

# Repeated Cocaine Augments Excitatory Amino Acid Transmission in the Nucleus Accumbens Only in Rats Having Developed Behavioral Sensitization

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Rats were pretreated with daily cocaine or saline injections for 1 week. The rats treated with daily cocaine were separated into two groups: a sensitized group of animals demonstrating >20% increase in motor activity on the last injection compared with the first injection of daily cocaine, and a nonsensitized group showing <20% elevation. At 2–3 weeks after the last daily injection, four experiments were performed to assess changes in excitatory amino acid (EAA) transmission in the nucleus accumbens produced by repeated cocaine administration. (1) Rats were challenged with a microinjection of AMPA into the shell or core of the nucleus accumbens. The sensitized rats demonstrated greater motor activity than did the saline-pretreated or nonsensitized animals after AMPA injection into either subnucleus. (2) It was shown that the behavioral distinction between sensitized, nonsensitized, and *control* rats in be-

havioral responsiveness to AMPA was not mediated by differences in AMPA-induced dopamine release. (3) The extracellular content of glutamate was measured after a cocaine challenge given at 21 d of withdrawal. Cocaine elevated the levels of glutamate in the core of sensitized rats, but not of nonsensitized or control rats. (4) Microinjection of the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione into the core abolished the augmented motor response to a cocaine challenge in sensitized rats, but was without effect on cocaine-induced motor activity in nonsensitized animals. These results indicate that repeated cocaine administration increases EAA transmission in the nucleus accumbens only in rats that develop behavioral sensitization to cocaine.

**Key words:** *glutamate; cocaine; dopamine; nucleus accumbens; sensitization; microdialysis*

Repeated administration of amphetamine-like psychostimulants causes a progressive and enduring augmentation in behavioral activation that is called *behavioral sensitization* (Segal and Schuckit, 1983; Robinson and Becker, 1986; Post and Weiss, 1988; Kalivas and Stewart, 1991). Both the behavioral hyperactivity and the reinforcing properties of these drugs arise, in part, from their capacity to increase dopamine neurotransmission (Kelly and Iversen, 1975; Clarke et al., 1988; Koob et al., 1993). As a result, research has focused on identifying alterations in dopamine transmission that may mediate behavioral sensitization (for review, see Robinson and Becker, 1986; Kalivas and Stewart, 1991; Nestler, 1992; White et al., 1995b). Because psychostimulant abuse in humans can manifest as paranoid psychosis (Angst and Gershon, 1970; Post and Weiss, 1988; Sato, 1992) and because the mesoaccumbens dopamine projection has been postulated as a pathophysiological substrate for idiopathic psychosis (Goldstein and Deutch, 1992), many investigators have focused on a role for dopamine cell bodies in the ventral tegmental area and their axon terminals in the nucleus accumbens in the expression of behavioral sensitization to psychostimulants. To this end, a growing body of biochemical, electrophysiological, and behavioral evidence indicates that the mesoaccumbens dopamine system is involved in the expression of psychostimulant-induced behavioral

sensitization (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Nestler, 1992; White et al., 1995b).

Although the mesoaccumbens dopamine system appears to have a role in behavioral sensitization to psychostimulants, other neurotransmitter systems also may be important. Recent evidence suggests that alterations in excitatory amino acid (EAA) transmission contribute to the expression of behavioral sensitization. Behavioral sensitization to psychostimulants is inhibited by systemic administration of non-NMDA receptor antagonists (Karler et al., 1991, 1994) and by lesions of EAA projections from the amygdala, hippocampus, or prefrontal cortex to the nucleus accumbens (Yoshikawa et al., 1991; Pert et al., 1992; Dahlin et al., 1994).

Four experiments were conducted to evaluate the effect of repeated cocaine administration on EAA transmission in the nucleus accumbens. The subnuclei of the nucleus accumbens, the shell and core, were examined independently, because recent studies have identified functional distinctions between these two accumbal compartments (Deutch and Cameron, 1992; Maldonado-Irizarry and Kelley, 1994; Pulvirenti et al., 1994; Kalivas and Duffy, 1995; Pierce and Kalivas, 1995). All rats were pretreated for 1 week with daily cocaine or saline, and four experiments were conducted between 14 and 23 d after discontinuing daily administration. The following steps were taken. (1) Motor activity elicited by the microinjection of the non-NMDA agonist AMPA into the shell and core of the nucleus accumbens was measured. (2) AMPA-induced increase in extracellular dopamine was measured in the core and shell. (3) Cocaine-induced changes in extracellular levels of glutamate were measured in the shell and core. (4) The capacity of microinjection of the AMPA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) into

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the core to alter the motor stimulant response to a cocaine challenge was determined.

## MATERIALS AND METHODS

**Animal housing and surgery.** Male Sprague–Dawley rats (Simmons Laboratories, Gilroy, CA) were housed individually with food and water available *ad libitum*. A 12 hr light/dark cycle was used, with the lights on at 6:30 A.M. All cocaine injections, behavioral testing, and microdialysis were performed during the light cycle.

Before surgery, rats weighing 250–350 gm were anesthetized with Equithesin (3.0 ml/kg) and mounted in a stereotaxic apparatus. For dialysis and microinjection experiments, cannulae (12 mm, 20-gauge stainless steel for dialysis; 14 mm, 26-gauge for microinjection) were implanted bilaterally 3 or 1 mm, respectively, dorsal to the nucleus accumbens shell or core (9.0 mm anteroposterior; 0.8 or 2.0 mm medio-lateral, respectively; 0.0 mm dorsoventral; relative to the interaural line) (Pellegrino et al., 1979) and cemented in place by affixing dental acrylic to three stainless steel screws that were tapped into the skull.

**Repeated cocaine or saline treatment.** In both behavior and dialysis experiments, all subjects were assigned to either the cocaine or the saline group after at least 7 d of postoperative recovery. The day before the start of the experiment, all animals were habituated to the photocell boxes (Omnitech Electronics, Columbus, OH) for 3 hr. On the first treatment day, all animals were habituated to the photocell boxes for 1 hr. After habituation, animals received either cocaine (15 mg/kg, i.p.; donated by the National Institute of Drug Abuse, Bethesda, MD) or saline (1.0 ml/kg, i.p.), and subsequent behavior was monitored for 2 hr. On days 2–6, rats received daily injections of cocaine (30 mg/kg, i.p.) or saline in the home cage. On day 7, all animals were habituated again to the photocell boxes for 1 hr, followed by the administration of 15 mg/kg, i.p., cocaine or saline, and behavior was monitored for 120 min after injection. This cocaine treatment protocol has been shown to induce behavioral sensitization as well as alterations in dopamine transmission (Kalivas et al., 1993). A lower dose of cocaine was administered for behavioral testing (i.e., days 1 and 7), because this dose is approximately the ED<sub>50</sub> for cocaine-induced increases in horizontal photocell counts (Kalivas et al., 1988). Thus, this dose permits accurate quantification of either increases or decreases in photocell counts between days 1 and 7 of treatment. The rats were treated at home on days 2–6 to avoid the photocell apparatus for other experiments.

**Microinjection procedure.** On the challenge day, after a 1 hr adaptation to the photocell cell apparatus (Omnitech), the obturators were removed from the microinjection guide cannulae and replaced by an injection needle (33-gauge stainless steel) that extended 1 mm below the tip of the guide cannulae into the nucleus accumbens. Bilateral infusions were made over 60 sec in a volume of 0.3  $\mu$ l/side (experiment 1) or 0.5  $\mu$ l/side (experiment 4). Twenty seconds later, the injector was removed and the rat was returned to the photocell cage. Behavior was monitored for 120 min after injection.

**Microdialysis and measurement of extracellular glutamate and dopamine.** The dialysis probes were constructed as described by Robinson and Wishaw (1988), with ~2.0 mm of active dialysis membrane exposed at the tip. The evening before the experiment, the probes were inserted through the guide cannulae into the nucleus accumbens, and the rats were placed in photocell chambers (Omnitech) permitting simultaneous quantification of behavior. The next day, dialysis buffer containing (in mM) KCl 5, NaCl 120, CaCl<sub>2</sub> 1.2, MgCl<sub>2</sub> 1.2, glucose 5, plus PBS 0.2 to give a pH value of 7.4 and a final sodium concentration of 120.7 mM was advanced through the probe at a rate of 1.9  $\mu$ l/min via a syringe pump (Harvard Instruments, Boston, MA). Two hours after starting perfusion, baseline samples of 20 min each were collected for 100 min before beginning drug treatment.

