The Journal of Neuroscience, January 1993, 13(1): 276–284

Time Course of Extracellular Dopamine and Behavioral Sensitization to Cocaine. II. Dopamine Perikarya

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Cocaine was administered daily (15 mg/kg, i.p. × 1 d followed by 30 mg/kg, i.p. × 5 d) to produce behavioral sensitization. Using microdialysis in the ventral tegmental area and medial substantia nigra, the effect of repeated cocaine was examined on the extracellular levels of dopamine. One day after discontinuing repeated cocaine injections, an acute challenge with cocaine (15 mg/kg, i.p.) produced a significant elevation in extracellular dopamine compared to rats pretreated with daily saline (×6 d). The augmentation in extracellular dopamine persisted longer than the sensitized behavioral response. In contrast, 14 d after discontinuing daily cocaine, the increase in extracellular dopamine produced by an acute cocaine challenge was not augmented, although behavioral sensitization was present. In separate animals, the basal concentration of dopamine in the ventral tegmental area/medial substantia nigra was measured by determining the concentration of dopamine at which no net flux occurred across the dialysis membrane *in vivo*. One day after discontinuing daily treatments, the basal level of extracellular dopamine in the cocaine pretreated rats was significantly elevated over the level in saline-pretreated animals (1.3 nM vs. 0.8 nM). By 14 d after the last daily injection, the basal levels of dopamine were equivalent in cocaine- and saline-pretreated animals. It is concluded that daily cocaine injections produce a transient alteration in the regulation of somatodendritic dopamine release. While such changes are not responsible for the long-term behavioral sensitization produced by repeated cocaine administration, they may be involved in the initiation of behavioral sensitization.

[Key words: cocaine, dopamine, ventral tegmental area, sensitization, locomotion, dialysis]

Behavioral augmentation resulting from the repeated administration of psychostimulants is generally found to be associated with enhanced extracellular dopamine content in the striatum and nucleus accumbens (see Kalivas and Stewart, 1991, for review). As shown in the preceding report, the association between extracellular dopamine and behavior varies with the treatment regimen and the time course of drug withdrawal (Kalivas and Duffy, 1992). Nonetheless, there is a concurrence in the literature that behavioral sensitization that endures for weeks or months after discontinuing daily psychostimulant administration is associated with an augmentation in extracellular dopamine content in axon terminal fields, such as the striatum and nucleus accumbens (Robinson et al., 1988; Akimoto et al., 1989, 1990; Kazahaya et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990; Patrick et al., 1991). These findings argue that a change in dopamine release or reuptake in dopamine axon terminals is important in behavioral sensitization. While presynaptic regulation by other transmitters has been postulated to play a role in altering axonal dopamine transmission (Benloucif and Galloway, 1991; Yoshikawa et al., 1991), it is also likely that alterations in the dopamine cell bodies may be critical. Pretreatment with the protein synthesis inhibitor anisomycin prevents the development of behavioral sensitization to amphetamine (Robinson, 1991), arguing for an alteration in perikarya. Furthermore, microinjection of amphetamine or opioids onto the dopamine cell bodies in the ventral mesencephalon, but not into the axon terminal fields, produces behavioral sensitization to a subseqent systemic drug challenge (Hitzemann et al., 1980; Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992). Finally, pretreatment of dopamine cell bodies with a D_1 antagonist or a $GABA_B$ agonist prevents behavioral sensitization to systemic amphetamine or cocaine, respectively (Stewart and Vezina, 1989; Kalivas and Stewart, 1991).

Based upon the fact that drug action in the dopamine cell body region is important in the initiation of behavioral sensitization to psychostimulants, the present report measured extracellular dopamine content in the vicinity of dopamine neurons in the ventral mesencephalon using *in vivo* microdialysis. The dopamine perikarya in this region are topographically organized with respect to ascending projection fields (Fallon and Moore, 1978; Swanson, 1982). The more medial cells in the ventral tegmental area and medial substantia nigra project to limbic and cortical structures such as the ventral striatum, while the lateral substantia nigra projects more exclusively to the dorsal striatum. Because of this topography, dialysis probes were placed into the ventral tegmental area and medial substantia nigra (VTA/SN) to estimate extracellular somatodendritic dopamine levels from neurons projecting preferentially to the ventral striatum where the dialysis experiments in the accompanying article were conducted (Kalivas and Duffy, 1992).

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

Received Feb. 11, 1992; revised Apr. 28, 1992; accepted July 17, 1992.

We thank Jenny Baylon for assistance in preparing the manuscript. The research was supported in part by U.S. Public Health Service Grants DA-03906 and MH-40817, and by Research Career Development Award DA-00158.

