

# Context-specific Enhancement of Glutamate Transmission by Cocaine

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*The repeated injection of cocaine causes an increase in the capacity of a subsequent acute injection to elevate extracellular glutamate levels in the nucleus accumbens, and the present study sought to determine if the elevation in extracellular glutamate is regulated by the pairing of environmental stimuli with drug administration. Three treatment groups were injected daily for seven days with saline or cocaine (15 mg/kg, ip); 1) injection of saline in the home cage, 2) injection of cocaine in the home cage (cocaine-unpaired), and 3) injection of cocaine in the test apparatus (cocaine-paired). Three weeks following the last daily injection dialysis probes were placed into the nucleus accumbens and all rats were injected with saline followed by cocaine. Basal levels of extracellular glutamate were significantly reduced in the cocaine-paired treatment group. Moreover, only in the cocaine-paired group did the cocaine injection elevate extracellular glutamate. Repeated administration of cocaine also produces an enduring increase in the motor stimulant response to an acute cocaine injection and it was previously found that administration of*

*the  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainic acid glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione inhibited the sensitized, but not the acute motor, response to cocaine. In this study it was found that the motor stimulant response elicited by cocaine was blunted by pretreatment of the nucleus accumbens with 6-cyano-7-nitroquinoxaline-2,3-dione only in animals receiving daily cocaine injections in the paired environment. In contrast, the N-methyl-D-aspartate glutamate receptor antagonist R-(-)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid did not significantly affect cocaine-induced motor activity in any treatment group. These data support a hypothesis that environmental stimuli previously associated with daily cocaine administration can modulate glutamate transmission in the nucleus accumbens in a manner affecting cocaine-induced behavior.*

**[Neuropsychopharmacology 23:335–344, 2000]**

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**KEY WORDS:** *Glutamate; Cocaine; Sensitization; Nucleus accumbens; AMPA; Dialysis*

The repeated administration of amphetamine-like psychostimulants produces enduring alterations in the be-

havioral response to subsequent drug administration. The behavioral adaptations include the induction of paranoia in humans, which has been modeled in rodents as the induction of a sensitized motor stimulant response to psychostimulant injection (Kokkinidis and Anisman 1980; Segal and Schuckit 1983; Robinson and Becker 1986). This animal model has been extensively employed over the last decade to elucidate the cellular adaptations that underlie the behavioral changes associated with repeated psychostimulant use. The research effort has been guided by two facts; 1) a critical molecular site of action for amphetamine-like psychostimulants is the dopamine transporter (Reith et al. 1986; Ritz et al. 1990; although see Rocha et al. 1998), and 2) dopa-

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Received December 1, 1999; revised February 1, 2000; accepted February 11, 2000.

mine transmission in the mesocorticolimbic dopamine projection from the ventral tegmental area to the nucleus accumbens is critical for the rewarding and motor stimulant actions of this class of drugs (Koob et al. 1993). Knowledge of the molecular action has precipitated the discovery of many cocaine-induced, dopamine-dependent neuroadaptations in the nucleus accumbens (see Nestler et al. 1993; White and Kalivas 1998, for reviews). Arising from this considerable research effort is the awareness that neither the emphasis on dopamine transmission nor the focus on the nucleus accumbens provides a complete profile of the neuroadaptations produced by repeated cocaine (Pierce and Kalivas 1997; Bardo 1998; Wolf 1998).

To some extent, exploration beyond molecular binding of cocaine to dopamine transporters is impelled by a realization that the expression of behavioral adaptations to repeated cocaine is potentially regulated by learned associations made between the pharmacological actions of the drug and environmental stimuli (Stewart and Vezina 1988; Robinson et al. 1998). Thus, the daily administration of psychostimulant in a test environment produces more robust behavioral sensitization than when the drug is given in the home environment (Weiss et al. 1989; Dietze and Kuschinsky 1994; Badiani et al. 1997). Due in part to the potent regulation of behavioral sensitization by learned associations, glutamatergic cortical and allocortical brain regions involved in learning and memory have become a focus in the search for substrates mediating behavioral sensitization to cocaine. This research tactic has resulted in the characterization of long-term changes by repeated cocaine administration in both pre- and postsynaptic glutamate transmission (White et al. 1995; Fitzgerald et al. 1996; Pierce et al. 1996; Reid and Berger 1996; Zhang et al. 1997; Ghasemzadeh et al. 1999; Churchill et al. 1999). Specifically, repeated administration of cocaine increases the capacity of a subsequent cocaine injection to elevate extracellular glutamate levels in the nucleus accumbens (Reid and Berger 1996; Pierce et al. 1996). In addition, the sensitized but not the acute motor stimulant effect of cocaine is inhibited by  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist microinjection into the nucleus accumbens (Pierce et al. 1996).

