, $F(4,224)=48.40$, $p<.001$], as was the Bottle $\times$ Drug interaction [$F(1,56)=13.23$, $p<.001$]. However, the Treatment $\times$ Drug $\times$ Bottle $\times$ Cycle interaction was not significant [$F(4,224)=0.33$, $p=.86$]. Given the significance of the main effects, separate one-way ANOVAs were conducted on the Drug variable to examine extinction of the avoidance behavior (i.e., difference in saccharin intake between the cocaine group and its saline control group). In the saline history condition, a one-way ANOVA for saccharin intake demonstrated significant difference between the saline and cocaine groups on cycles 1 [$F(1,28)=11.43$, $p<.01$], 2 [$F(1,28)=6.02$, $p<.05$], 4 [$F(1,28)=6.86$, $p<.05$], and 5 [$F(1,28)=4.31$, $p<.05$]; in the

ceftriaxone history treatment, the saline and cocaine groups differed on cycles 1 [$F(1,28)=4.69$, $p<.05$] and 2 [$F(1,28)=4.48$, $p<.05$]. One-way ANOVAs for water intake demonstrated significant difference between the groups in the saline history treatment on cycles 1 [$F(1,28)=6.08$, $p<.05$] and 2 [$F(1,28)=5.94$, $p<.05$] and in the ceftriaxone history treatment for cycle 1 [$F(1,28)=5.02$, $p<.05$]. In addition, extinction within each cocaine group (i.e., increasing saccharin intake across cycles for each group) was evaluated using t-test comparisons for each cycle against cycle 1 intake. Saccharin intake compared with cycle 1 intake was significantly higher on cycles 3–5 in both the saline [$p<.05$] and ceftriaxone [$p<.05$] history treatments. The results demonstrate that, under both the saline and ceftriaxone history treatments, mice that experienced saccharin-cocaine pairings initially drank less saccharin than saline controls but increased saccharin intake over cycles. However, whereas saline history treatment mice do not fully extinguish by the terminal test cycle, ceftriaxone history treatment mice fully extinguish by cycle 3.

3.6 Individual Differences

A $2 \times 3 \times 2 \times 5$ repeated measures ANOVA varying Treatment (saline, ceftriaxone), Drug (saline, cocaine [low suppressors], cocaine [high suppressors]), Bottle (saccharin, water), and Cycle (cycles 1–5) was conducted to evaluate individual differences in saccharin and water intake over extinction cycles (Figure 5). As with the undivided groups, all main effects were significant [Treatment, $F(1,54)=9.45$, $p<.01$, Drug, $F(2,54)=14.83$, $p<.001$, Bottle, $F(1,54)=153.93$, $p<.001$, and Cycle, $F(4,216)=39.95$, $p<.001$], as were the Bottle $\times$ Drug [$F(2,54)=20.75$, $p<.001$], Bottle $\times$ Treatment [$F(1,54)=4.43$, $p<.05$], and Bottle $\times$ Cycle [$F(4,216)=11.07$, $p<.001$] interactions, but the Treatment $\times$ Drug $\times$ Bottle $\times$ Cycle interaction was not significant [$F(4,216)=0.40$, $p=.92$]. One-way ANOVAs on Drug indicated that, for the saline and ceftriaxone history treatments, the saline and low suppressor cocaine groups were not significantly different in saccharin or water intake [$p>.05$]. High suppressor mice demonstrated a similar pattern to that observed in the undivided groups. That is, in the saline history condition, saccharin intake of the saline and higher suppressor cocaine groups were significantly different on all cycles [1: $F(1,20)=37.68$, $p<.001$, 2: $F(1,20)=16.35$, $p<.0001$, 3: $F(1,20)=18.17$, $p<.001$, 4: $F(1,20)=20.66$, $p<.001$, 5: $F(1,20)=18.90$, $p<.001$]; in the ceftriaxone history condition, the saline and high suppressor cocaine groups differed on cycles 1–4 [1: $F(1,21)=10.78$, $p<.01$, 2: $F(1,21)=11.27$, $p<.01$, 3: $F(1,21)=13.32$, $p<.01$, 4: $F(1,21)=7.33$, $p<.05$]. Water intake differed on cycle 1 [$F(1,20)=13.01$, $p<.01$] and 2 [$F(1,20)=6.29$, $p<.05$] in the saline history treatment and cycle 1 [$F(1,21)=5.03$, $p<.05$] in the ceftriaxone history treatment. The results demonstrate that low suppressors extinguished cocaine-induced suppression of saccharin intake on the first extinction test cycle regardless of saline or ceftriaxone history. High suppressors, however, demonstrated significant cocaine-induced suppression of saccharin intake in the initial cycle under both conditions but fully extinguished the avoidance behavior by the terminal cycle in the ceftriaxone history condition.