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Table 1. Treatment groups for dialysis in the VTA

	Treatment day			
Group	1	2–6	7	20
1	Saline	Saline	Saline (7)	Saline (4)
2	Cocaine	Cocaine	Saline (8)	Saline (6)
3	Saline	Saline	Cocaine (6)	Cocaine (6)
4	Cocaine	Cocaine	Cocaine (8)	Cocaine (8)
5	Saline	Saline	Basal (7)	Basal (6)
6	Cocaine	Cocaine	Basal (8)	Basal (8)

Days 7 and 20 were in the photocell/dialysis apparatus, and days 1–6 were in the home cage. On days 1, 7, and 20 the intraperitoneal dose of cocaine was 15 mg/kg, and on days 2–6 it was 30 mg/kg. "Basal" refers to studies where dopamine was titrated through the dialysis probe *in vivo* to determine extracellular dopamine content. The number of animals is shown in parentheses.

were made between 11:00 and 13:00 hr. Rats weighing between 260 and 320 gm were anesthetized with Equithesin and mounted in a stereotaxic apparatus (David Kopf, Torrance, CA). A chronic unilateral dialysis guide cannula (20 gauge stainless steel, 14 mm long) was implanted 3 mm dorsal to the VTA/SN (A/P 2.6 mm, D/V −2.5 mm, M/L 0.7 mm; relative to the interaural line according to the atlas of Pellegrino et al., 1979). The cannula was 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 microdialysis experiments were conducted as described in the accompanying article (Kalivas and Duffy, 1992). The dialysis probes were inserted through the guide cannulas into the VTA/SN the night prior to the experiment. The next day, baseline samples (20 min each) were collected for 60–80 min, then cocaine (15.0 mg/kg, i.p.) or saline (1.0 ml/kg, i.p.) was administered and six 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 and killed within 7 d for histological verification of the dialysis probe placement (see below).

Treatment groups. Table 1 shows the treatment groups used. Groups 1–4 were challenged after daily saline or cocaine with acute saline or cocaine injections, while groups 5 and 6 were examined for basal levels of extracellular dopamine after daily pretreatment with cocaine or saline. The rats were administered either daily saline (1.0 ml/kg, i.p. × 6 d; groups 1, 3, and 5) or given cocaine (15 mg/kg, i.p.; groups 2, 4, and 6) on day 1 followed by daily cocaine injections (30 mg/kg, i.p. × 5 d) on days 2–6. In groups 1 and 2, the daily saline- and cocaine-pretreated animals were challenged with an acute saline injection 24 hr (day 7) or 2 weeks (day 20) after discontinuing the daily treatment regimen. In groups 3 and 4, the rats were challenged with acute cocaine (15 mg/kg, i.p.) on day 7 or day 20. A single dialysis experiment was conducted in each rat; therefore, separate animals were used for each day.

In groups 5 and 6 (see Table 1), the basal concentration of extracellular dopamine was determined by adding dopamine to the dialysis perfusate at concentrations above and below the expected extracellular concentration (0, 1, 3, and 10 nM) 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; Parsons et al., 1991; see accompanying article, Kalivas and Duffy, 1992).

Measurement of dopamine in dialysis samples. The collection of the dialysis samples and quantification of dopamine content were carried out as described in the accompanying article (Kalivas and Duffy, 1992).

Histology and data analysis. Rats were killed with an overdose of pentobarbital, and the location of the dialysis probes in the brain was histologically verified as described in the accompanying article (Kalivas and Duffy, 1992). In addition, some tissue sections (50 μm thick) were prepared for tyrosine hydroxylase immunocytochemistry as described elsewhere (Kalivas and Duffy, 1991).

The neurochemical and behavioral time course data were statistically evaluated using a two-way analysis of variance (ANOVA) with repeated measures over time. Dopamine content was normalized to percentage change from the average of three baseline samples for each experiment prior to statistical analysis. Post hoc evaluation of statistical differences was performed using a least significant difference test, as described by Milliken and Johnson (1984). To determine the concentration of no net flux of dopamine through the dialysis probe, regression analysis was performed and the basal concentration compared between cocaine and saline treatment groups using a two-tailed Student's *t* test.