The present study was designed to determine if the changes in glutamate transmission can be regulated by specifically pairing or not pairing an environment with the daily injection of cocaine. The basal and cocaine altered levels of extracellular glutamate in the nucleus accumbens were measured in rats pretreated three weeks earlier with five daily injections of cocaine. In addition, the capacity of glutamate receptor blockade in the nucleus accumbens to inhibit the motor response to cocaine in the paired versus the unpaired environment was examined.

## METHODS

### Animals and Surgery

Male Sprague-Dawley rats weighing between 250–300 g (Simonsen Laboratories, Gilroy, CA) were individually housed with food and water made available *ad libitum*. A 12 hour light/dark cycle (7 a.m.–7 p.m. light) was used and all experimentation was carried out during the light cycle.

Surgeries were performed five to seven days after the arrival of the subjects to the AAALAC approved housing facility and all experimentation began one week following the operative procedure. Animals were anesthetized with Equithesin (3.0 ml/kg. ip) and a chronic indwelling guide cannula (20 gauge, 14 mm; Small Parts, Roanoke, VA) was aimed 3 mm above the dialysis site in the core of the nucleus accumbens (+1.2mm rostral/caudal,  $\pm 1.5$ mm medial/lateral,  $-0.5$ mm dorsal/ventral from interaural zero according to the atlas of Pellegrino et al. 1979). The core was targeted because a previous study indicated that the cocaine-induced changes in extracellular glutamate were more robust in the core than in the shell compartment of the nucleus accumbens (Pierce et al. 1996). In another study the same coordinates were employed except chronic bilateral microinjection cannulae (26 gauge, 14 mm; Small Parts) were stereotactically implanted one mm over the nucleus accumbens.

### Repeated Systemic Treatments

Following surgery each rat was randomly assigned to one of three treatment groups: 1) rats to receive repeated saline in the home cage (saline-home), 2) rats to receive repeated cocaine in the home cage (cocaine-unpaired) or 3) rats to receive repeated cocaine in the test apparatus (cocaine-paired). On the day prior to the start of daily drug injection (day 1), each subject was habituated to a photocell box (Omni-techn Electronics, Columbus, OH) that was located in a wooden box modified for conducting microdialysis experiments (see below). Each box was equipped with an air and light source, and food and water were present at all times. All rats were attached to the dialysis probe connector; however a dialysis probe was not inserted through the guide cannula into the nucleus accumbens. The adaptation lasted for three hours and the animals were disconnected from the dialysis probe connector and returned to the home cage. On each of the next seven days (days 2 through 8), all subjects were habituated to the dialysis apparatus for one hour. Following habituation, saline-home and cocaine-unpaired subjects were disconnected from the probe connector, returned to their respective home cages and injected one hour later with saline (1.0 ml/

kg, i.p.) or cocaine (15 mg/kg, i.p.), respectively. In contrast, the cocaine-paired subjects were left in the dialysis apparatus attached to the connector, were injected with cocaine (15 mg/kg, ip) and remained in the dialysis apparatus for three hours after injection. In another set of experiments the treatment groups and repeated saline or cocaine injection regimens were conducted as described above except subjects were not connected daily to the dialysis probe connector. These animals were used for the microinjection study described below.

### Microinjection and Glutamate Receptor Blockade

The day prior to the first microinjection, the rats were placed into the photocell box and given a sham microinjection that consisted of inserting an injector into the nucleus accumbens but not injecting any fluid. Fourteen to 23 days after discontinuing daily treatment with cocaine, rats were adapted to the photocell apparatus for 60 min and then microinjected with the AMPA/kainate glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 0.1 nmol; Tocus Neuramin, Essex, UK), the NMDA receptor antagonist R(-)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; 0.1 nmol; Tocus Neuramin) or vehicle (1% DMSO in saline) into the core of the nucleus accumbens. The doses of glutamate antagonist were chosen based upon previous studies demonstrated the effectiveness of these doses after intracranial microinjection (Kalivas and Alesdatter 1993; Pierce et al. 1996; Bell and Kalivas 1996). Following a 1-hour adaptation to the photocell cell apparatus (Omnitech Electronics), the obturators were removed from the microinjection guide cannulae and replaced by an injection needle (33 ga stainless steel) which extended 1 mm below the tip of the guide cannulae into the nucleus accumbens. Bilateral infusions were made over 60 seconds in a volume of 0.3  $\mu$ l/side. Twenty seconds after completing the microinjection the injector was removed and the obturators replaced. Five minutes later the rats were injected with saline (1.0 ml/kg, ip) or cocaine (15 mg/kg, ip) and were returned to the photocell apparatus where motor activity was monitored for 120 minutes. After repeated injections rats were divided into either a CNQX or CPP treatment group. At a minimum of three day intervals, each rat received each of four microinjections in random order including: 1) either CNQX (0.1 nmol) or CPP (0.1 nmol) in the accumbens core plus systemic saline; 2) DMSO (1% v/v) vehicle in the core plus systemic saline; 3) either CNQX or CPP plus cocaine (15 mg/kg, ip) or 4) DMSO vehicle plus cocaine.