4. DISCUSSION

Given the current literature demonstrating that the beta-lactam antibiotic ceftriaxone attenuates a number of drug-related behaviors in rodent models (Knackstedt *et al.*, 2010,

Ward *et al.*, 2011), we hypothesized that ceftriaxone also would attenuate cocaine-induced suppression of saccharin intake, a model for the avoidance of natural rewards by drugs of abuse. The data in the current manuscript demonstrate that ceftriaxone delays acquisition of cocaine-induced suppression in C57BL/6J mice (Figure 2). Published literature of suppression in mice (e.g., Freet *et al.*, 2013, Freet *et al.*, 2014, Freet *et al.*, 2009) demonstrates that saline controls and drug groups start with similar saccharin intake on Trial 1. This makes sense, of course, given that drug (or saline) has yet to be experienced on that trial. Oddly, in the current set of experiments, the drug group in the saline treatment began below control intake. This group had not yet experienced either cocaine or ceftriaxone and appears to be anomalous in the literature. In addition, observation of individual differences in this group demonstrates that this effect was carried by only half of the mice (specifically high suppressors). Low suppressors did not start significantly different from controls. While this muddles interpretation somewhat, the authors believe it does not invalidate the extension of the drug-attenuating effects of ceftriaxone to drug-induced suppression of saccharin intake. We have observed in our lab that, with a higher dose of cocaine than that used in the current study, intake is not different from saline controls on Trial 1 and that significant suppression starts on Trial 2 for the saline treatment group and on Trial 3 for the ceftriaxone treatment group (unpublished findings). In addition, ceftriaxone prevents the development of cocaine-induced suppression in a population of C57BL/6J mice with a relatively low degree of suppression (low suppressors, Figure 3) in the current study.

If ceftriaxone does indeed attenuate cocaine-induced suppression of saccharin intake, it would be predicated that extinction of the avoidance behavior would be facilitated in mice with a history of ceftriaxone administration. One week following saccharin-cocaine pairings, mice were run through extinction testing (Figure 4). Mice with the saline treatment history demonstrated extinction of saccharin avoidance over the testing period but did not fully extinguish the behavior by the end of testing (i.e., the cocaine group was still significantly lower than saline controls). In contrast, mice with the ceftriaxone treatment history demonstrated avoidance of saccharin at the start of testing but fully extinguished the behavior by the end. Figure 5 presents individual differences (i.e., low and high suppressors) in the extinction testing; while low suppressor mice appear to have fully extinguished at the start of testing regardless of saline or ceftriaxone treatment history, high suppressor mice displayed a pattern similar to that observed with the undivided groups. That is, high suppressor mice with a saline treatment history did not fully extinguish while the ceftriaxone treatment history mice did fully extinguish the avoidance behavior.

A number of interpretations for the current results can be excluded based on the literature and current data. First, the attenuation of the cocaine-induced suppression is not likely due to hyperphagic effects of ceftriaxone. Although the data in the current manuscript demonstrates elevated intake of naturally rewarding saccharin in the ceftriaxone groups, this effect did not support a significant interaction across trials and was not evident in previous studies (unpublished data). In addition, baseline water intake was not elevated by ceftriaxone suggesting the antibiotic does not generally elevate fluid intake. In the literature, ceftriaxone does not produce appetite-stimulating effects or increase food intake. Ceftriaxone has been shown to alter acquisition and motivation for cocaine but not food in