For the measurement of extracellular dopamine, samples were collected into microfuge tubes containing 20  $\mu$ l of mobile phase [0.1 M citric acid, 75 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM heptane sulfonic acid, 0.1 mM EDTA, 15% methanol (v/v), pH 4.2] plus 100 nmol of dihydroxybenzylamine as the internal standard. After collection, all samples were frozen at –80°C until analyzed. The samples were thawed subsequently and placed in an autosampler (Gilson Medical supplies, Middleton, WI) connected to an HPLC system with electrochemical detection. The dopamine was separated using a 25 cm C-18 reversed-phase column (Bioanalytical Systems, West Lafayette, IN) and oxidized/reduced using coulometric detection (ESA, Bedford, MA). Three electrodes were used: a preinjection port guard cell (+0.4 V) to oxidize the mobile phase, an oxidation analytical electrode (+0.3 V), and a reduction analytical electrode (–0.14 V). Peaks

were recorded on a chart recorder and compared with an external standard curve (10–1000 fmol).

For the measurement of extracellular glutamate, samples were collected into 10  $\mu$ l of mobile phase [0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.13 mM Na<sub>2</sub>EDTA, 25% methanol (v/v), pH 6.4] plus 2.0 pmol of homoserine as an internal standard. Precolumn derivatization with *o*-phthaldehyde was performed by an autosampler, and the chromatography was conducted as described above for dopamine, except that a 10 cm column was used and the electrode currents were set as follows: preinjection port guard cell, +0.7 V; reduction analytical electrode, –0.25 V; oxidation analytical electrode, +0.65 V.

**Experiment 1: response to AMPA.** Twenty days after discontinuing daily saline or cocaine, rats were microinjected with saline into the shell or core of the nucleus accumbens. On days 21 and 22, after 1 hr of habituation to the behavioral chamber, rats were given two doses of AMPA (0.03 or 0.1  $\mu$ g) in random order, and motor activity was monitored for 2 hr. Thus, the rats were given a total of three microinjections, each separated by a 24 hr intertrial interval.

**Experiment 2: dopamine dialysis.** On days 21 or 22 after discontinuing repeated cocaine or saline injection, 100 min of baseline data were collected in either the shell or core followed by the addition of four concentrations of AMPA (0.1, 1.0, 10.0, and 100.0  $\mu$ M) to the dialysis buffer. AMPA doses were administered sequentially in ascending order through the probe in the core for 100 min at each concentration. During both baseline and AMPA administration, samples were taken every 20 min.

**Experiment 3: glutamate dialysis.** On day 21, after discontinuing repeated cocaine or saline, microdialysis was conducted in the shell or core to measure the levels of extracellular glutamate. After collecting 100 min of baseline data, rats were challenged with saline (1.0 ml/kg, i.p.) followed 80 min later by cocaine (15 mg/kg, i.p.). Samples were collected for 180 min after cocaine administration before terminating the experiment.

**Experiment 4: CNQX pretreatment.** Fourteen to twenty-three days after discontinuing daily treatment with cocaine, rats were adapted to the photocell apparatus for 60 min and then microinjected with CNQX (0.01, 0.1, or 1.0 nmol; Tocris Neuramin, Essex, UK) or vehicle [0.1, 1.0, or 10.0% dimethylsulfoxide (DMSO) in saline] into the core. Five minutes later, rats received an injection of cocaine (15 mg/kg, i.p.) and were returned to the photocell apparatus where motor activity was monitored for 120 min. At a minimum of 3 d intervals, each rat received a maximum of four combinations of CNQX or DMSO and cocaine. A control experiment was conducted in which animals that did not receive daily cocaine pretreatment were challenged in random order with one of three treatments: (1) CNQX (0.1 nmol) in the core plus systemic saline; (2) DMSO (1% v/v) vehicle in the core plus systemic saline; or (3) either CNQX plus cocaine (15 mg/kg, i.p.) or DMSO vehicle plus cocaine. The latter cocaine treatments were administered to separate groups of animals to avoid possible confounds of sensitization to a single injection of cocaine (Weiss et al., 1989).

**Histology.** After the dialysis and behavioral experiments, the rats were given an overdose of pentobarbital (>100 mg/kg, i.p.) and perfused intracardially with PBS followed by 10% formalin. The brain was removed and stored in 10% formalin for at least 1 week. The brains then were blocked, and coronal sections (100  $\mu$ m) were taken at the level of the nucleus accumbens with a vibratome. The sections were mounted on gelatin-coated slides and stained with cresyl violet. Probe and cannula placements were determined according to the atlas of Paxinos and Watson (1986) by an individual blind to the rat's behavioral or neurochemical response.

**Data analysis.** Separate two-way ANOVAs were performed on the totals (horizontal and vertical counts summed across 120 min) of the behavioral data. In time-course analyses of behavioral data, a two-way ANOVA with repeated measures over time was conducted. The content of glutamate was expressed both as raw data and as percent change from baseline and evaluated statistically using a two-way ANOVA with repeated measures over time. Based on the average of the last two dialysis samples at each AMPA concentration, extracellular dopamine levels were compared using a two-way ANOVA with repeated measures over dose. Pairwise comparisons were made via a least significant difference or a Scheffé's test (Milliken and Johnson, 1984).

## RESULTS

### Sensitization to repeated cocaine

Shortly after the studies were begun, it became clear that within each experiment a portion of rats receiving daily cocaine did not

**Table 1.** Effect of daily administrations of cocaine on horizontal activity

	N	Day 1 <sup>a</sup>	Day 7	Statistics <sup>b</sup>
Experiment 1: AMPA behavior				
Total <sup>c</sup>	29	16752 (1733) <sup>d</sup>	22156 (2362)	$t_{(28)} = 2.43, p = 0.022$
Core				
Sensitized	10	12577 (1956)	26633 (2696)	$t_{(9)} = 4.95, p < 0.001$
Nonsensitized	6	18648 (4566)	11920 (5555)	$t_{(5)} = 2.27, p = 0.073$
Shell				
Sensitized	7	15088 (3784)	27194 (4566)	$t_{(6)} = 4.51, p = 0.004$
Nonsensitized	6	23789 (3437)	19050 (5395)	$t_{(5)} = 2.06, p = 0.094$
Experiment 2: Dopamine dialysis				
Total	20	16154 (1915)	22864 (2168)	$t_{(19)} = 2.32, p = 0.026$
Core				
Sensitized	5	9839 (1934)	17864 (2751)	$t_{(4)} = 2.34, p = 0.044$
Nonsensitized	4	19057 (2823)	16306 (2384)	$t_{(3)} = 0.55, p = 0.485$
Shell				
Sensitized	8	17545 (3578)	30003 (3575)	$t_{(7)} = 2.46, p = 0.027$
Nonsensitized	3	19096 (6211)	20902 (4747)	$t_{(2)} = 0.23, p = 0.829$
Experiment 3: Glutamate dialysis				
Total	21	22074 (3093)	25828 (3034)	$t_{(20)} = 0.88, p = 0.387$
Core				
Sensitized	6	12795 (2419)	33118 (3607)	$t_{(5)} = 10.97, p < 0.001$
Nonsensitized	6	32778 (6350)	19553 (6108)	$t_{(5)} = 2.69, p = 0.043$
Shell				
Sensitized	5	19199 (2683)	32077 (3449)	$t_{(4)} = 3.66, p = 0.022$
Nonsensitized	4	29531 (6317)	16493 (9222)	$t_{(3)} = 1.79, p = 0.171$
Experiment 4: CNQX behavior				
Total	26	20650 (2494)	30685 (2839)	$t_{(25)} = 3.19, p = 0.004$
Sensitized	16	16211 (2666)	36027 (3199)	$t_{(15)} = 7.69, p < 0.001$
Nonsensitized	10	27751 (4105)	22136 (4215)	$t_{(9)} = 1.84, p = 0.099$

<sup>a</sup>Cocaine was administered daily for 1 week. Day 1 corresponds to the first, and day 7 to the last, injection of cocaine.