### Microdialysis

The dialysis probes were constructed as described by Robinson and Wishaw (1988), with 1.5 to 2.5 mm of ac-

tive dialysis membrane exposed at the tip. The probes were inserted through the guide cannula into the nucleus accumbens the night prior to the experiment. The next day, dialysis buffer (5 mM KCl, 140 mM NaCl, 1.4 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgCl}_2$ , 5.0 mM d-glucose, plus 0.2 mM phosphate-buffered saline to give a pH value of 7.4 and a final sodium concentration of 140.7 mM) was advanced via a syringe pump (Harvard Instruments, Boston, MA) through the probe at a rate of 1.9  $\mu$ l/minute for two hours prior to collecting baseline dialysis samples. 20-minute baseline samples were collected, followed by an injection of saline (1.0 ml/kg, ip). After collecting six more samples (120 minutes), cocaine (15 mg/kg, ip) was injected and eight more dialysis samples (160 minutes) were collected.

### Quantification of Glutamate Content

The concentration of glutamate was determined using HPLC with fluorometric detection. The dialysis samples were collected into 10  $\mu$ l of mobile phase containing 2.0 pmoles of homoserine as the internal standard. The mobile phase for glutamate is described by Donzanti and Yamamoto (1988). A reversed-phase column (10 cm; ODS) was used to separate the amino acids and precolumn derivatization of amino acids with o-phthaldehyde was performed using an autosampler. Glutamate was detected by a fluorescence spectrophotometer (Shimadzu, Columbia, MD) using an excitation wavelength of 300 nm and an emission wavelength of 400 nm. Peak heights were measured and compared to an external standard curve for quantification.

### Histology

At the end of each study, all subjects were sacrificed for histological verification of cannula placement. Subjects were given an overdose of pentobarbital and sequentially perfused transcardially with warm PBS and a 4% formaldehyde solution. The brains were removed and stored in formaldehyde overnight, then coronally-sectioned at 100  $\mu$ m through the site of microinjection with a vibratome. The sections were mounted on gelatin-coated slides and stained with cresyl violet. An animal was used for data analysis if at least 50% of the active region of the probe traversed the core of the nucleus accumbens. In some subjects as much as 50% of the probe was dorsal to the nucleus accumbens in the striatum or ventral in the olfactory tubercle. In order to be included in data analysis it was required that the tips of the microinjection cannulae were bilaterally located in the core of the nucleus accumbens, similar to what is shown elsewhere (Pierce et al. 1996).

**Table 1.** Basal Levels of Extracellular Glutamate in the Nucleus Accumbens

Treatment Group	N	Glutamate (Pmol/sample)
Saline/home	10	111.8 ± 31.2
Cocaine/unpaired	14	68.0 ± 15.9
Cocaine/paired	19	45.3 ± 8.6*

Basal values were determined by averaging the three baseline samples for each animal. The data were statistically evaluated using a one-way ANOVA followed by a Fisher's PLSD post hoc comparison ( $F_{(2,40)} = 3.72$ ,  $p = 0.033$ ).

\* $p < .05$ , compared with saline/home

### Data Analyses

In the microdialysis experiment the basal levels of glutamate were significantly different between treatment groups (see Table 1). Therefore, the raw data were statistically evaluated using a two-way ANCOVA. In addition, the data were normalized to percent change from the average basal level of glutamate in each animal and analyzed using a two-way ANOVA with repeated measures over time. In the microinjection study comparisons of total photocell beam breaks over one hour after injection were made using a two-way ANOVA and the time-courses of behavioral data were evaluated using a two-way ANOVA with repeated-