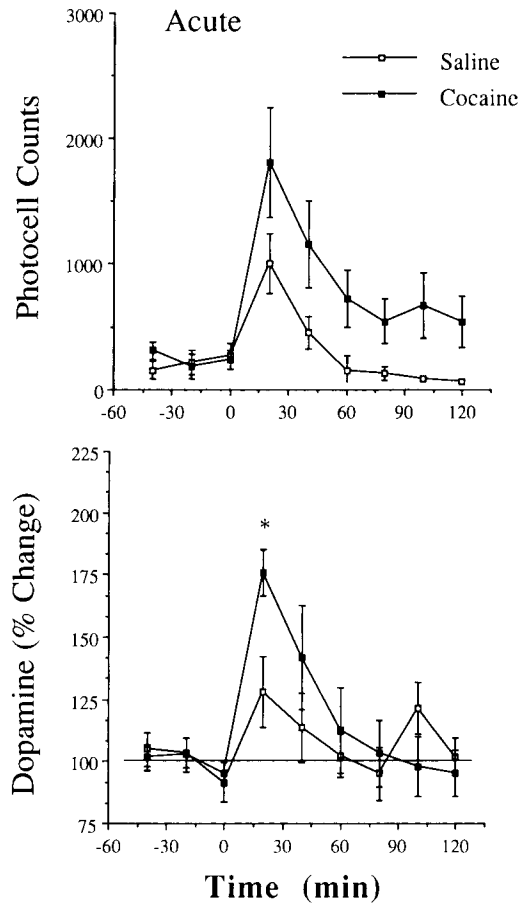

Figure 1. Effect of acute cocaine and saline on motor activity and extracellular dopamine in the VTA/SN. The data are the pooled response to saline ($N = 11$) or cocaine ($N = 12$) on days 7 or 20 from rats pretreated with daily saline in groups 1 and 3. For dopamine values, the data were divided by the average of the three baseline measurements obtained prior to injecting saline at time 0. The basal values were not statistically different between the cocaine (47 ± 9 fmol/min) and saline (29 ± 6 fmol/min) pretreatment groups ($F = 2.62$, $p = 0.121$). The data are shown as mean ± SEM photocell counts or percentage change in dopamine. All data were evaluated using a two-way ANOVA with repeated measures over time. F scores for behavior: treatment $F = 4.96$, $p = 0.044$; time $F = 10.24$, $p < 0.001$; interaction $F = 0.80$, $p = 0.601$. Dopamine F scores: treatment $F = 0.56$, $p = 0.466$; time $F = 1.65$, $p < 0.121$; interaction $F = 2.34$, $p = 0.024$. *, $p < 0.05$, comparing cocaine to saline pretreatment groups at each time using a least significant difference test (Milliken and Johnson, 1984).

Results

Effect of acute cocaine and saline on extracellular dopamine. Figure 1 shows the response to acute cocaine (15 mg/kg, i.p.) or saline (1.0 ml/kg, i.p.) on motor activity and the extracellular concentration of dopamine in the VTA/SN. The data were pooled from rats challenged with saline or cocaine on days 7 or 20 after receiving daily saline injections (i.e., groups 1 and 3). Cocaine injection resulted in a significant decrease in extracellular dopamine concentration compared to saline administration. Although a significant effect of treatment was produced on photocell counts, there was not a significant difference at individual

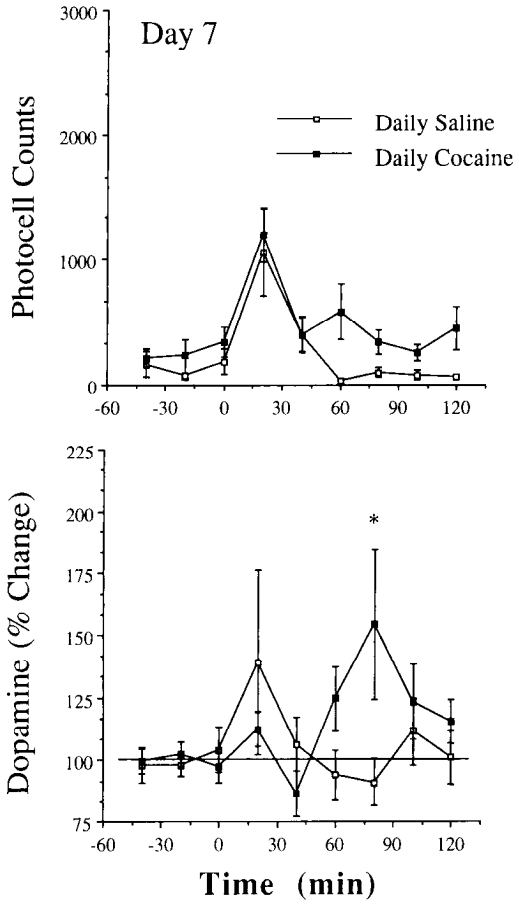

Figure 2. Effect on motor activity and extracellular dopamine content in the VTA/SN of acute saline administration made on day 7. Rats were pretreated with either daily saline × 6 d or cocaine × 6 d. For dopamine values, the data were divided by the average of the three baseline measurements obtained prior to injecting saline at time 0. The basal values were not statistically different between the cocaine (28 ± 5 fmol/min; $N = 8$) and saline (38 ± 8 fmol/min; $N = 7$) treatment groups ($F = 1.36$, $p = 0.265$). The data are shown as mean ± SEM photocell counts or percentage change in dopamine. All data were evaluated using a two-way ANOVA with repeated measures over time. F scores for behavior: pretreatment $F = 5.45$, $p = 0.030$; time $F = 12.41$, $p < 0.001$; interaction $F = 1.86$, $p = 0.070$. Dopamine F scores: pretreatment $F = 0.76$, $p = 0.393$; time F = 6.62, $p < 0.001$; interaction $F = 2.03$, $p = 0.046$. *, $p < 0.05$, comparing cocaine to saline pretreatment groups at each time using a least significant difference test (Milliken and Johnson, 1984).