C57BL/6 mice (Ward *et al.*, 2011). Second, the decrease in avoidance of the palatable cue may be due to a general disruptive effect on learning and memory by ceftriaxone. This also seems unlikely, although cognitive effects of ceftriaxone are under investigation. Evidence shows no effect of ceftriaxone on spatial memory (Morris water maze, Karaman *et al.*, 2013) or on motor skill learning (pellet retrieval task, Kim and Jones, 2013). Ceftriaxone has, however, been shown to rescue hippocampal memory deficits in AQP4 knockout mice (Yang *et al.*, 2013). That said, somewhat paradoxically, ceftriaxone may also disrupt certain hippocampal learning, specifically in novel object recognition tasks (Matos-Ocasio *et al.*, 2014). The degree to which such an effect would influence the observed attenuation of suppression (i.e., increased intake) in the current data is minimal as neophobia to a novel gustatory cue dissipates between the first and second taste access periods (Domjan, 1976, Monk *et al.*, 2014). Finally, the results presented are not likely due to increased locomotor activity in response to ceftriaxone as the antibiotic does not alter locomotor activity (open field test, Knackstedt *et al.*, 2010) and attenuates locomotor sensitization in response to cocaine (Sondheimer and Knackstedt, 2011, Tallarida *et al.*, 2013).

Although much remains unclear, the data suggest at least two novel concepts regarding the development and expression of cocaine-induced suppression of a palatable taste cue such as saccharin. First, the fact that ceftriaxone had an effect suggests that there may be at least transient changes in glutamate homeostasis during the formation and expression of drug-induced suppression. The involvement of glutamate in this process is supported by evidence linking glutamatergic activity to other forms of associative learning. For example, blockade of metabotropic glutamate receptor 5 (mGluR5) attenuates conditioned taste aversion (CTA, i.e., suppression of a palatable cue when paired with an aversive unconditioned stimulus such as LiCl) in rats (Schachtman *et al.*, 2003, Simonyi *et al.*, 2009) and increased phosphorylated NMDA subunit NR1 (p-NR1) expression in the prefrontal cortex has been observed during the acquisition, but not expression, of CTA (Marotta *et al.*, 2014). Similarly, inhibition of the vesicular glutamate transporter (VGLUT) with intracerebroventricular Chicago sky blue 6B (CSB6B) attenuates conditioned place preference (CPP) for methamphetamine (He *et al.*, 2014) and a similar disruption in morphine, methamphetamine, and cocaine CPP learning has been observed with activation of GLT-1 using gene transfer (Fujio *et al.*, 2005) or MS-153 administration (Nakagawa *et al.*, 2005). Future studies, however, would need to determine if similar mechanisms are involved in drug-induced suppression of a taste cue and begin to elucidate the contribution of the various elements of the glutamate system (e.g., ionotropic vs metabotropic receptors, synaptic vs perisynaptic mechanisms, etc.). A working hypothesis for the current data set would be that an initial decrease in glutamatergic tone (which would allow increased glutamatergic signaling) facilitates acquisition of drug-induced suppression of a saccharin cue. In low suppressors, normalization by ceftriaxone may be sufficient to block acquisition and prevent subsequent learning of the behavior; glutamate homeostasis in high suppressors, however, may not be normalized to the same degree and acquisition would proceed, although delayed and somewhat weakened as indicated by the facilitated extinction observed in the mice with a ceftriaxone history. Future studies will be needed to test the validity of this hypothesis and to characterize any molecular differences that may account for the individual differences.

It should be noted, however, that although the data in the current manuscript suggests the involvement of disruptions in glutamate homeostasis, the time course and modesty of the effect also suggests that it may not be the entire or only mechanism involved. The majority of the research to date has evaluated ceftriaxone and glutamate homeostasis in the context of chronic exposure to cocaine (i.e., weeks). There is little data demonstrating when cocaine begins to disrupt glutamate homeostasis; consequently, it is unknown whether acute exposure is sufficient to produce a homeostatic disruption that could be normalized by ceftriaxone. There are, however, a few studies that demonstrate attenuating effects of ceftriaxone in more acute designs. For example, ceftriaxone attenuates acute locomotor effects of cocaine [Tallarida, et al., 2013] and ceftriaxone begins to attenuate cocaine self-administration in mice by the 3rd day [Ward et al., 2011, but see Sondheimer et al., 2011]. The design in the current manuscript would probably more closely align with these studies but the possibility exists that the protective effect of ceftriaxone in drug-induced suppression may be due to, or at least includes, an additional mechanism.