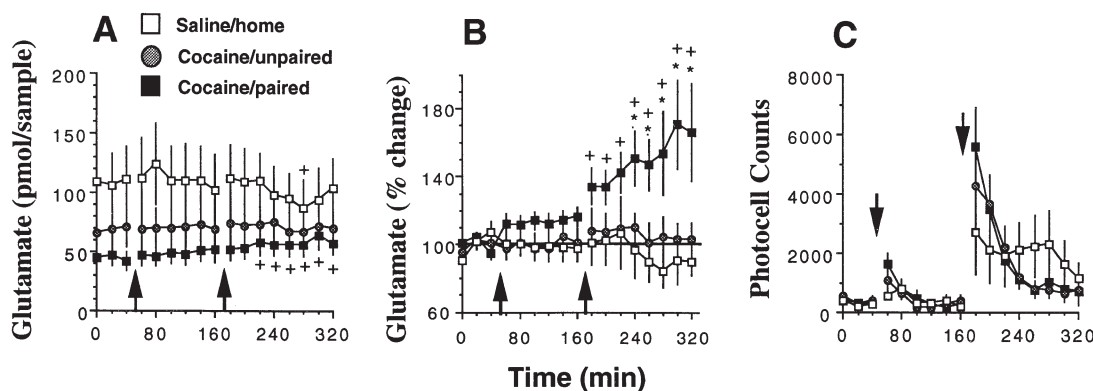
measures over time. When significant interaction F scores were identified, *post hoc* comparisons between acute treatments within each chronic treatment condition were performed using a one-way repeated measures ANOVA, while *post hoc* comparisons between chronic treatment groups were made using a least significant difference test (Milliken and Johnson 1984).

## RESULTS

### Cocaine Pairing with the Environment Augments Extracellular Glutamate in the Nucleus Accumbens

Figure 1 shows the effect of saline and cocaine (15 mg/kg, ip) administration on extracellular glutamate levels in the nucleus accumbens. The raw data shown in panel A reveal that the basal levels of extracellular glutamate differ between the treatment groups. The baseline data are summarized in Table 1, which shows that the level of extracellular glutamate was significantly reduced in the animals treated with cocaine in the paired environment. The group injected with cocaine in the home cage (cocaine-unpaired) showed intermediate values and did not significantly differ from either of the other two groups.

Evaluation of the raw data in Figure 1A using a two-way ANCOVA with basal values as the covariant re-



**Figure 1.** Cocaine increases extracellular glutamate only in the cocaine-paired subjects. Rats were administered daily cocaine or saline and three weeks later microdialysis was conducted in the nucleus accumbens. After collecting baseline samples, the subjects were injected with saline (first arrow; 1 ml/kg, ip), followed by an injection of cocaine (second arrow; 15 mg/kg, ip). Panel A shows the raw data as mean  $\pm$  sem pmol/sample, panel B shows the data normalized to basal levels as mean  $\pm$  sem percent change, and panel C shows the accumulation of horizontal photocell beam breaks obtained simultaneously with collecting the dialysis samples. The data are shown as mean  $\pm$  sem horizontal photocell counts accumulated over 20-minute intervals. For panels A and B the number of determinations in each group is indicated in Table 1. Behavioral data were not obtained for all animals due to technical difficulties and the N in each treatment group for panel D is as follows: saline/home = 9; cocaine/unpaired = 11; cocaine/paired = 18. The data were statistically evaluated using a two-way ANOVA (panels B and C) or ANCOVA (panel A) with repeated measures over time, and the significant F scores are shown in the text.

\*  $p < .05$ , comparing all groups with saline-home using a least significant difference *post hoc* comparison (Milliken and Johnson 1984).

+  $p < .05$ , comparing baseline to times after saline or cocaine injection within each chronic treatment group using a one-way ANOVA with repeated measures over time.

vealed a significant covariance ( $F[1,39] = 332.0$ ;  $p < .001$ ), as well as a significant effect of acute treatment ( $F[1,39] = 5.50$ ;  $p = .024$ ) and interaction between acute treatment and basal values ( $F[1,39] = 10.72$ ;  $p = .002$ ). *Post hoc* evaluation revealed a significant difference from baseline beginning at 60 minutes after cocaine administration (i.e. time = 220 minutes in Figure 1A) in the cocaine-paired treatment group that continued for the duration of the experiment. A significant difference was also measured between basal values and 120 minutes after cocaine injection (i.e. time = 280 minutes) in the saline-home group. No significant differences between baseline and saline or cocaine treatments were measured in the cocaine-unpaired group.