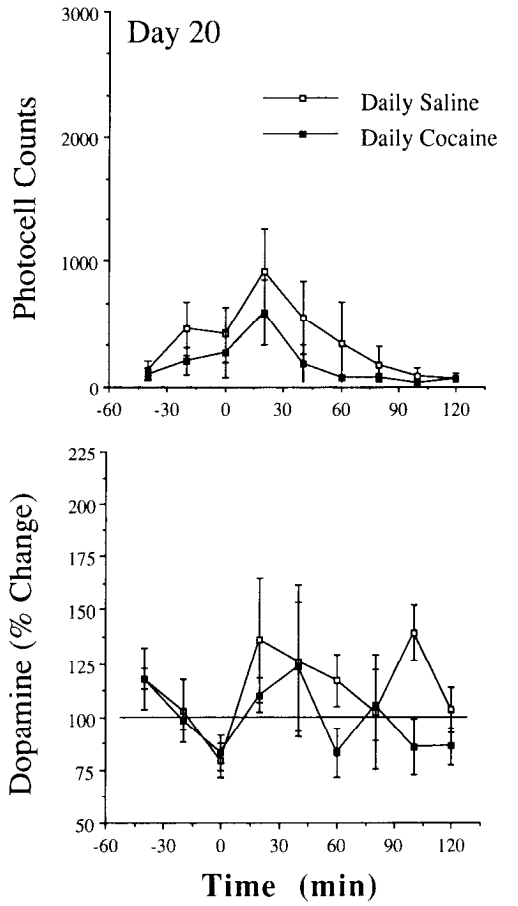

Figure 3. Effect on motor activity and extracellular dopamine content in the VTA/SN of acute saline administration made on day 20. Rats were pretreated with either daily saline × 6 d or cocaine × 6 d. For dopamine values the data were divided by the average of the three baseline measurements obtained prior to injecting saline at time 0. The basal values were not statistically different between the cocaine (20 ± 5 fmol/min; $N = 6$) and saline (13 ± 7 fmol/min; $N = 4$) pretreatment groups ($F = 0.65$, $p = 0.443$). The data are shown as mean ± SEM photocell counts or percentage change in dopamine. All data were evaluated using a two-way ANOVA with repeated measures over time. F scores for behavior: pretreatment $F = 0.17$, $p = 0.693$; time F = 4.12, $p < 0.001$; interaction $F = 1.38$, $p = 0.222$. Dopamine F scores: pretreatment $F = 9.35$, $p = 0.016$; time $F = 2.74$, $p = 0.012$; interaction $F = 1.38$, $p = 0.225$.

times after injection. The increase in motor activity produced by cocaine in rats with dialysis probes in the VTA/SN was less than that observed in rats with probes in the nucleus accumbens (e.g. see Fig. 1 in accompanying article, Kalivas and Duffy, 1992). Rats with probes in the VTA/SN demonstrated a maximum 1800 photocell counts in response to cocaine, and those with probes in the ventral striatum responded with 3500 counts. By itself, saline injection produced a moderate increase in motor activity and extracellular dopamine.

Effect of saline challenge on extracellular dopamine, groups 1 and 2. Figure 2 shows the effect of acute saline (1.0 ml/kg, i.p.) injected on day 7, 1 d after discontinuing daily cocaine or saline injections. Although a significant overall effect was measured between pretreatment groups on motor activity, at no individual time point did the post hoc analysis reveal a significant difference. However, it appears that the difference between the two groups resulted primarily from an elevation in photocell counts in the cocaine-pretreated rats between 60 and 120 min after injection of saline. This late increase in behavioral activity was associated with a similar increase in extracellular dopamine in the VTA/SN in cocaine-pretreated rats that reached statistical significance at 80 min after saline administration. In contrast, Figure 3 shows that on day 20 there was no difference between the cocaine and saline pretreatment groups in either photocell counts or extracellular dopamine after the acute administration of saline.

Effect of cocaine challenge on extracellular dopamine, groups 3 and 4. Figure 4 shows the effect of acute cocaine (15 mg/kg, i.p.) given on day 7 in rats pretreated with daily saline or cocaine on days 1–6. The behavioral response to acute cocaine was significantly augmented in the rats pretreated with daily cocaine (Fig. 4, top). The augmentation was statistically significant at 20 min after injection. Likewise, Figure 4 (bottom) shows that