Second, the fact that GLT-1 and the cystine-glutamate exchanger are astrocytic supports a possible role for glia in drug-induced suppression of a palatable taste cue. To the authors' knowledge, this is a novel area of research that remains unexplored. Current evidence, however, suggests that (as with glutamate in general) astrocytic factors also may be important in associative learning. For example, inhibition of glutamine synthetase, an astrocytic enzyme that converts glutamate to glutamine and is involved in normal neuronal glutamate transmission (Rothstein and Tabakoff, 1984, Zhou and Danbolt, 2014), disrupts temporal, but not spatial, memory in an associate trace learning task (Kant *et al.*, 2014). In addition, transgenic knockout mice deficient for gliotransmission also demonstrate decreased reinstatement of cocaine CPP, self-administration of cocaine, and cue-induced reinstatement (Turner *et al.*, 2013). With the paucity of literature available, it is difficult to characterize the role of astrocytes, or glia in general, in drug-induced suppression of a taste cue. However, the current data suggests that the phenomenon is influenced by non-neuronal cells and this is a novel avenue of research that also needs to be explored.

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Highlights

- We evaluated ceftriaxone in cocaine-induced suppression of saccharin intake.
- Ceftriaxone delays suppression of saccharin intake paired with cocaine.
- Ceftriaxone facilitated extinction of the saccharin avoidance behavior.

Figure 1. Experimental design

Baseline water intake occurred for 8 trials (Trials B1–B8) followed by 5 taste-drug pairings and a CS only test trial (Trials 1–6). Ceftriaxone was administered daily over 8 trials (Trials B6–5). Following 1 week, extinction testing began (Trials E1–E15). Extinction testing was comprised of five 3-day cycles consisting of two 1-bottle and a 2-bottle saccharin intake test.

Figure 2. Cocaine-induced suppression of saccharin intake

Mean ($\pm$ SEM) intake (ml/hr) of 0.15% saccharin paired with saline (open squares) or cocaine (closed squares) in C57BL/6J mice with saline treatment (left panel) or 200 mg/kg ceftriaxone treatment (right panel). Asterisks (*) indicate significant difference compared with saline controls, $p < .05$.

Figure 3. Individual differences in cocaine-induced suppression of saccharin intake
Mean ($\pm$ SEM) intake (ml/hr) of 0.15% saccharin paired with saline (open squares) or cocaine (low suppressors, closed circles, top panels; high suppressors, closed triangles, bottom panels) in C57BL/6J mice with saline treatment (left panels) or 200 mg/kg ceftriaxone treatment (right panels). Asterisks (*) indicate significant difference compared with saline controls, $p < .05$.

Figure 4. Two-bottle extinction test for saccharin intake

Mean ($\pm$ SEM) intake (ml/hr) of 0.15% saccharin and water over extinction cycles in C57BL/6J mice previously paired with saline (solid squares for saccharin, open squares for water) or cocaine (solid circles for saccharin, open circles for water) and with a treatment history of saline (left panel) or 200 mg/kg ceftriaxone (right panel). Asterisks (*) indicate significant difference between saccharin groups, $p < .05$. Pluses (+) indicated significant difference between water groups.

Figure 5. Individual differences in two-bottle extinction test for saccharin intake

Mean ($\pm$ SEM) intake (ml/hr) of 0.15% saccharin and water over extinction cycles in C57BL/6J mice previously paired with saline (solid squares for saccharin, open squares for water) or cocaine (low suppressors: solid circles for saccharin, open circles for water, top panels; high suppressors: solid triangles for saccharin, open triangles for water, bottom panels) and with a treatment history of saline (left panels) or 200 mg/kg ceftriaxone (right panels). Asterisks (*) indicate significant difference between saccharin groups, $p < .05$. Pluses (+) indicated significant difference between water groups.