<sup>b</sup>A two-tailed paired Student's *t*-test was employed to compare day 1 with day 7 in each group. *Df* is shown in parenthesis.

<sup>c</sup>Total refers to all rats in an experiment that were treated with daily cocaine; sensitized refers to the subpopulation showing a greater than 20% increase in photocell counts between day 1 and day 7; nonsensitized corresponds to rats demonstrating a less than 20% increase.

<sup>d</sup>Data are presented as the mean (SEM) horizontal photocell counts over the first 120 min after cocaine injection.

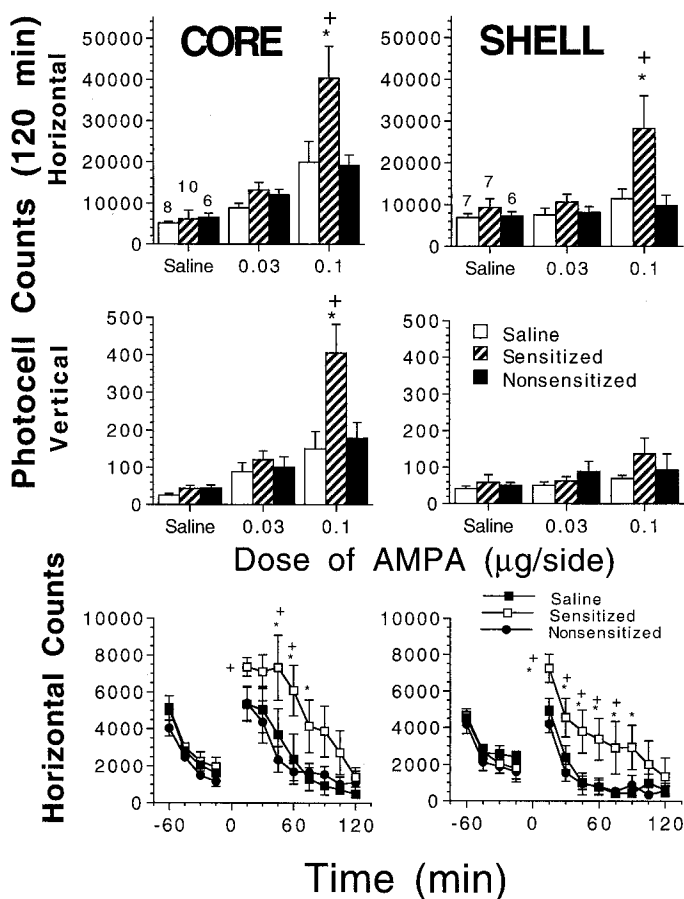
develop behavioral sensitization as defined by an increase in horizontal photocell counts on day 7 compared with day 1 of daily injection. As detailed below, the subpopulation of rats that demonstrated an increase in photocell counts on day 7 versus day 1 showed different alterations in EAA transmission than rats that did not demonstrate an increase in horizontal photocell counts. By setting a criterion of a 20% increase in horizontal photocell counts on day 7 compared with day 1, the rats receiving daily cocaine were divided into sensitized (>20% increase) and nonsensitized (<20% increase) experimental groups. Using this criterion, between 50 and 75% of all rats in each experiment were placed in the sensitized group. Table 1 shows the effect of cocaine on horizontal photocell counts on day 1 and day 7 of the daily pretreatment regime. Photocell counts for cocaine-pretreated rats are shown for each experiment as the value for all animals (Total) and after the animals had been divided into sensitized and nonsensitized groups. Table 1 reveals distinguishing characteristics of the sensitized and nonsensitized rats. The rats in the sensitized group demonstrated robust sensitization, with a mean increase in photocell counts ranging from 67.1 to 158.8% on day 7 compared with day 1, depending on the experiment. Rats in the nonsensitized group demonstrated no significant difference in photocell counts on day 7 versus day 1; however, the counts on day 7 tended to be lower. Rats in the sensitized group tended to have lower

photocell counts after the first injection of cocaine compared with the nonsensitized animals. Finally, rats treated with daily saline did not show significant differences in photocell counts between day 1 and day 7 (data not shown).

### Experiment 1: microinjection of AMPA

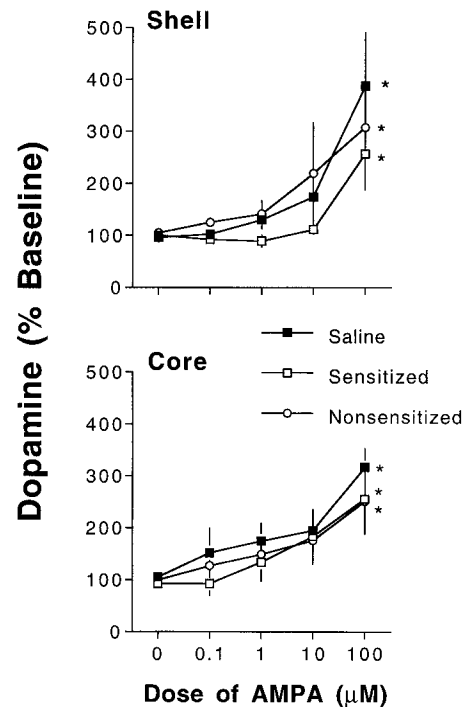
Figure 1 shows the behavioral response to AMPA microinjection into the core of the nucleus accumbens. AMPA produced a dose-related increase in both horizontal and vertical photocell counts in all three treatment groups. After the highest dose of AMPA (0.1  $\mu$ g), the increase in both horizontal and vertical counts was greater in the sensitized than in the nonsensitized or saline rats. Figure 1 shows the time course for horizontal photocell counts after microinjection of 0.1  $\mu$ g of AMPA into the core. A significant difference between treatment groups was measured that was reflected in the larger behavioral response to AMPA in the sensitized rats, compared with saline-pretreated and nonsensitized animals, between 45 and 90 min after microinjection into the core. At no time point did the behavioral response of the nonsensitized animals differ from controls.

Figure 1 also shows the behavioral response to AMPA microinjection into the shell. Consistent with findings in a previous report (Johnson et al., 1995), AMPA was not as effective at inducing motor activity in the shell compared with the core.



**Figure 1.** Behavioral effect of AMPA microinjection into the core and shell of the nucleus accumbens of saline (open bars), sensitized (hatched bars), and nonsensitized (filled bars) rats. All animals that displayed a >20% increase in behavior between day 7 and day 1 of repeated cocaine administrations were classified as sensitized; all others were defined operationally as nonsensitized. The data are represented as mean  $\pm$  SE horizontal and vertical photocell counts. The number of rats in each group is indicated in the top panel. **Top panel,** Total horizontal photocell counts (120 min after microinjection). The data were evaluated with a two-way ANOVA (saline/sensitized/nonsensitized  $\times$  AMPA dose) with repeated measures over dose. **CORE:** treatment,  $F_{(2,21)} = 4.31, p = 0.027$ ; dose,  $F_{(2,42)} = 24.77, p < 0.001$ ; interaction,  $F_{(4,42)} = 2.54, p < 0.051$ . **SHELL:** treatment,  $F_{(2,17)} = 6.34, p = 0.009$ ; dose,  $F_{(2,34)} = 6.79, p = 0.003$ ; interaction,  $F_{(4,34)} = 2.31, p = 0.077$ . **Middle panel,** Total vertical photocell counts (120 min after microinjection). The data were evaluated with a two-way ANOVA (saline/sensitized/nonsensitized  $\times$  AMPA dose) with repeated measures over dose. **CORE:** treatment,  $F_{(2,21)} = 5.06, p = 0.016$ ; dose,  $F_{(2,42)} = 22.48, p < 0.001$ ; interaction,  $F_{(4,42)} = 2.75, p < 0.041$ . **SHELL:** treatment,  $F_{(2,17)} = 1.34, p = 0.288$ ; dose,  $F_{(2,34)} = 3.49, p = 0.042$ ; interaction,  $F_{(4,34)} = 0.69, p = 0.602$ . **Bottom panel,** Time course of the horizontal photocell counts shown in the top panel corresponding to 0.1  $\mu$ g of AMPA. Time: -60 to -15 min corresponds to the 60 min adaptation before administering AMPA at time 0. The data were evaluated using a two-way ANOVA (saline/sensitized/nonsensitized  $\times$  time) with repeated measures over time. **CORE:** treatment,  $F_{(2,21)} = 5.46, p = 0.013$ ; time,  $F_{(11,231)} = 14.57, p < 0.001$ ; interaction,  $F_{(22,231)} = 1.03, p = 0.425$ . **SHELL:** treatment,  $F_{(2,17)} = 3.19, p = 0.067$ ; time,  $F_{(11,187)} = 22.16, p < 0.001$ ; interaction,  $F_{(22,187)} = 2.12, p = 0.004$ . \* $p < 0.05$  comparing treatments to saline using a least significant difference test (Milliken and Johnson, 1984); + $p < 0.05$  comparing sensitized to nonsensitized.

