Time Course of Extracellular Dopamine and Behavioral Sensitization to Cocaine. I. Dopamine Axon Terminals

Peter W. Kalivas and Patricia Duffy

Alcohol and Drug Abuse Program and Department of Veterinary Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, Washington 99164-6520

Repeated administration of cocaine to rodents produces a progressive augmentation in motor activity known as behavioral sensitization. By using microdialysis in the ventral striatum, some studies have found that the development of behavioral sensitization is associated with a similar augmentation in dopamine release, while others have not. It was postulated that differences in doses and withdrawal periods may account for the discrepancies between studies. Rats were behaviorally sensitized to daily peripheral injections using two cocaine treatment regimens (15 mg/kg, i.p. $\times$ 5 d or 30 mg/kg, i.p. $\times$ 5 d). Using *in vivo* microdialysis in the ventral striatum, the effect of acute cocaine (15 mg/kg, i.p.) on extracellular dopamine content and motor behavior was examined at various times after discontinuing daily treatments. Twenty-four hours after discontinuing the low dose of daily cocaine, the increase in motor activity and extracellular dopamine elicited by an acute cocaine challenge was significantly elevated. In contrast, following the higher daily treatment regimen there was a significant augmentation in motor activity, but the increase in extracellular dopamine produced by cocaine was significantly reduced. When rats were challenged 10–14 d after discontinuing either dosage regimen of daily cocaine, the increase in both motor activity and extracellular dopamine was augmented. In general, the increase in extracellular dopamine by an acute cocaine challenge increased over time when rats were challenged between 1 and 22 d after discontinuing daily cocaine. Basal concentrations of extracellular dopamine were determined by measuring the *in vivo* flux of dopamine across the dialysis membrane, and there was no significant difference at 24 hr or 2 weeks following the last daily injection of saline or cocaine. It is concluded that behavioral sensitization to cocaine is generally associated with an augmentation in extracellular dopamine in the ventral striatum, but that high doses of daily cocaine produce apparent tolerance to the augmentation in extracellular dopamine during the early withdrawal period.

[Key words: cocaine, dopamine, nucleus accumbens, sensitization, locomotion, dialysis]

The acute motor stimulant effect of systemic cocaine is thought to be mediated by increased dopamine transmission in the forebrain resulting from a blockade of presynaptic dopamine reuptake from the synaptic cleft (Hadfield and Nugent, 1983; Reith et al., 1986; Izenwasser et al., 1990). Thus, cocaine-induced motor excitation is prevented by the microinjection of dopamine antagonists into the nucleus accumbens, or by destruction of accumbal dopamine terminals (van Rossum et al., 1962; Kelly and Iversen, 1975; Scheel-Kruger et al., 1977). Motor activity is also produced by direct cocaine administration into the nucleus accumbens (Delfs et al., 1990). Finally, the motor stimulation produced by acute peripheral cocaine injection is associated with an increase in extracellular dopamine in the ventral striatum, including the nucleus accumbens, as measured by *in vivo* dialysis (Di Chiara and Imperato, 1988; Hurd et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990).

When cocaine is injected repeatedly, the motor stimulant response increases with each subsequent injection (Downs and Eddy, 1932; Post and Rose, 1976; Kalivas et al., 1988). This phenomenon, termed behavioral sensitization, has been shown to occur with as little as a single injection of cocaine (Weiss et al., 1989), and to persist for weeks after discontinuing cocaine treatment (Kalivas and Stewart, 1991). Like the acute motor stimulant effect of cocaine, the sensitized motor response has been postulated to result from augmented dopamine release in the forebrain. Because cocaine potentiates dopamine transmission by blocking the reuptake of dopamine into axon terminals, the use of traditional postmortem measures of dopamine turnover such as tissue levels of dopamine metabolites and dopamine accumulation has not proven very useful in examining this hypothesis (Taylor and Ho, 1977; Hanson et al., 1987; Kalivas et al., 1988). More recently, *in vivo* brain microdialysis has been used to measure directly the extracellular concentration of dopamine in the nucleus accumbens and striatum. In general, this technique has verified the involvement of dopamine in behavioral sensitization to cocaine by showing that an increase in extracellular dopamine is associated with the augmented behavioral response to repeated cocaine injections (Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990). However, in one study where rats self-administered cocaine, there was a reduction, not an augmentation, in extracellular dopamine in the nucleus accumbens in response to an acute cocaine challenge (Hurd et al., 1989). Also, Segal and Kuczenski (1992b) recently observed that the daily administration of cocaine produced behavioral sensitization without augmenting the extracellular content of dopamine in the nucleus accumbens. Similar to cocaine, many dialysis studies conducted in amphetamine- or methamphetamine-sensitized rats reveal that augmented extracel-

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Correspondence should be addressed to Peter Kalivas, Ph.D., Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

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Table 1. Treatment groups for dialysis in the ventral striatum

Group	Treatment day					
	1	2–6	7	10	17–21	27–29
1	Saline (10)	Cocaine, 30 mg/kg	Saline (11)	—	Saline (13)	—
2	Saline (7)	Saline	Cocaine (9)	—	Cocaine (8)	—
3	Cocaine (30)	Cocaine, 15 mg/kg	Cocaine (8)	Cocaine (8)	Cocaine (13)	Cocaine (9)
4	Cocaine (22)	Cocaine, 30 mg/kg	Cocaine (12)	Cocaine (6)	Cocaine (9)	Cocaine (8)
5	Saline	Saline	Basal (6)	—	Basal (6)	—
6	Cocaine	Cocaine, 30 mg/kg	Basal (6)	—	Basal (8)	—