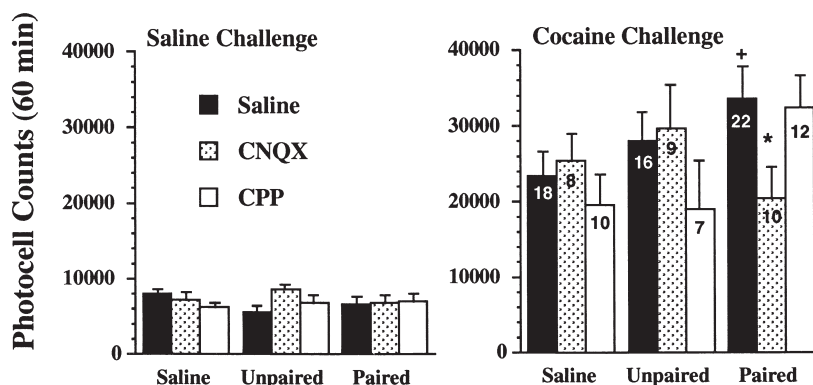
Because the baseline values were significantly different from each other, the data were normalized to percent change from the average baseline value for each animal. Figure 1B reveals that there was a significant treatment effect ( $F[2,40] = 3.86$ ;  $p = .030$ ), a significant change over time ( $F[17,680] = 3.67$ ;  $p < .001$ ), and a significant interaction between time and treatment ( $F[34,680] = 2.20$ ;  $p < .001$ ). Injection of saline (1.0 ml/kg, ip) did not alter the levels of glutamate in any treatment group. However, *post hoc* analysis revealed that the cocaine-paired group showed an increase in extracellular glutamate in response to an injection of cocaine (15 mg/kg, ip) beginning in the first 20 minutes after injection for the duration of the experiment. Cocaine produced no significant

change in the other chronic treatment groups, and the cocaine-induced elevation in glutamate in the cocaine-paired group was significantly greater than glutamate levels in the other two treatment groups beginning at 80 minutes after injection of cocaine.

Figure 1C shows the accumulation of photocell beam breaks obtained in association with collecting dialysis samples. A two-way ANOVA with repeated measures over time revealed a significant effect of time ( $F[17,578] = 17.59$ ;  $p < .001$ ), but no difference between treatment groups or an interaction between treatment group and time.

### Effect of CNQX and CPP on the Behavioral Response to Cocaine

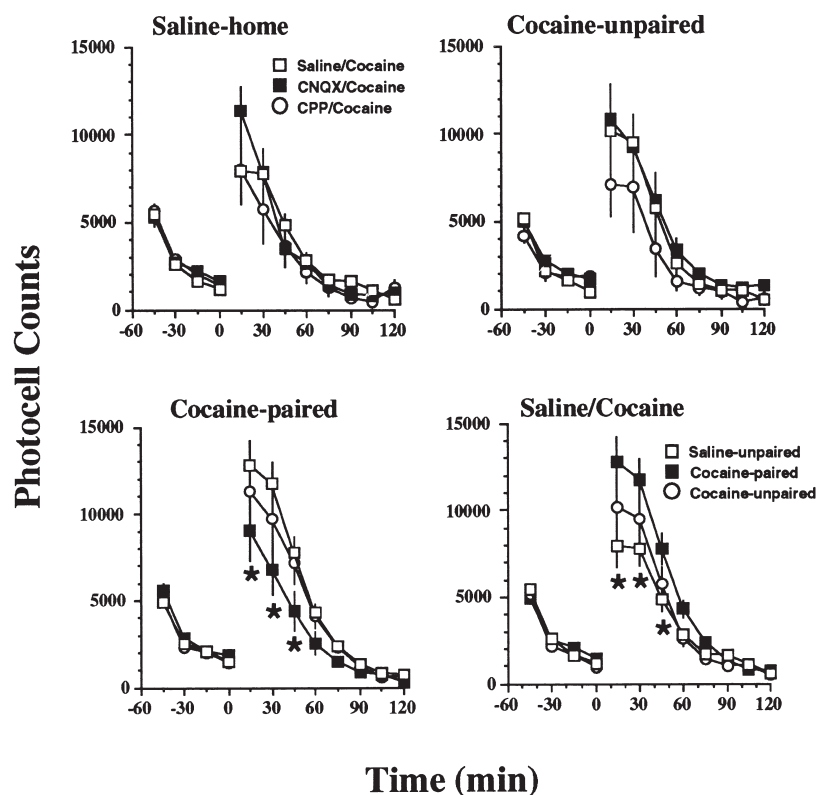
Figures 2 and 3 show the effect of blocking AMPA and NMDA receptors in the nucleus accumbens on the behavioral response to an injection of cocaine or saline made two to three wks after discontinuing daily cocaine or saline injections. Separate two-way ANOVAs with repeated measures over acute injection were used to evaluate the CNQX and CPP treatment groups. There was a significant effect of acute treatment in the CNQX group ( $F[3,72] = 39.47$ ,  $p < .001$ ), but no effect of chronic treatment group nor any interaction between chronic and acute treatments. *Post hoc* analysis was conducted using a one-way repeated measures ANOVA in over each