Although the ANOVA did not reveal a significant interaction between dose and treatment group in either horizontal or vertical photocell counts, there was a significant effect of dose in both behavioral parameters and a significant effect of treatment group in horizontal photocell counts. As with the core, the difference

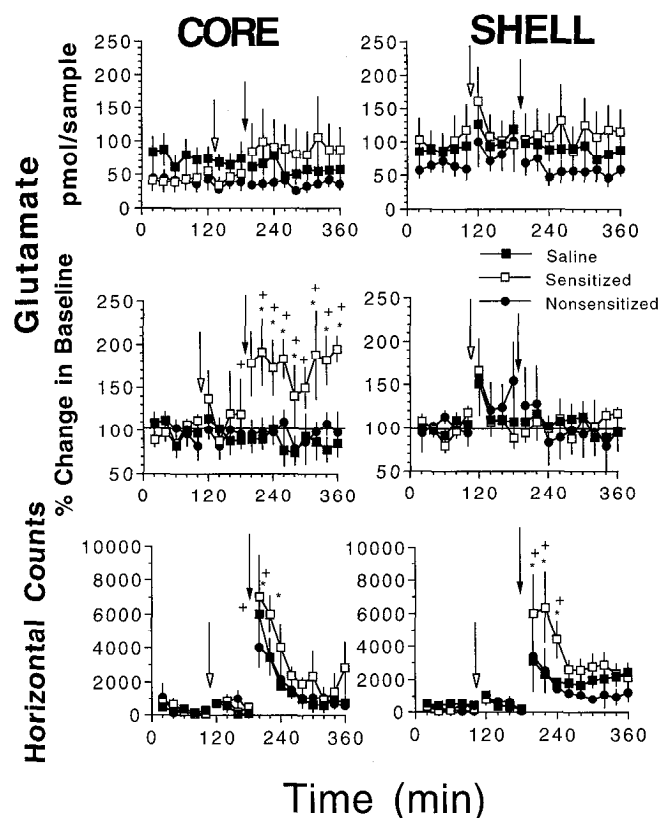


**Figure 2.** Effect of AMPA administration through the dialysis probe in the core and shell on extracellular dopamine content. The experiment was conducted at 21 or 22 d after discontinuing repeated cocaine or saline. The data were normalized to percent change from baseline, and the last two samples taken at baseline and at each AMPA concentration were averaged. The top panel shows data obtained from the Shell, and the bottom panel shows data obtained from the Core. The data were analyzed using a two-way ANOVA (saline/sensitized/nonsensitized  $\times$  dose) with repeated measures over dose. **Core:** treatment,  $F_{(2,11)} = 0.41, p = 0.671$ ; time,  $F_{(4,44)} = 15.65, p < 0.001$ ; interaction,  $F_{(8,44)} = 0.24, p = 0.982$ . **Shell:** treatment,  $F_{(2,15)} = 0.17, p = 0.843$ ; time,  $F_{(4,60)} = 5.53, p < 0.001$ ; interaction,  $F_{(8,60)} = 0.58, p = 0.793$ . \* $p < 0.05$  comparing each group to its own basal value with a least significant difference test (Milliken and Johnson, 1984).

between treatment groups in horizontal counts was attributable primarily to the increased response to 0.1  $\mu$ g of AMPA microinjected into the shell of the sensitized compared with nonsensitized or saline rats. Figure 1 shows the time course of the effect of AMPA (0.1  $\mu$ g) microinjection into shell on horizontal photocell counts. The behavioral response to AMPA was significantly greater in sensitized rats, compared with saline-pretreated and nonsensitized animals, for the first 90 min after microinjection. At no time point did the behavioral response of the nonsensitized animals differ from that of controls.

## Experiment 2: AMPA-induced dopamine release

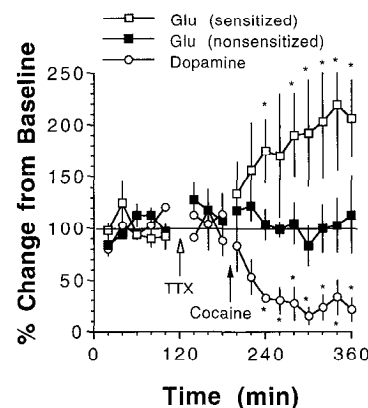
The perfusion of AMPA through a microdialysis probe in the core or shell produced a dose-dependent increase in extracellular dopamine in the nucleus accumbens (Fig. 2). There were no significant differences in the dopamine response between the sensitized, nonsensitized, and saline groups. The baseline values for dopamine did not differ significantly among the three treatment groups in either the core ( $F_{(2,15)} = 0.12, p = 0.892$ ; saline,  $0.136 \pm 0.054$  pmol/sample; sensitized,  $0.124 \pm 0.062$  pmol/sample; nonsensitized,  $0.094 \pm 0.047$  pmol/sample) or the shell ( $F_{(2,16)} = 0.59, p = 0.567$ ; saline,  $0.019 \pm 0.004$  pmol/sample; sensitized,  $0.023 \pm 0.003$  pmol/sample; nonsensitized,  $0.027 \pm 0.010$  pmol/sample).



**Figure 3.** Effect of saline and cocaine (15 mg/kg, i.p.) on extracellular glutamate in the core and shell of saline-pretreated and cocaine-pretreated rats at 21 d of withdrawal. The data are presented as mean  $\pm$  SEM. The *top panel* shows the level of extracellular glutamate (pmol/sample), and the *middle panel* shows percent change from basal levels of glutamate. The *bottom panel* shows the motor response obtained simultaneously with the neurochemistry. In each panel, the data were evaluated using a two-way ANOVA (saline/sensitized/nonsensitized  $\times$  time) with repeated measures over time. The *open arrow* indicates the administration of saline (after 100 min), and the *closed arrow* indicates the injection of cocaine (after 180 min). *Top panel, CORE:* treatment,  $F_{(2,15)} = 0.77$ ,  $p = 0.482$ ; time,  $F_{(17,255)} = 0.96$ ,  $p = 0.501$ ; interaction,  $F_{(34,255)} = 2.21$ ,  $p < 0.001$ ; *SHELL:* treatment,  $F_{(2,15)} = 0.72$ ,  $p = 0.504$ ; time,  $F_{(17,255)} = 2.49$ ,  $p < 0.001$ ; interaction,  $F_{(34,255)} = 0.68$ ,  $p = 0.913$ . *Middle panel, CORE:* treatment,  $F_{(2,15)} = 7.44$ ,  $p = 0.006$ ; time,  $F_{(17,255)} = 1.37$ ,  $p = 0.153$ ; interaction,  $F_{(34,255)} = 1.91$ ,  $p = 0.003$ . *SHELL:* treatment,  $F_{(2,15)} = 0.02$ ,  $p = 0.981$ ; time,  $F_{(17,255)} = 2.71$ ,  $p < 0.001$ ; interaction,  $F_{(34,255)} = 0.75$ ,  $p = 0.846$ . *Bottom panel, CORE:* treatment,  $F_{(2,15)} = 4.56$ ,  $p = 0.028$ ; time,  $F_{(17,255)} = 14.22$ ,  $p < 0.001$ ; interaction,  $F_{(34,255)} = 0.90$ ,  $p = 0.632$ . *SHELL:* treatment,  $F_{(2,15)} = 3.82$ ,  $p = 0.046$ ; time,  $F_{(17,255)} = 11.74$ ,  $p < 0.001$ ; interaction,  $F_{(34,255)} = 1.47$ ,  $p = 0.051$ . \* $p < 0.05$  comparing sensitized with saline at a given time using a least significant difference test (Milliken and Johnson, 1984); + $p < 0.05$  comparing sensitized with nonsensitized.