Days 1, 7, 10, 17–21, and 27–29 were conducted in the photocell/dialysis apparatus and rats were injected with cocaine (15 mg/kg, i.p.) or saline, or different concentrations of dopamine were passed through the probe *in vivo* (groups 5 and 6). On days 2–6 rats received a daily intraperitoneal injection of saline or 15 or 30 mg/kg of cocaine in the home cage. The number of determinations at each time is shown in parentheses.

lular dopamine levels in the nucleus accumbens and striatum are associated with behavioral sensitization (Robinson et al., 1988; Kazahaya et al., 1989; Akimoto et al., 1990; Patrick et al., 1991). However, one laboratory found that daily amphetamine administration resulted in robust behavioral sensitization without an accompanying augmentation in extracellular dopamine in the nucleus accumbens or striatum (Kuczenski and Segal, 1990; Segal and Kuczenski, 1992a).

One possible explanation for the negative reports is revealed in previous studies demonstrating that after longer drug withdrawal periods behavioral sensitization increases (Kolta et al., 1985; Robinson and Becker, 1986; Antelman, 1988). This seems especially true if large doses of psychostimulants are employed. The negative reports with microdialysis examined a relatively short withdrawal period of 24–48 hr, and in one of the cocaine studies, high doses of cocaine were being self-administered (approximately 30 mg/kg, i.v. $\times$ 10 d) (Hurd et al., 1989; Kuczenski and Segal, 1990; Segal and Kuczenski, 1992a,b). Based upon the possible role of withdrawal period and dose on the association between dopamine release and psychostimulant-induced behavioral sensitization, the following experiments examined the effect of two different daily doses of cocaine on extracellular dopamine in the nucleus accumbens and motor activity in response to a cocaine challenge made between 1 and 21 d after discontinuing daily drug treatment.

A small portion of these data were reported previously in a review (Kalivas and Stewart, 1991).

Materials and Methods

Animal housing and surgery. Male Sprague–Dawley rats (Laboratory Animal Resource Center, Pullman, WA) were individually housed with food and water made available *ad libitum*. A 12 hr/12 hr light/dark cycle was used with the lights on at 6:30 hr. All injections of cocaine were made between 11:00 and 13:00 hr.

Rats weighing between 260 and 320 gm were anesthetized with Equithesin (3.0 ml/kg; 9.7 gm of sodium pentobarbital, 42.5 gm of chloral hydrate, 21.3 gm of MgSO₄ dissolved in one liter of 11% ethanol, 42% propylene glycol, v/v) and mounted in a stereotaxic apparatus (David Kopf, Torrance, CA). Either chronic unilateral or bilateral dialysis (20 gauge stainless steel, 12 mm long) guide cannulas were implanted 3 mm dorsal to the nucleus accumbens (A/P 8.7 to 9.0 mm, D/V –0.5 to 0.0 mm, M/L 1.7 mm; relative to the interaural line according to the atlas of Pellegrino et al., 1979). The cannulas were cemented into place by affixing dental acrylic to three stainless steel screws tapped into the skull. The wounds were sutured, and the rats were allowed a minimum of 1 week recovery prior to beginning experimentation.

Microdialysis. All rats were placed into individual photocell cages (Omnitech Electronic, Inc., Columbus, OH) that had been modified to conduct intracranial dialysis experiments (Kalivas and Duffy, 1990). The dialysis probes were constructed as described by Robinson and Whishaw (1988), with 2.0–3.0 mm of active dialysis membrane exposed

at the tip. The probes were inserted through one of the guide cannulas into the ventral striatum the night prior to the experiment. The next day, dialysis buffer (5 mM KCl, 120 mM NaCl, 1.8 mM CaCl₂, 1.2 mM MgCl₂, plus 0.2 mM phosphate-buffered saline 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 μ l/min (Harvard Instruments, Boston, MA) for 2 hr. Baseline samples (20 min each) were collected for 60–80 min, and then cocaine (15.0 mg/kg, i.p.) or saline (1.0 ml/kg, i.p.) was administered and between six and nine additional 20 min dialysis samples were obtained. Behavioral data were collected in 20 min intervals simultaneous with the dialysis samples. When the experiment was terminated, the dialysis probe was removed, and the animal returned to its home cage or killed for histological verification of dialysis probe placement (see below).

Treatment groups 1–4. Table 1 shows the cocaine treatment and dialysis schedule. In the initial experiments, rats were implanted with a unilateral guide cannula ($N = 22$), and were examined at a single time point. However, in later experiments ($N = 108$), rats were implanted with bilateral guide cannulas and two experiments were performed in each rat. In all cases, each rat was acutely implanted with a dialysis probe only once in each guide cannula. Thus, rats receiving bilateral guide cannulas underwent two separate dialysis experiments (i.e., on days 1, 7, 10, 17–21, 27–29; see Table 1).