**Figure 2.** Blockade of AMPA glutamate receptors in the nucleus accumbens inhibits the behavioral response to cocaine only in the cocaine-paired subjects. Rats were divided into three daily drug treatment groups, including those given saline or cocaine in the home environment (home and unpaired, respectively) and those given cocaine in the photocell apparatus (paired). At two to three weeks after the last injection of daily cocaine or saline subjects were administered four different treatments given in random order using a 72-hour intertrial interval. The treatments consisted of all possible combinations of 1) 1% DMSO vehicle (0.5  $\mu$ l/side) + saline (1 ml/kg, ip), 2) CNQX (0.1 nmol/side) + saline, 3) 1% DMSO vehicle + cocaine (15 mg/kg, ip) or 4) CNQX + cocaine. In another group of animals the NMDA antagonist CPP (0.1 nmol) was substituted for CNQX. The data are shown as the total number of horizontal photocell counts measured during the first 60 minutes after treatment and the number of determinations in each group is indicated in the bar. All data were statistically evaluated using a two-way ANOVA (see text for significant F scores).

\*  $p < .05$ , comparing Sa/Cocaine with CNQX/Cocaine or CPP/Cocaine using a one-way ANOVA with repeated measures over microinjection treatments.

+  $p < .05$ , comparing Sa/Cocaine in the saline-home group to the other two daily treatment groups using a one-way ANOVA.



**Figure 3.** The time course of the behavioral data showing the capacity of CNQX to inhibit cocaine induced motor activity in the group of rats that were pretreated with daily cocaine in the test environment. An additional panel (Saline/Cocaine) shows the time course for the response to cocaine alone in each of the three chronic treatment groups. The data were derived from those shown in Figure 2 and are shown as mean  $\pm$  sem horizontal photocell counts. The data were statistically evaluated using a two-way ANOVA with repeated measures over time.

\*  $p < .05$ , comparing Saline/Cocaine with CNQX/Cocaine using a least significant difference *post hoc* analysis (Milliken and Johnson 1984).

chronic treatment group. In all groups the cocaine significantly elevated motor activity, but only in the cocaine-paired group did CNQX reduce cocaine-induced motor activity. In the CPP treatment group the only significant effect was acute treatment ( $F[3,72] = 39.23$ ,  $p < .001$ ). The *post hoc* analysis revealed that cocaine elevated motor activity in all groups, but in no group did CPP alter the stimulant effect of cocaine.

Figure 3 shows the time course of the behavioral response produced by cocaine in each of the treatment groups. Pretreatment of the nucleus accumbens with CNQX significantly reduced the motor response to cocaine only in the cocaine-paired group (drug  $F(2,42) = 2.24$ ,  $p = .119$ ; time  $F(11,462) = 95.50$ ;  $p < .001$ ; interaction  $F(22,462) = 1.92$ ,  $p = .008$ ). A least significant difference *post hoc* analysis revealed a significant reduction by CNQX during the first 60 minutes after cocaine administration. Neither glutamate receptor antagonist altered cocaine-induced motor activity in the saline-home treatment group (drug  $F(2,29) = 0.32$ ,  $p = .730$ ; time  $F(11,319) = 68.71$ ,  $p < .001$ ; interaction  $F(22,319) = 1.54$ ,  $p = .059$ ) nor in the cocaine-unpaired treatment group (drug  $F(2,29) = 1.54$ ,  $p = .232$ ; time  $F(11,319) = 50.96$ ;  $p < .001$ ; interaction  $F(22,319) = 0.70$ ,  $p = .814$ ). Figure 3 also compares the response to cocaine between the three chronic treatment groups and reveals that behavioral sensitization was present during the first 60 minutes after cocaine administration compar-

ing the saline-home with the cocaine-paired group (chronic  $F(2,53) = 3.59$ ,  $p = .035$ ; time  $F(11,583) = 116.06$ ,  $p < .001$ ; interaction  $F(22,583) = 2.87$ ,  $p < .001$ ). The cocaine-unpaired group showed intermediate values indicating that the sensitized motor response was only partly a conditioned response.