### Experiment 3: extracellular glutamate after a cocaine challenge

Figure 3 shows the effect of a saline and cocaine injection on extracellular levels of glutamate in the core of the nucleus accumbens. Injection of saline did not alter extracellular glutamate levels in the core in any treatment group. There was a significant difference between treatment groups in response to a subsequent injection of cocaine (15 mg/kg, i.p.). Cocaine administration elevated the levels of extracellular glutamate significantly in the sensitized group compared with both the nonsensitized and saline-pretreated rats. Significant elevations occurred immediately after administering cocaine (200 min) and continued until

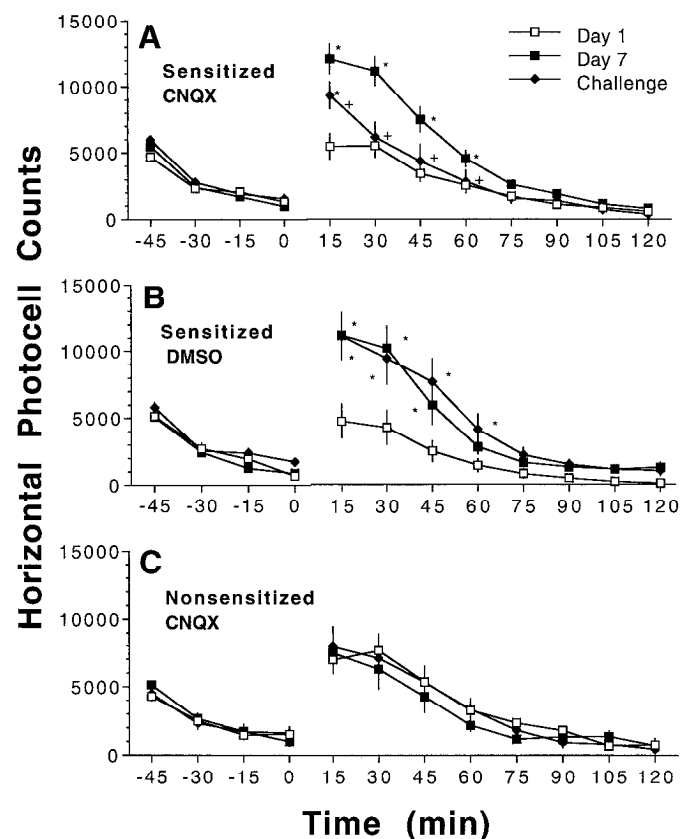


**Figure 4.** Effect of TTX (1.0  $\mu$ M) on extracellular levels of glutamate and dopamine in the core of the nucleus accumbens of rats pretreated 21 d earlier with daily cocaine. TTX was introduced into the dialysis buffer at the *open arrow* (120 min), and Cocaine (15 mg/kg, i.p.) was administered at the *closed arrow* (after 180 min). The dopamine values were obtained from samples of three animals (2 sensitized, 1 nonsensitized) used to measure glutamate. The data are shown as mean  $\pm$  SEM percent change in baseline and were analyzed using a one-way repeated-measures ANOVA. *Glu (sensitized):* baseline =  $81 \pm 25$  pmol/sample,  $n = 3$ ,  $F_{(16,50)} = 3.13$ ,  $p = 0.003$ . *Glu (nonsensitized):* baseline =  $81 \pm 20$  pmol/sample,  $n = 3$ ,  $F_{(16,50)} = 0.76$ ,  $p = 0.715$ . *Dopamine:* baseline =  $135 \pm 20$  fmol/sample,  $n = 3$ ,  $F_{(16,50)} = 10.07$ ,  $p < 0.001$ . \* $p < 0.05$  compared with baseline using Scheffe's test for multiple comparisons.

the end of the experiment. The average basal levels of glutamate in both groups of cocaine-pretreated animals tended to be lower than in saline-pretreated rats ( $F_{(2,15)} = 1.68$ ,  $p = 0.080$ ; saline,  $77 \pm 19$  pmol/sample; sensitized,  $42 \pm 15$  pmol/sample; nonsensitized,  $41 \pm 13$  pmol/sample). Figure 4 also shows the behavioral response to saline and cocaine administration in the three treatment groups. Although there were significant main effects of time and treatment, there was not a significant interaction. However, if the sums of the photocell counts obtained after the injection of cocaine were compared with a one-way ANOVA, the sensitized group demonstrated more photocell counts in response to the cocaine challenge than did either the nonsensitized or the saline group ( $F_{(2,15)} = 5.46$ ,  $p = 0.017$ ; saline ( $n = 6$ ),  $16,552 \pm 3321$ ; sensitized ( $n = 6$ ),  $28,460 \pm 3289$ ; nonsensitized ( $n = 6$ ),  $14,090 \pm 3260$ ).

Figure 3 also illustrates that there were no significant differences in extracellular glutamate levels in the shell of the nucleus accumbens among the sensitized, nonsensitized, and saline treatment groups after an injection of saline or cocaine. Likewise, there were no differences in the basal levels among the three groups ( $F_{(2,15)} = 0.57$ ,  $p = 0.567$ ; saline,  $89 \pm 15$  pmol/sample; sensitized,  $80 \pm 18$  pmol/sample; nonsensitized,  $63 \pm 10$  pmol/sample). In contrast to the extracellular glutamate levels, analysis of the corresponding behavioral data revealed significant treatment and time effects, with a near-significant interaction. If the sums of the horizontal photocell counts obtained after the injection of cocaine were compared with a one-way ANOVA, the sensitized group demonstrated more photocell counts in response to the cocaine challenge than did either the nonsensitized or the saline group ( $F_{(2,15)} = 5.49$ ,  $p = 0.016$ ; saline ( $n = 9$ ),  $19,497 \pm 2525$ ; sensitized ( $n = 5$ ),  $32,175 \pm 4850$ ; nonsensitized ( $n = 4$ ),  $13,820 \pm 3207$ ).

To determine whether the extracellular glutamate was derived from cytosolic or vesicular pools, tetrodotoxin (TTX; 1.0  $\mu$ M) was included in the dialysis buffer. Figure 4 demonstrates that with the



**Figure 5.** Time course of the effects of CNQX (0.1 nmol) or DMSO vehicle (1% v/v) microinjection into the core of the nucleus accumbens on the behavioral effect of cocaine given 2–3 weeks after discontinuing daily cocaine. Time: –45 to 0 min corresponds to the adaptation period before cocaine (15 mg/kg, i.p.) and CNQX administration, and 15–120 min reflects the behavioral response to these drugs. Also shown are the responses to daily cocaine on day 1 (open squares) and day 7 (filled squares). The data are presented as mean  $\pm$  SEM horizontal photocell counts and were evaluated using a two-way ANOVA with repeated measures over time. *A*, Sensitized animals were injected with CNQX and cocaine: day,  $F_{(2,34)} = 7.33$ ,  $p < 0.003$ ; time,  $F_{(11,374)} = 88.82$ ,  $p < 0.001$ ; interaction,  $F_{(22,374)} = 6.27$ ,  $p < 0.001$ . *B*, Sensitized animals were injected with DMSO and cocaine: day,  $F_{(2,21)} = 5.29$ ,  $p < 0.02$ ; time,  $F_{(11,231)} = 48.34$ ,  $p < 0.014$ ; interaction,  $F_{(22,231)} = 3.51$ ,  $p < 0.001$ . *C*, Nonsensitized animals were injected with CNQX and cocaine: day,  $F_{(2,20)} = 0.13$ ,  $p > 0.05$ ; time,  $F_{(21,220)} = 45.01$ ,  $p < 0.001$ ; interaction,  $F_{(22,220)} = 0.54$ ,  $p > 0.05$ . \* $p < 0.05$  comparing treatments to day 1 using a least significant difference test (Milliken and Johnson, 1984); + $p < 0.05$  comparing day 7 to CNQX or DMSO.

exception of the first time bin, the increase in extracellular glutamate observed in the core after a cocaine challenge in the sensitized group of rats was not inhibited by TTX. Likewise, the level of glutamate in the nonsensitized rats was not altered. However, as noted in previous reports (Westerink et al., 1987), the concentration of extracellular dopamine in the same samples was reduced significantly below basal levels by TTX.