All rats were placed into the photocell/dialysis apparatus the night prior to day 1 and connected to a dialysis probe connector. However, only a portion of rats had a dialysis probe inserted into the ventral striatum ($N = 69$ out of 130 rats). Daily injections on days 2–6 were made in the home cage. All acute challenges of cocaine or saline made following the daily treatments (i.e., days 7–29) were in the photocell/dialysis chamber, and the rats were attached to a dialysis connector with a dialysis probe inserted into the ventral striatum. In group 1, all rats were injected with saline (1.0 ml/kg, i.p.) in the photocell/dialysis apparatus on day 1, and on days 2–6 were given daily cocaine (30 mg/kg, i.p.) in the home cage. Then the rats were injected with saline (1.0 ml/kg, i.p.) in the photocell/dialysis chamber on days 7 and/or 20. The rats placed in group 2 were injected with saline on days 1–6, and then challenged with cocaine (15 mg/kg, i.p.) on days 7 and/or 20. The treatments in group 3 consisted of cocaine (15 mg/kg, i.p.) on days 1–6 followed by an acute cocaine challenge (15 mg/kg, i.p.) on days 7, 10, 17–21, or 27–29. The fourth treatment group received cocaine (15 mg/kg, i.p.) on day 1, cocaine (30 mg/kg, i.p.) on days 2–6, and an acute cocaine challenge (15 mg/kg, i.p.) on days 7, 10, 17–21, or 27–29. In 68 rats treated with the high dose of cocaine (i.e., groups 1 and 4), 9 died from convulsions occurring within 60 sec after administering the cocaine. No convulsive activity was observed in the other rats. Of 238 possible dialysis experiments from 130 rats in groups 1–4, 183 (77%) were used in data analysis. In addition to cocaine-induced convulsions, experiments were excluded or not performed for one of the following reasons: probe placement was outside the ventral striatum, the guide cannula was obstructed, the acrylic implant was loose, the basal levels of dopamine were unstable, or chromatographic difficulties occurred.

Determination of basal levels of extracellular dopamine (groups 5 and 6). In separate animals, the basal concentration of extracellular dopamine was determined by adding dopamine to the dialysis perfusate at concentrations above and below the expected extracellular concentration to generate a series of points that were interpolated to measure the concentration at which no net flux of dopamine occurred across the dialysis membrane (Lonroth et al., 1987; Parsons and Justice, 1991;

Figure 1. The effect of acute cocaine and saline in naive rats on motor activity and extracellular dopamine in the ventral striatum. Data were pooled from day 1 of treatment groups 1–4. The neurochemical data were normalized by dividing all values by the average of the three baseline measurements made before the injection of acute saline. The data are shown as mean $\pm$ SEM photocell counts or percentage change in dopamine. Basal values (fmol/20 min) and number of determinations: saline, 62 ± 8 , $N = 17$; cocaine, 89 ± 11 , $N = 52$ ($F = 3.70$, $p = 0.059$). All data were analyzed using a two-way ANOVA with repeated measures over time. F scores for the photocell counts: treatment $F = 11.87$, $p < 0.001$; time $F = 46.51$, $p < 0.001$; interaction $F = 4.49$, $p < 0.001$. Dopamine F scores: treatment $F = 6.13$, $p = 0.016$; time $F = 33.22$, $p < 0.001$; interaction $F = 5.08$, $p < 0.001$. *, $p < 0.05$, comparing cocaine to saline using a least significant difference post hoc analysis, as described by Milliken and Johnson (1984).

Parsons et al., 1991a). A total of 18 rats with bilateral guide cannulas over the ventral striatum were used. As shown in Table 1, group 5 received saline (1.0 ml/kg, i.p.) on days 1–6 and group 6 received cocaine (15 mg/kg, i.p.) on day 1 and cocaine (30 mg/kg, i.p.) on days 2–6. The probes were inserted the night before the experiment. Perfusion of dialysis buffer (containing 0.25 mM ascorbic acid to inhibit dopamine oxidation) was begun in the morning, and 3 hr later 0, 1, 3, or 10 nM dopamine was advanced through the probe. Five 20 min dialysis samples were obtained at each concentration of dopamine, and the last two samples were averaged for a determination of the net flux of dopamine into or out of the dialysis buffer. A steady state between the perfusate and extracellular dopamine concentration was achieved more rapidly than has been reported previously (Parsons and Justice, 1991; Parsons et al., 1991a) because of the higher flow rate employed in the present study (1.9 vs. 0.2 μ l/min).

Measurement of dopamine in dialysis samples. The dialysis samples were collected into microfuge tubes in 20 μ l of mobile phase (0.1 M citric acid, 75 mM Na_2HPO_4 , 0.6–1.0 mM heptane sulfonic acid, 0.1 mM EDTA, 13% methanol, v/v, pH 3.8–4.2) plus 2.0 pmol of dihydroxybenzylamine as the internal standard, and placed in a freezer (-70°C) until analyzed for dopamine content. The samples were placed in a refrigerated autosampler (Gilson Medical Supplies, Middleton, WI) and dopamine content measured using HPLC 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 Inc., 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 (-0.2 V). Peaks were recorded on a chart recorder and compared to an external standard curve (10–1000 fmol). For experiments using no net flux of dopamine, the pH of the mobile phase was 6.0 to avoid coelution of dopamine and ascorbic acid.