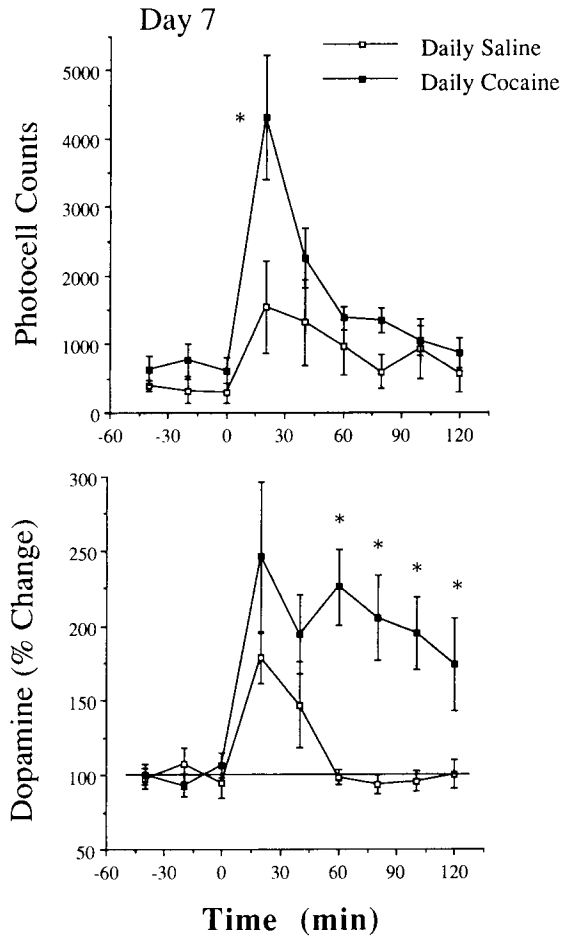

Figure 4. Effect on motor activity and extracellular dopamine content in the VTA/SN of acute saline administration made on day 7. Rats were pretreated with either daily saline (×6 d, $N = 7$) or cocaine (×6 d, $N = 8$). For dopamine values, the data were divided by the average of the three baseline measurements obtained prior to injecting saline at time 0. The basal values were not statistically different between the cocaine (36 ± 3 fmol/min) and saline (42 ± 8 fmol/min) pretreatment groups ($F = 0.51$, $p = 0.490$). The data are shown as mean ± SEM photocell counts or percentage change in dopamine. All data were evaluated using a two-way ANOVA with repeated measures over time. F scores for behavior: pretreatment $F = 4.63$, $p = 0.053$; time $F = 12.62$, $p < 0.001$; interaction $F = 2.87$, $p = 0.007$. Dopamine F scores: pretreatment $F = 12.94$, $p = 0.004$; time $F = 7.06$, $p < 0.001$; interaction $F = 2.69$, $p = 0.010$. *, $p < 0.05$, comparing cocaine to saline pretreatment groups at each time using a least significant difference test (Milliken and Johnson, 1984).

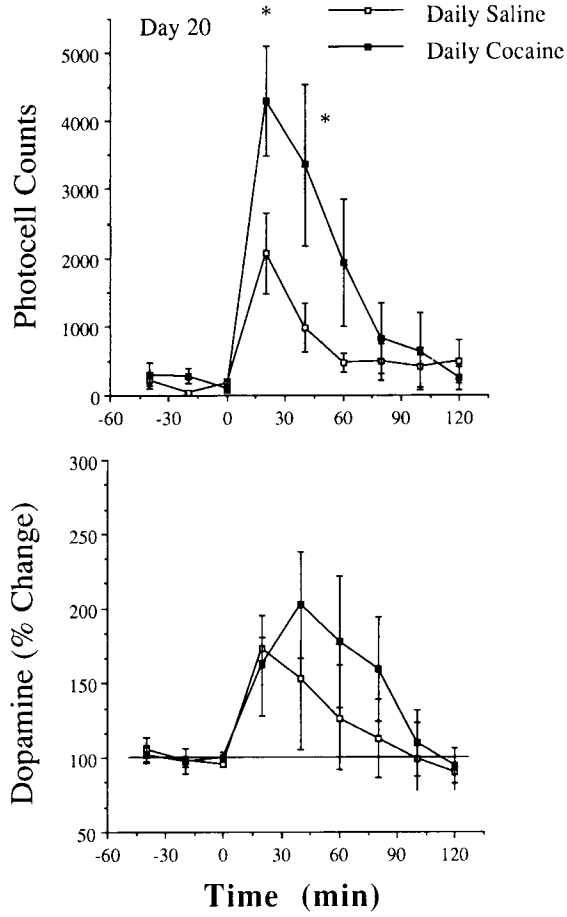

Figure 5. Effect on motor activity and extracellular dopamine content in the VTA/SN of acute cocaine administration made on day 20. Rats were pretreated with either daily saline (×6 d, $N = 6$) or cocaine (×6 d, $N = 8$). For dopamine values, the data were divided by the average of the three baseline measurements obtained prior to injecting saline at time 0. The basal values were not statistically different between the cocaine (38 ± 6 fmol/min) and saline (53 ± 17 fmol/min) pretreatment groups ($F = 1.62$, $p = 0.228$). The data are shown as mean ± SEM photocell counts or percentage change in dopamine. All data were evaluated using a two-way ANOVA with repeated measures over time. F scores for behavior: pretreatment $F = 2.29$, $p = 0.156$; time $F = 14.19$, $p < 0.001$; interaction $F = 2.82$, $p = 0.008$. Dopamine F scores: pretreatment $F = 0.58$, $p = 0.463$; time $F = 4.87$, $p < 0.001$; interaction $F = 0.63$, $p = 0.751$. *, $p < 0.05$, comparing cocaine to saline pretreatment groups at each time using a least significant difference test (Milliken and Johnson, 1984).

the level of extracellular dopamine in the VTA/SN was enhanced in daily cocaine-pretreated rats. However, in contrast to the behavioral sensitization, the augmentation in extracellular dopamine was between 60 and 120 min after acute cocaine administration when motor activity had returned to control levels.

Figure 5 shows the effect of acute cocaine given on day 20. The behavioral response to cocaine was significantly augmented in the daily cocaine pretreatment group at 20 and 40 min after injection. In contrast, no significant augmentation in extracellular dopamine was measured in the VTA/SN of daily cocaine-treated rats compared to rats pretreated with daily saline injections.