#### Experiment 4: effect of CNQX on the expression of sensitization

Figure 5 shows that CNQX (0.1 nmol) pretreatment in the core of the nucleus accumbens reduced the motor stimulant effect of cocaine in sensitized rats. Pretreatment with CNQX (0.1 nmol) before a cocaine challenge at 14–23 d after the last repeated cocaine injection reduced the augmented level of motor activity. From 15 to 60 min after injection, the motor response was

**Table 2.** Effect of CNQX pretreatment (0.1 nmol) into the core on the behavioral response to a cocaine challenge (15 mg/kg, i.p.) in control rats

Treatment <sup>a</sup>	DMSO/cocaine ( <i>n</i> = 15)	CNQX/cocaine ( <i>n</i> = 8)
DMSO/Saline	7809 (997) <sup>b</sup>	7232 (1754)
CNQX/Saline	9266 (1186)	7107 (1508)
DMSO or CNQX plus cocaine	18405 (3322) <sup>c</sup>	19709 (3292)

<sup>a</sup>Rats were pretreated with daily saline (1.0 ml/kg, i.p.) for 7 d (*N* = 16) or were naive (*N* = 7). Beginning at 2 weeks after the last daily injection, rats were divided into two groups, DMSO/Cocaine and CNQX/Cocaine. Each group of rats were given all three treatments in random order separated by a 72 hr intertrial interval. DMSO (1% v/v) or CNQX was microinjected into the core 5 min before systemic saline or cocaine (15 mg/kg, i.p.). Two treatments were identical in each group (DMSO/saline and CNQX/saline) and for the other treatment, one group received DMSO/cocaine and the other received CNQX/cocaine. The data were not significantly different between the rats pretreated with daily saline and the naive animals. These data were pooled in Table 2 for statistical analysis.

<sup>b</sup>The data are shown as mean (SEM) horizontal photocell counts for 120 min after administering systemic cocaine or saline. The data were evaluated with a two-way ANOVA with repeated measures over treatment. Group  $F_{(1,21)} = 0.06$ ,  $p = 0.816$ ; treatment  $F_{(2,42)} = 15.87$ ,  $p < 0.001$ ; interaction  $F_{(2,42)} = 0.28$ ,  $p = 0.760$ .

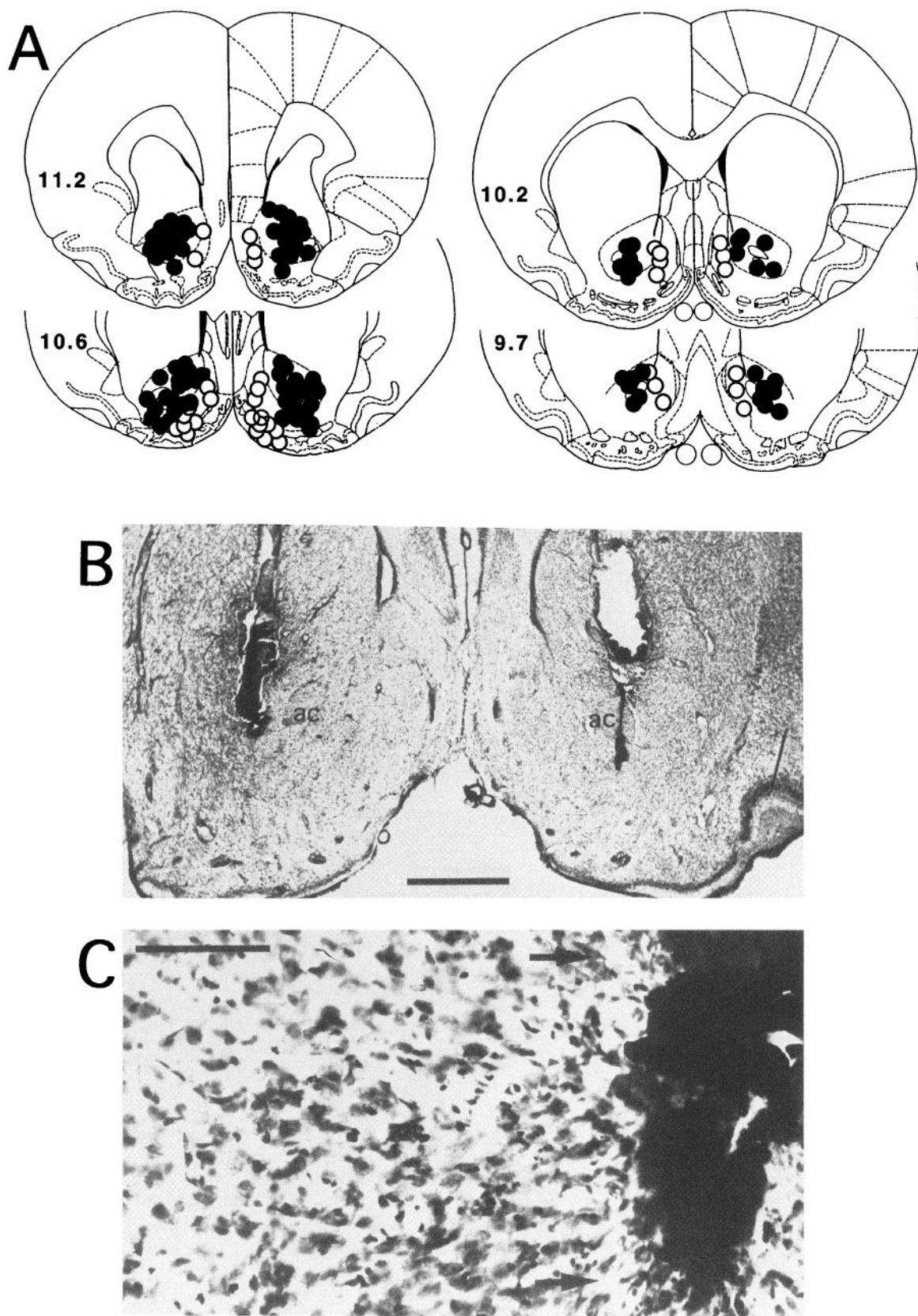
<sup>c</sup> $p < 0.05$ , compared with DMSO/saline and CNQX/saline. No other comparisons were statistically significant.

reduced by CNQX compared with the response produced by cocaine on day 7 and was equivalent to the motor response produced on day 1 at all time points except the first time bin. When sensitized rats were pretreated with DMSO vehicle (1% v/v) in the core before a cocaine challenge, the motor response was equivalent to the augmented levels on day 7 and significantly elevated over the day 1 cocaine response. Figure 5 also shows the response to CNQX in nonsensitized rats. Consistent with behavior defined as nonsensitized, the response to cocaine on day 7 was not greater than on day 1. Further, pretreatment with CNQX did not alter the response to a cocaine challenge compared with the response on either day 1 or day 7. Table 2 shows that this dose of CNQX did not alter the response to an acute systemic cocaine challenge in rats that did not receive daily pretreatment with cocaine. Likewise, CNQX did not alter the response to a systemic saline injection.

Two other doses of CNQX were examined (data not shown). Whereas pretreatment of sensitized rats with 0.01 nmol of CNQX was ineffective, 1.0 nmol of CNQX reduced the number of photocell counts on the cocaine challenge day compared with day 7 of repeated cocaine pretreatment. In nonsensitized animals, there was not a significant alteration in either day 1 or day 7 cocaine-induced activity by any dose of CNQX. Concentrations of the DMSO vehicle used to solubilize the corresponding doses of CNQX did not alter motor activity in sensitized rats.