Histology and data analysis. Rats were killed with an overdose of pentobarbital, and their brains were removed and placed in 10% formalin for at least 1 week. The brains were then blocked and coronal sections (100 μ m thick) made with a vibratome. The sections were mounted on gelatin-coated slides and stained with cresyl violet, and probe sites were determined by an individual unaware of the rats' behavioral or neurochemical response according to the atlas of Paxinos and Watson (1986).

Some rats were used twice while others only once (see above). To determine if the repeated measurements could be pooled with the single measurements, the variance between rats used twice and those used once was compared at each day with an F test. In no instance was the F score significant ($p < 0.05$), and the data were pooled. The neurochemical and behavioral time course data were statistically evaluated using a two-way analysis of variance (ANOVA) with repeated measures over time. The dopamine content was normalized to percentage change from the average of three baseline samples for each experiment prior to statistical analysis. Pos hoc evaluation of statistical differences was performed using a least significant difference test, as described by Milliken and Johnson (1984). To determine the concentration corresponding to no net flux of dopamine through the dialysis probe, regression analysis was performed (Lonroth et al., 1987; Parsons and Justice, 1991; Parsons et al., 1991), and basal concentrations compared between cocaine and saline treatment groups using a two-tailed Student's t test.

Results

Effect of acute cocaine and saline in untreated rats. Figure 1 shows the pooled data from groups 1–4 for animals undergoing dialysis on day 1. The behavioral response to cocaine (15 mg/kg, i.p.) reached a maximum during the first 20 min after injection and returned to baseline levels by 100 min after injection. Cocaine significantly increased the level of extracellular dopamine in the ventral striatum, with a peak response between 20 and 40 min after injection, and returned to baseline levels by 80 min after injection.

Effect of daily cocaine on the response to a saline challenge, group 1. Figure 2 shows the behavioral and neurochemical effects of saline (1.0 ml/kg, i.p.) in rats treated with daily cocaine (30 mg/kg, i.p. $\times$ 5 d; group 1). This cocaine treatment regimen did not significantly alter the response to saline. A modest increase in horizontal photocell counts and no alteration in extracellular dopamine in the ventral striatum was produced on day 1 before daily cocaine administration and on days 7 and 20 after discontinuing the daily injections.

Effect of daily cocaine on the response to a cocaine challenge, groups 2–4. Figure 3 shows the effect of the three daily treatment regimens on extracellular dopamine and motor activity following an acute cocaine challenge (15 mg/kg, i.p.) on day 7 (i.e., 24 hr after discontinuing daily treatments). Compared to the rats pretreated with daily saline (group 2), rats pretreated with daily cocaine demonstrated an increase in cocaine-induced motor activity. However, only at 20 min after cocaine administration

Figure 2. Effect of daily cocaine on the behavioral and neurochemical response to an acute saline challenge on day 1 (■), day 7 (◆), or day 20 (□). These data were derived from group 1 shown in Table 1. The neurochemical data were normalized by dividing all values by the average of the three baseline measurements made before the injection of acute saline. The data are shown as mean $\pm$ SEM photocell counts or percentage change in dopamine. Basal values (fmol/20 min) and number of determinations for each day: day 1, 48 ± 10 , $N = 10$; day 7, 79 ± 19 , $N = 11$; day 20, 95 ± 16 , $N = 13$ ($F = 2.09$, $p = 0.141$). All data were analyzed using a two-way ANOVA with repeated measures over time. F scores for the photocell counts: day $F = 2.24$, $p = 0.123$; time $F = 15.39$, $p < 0.001$; interaction $F = 1.40$, $p = 0.142$. Dopamine F scores: day $F = 0.15$, $p = 0.866$; time $F = 1.52$, $p = 0.149$; interaction $F = 0.47$, $p = 0.961$. *, $p < 0.05$, comparing the effect of cocaine on days 7 and 20 to the effect on day 1 within each time bin using a least significant difference post hoc analysis, as described by Milliken and Johnson (1984).

was the increase significant. The rats pretreated with the lower dose of daily cocaine (15 mg/kg, i.p.; group 3) also showed a significant elevation in extracellular dopamine content in the first 20 min after cocaine injection. In contrast, rats receiving the highest dose of daily cocaine (30 mg/kg, i.p.; group 4) demonstrated a significant decrease in extracellular dopamine during the first 20 min after injection.

Figure 4 shows the effect of the three daily treatment regimens on the response to a cocaine challenge (15 mg/kg, i.p.) given on days 17–21 (i.e., 11–15 d after discontinuing the daily injections). Compared to rats pretreated with daily saline (group 2), both daily cocaine pretreatment groups demonstrated behavioral sensitization. Following the lower dose of daily cocaine

Figure 3. Effect of daily cocaine pretreatment on the effect of an acute cocaine challenge made 24 hr after discontinuing daily treatment. Rats were pretreated with daily saline (group 2, $N = 9$), daily cocaine, (15 mg/kg; group 3, $N = 8$) or daily cocaine (30 mg/kg; group 4, $N = 12$), and challenged with acute cocaine (15 mg/kg, i.p.) 1 d after discontinuing the daily treatments (i.e., day 7). **A.** Photocell counts are shown as mean $\pm$ SEM. Treatment $F = 1.40$, $p = 0.26$; time $F = 41.26$, $p < 0.001$; interaction $F = 1.69$, $p = 0.051$. **B.** Extracellular dopamine is shown as the mean $\pm$ SEM percentage change from the average of the three baseline samples. Treatment $F = 1.20$, $p = 0.32$; time $F = 24.14$, $p < 0.001$; interaction $F = 1.95$, $p = 0.017$. Basal values (fmol/20 min): group 1, 72 ± 12 ; group 2, 77 ± 14 ; group 3, 76 ± 17 ($F = 0.026$, $p = 0.974$). *, $p < 0.05$, comparing the effect of cocaine in daily cocaine-treated rats (groups 3 and 4) with daily saline-treated rats (group 2) within each time bin using a least significant difference post hoc analysis as described by Milliken and Johnson (1984).