## DISCUSSION

The data in this study demonstrate that pairing daily cocaine administration with a specific environment augments the capacity of cocaine to elevate extracellular glutamate in the nucleus accumbens. Moreover, the lack of effect by AMPA/kainate receptor blockade in the cocaine-unpaired or saline control treatment groups indicates that glutamate transmission is consequential in the motor response only when cocaine-associated environmental cues are present.

### Glutamate Transmission and Cocaine

Previous studies have shown that cocaine alters glutamate transmission in the nucleus accumbens. The acute systemic administration of a high dose of cocaine increases extracellular glutamate in the nucleus accumbens (Smith et al. 1995; Reid et al. 1997), and after repeated cocaine treatment a lower dose of cocaine produces a rapid and enduring elevation in extracellular

glutamate (Pierce et al. 1996, 1998; Reid and Berger 1996). Moreover, by employing an arbitrary criterion to separate daily cocaine-treated subjects that developed behavioral sensitization from those that did not, Pierce et al. (1996) found that the most robust effect of cocaine on extracellular glutamate was measured in the animals developing sensitization. Since previous studies clearly show that the expression of behavioral sensitization is promoted by the presence of environmental stimuli specifically associated with previous drug injections (Weiss et al. 1989; Stewart 1991), it was hypothesized that the changes in glutamate transmission might mediate this learned association. The present data provide partial support for this postulate since only animals given cocaine paired with the test environment showed elevated extracellular glutamate levels. Moreover, blocking AMPA/kainate receptors in the nucleus accumbens attenuated the motor response in animals receiving daily cocaine in the test environment while it was without effect in the other treatment groups.

In spite of differences in extracellular glutamate there was no difference in the behavioral response to cocaine between treatment groups in the dialysis study. This is inconsistent with the concept that the elevation in extracellular glutamate is mediating behavioral sensitization in the paired environment. Moreover, the most robust differences in extracellular glutamate occurred beyond one hour after cocaine administration, which differs from the peak behavioral response elicited by cocaine that occurs during the first hour. Thus, while the elevation in extracellular glutamate may be permissive to the expression of behavioral sensitization, it is not a sufficient stimulus to elicit behavioral augmentation. However, the fact that CNQX microinjection inhibited the motor response to cocaine only in the cocaine-paired group supports a necessary role for glutamate transmission in the nucleus accumbens in the regulation of cocaine-induced motor behavior by environmental stimuli. Moreover, this dose of CNQX did not affect the acute motor response to cocaine in the saline-home group indicating a more critical role for glutamate in the repeated versus the acute effects of cocaine. Finally, it was reported that the motor response elicited by AMPA receptor stimulation in the nucleus accumbens is augmented in rats pretreated with daily cocaine (Pierce et al. 1996), and Bell and Kalivas (1996) demonstrated that the cross-sensitization between cocaine and AMPA is manifested only in a cocaine-paired environment. Taken together these data provide evidence for involvement of glutamate transmission in the nucleus accumbens when cocaine is administered in the presence of environmental stimuli previously associated with cocaine. However, the data also indicate that the in-

crease in glutamate transmission alone is not sufficient to elicit behavioral sensitization.

While used primarily as an AMPA/kainate antagonist, CNQX has some affinity for the NMDA receptors, posing possible involvement of this ionotropic glutamate receptor subtype. However, the selective competitive NMDA antagonist CPP was without effect on cocaine-induced motor activity in the cocaine-paired treatment group. Interestingly, CPP produced a trend towards a reduction in motor activity in the cocaine-unpaired and saline-home treatment groups. This may indicate a role for NMDA receptors in the unconditioned motor responses to cocaine and is consistent with previous studies showing that relatively high doses of NMDA antagonists in the nucleus accumbens inhibit the motor response to an acute injection of cocaine (Pulvirenti et al. 1991).