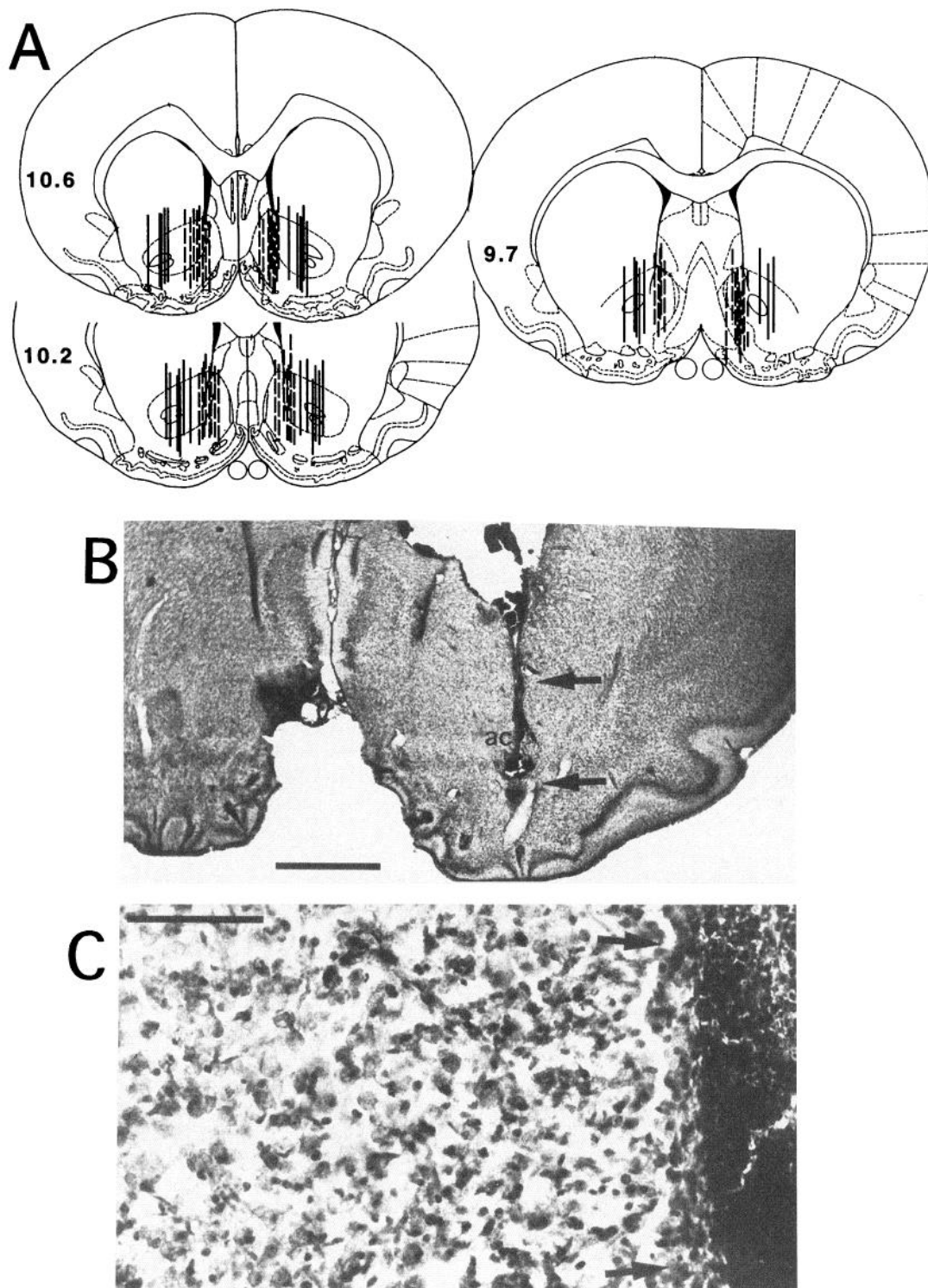
#### Histology

Figure 6 depicts the location of the ventral tip of the microinjection cannulae in the nucleus accumbens for the behavioral experiments. The cannulae were distributed between the core and the medial shell regions of the nucleus accumbens. All cannulae used in experiment 4 were in the core, whereas placements from experiment 1 were in either the shell or the core. The micrograph shows histology from an animal used in experiment 1 that was microinjected with the highest dose of AMPA (0.1  $\mu$ g) and illustrates the lack of neurotoxicity beyond the mechanical destruction associated with the injection procedure. Figure 7 depicts the dialysis probe placements in the nucleus accumbens. In experiments 2 and 3, the probes were located in both the shell and



**Figure 6.** Location of the microinjection cannula tips in the nucleus accumbens from experiments 1 and 4 (*A*). The numbers indicate millimeters rostral to the interaural line according to the atlas of Paxinos and Watson (1986). The open circles represent cannula placements in the shell, and the filled circles represent cannula placements in the core. The micrographs (*B* and *C*) are representative of an animal microinjected with AMPA in experiment 2. Note the lack of neurotoxicity outside the mechanical damage produced by penetration of the injection cannulae. Scale bars: *B*, 2 mm; *C*, 0.2 mm.





**Figure 7.** Location of dialysis probe placements in the nucleus accumbens from experiments 2 and 3 (*A*). The numbers indicate millimeters rostral to the interaural line according to the atlas of Paxinos and Watson (1986). The solid lines represent probe placements in the core, and the dashed lines depict placements in the shell. The micrographs (*B* and *C*) are from an animal used in experiment 2 and show the lack of neurotoxicity outside of the mechanical damage produced by the probe after perfusion of AMPA through the dialysis probe. The arrows approximate the dorsal and ventral extent of the active portion of the dialysis membrane. Scale bars: *B*, 2 mm; *C*, 0.2 mm.

core. Note that some dialysis probes placed in the core had active membrane dorsal to the core in the ventral striatum or ventral to the core in the lateral limb of the shell. Also, some probe placements in the shell penetrated the medial edge of the core or the

diagonal band of Broca. The micrograph was from an animal used in experiment 2 in which 100  $\mu$ M AMPA was passed through the dialysis probe. Note the lack of neurotoxicity outside the mechanical destruction produced by the dialysis probe and guide cannula.



## DISCUSSION

These experiments demonstrate that augmented EAA transmission in the nucleus accumbens is associated with the expression of behavioral sensitization in rats pretreated with daily cocaine injections. Augmented EAA transmission was observed only in rats developing behavioral sensitization to repeated cocaine pretreatment (sensitized) and was not present in rats pretreated with cocaine that did not develop behavioral sensitization to criterion (nonsensitized) or in control animals. Three aspects of EAA transmission were augmented: (1) The behavioral response to microinjection of the non-NMDA agonist AMPA into either the shell or core of the nucleus accumbens was increased in rats sensitized to cocaine versus nonsensitized or saline-pretreated rats. (2) A cocaine challenge elevated extracellular glutamate levels in the core, but not the shell, only in rats sensitized to cocaine. (3) The sensitized motor response to cocaine was prevented by pretreatment of the core with the AMPA antagonist CNQX.

### Neurotransmission in the nucleus accumbens in the expression of behavioral sensitization to cocaine

The nucleus accumbens is thought to mediate the transfer of information from the limbic system to motor systems, and both EAA and dopamine transmission are considered to be important modulators in this process (Mogenson et al., 1993; LeMoal and Simon, 1991; Kalivas et al., 1993). It has been proposed that the expression of behavioral sensitization to psychostimulants is associated with alterations in EAA and dopamine transmission within the nucleus accumbens (Kalivas and Stewart, 1991; Nestler, 1992; White et al., 1995b). Supporting this proposition, the present data demonstrate that there are changes in presynaptic and postsynaptic EAA transmission in the nucleus accumbens. Because the behavioral effects of AMPA and CNQX, and the elevation in extracellular glutamate, are present only in the subgroup of rats that develop behavioral sensitization to repeated cocaine administration, the changes in EAA transmission in the nucleus accumbens may be critical determinants in the expression of behavioral sensitization.