(15 mg/kg, i.p.; group 3), the increase was significant during the first 20 min after injection. In group 4, which was pretreated with the high dose of daily cocaine (30 mg/kg, i.p.), the augmentation in photocell counts was significant at 20 and 40 min after injection. In comparison, the effect of daily cocaine pretreatment on extracellular dopamine was more marked. The lowest dose of daily cocaine resulted in a significant augmentation at 40, 60, and 100 min after the acute cocaine challenge, and in rats pretreated with the high dose of daily cocaine, extracellular dopamine was significantly augmented at 20, 40, and 60 min after injection.

Figure 4. Effect of daily cocaine pretreatment on the effect of an acute cocaine challenge made 11–15 d after discontinuing daily treatment. Rats were pretreated with daily saline (group 2, $N = 8$), daily cocaine, (15 mg/kg; group 3, $N = 13$) or daily cocaine (30 mg/kg; group 4, $N = 9$), and challenged with acute cocaine (15 mg/kg, i.p.) 11–15 d after discontinuing the daily treatments (i.e., days 17–21). **A**, Photocell counts are shown as mean $\pm$ SEM. Treatment $F = 1.19$, $p = 0.32$; time $F = 47.31$, $p < 0.001$; interaction $F = 3.24$, $p < 0.001$. **B**, Extracellular dopamine is shown as the mean $\pm$ SEM percentage change from the average of the three baseline samples. Treatment $F = 5.87$, $p = 0.008$; time $F = 38.06$, $p < 0.001$; interaction $F = 2.73$, $p < 0.001$. Basal values (fmol/20 min): group 2, 67 ± 9 ; group 3, 65 ± 16 ; group 4, 62 ± 14 ($F = 0.31$, $p = 0.737$). *, $p < 0.05$, comparing the effect of cocaine in daily cocaine-treated rats (groups 3 and 4) with daily saline-treated rats (group 2) within each time bin using a least significant difference post hoc analysis as described by Milliken and Johnson (1984).

Figure 5 shows the cumulative photocell counts and extracellular dopamine over 120 min after the injection of cocaine in groups 3 and 4. Comparisons were made between the injection of cocaine on day 1 and the cocaine challenges made after discontinuing daily cocaine treatment (i.e., days 7, 10, 17–21, and 27–29). Daily treatment with 15 mg/kg (group 3) significantly enhanced the motor stimulant effect of cocaine at all times compared to day 1 (Fig. 5A). However, extracellular dopamine was augmented only following cocaine administration in the last two challenge periods, days 17–21 and 27–29 (Fig. 5B). After daily treatment with 30 mg/kg (group 4), photocell

Figure 5. Cumulative photocell counts and area under the curve for extracellular dopamine during the first 120 min after injection of cocaine. Data are from groups 3 (daily 15 mg/kg, i.p.) and 4 (daily 30 mg/kg, i.p.). The number of determinations is shown within each bar, and the data are shown as mean $\pm$ SEM. The dopamine values were converted to percentage change from the average of three basal values in each dialysis experiment and analyzed with a one-way ANOVA. **A**, $F(4,67) = 6.93$, $p < 0.001$. **B**, $F(4,67) = 4.64$, $p = 0.002$. Basal values (fmol/20 min): day 1, 83 ± 8 ; day 7, 77 ± 14 ; day 10, 122 ± 38 ; days 17–21, 65 ± 16 ; days 27–29, 43 ± 9 ($F = 1.97$, $p = 0.113$). **C**, $F(4,56) = 4.51$, $p = 0.003$. **D**, $F(4,56) = 3.45$, $p = 0.014$. Basal values (fmol/20 min): day 1, 97 ± 14 ; day 7, 76 ± 17 ; day 10, 55 ± 13 ; days 17–21, 62 ± 14 ; days 27–29, 94 ± 21 ($F = 1.59$, $p = 0.189$). *, $p < 0.05$, comparing all days to day 1 with a Dunnett's test.

counts were elevated at all times except on day 10 (Fig. 5C). Similar to the lower dose of daily cocaine, extracellular dopamine was augmented only during the last two challenge periods (Fig. 5D).