Basal levels 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 concentration of dopamine was significantly elevated in rats pretreated with daily cocaine compared to rats pretreated with daily saline (cocaine, 1.31 ± 0.15 nM; saline, 0.79 ± 0.14 nM; $t_{(13)} = 3.73$, $p < 0.01$). There was no significant difference in the slopes of the lines (cocaine, 0.64 ± 0.08; saline, 0.60 ± 0.07). In contrast, on day 20 the concentration of dopamine in the cocaine-pretreated rats had returned to the values obtained in the control group (cocaine, 1.52 ± 0.28 nM; saline, 1.33 ± 0.32 nM). Likewise, there was no difference in the slopes (cocaine, 0.59 ± 0.03; saline, 0.66 ± 0.08). The correlation coefficient for the linear regression from each experiment was >0.94, and none of the mean correlation coefficients differed significantly for the lines shown in Figure 6.

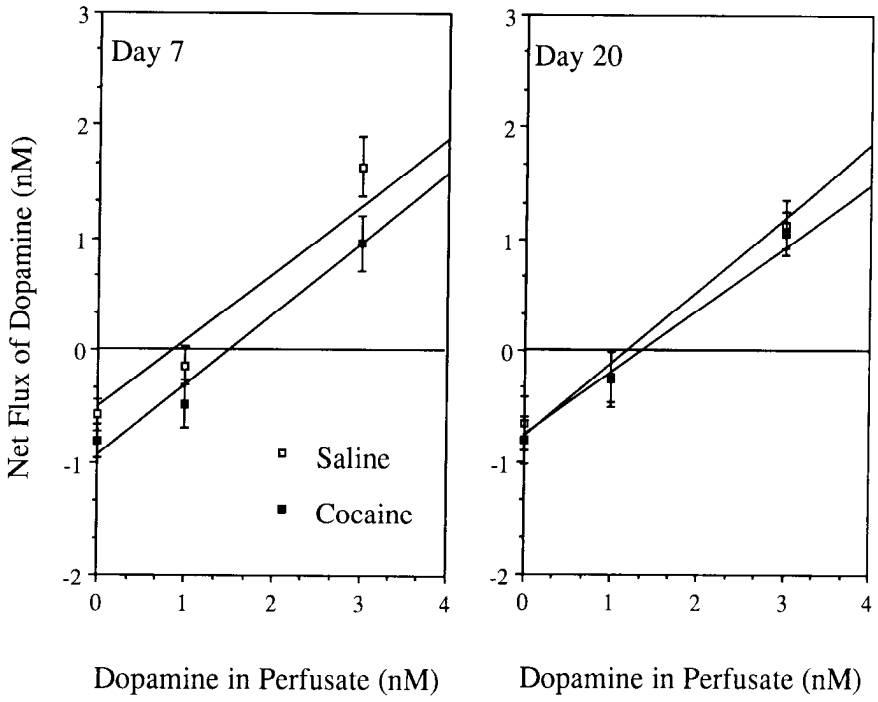

Figure 6. Basal extracellular concentration of dopamine in the VTA/SN determined using *in vivo* net flux of dopamine. Dialysis was performed on day 7 or day 20 after the rats had been pretreated with saline (group 5) or cocaine (group 6) on days 1–6. Each line was derived from six to eight animals, and the data are shown as the mean ± SEM difference between the concentration of dopamine added 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. Although four concentrations of dopamine (0, 1, 3, and 10 nM) were used to generate the line, for illustrative purposes the three concentrations nearest the point of no net flux are shown. The basal concentration of dopamine was greater in cocaine-pretreated rats on day 7 (cocaine, 1.31 ± 0.15 nM; saline, 0.79 ± 0.14 nM; $t_{(13)} = 3.73$, $p < 0.01$), but not on day 20 (cocaine, 1.52 ± 0.28 nM; saline, 1.33 ± 0.32 nM). The slopes and correlation coefficients for all lines were not statistically different.

Histology. Figure 7 illustrates the location of the dialysis probes in the VTA/SN from all the experiments conducted in which animals received an acute injection of saline or cocaine (groups 1–4). From 72 rats, there were a total of 55 successful dialysis experiments. Eight rats died from convulsions immediately following the administration of cocaine (30 mg/kg, i.p.). No other convulsive behavior was noted. In 10 experiments, other difficulties prevented using the data, including the chronic implant coming loose, decreased flow through the dialysis probe, or chromatographic problems (coelution of dopamine with an electroactive contaminant or dopamine was below the detection limit). Three rats were excluded because the cannulas were outside the VTA/SN, which was defined as the ventral tegmental area, medial substantia nigra (within 0.5 mm of the ventral tegmental area), nucleus linearis, and nucleus interfascicularis (Phillipson, 1979; Swanson, 1982). The inset in Figure 7 shows a micrograph of tyrosine hydroxylase–immunoreactive neurons in the vicinity of the glial scar produced by the insertion of a dialysis probe into the VTA. Note the abundance of tyrosine hydroxylase–immunoreactive perikarya, indicating that the dialysis experiment was not neurotoxic to the tissue adjacent to the probe. However, in spite of the lack of apparent toxicity adjacent to the dialysis probe, the mechanical destruction produced by the dialysis probe may account for the relatively low behavioral response to an acute cocaine challenge in rats with probes in the VTA/SN compared to rats with dialysis probes in the nucleus accumbens (compare Fig. 1 with Fig. 1 in the accompanying article, Kalivas and Duffy, 1992).