The absence of changes in nonsensitized rats indicates that repeated cocaine administration is not a sufficient condition for producing augmented EAA transmission. Another important condition permitting effects on EAA transmission may be genetic variation in responsiveness to cocaine (Nestler et al., 1993; Hooks and Kalivas, 1994). Also, differential development of conditioning to environmental stimuli may be involved. There is substantial evidence for a conditioned component of psychostimulant-induced behavioral sensitization (Tilson and Rech, 1973; Post and Weiss, 1988; Stewart and Vezina, 1988; Pert et al., 1992). Supporting a possible role for EAA transmission in the nucleus accumbens in psychostimulant-conditioned behaviors, the nuclei supplying EAA afferents to the nucleus accumbens, including the prefrontal cortex, hippocampus, and amygdala (Kelley and Domesick, 1982; Kelley et al., 1982; Berendse and Groenewegen, 1990; Berendse et al., 1992; Zahm and Brog, 1992), contribute to both the development of conditioned behaviors (Mishkin and Appenzeller, 1987; Goldman-Rakic, 1988; Dudai, 1989) and the behavioral sensitization to psychostimulants (Yoshikawa et al., 1991; Pert et al., 1992; Dahlin et al., 1994).

Figure 4 reveals a dissociation in the time course of the increase in extracellular glutamate in the core and the behavioral response to a cocaine challenge. This indicates that augmented extracellular glutamate is not the sole determinant of the sensitized behavioral response. The involvement of multiple transmitters in me-

diating a change in complex motor behaviors is not surprising. Although experiment 2 demonstrated that the augmented behavioral responsiveness to AMPA was independent of AMPA-induced release of dopamine, this monoamine has been linked consistently to the expression of behavioral sensitization to psychostimulants (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Nestler, 1992; White et al., 1995b). In addition to glutamate and dopamine, serotonin, dynorphin, and enkephalin have been identified in recent studies as possibly playing a role in the expression of behavioral sensitization to cocaine (Cunningham et al., 1992; Heidbreder et al., 1993a,b; Parsons and Justice, 1993; Sala et al., 1995). None of the investigations supporting a role for dopamine or the other neurotransmitters divided rats treated with repeated cocaine into sensitized and nonsensitized groups. Thus, it is not known whether the alterations in these neurotransmitters, as in glutamate, are associated only with animals that develop behavioral sensitization.

### Mechanisms underlying augmented extracellular glutamate in the core

With the exception of during the first 20 min after injecting cocaine, the increase in extracellular glutamate was not affected by blocking voltage-dependent sodium channels with TTX. This indicates that the increase in extracellular glutamate was not attributable to impulse-dependent depolarization (Westerink et al., 1987) and argues that the elevation in extracellular glutamate produced by a cocaine challenge in cocaine-sensitized rats is attributable either to a decrease in glutamate uptake by neurons and glia or to impulse-independent, nonvesicular glutamate release. The former seems unlikely, because a reduction in uptake would be expected to elevate basal levels of extracellular glutamate, and the basal concentration of glutamate was somewhat reduced in the core of cocaine-pretreated rats (see Fig. 4). On the other hand, impulse-independent release of glutamate via reversal of the uptake transporter is well documented in pathophysiological situations, such as brain ischemia (Choi, 1988; Attwell et al., 1993; Nicholls, 1993). The fact that the cocaine-induced increase in the extracellular concentration of glutamate in the core of sensitized rats continued for the duration of the experiment may indicate that cocaine is triggering pathophysiological release of cytoplasmic glutamate. However, consistent with the lack of overt neurotoxicity in the nucleus accumbens of cocaine-treated animals (Kleven et al., 1988; Yeh and De Souza, 1991), the increased levels of glutamate produced by cocaine were at least 10-fold lower than the extracellular levels achieved after brain ischemia (Nilsson et al., 1990; Simpson et al., 1992). Ischemia-induced carrier-mediated efflux of glutamate is attributable to decreased  $\text{Na}^+/\text{K}^+$ -ATPase activity, which degrades the electrochemical gradient maintaining the inward directional bias for glutamate transport (Attwell et al., 1993; Nicholls, 1993). Three studies support a direct or indirect interaction between cocaine and  $\text{Na}^+/\text{K}^+$ -ATPase. (1) Cocaine inhibits neuronal  $\text{Na}^+/\text{K}^+$ -ATPase in fetal neurons (Lien et al., 1994). (2) Dopamine inhibits  $\text{Na}^+/\text{K}^+$ -ATPase in striatal cells (Bertorello et al., 1990), and the augmentation in extracellular dopamine (Pettit et al., 1990; Kalivas and Duffly, 1993; Parsons and Justice, 1993; Hooks et al., 1994) and postsynaptic dopamine responsiveness (Henry and White, 1991; Terwilliger et al., 1991) after repeated cocaine administration may inhibit  $\text{Na}^+/\text{K}^+$ -ATPase sufficiently to promote carrier-mediated efflux of glutamate. (3) Repeated treatment with another psychostimulant, methamphetamine, reduces ATP levels in the striatum (Chan et al., 1994).

## Mechanisms underlying augmented AMPA receptor responsiveness

One explanation for the augmented behavioral response to AMPA in rats sensitized to repeated cocaine is the regulation of non-NMDA ionotropic receptors by dopamine receptor stimulation. Electrophysiological studies show that dopamine modulates the excitatory effects of amygdala and hippocampal stimulation on accumbal neurons (for review, see Mogenson et al., 1993) and that EAAergic afferents from the hippocampus, amygdala, and prefrontal cortex form synaptic contacts on the same accumbal cells as dopaminergic axon terminals (Sesack and Pickel, 1990, 1992; Meredith et al., 1993). Also,  $D_1$ -receptor stimulation enhances glutamate-induced membrane polarizations in retinal bipolar cells (Maguire and Werblin, 1994). The marked increase in the inhibitory effects of  $D_1$ -receptor agonists in the nucleus accumbens of rats pretreated with daily cocaine or amphetamine (Henry et al., 1989; Higashi et al., 1989; Wolf et al., 1993) supports the possibility that  $D_1$ -receptor stimulation may influence AMPA receptor-mediated neurotransmission.

Although the present results show that AMPA-induced behavioral hyperactivity is increased in cocaine-sensitized animals, White et al. (1995a) demonstrated recently that the stimulation of accumbal neurons by iontophoretically applied glutamate is blunted after repeated cocaine. It is not precisely clear how EAA afferents to the nucleus accumbens influence neuronal activity to modulate motor behaviors. This lack of clarity is reflected by two paradoxes (for review, see Kalivas et al., 1993; Mogenson et al., 1993). (1) Microinjection of dopamine or EAA agonists into the nucleus accumbens elicits motor activity, yet dopamine antagonizes the electrophysiological response to the stimulation of EAA afferents. (2) Despite the fact that activation of all EAA afferents stimulates neuronal activity in the nucleus accumbens, stimulation of EAA afferents from the hippocampus produces motor activity and stimulation of EAA afferents from the amygdala causes freezing behavior. These discrepancies indicate that responses produced by AMPA administration to single neurons may not reflect the behavioral response elicited when many neurons are affected by microinjected AMPA. Another possible explanation rests on the fact that the electrophysiological data were obtained from anesthetized animals (White et al., 1995a). Considering the distinction in AMPA responsiveness between sensitized and nonsensitized rats in the present report, electrophysiological measurements made in conscious rats may reflect more adequately the behavioral output of the nucleus accumbens (Henriksen and Giachino, 1993).

## CONCLUSIONS

These studies reveal that augmented EAA transmission in the nucleus accumbens may have a role in the expression of behavioral sensitization to cocaine. This possibility is buttressed by the fact that the increases in extracellular glutamate and behavioral responsiveness to AMPA receptor stimulation occurred only in rats pretreated with daily cocaine that developed behavioral sensitization. Although changes in many transmitters in the nucleus accumbens are likely to be involved, the present data suggest that glutamate transmission acts as one of the primary determinants in the expression of the behavioral sensitization to cocaine.

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