Basal concentration of dopamine, groups 5 and 6. Regression curves were obtained from the titration of increasing concentrations of dopamine through the dialysis probe. The point of no net flux is indicative of the basal concentration of extracellular dopamine. Figure 6 shows that on day 7 the basal value of dopamine was slightly, but not significantly, reduced in rats pretreated with daily cocaine compared to daily saline rats (saline, 3.62 ± 0.50 nM; cocaine, 3.14 ± 0.56 nM). However, the slope of the regression line obtained from cocaine-pretreated rats was significantly elevated (saline, 0.26 ± 0.03 ; cocaine, 0.47 ± 0.07 ; $t_{10} = 36.2$, $p < 0.001$). While the slopes were not significantly different on day 20 between daily saline- and cocaine-pretreated rats (saline, 0.31 ± 0.08 ; cocaine, 0.39 ± 0.05), the basal extracellular concentration of dopamine in cocaine-pretreated rats approached a significant increase compared to controls (saline, 3.35 ± 0.26 nM; cocaine, 4.85 ± 0.87 nM; $t_{12} = 1.82$, $p < 0.1$). The slopes of the lines are less than those previously reported in the ventral striatum of control animals using this method (Parsons and Justice, 1991, 0.67 ± 0.08 and 0.64

± 0.09 ; Parsons et al., 1991a, 0.60 ± 0.03 ; Parsons et al., 1991b, 0.60 ± 0.03), because of the higher flow rate employed in the present study ($1.9 \mu\text{l}/\text{min}$ vs. $0.2 \mu\text{l}/\text{min}$). The mean correlation coefficient for each of the lines in Figure 6 was greater than 0.96.

Histology. Figure 7 illustrates the placement of the dialysis probes in the ventral striatum from groups 1–4. The majority of probes were placed in the rostral nucleus accumbens, medial to the anterior commissure. While at least 50% of the dialysis membrane of most probes was in the nucleus accumbens, all probes were also located partly in the striatum and/or olfactory tubercle. Six probes in the ventral striatum were caudal to the illustrations in Figure 7 at A/P = 10.0 ± 0.2 mm. Eleven of a total of 194 probe placements from completed experiments were determined to be outside of the ventral striatum, 7 of which were rostral to the diagrams in Figure 7. The probe placements for groups 5 and 6 were also located predominantly in the rostromedial ventral striatum. One probe placement from 31 completed experiments was rostral to the ventral striatum. In four experiments, the correlation coefficient of the regression line was <0.90 and these data were excluded from statistical analysis.

Discussion

The present study demonstrates that after repeated daily cocaine injections the sensitized behavioral response to an acute cocaine challenge is generally associated with an augmentation in extracellular dopamine concentrations in the ventral striatum. This is in agreement with recent studies examining this behavioral sensitization to repeated administration of cocaine (Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990) and other psychostimulants such as amphetamine, methamphetamine, and methylphenidate (Robinson et al., 1988; Kazahaya et al., 1989; Akimoto et al., 1990; Patrick et al., 1991). However, during the first 1–4 d after discontinuing daily treatments with the higher dose of cocaine, the behavioral augmentation was not associated with elevated extracellular dopamine. This apparent dissociation at early withdrawal time points from high doses of cocaine is consistent with the finding of Hurd et al. (1989) that 24 hr after rats had been self-administering cocaine (approximately 30 mg/kg, i.v. $\times 10$ d), a decrease in extracellular dopamine was produced by an acute cocaine challenge compared to a cocaine challenge in naive rats. Kuczenski and Segal (1990) and Segal and Kuczenski (1992a,b) have also observed that behavioral sensitization to repeated amphetamine or cocaine treatments was associated with a significant reduction in extracellular dopamine in both the striatum and nucleus accumbens. In these studies, moderate doses of daily amphetamine (2.5 mg/kg, i.p. $\times 6$ d) or cocaine (10 mg/kg, i.p. $\times 6$ d) were given, and the rats challenged with amphetamine or cocaine 2 d after discontinuing the daily treatments. In contrast, Robinson et al. (1988) observed behavioral sensitization associated with augmented dopamine release in the ventral striatum using a much more aggressive repeated amphetamine treatment regimen in which intraperitoneal doses of amphetamine were escalated from 1.0 to 10.0 mg/kg over a 35 d treatment period. However, the test for sensitization was not made until 15–21 d after discontinuing the sensitizing treatment regimen.

The data to date with daily cocaine and amphetamine indicate that tolerance may develop to psychostimulant-induced elevation in extracellular dopamine that persists for a few days after discontinuing administration. Thus, Hurd et al. (1989) and Segal and Kuczenski (1992a,b) observed a decrease in the ca-

Figure 6. Basal extracellular concentration of dopamine in ventral striatum determined using *in vivo* net flux of dopamine. Dialysis was performed on day 7 or day 20 after the rats had been pretreated with saline (group 5 in Table 1) or cocaine (group 6) on days 1–6. Each line was derived from six to eight animals, and the data are shown as the mean $\pm$ SEM difference between the concentration of dopamine applied to the dialysis probe in the perfusate and that collected at the probe effluent (Parsons and Justice, 1991). Zero on the y-axis is the interpolated concentration of dopamine in the perfusate at which no net flux with the extracellular fluid occurs and corresponds to the basal concentration of dopamine. The basal concentration of dopamine was unaltered in cocaine-pretreated rats at either withdrawal time, but on day 7 the slope of the line was significantly elevated.

capacity of an acute challenge with cocaine or amphetamine to elevate extracellular dopamine during the early withdrawal from a daily drug treatment regimen. Likewise, we found a reduction in the effect of cocaine on day 7 in rats pretreated daily with 30 mg/kg intraperitoneal cocaine (group 4), but also observed that the apparent tolerance subsided with time such that an augmentation in extracellular dopamine was manifest by 11–15 d following the discontinuation of daily cocaine. One study reported a similar increase in the sensitization of *in vitro* dopamine release with the passage of time after discontinuing a sensitizing psychostimulant treatment regimen (Kolta et al., 1985). Also, many studies have revealed an increase in behavioral sensitization over time (Hitzemann et al., 1980; Hirabayashi and Alam, 1981; Kolta et al., 1985; Antelman, 1988; Kalivas and Duffy, 1989; Robinson, 1991).