### Possible Anatomical Substrates

There are many possible neuronal sources of glutamate in the nucleus accumbens, including the prefrontal cortex, amygdala, hippocampus and periventricular thalamus (Deutch et al. 1993). Lesioning these brain nuclei either before or after administering repeated psychostimulant injections reveals that the prefrontal cortex may play a role in both the development and expression of behavioral sensitization, while lesions of the amygdala attenuated the development but not the expression of sensitization (Wolf et al. 1995; Browman et al. 1996; Pierce et al. 1998; however, see Li et al. 1999). Moreover, electrical stimulation of the prefrontal cortex, but not the hippocampus, produced behavioral sensitization to cocaine (Schenk and Snow 1994), and lesions of the dorsal prefrontal cortex (e.g. prelimbic and anterior cingulate cortices; Paxinos and Watson 1986) inhibited the capacity of cocaine to elevate extracellular glutamate in the nucleus accumbens in rats pretreated with repeated cocaine injections (Pierce et al. 1998). Taken together, these data are consistent with a hypothesis that the projection from the dorsal prefrontal cortex to the nucleus accumbens is important in the expression of behavioral sensitization to cocaine. That cortical glutamatergic projections may be especially important in the interaction between pharmacological effects of cocaine and drug paired environmental stimuli is supported by imaging studies in human cocaine addicts. Recent studies reveal that craving for cocaine induced by the presentation of stimuli previously paired with cocaine use increases metabolic activity in the anterior cingulate cortex and amygdala (Grant et al. 1996; Childress et al. 1999).

### The Source of Extracellular Glutamate and Technical Concerns with Dialysis

The relevance of dialysis measures of extracellular glutamate to ongoing neuronal activity is of some con-



cern. Most studies find that the basal level of extracellular glutamate is not reduced by blocking voltage-dependent calcium or sodium channels (Timmerman and Westerink 1997). In a previous study, we found that while the basal levels of extracellular glutamate were not sensitive to tetrodotoxin (TTX) blockade of sodium channels, the initial 20 to 40 minutes of the increase in extracellular glutamate elicited by an injection of cocaine was antagonized by TTX (Pierce et al. 1996). Further supporting neuronal involvement, the rise in extracellular glutamate produced by systemic cocaine administration was inhibited by lesions of the glutamatergic afferents to the nucleus accumbens arising from the prefrontal cortex (Pierce et al. 1998). Nonetheless, a contribution by nonvesicular glutamate is likely and probably accounts for the majority of the increase during the last two hours after administering cocaine (see Pierce et al. 1996; Xue et al. 1996; Wolf 1998, for further discussion of this possibility).

The source of the reduction in basal extracellular glutamate in the cocaine-paired environment is also difficult to discern with microdialysis. Given the apparently small contribution by vesicular glutamate towards the basal extracellular levels of glutamate (Timmerman and Westerink 1997), it seems likely that the reduction may arise from enhanced elimination of glutamate from the extracellular space via increased re-uptake. Glial transporters provide the greatest quantitative contribution to glutamate uptake in the brain (Rothstein et al. 1996). However, there is no clear mechanism by which different environmental stimuli can modulate glial function. Alternatively, cysteine-glutamate exchange makes a contribution towards maintaining extracellular glutamate levels in the brain (Warr et al. 1999; Jabaudon et al. 1999), and it is possible that the environment may modify this process.

Regardless of the source of extracellular glutamate, three observations in the present report support the potential functional relevance of the changes in extracellular glutamate. 1) The enduring reduction in basal extracellular levels of glutamate associated with placement of daily cocaine pretreated subjects in the cocaine-paired environment indicates that environmental context contributes to an alteration in glutamate disposition in the nucleus accumbens. 2) Only rats receiving cocaine in the drug-paired environment demonstrate enhanced responsiveness of extracellular glutamate to a cocaine injection. 3) Susceptibility of the cocaine induced motor responses to AMPA/kainate receptor blockade is present only in the cocaine-paired animals indicating that the cocaine-induced rise in extracellular glutamate may be functionally significant.

## Summary

This study reveals that extracellular glutamate is elevated by a cocaine injection only in the environment

where rats were pretreated with daily cocaine. Furthermore, only in the paired environment was the cocaine motor response inhibited by blocking AMPA/kainate receptors in the nucleus accumbens. The occurrence of these cocaine-induced effects only in the presence of environmental stimuli previously paired with repeated cocaine administration indicates a role for glutamate transmission in the nucleus accumbens in the environmental regulation of cocaine-induced behavior. In support of this notion, the elicitation of locomotor activity in experimental animals and of craving in cocaine addicts by cocaine-conditioned cues is associated with increased metabolism in cortical nuclei having glutamatergic projections to the nucleus accumbens (Brown et al. 1992; Grant et al. 1996; Childress et al. 1999). Taken together these data are consistent with a role for corticofugal glutamatergic afferents to the nucleus accumbens in modulating cocaine-induced motor activity by environmental stimuli.

## ACKNOWLEDGMENTS

We wish to thank Carol Heissenbottle for assisting in the preparation of this manuscript. This research was supported in part by USPHS grants MH-40817 and DA-03906, as well as a predoctoral NRSA DA-05642 (KB).

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