Discussion

This study reveals that the capacity of acute cocaine to elevate extracellular dopamine content in the VTA/SN is augmented in rats behaviorally sensitized to cocaine 24 hr after discontinuing daily cocaine (i.e., day 7), but not after 14 d of withdrawal (i.e., day 20). Likewise, the basal levels of extracellular dopamine were greater in cocaine-pretreated rats at 24 hr, but not at 14 d after discontinuing daily injections. Also at 24 hr but not 14 d, extracellular dopamine was increased in response to an acute saline injection in cocaine-pretreated rats. The time course of the augmentation in cocaine-induced extracellular dopamine levels at 24 hr of withdrawal did not fully parallel the behavioral response. Although the maximum in both responses occurred at 20 min after injection, the neurochemical response persisted for at least 120 min while the behavioral response returned to near baseline by 60 min. The prolonged release indicates that the normal mechanisms for regulating somatodendritic dopamine release in the VTA/SN have been altered by repeated cocaine treatments.

One mechanism whereby repeated cocaine administration may augment the basal and cocaine-induced extracellular somatodendritic dopamine is via an alteration in inhibitory regulation of the dopamine neurons. Short-loop negative feedback is derived from the stimulation of somatodendritic D_2 autoreceptors, which inhibits dopamine cell firing (Bunney et al., 1973; Wang, 1981; White and Wang, 1984a; Kapoor et al., 1989; Kalivas and Duffy, 1991). *In vivo* and *in vitro* administration of acute cocaine have been shown to elicit D_2 receptor–mediated inhibition of dopamine neurons (Einhorn et al., 1988; Brodie and Dunwiddie, 1990; Lacey et al., 1990). Systemic or *in vitro* exposure to cocaine or amphetamine reduces the capacity of D_2 receptor agonists to inhibit dopamine cell firing frequency (Antelman and Chiodo, 1981; White and Wang, 1984b; Kamata and Rebec, 1985; Lee et al., 1988; Henry et al., 1989; Seutin et al., 1991). Under conditions where D_2 autoreceptor inhibition is diminished, the neuron would be more easily depolarized. This would result in greater activation of voltage-dependent