There are a number of possible mechanisms for the development of short-term tolerance to the effects of cocaine on extracellular dopamine. First, it has been proposed that, similar to high doses of amphetamines (Seiden et al., 1988), repeated cocaine administration may reduce the concentration of dopamine in various terminal fields (Dackis and Gold, 1985). In general, the measurement of tissue levels of dopamine in numerous laboratories has failed to verify this hypothesis (Kalivas et al., 1988; Kleven et al., 1988; Yeh and De Souza, 1991; but see Karoum et al., 1990). Likewise, there is not a reduction in basal levels of extracellular dopamine in the early withdrawal period after repeated cocaine or amphetamine (Robinson et al., 1988; Kalivas and Duffy, 1990; Parsons et al., 1991; Segal and Kuczenski, 1992a,b; present results). However, there have been reports of diminished levels of dopamine synthesis and tyrosine hydroxylase phosphorylation in the nucleus accumbens after

Figure 7. Illustration of dialysis probe placement in the ventral striatum, including the nucleus accumbens, olfactory tubercle, and striatum. Although the active portion of the probe varied from 2.0 to 3.0 mm in length, all probes were drawn as 2.5 mm. The *broken track lines* refer to probe placements excluded from data analysis. An additional seven were located rostral to the illustration. The *number* on each section refers to millimeters rostral to the interaural line according to the atlas of Paxinos and Watson (1986).

repeated cocaine administration (Kalivas et al., 1988; Brock et al., 1990; Beitner-Johnson and Nestler, 1991). Thus, while basal levels of intra- and extracellular dopamine are not significantly affected during withdrawal from cocaine, it is possible that dopamine terminals may be compromised in the ability to regulate dopamine synthesis in response to a cocaine challenge.

Another possible explanation for the apparent tolerance is that an increase in dopamine reuptake may compensate for the augmented release such that the amount of dopamine escaping the synaptic cleft and captured by the dialysis probe may be unaltered or decreased. The effects of repeated cocaine on *in vitro* measurements of uptake carrier function have been inconsistent (Izenwasser and Cox, 1990; Peris et al., 1990; Yi and Johnson, 1990). However, *in vivo* voltammetry was recently employed to demonstrate that after 24 hr, but not 10 d, after discontinuing daily cocaine there is a significant increase in dopamine uptake in the striatum (Ng et al., 1991). This is consistent with an increase in the slope of the dopamine titration curve in the cocaine-pretreated rats (Parsons et al., 1991; present results), which has been postulated to reflect increased elimination of dopamine from the synaptic cleft (Parsons and Justice, 1991; Parsons et al., 1992). Thus, enhanced elimination of dopamine, presumably resulting from augmented reuptake, may partly mask the appearance at the dialysis probe of increased

concentrations of dopamine in the synaptic cleft. However, Ng et al. (1991) demonstrated in rats pretreated with daily cocaine that electrical stimulation of the medial forebrain bundle significantly augmented dopamine overflow compared to control rats, even in the presence of the enhanced dopamine uptake. Furthermore, similar to the present report, this group found that after 24 hr of withdrawal from repeated cocaine, the basal levels of extracellular dopamine were unchanged in the presence of increased dopamine uptake (Parsons et al., 1991). Thus, enhanced reuptake does not offer a complete explanation for the lack of an augmentation in synaptic dopamine levels during the early withdrawal period from daily cocaine.

A final explanation for the apparent tolerance is that there may be an alteration in the presynaptic regulation of dopamine release. Likely candidates include changes in presynaptic inhibition produced by D_2 autoreceptors or changes in the presynaptic stimulation of dopamine release by excitatory amino acids or 5-HT. Data suggesting an alteration in D_2 autoreceptor function following repeated cocaine administration have been inconsistent (Dwoskin et al., 1988; Peris et al., 1990; Yi and Johnson, 1990). Supporting a role for excitatory amino acids is the observation that systemic administration of the noncompetitive NMDA receptor antagonist MK-801 with cocaine prevents the development of behavioral sensitization (Karler et al.,

1989). Also, lesions of the fimbria, which would disrupt the excitatory amino acid projection from the hippocampus to the nucleus accumbens, prevent the development of behavioral sensitization to cocaine (Yoshikawa et al., 1991). A role for 5-HT is indicated by the fact that cocaine augments 5-HT transmission by binding the 5-HT uptake carrier (Reith et al., 1986), and the recent observation that the *in vivo* administration of 5-HT into the nucleus accumbens increases the extracellular level of accumbal dopamine (Benloucif and Galloway, 1991; Chen et al., 1991).

Regardless of the mechanism mediating the lack of augmented extracellular dopamine during the early withdrawal period, it remains unknown how behavioral sensitization could occur in the absence of enhanced dopamine transmission in the nucleus accumbens. A number of factors may be involved. First, behavioral sensitization may be mediated by nondopaminergic substrates or dopamine terminals outside the ventral striatum. 5-HT augments dopamine release (Imperato and Angelucci, 1989; Jiang et al., 1990; Benloucif and Galloway, 1991; Chen et al., 1991; Yi et al., 1991), and a role for 5-HT and norepinephrine in modulating the acute motor effect of psychostimulants has been described (Segal, 1976; Dickinson et al., 1988; Kuczenski and Segal, 1988; Taghzouti et al., 1988). Also, sensitization of adrenergic transmission in the hippocampus can be produced by prior exposure to chronic stress (Nisenbaum et al., 1991). Although the striatum, nucleus accumbens, and olfactory tubercles are the dopamine terminal fields most frequently associated with the modulation of motor activity by amphetamine-like psychostimulants, other dopamine terminal fields may be important. Notably, pharmacologically stimulated dopamine transmission in the ventral pallidum (Napier et al., 1988; Klitenick et al., in press) and prefrontal cortex (Loulot et al., 1989) has been shown to modulate locomotor activity and/or dopamine release in the nucleus accumbens. While enhanced dopamine transmission in the ventral pallidum increases motor activity, dopamine appears to have an inhibitory influence in the prefrontal cortex (Deutch and Roth, 1990). Thus, although acute cocaine injection increases the extracellular content of dopamine in the prefrontal cortex (Moghaddam and Bunney, 1989), perhaps behavioral sensitization could be associated with a decrease in extracellular dopamine in the prefrontal cortex (Sorg, in press).

A second explanation for the apparent lack of an association between extracellular dopamine and behavioral sensitization may be the presence of environmental conditioning previously identified with single and repeated administration of psychostimulants (Tilson and Rech, 1973; Post et al., 1981; Weiss et al., 1989; see Stewart and Vezina, 1988, for review). Furthermore, Beninger et al. (1983, 1986) found that amphetamine- and cocaine-conditioned locomotor behavior does not depend on intact dopamine transmission. Although in the present study the majority of cocaine injections were made in the home cage, all rats showing behavioral sensitization received a single, potentially conditioning exposure to cocaine in the dialysis chamber on day 1 prior to the cocaine challenge during the withdrawal period. Also, the rats could have become conditioned to the injection procedure or interoceptive cues, such as local anesthesia at the injection site, regardless of the environment where cocaine was administered (see Stewart and Vezina, 1988, 1991, for discussion of these issues).

A final possible explanation is that the postsynaptic response to dopamine has been augmented such that the same or less

dopamine release could elicit a greater behavioral stimulant response. This possibility is consistent with reports showing that the inhibitory effect of dopamine on the firing frequency of neurons in the striatum (Rebec and Groves, 1976) and nucleus accumbens (Henry et al., 1989) is enhanced in rats pretreated with repeated injections of cocaine or amphetamine (but see Kamata and Rebec, 1985). The augmented response to iontophoretic dopamine in the nucleus accumbens of rats pretreated with repeated cocaine injections appears to result from an enhanced responsiveness of the D_1 receptor and endures for up to 30 d after discontinuing the repeated cocaine treatments (Henry and White, 1991). This is consistent with reports showing that an augmentation in dopamine stimulated adenylyl cyclase in the striatum of rats pretreated with daily amphetamine (Roseboom et al., 1990; Beitner-Johnson et al., in press; but see Barnett et al., 1987). In addition, the density of D_2 receptors is upregulated in the nucleus accumbens during the early withdrawal period after daily treatment with cocaine (Goeders and Kuhar, 1987; Kleven et al., 1990; Peris et al., 1990; Zeigler et al., in press).

Parsons et al. (1991a) examined the effect of repeated cocaine (20 mg/kg, i.p. $\times$ 10 d) on basal dopamine levels using the *in vivo* dopamine flux method and found that at 10 d of withdrawal, extracellular dopamine in the nucleus accumbens was reduced. In contrast, we observed no change 14 d after discontinuing repeated cocaine treatments (group 6). An explanation for this discrepancy is not readily apparent, but may involve differences in treatment and withdrawal parameters. Also, differences in dialysis buffer and flow rate may affect the basal levels of extracellular dopamine. Notably, the higher flow rate employed in the present study (1.9 μ l/min vs. 0.2 μ l/min) may cause a modest depletion of extracellular dopamine, as reflected by the lower basal levels of dopamine in the present study (3.35–3.62 nM) versus those obtained by Justice and coworkers (Parsons and Justice, 1991, 3.8–4.5 nM; Parsons et al., 1991, 3.9 nM; Parsons et al., 1991b, 6.9 nM). It is possible that a modest depletion may have masked the reduction in daily cocaine-pretreated rats.

In conclusion, long-term behavioral sensitization to repeated cocaine administration was associated with an augmentation in extracellular dopamine content in the ventral striatum. However, behavioral sensitization was present in the absence of augmented dopamine levels during the early withdrawal period from a relatively high-dosage, repeated cocaine pretreatment regimen. Thus, while alterations in dopamine release in the ventral striatum are likely to contribute to the long-term expression of behavioral sensitization produced by repeated cocaine administration, other factors need to be considered during the early withdrawal period.

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