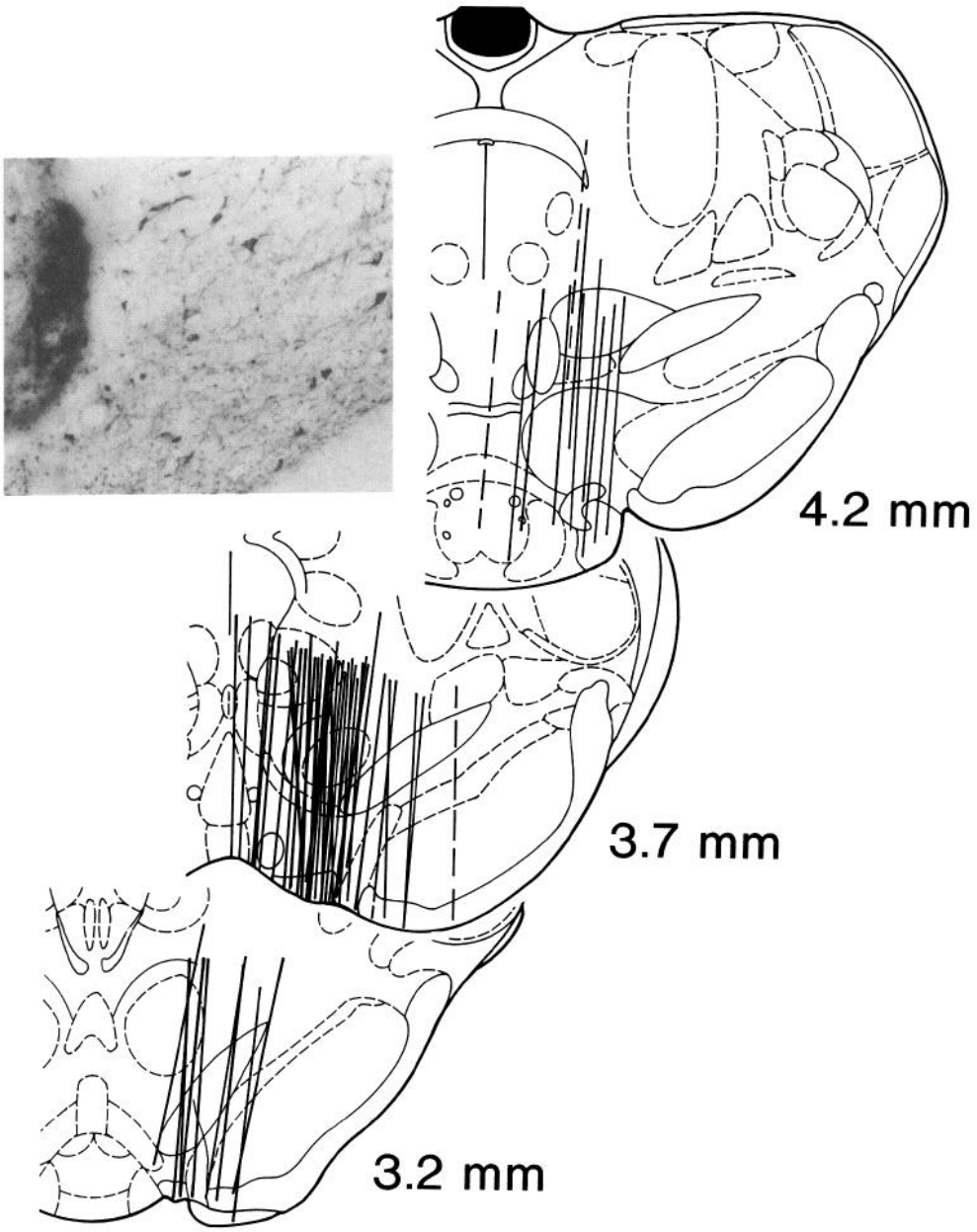

Figure 7. Location of dialysis probes in the ventromedial mesencephalon. The bottom 2.5 mm of each cannula track is shown. The inset shows the medial edge of the glial scar remaining 5 d after removing the dialysis probe from the VTA. Note the many tyrosine hydroxylase–immunoreactive neurons adjacent to the glial scar. The *dashed* cannula tracks correspond to probe placement outside the VTA/SN. The drawings were made according to the atlas of Paxinos and Watson (1986).

calcium channels and increased calcium-dependent dopamine release (Llinas et al., 1984; Grace and Onn, 1989). Supporting D_2 receptor desensitization as a mechanism for augmenting somatodendritic dopamine release, Ackerman and White (1990) demonstrated that D_2 receptor desensitization in response to repeated cocaine is transitory and not present by 8 d after discontinuing daily cocaine treatments, as was observed with extracellular dopamine in the VTA/SN in the present report. Furthermore, this is consistent with the finding that more spontaneously firing neurons are present and the overall firing rate is increased in the VTA of rats pretreated with daily cocaine (Henry et al., 1989). The elevation in basal levels of extracellular dopamine in the VTA after 24 hr, but not 14 d, of withdrawal also indicates a loss of autoreceptor regulation.

In spite of D_2 receptor desensitization, there is no alteration in D_2 receptor density in the VTA/SN after cocaine pretreatment (Peris et al., 1990; Ziegler et al., 1991). However, D_2 receptor-induced hyperpolarization of dopamine neurons results from a pertussis toxin–sensitive G-protein coupling to an ATP-sensitive potassium channels (Innis and Aghajanian, 1987; Lacey et al., 1987), and repeated cocaine reduces the content of Giα and Goα and the pertussis toxin–catalyzed *in vitro* ADP-ribosylation of G-proteins in the VTA/SN (Nestler et al., 1990; Striplin and Kalivas, 1992). Supporting the possible role of G-proteins in the desensitization of the D_2 receptor, the reduction in G-proteins in the VTA/SN by an acute cocaine challenge in cocaine-pretreated rats is transitory and not present by 14 d after discontinuing daily treatments (Kalivas et al., 1992). However, $GABA_B$ receptors are coupled via G-proteins to the same K^+ conductance as D_2 receptors (Lacey et al., 1988), and the inhibitory effect of iontophoretic GABA is not altered in rats pretreated with daily cocaine (Henry et al., 1989). Thus, if a reduction in G-proteins is involved in D_2 receptor desensitization, it is specific for D_2 receptor coupling mechanisms. Alternatively, iontophoretic GABA also stimulates $GABA_A$ receptors, which hyperpolarize dopamine cells and may mask a

reduction in $GABA_B$ receptor–mediated inhibition (Sugita et al., 1992).

In addition to D_2 receptor desensitization, it is possible that repeated cocaine may alter long-loop inhibitory feedback. Long-loop negative feedback is derived from neurons in the nucleus accumbens, striatum, and pallidum that have descending GABAergic projections to the VTA/SN (Walaas and Fonnum, 1980; Yim and Mogenson, 1980; Grace and Bunney, 1985; Haber et al., 1985; Smith and Bolam, 1990; Johnson et al., 1992; Sugita et al., 1992), and cocaine-induced inhibition of VTA dopamine cell firing frequency *in vivo* results partly from the disinhibition of GABAergic afferents (Einhorn et al., 1988). Although Henry et al. (1989) found that repeated cocaine pretreatment did not alter the capacity of iontophoretic GABA to inhibit dopamine neurons, it remains possible that GABA release may be altered by repeated cocaine treatments. One mechanism by which this could occur is via stimulation of D_1 receptors. In the substantia nigra, D_1 receptors are located primarily on nondopaminergic terminals in the ventral mesencephalon (Savasta et al., 1986; Altar and Hauser, 1987). Although a modest density of D_1 receptors was recently identified in the ventral tegmental area (Mansour et al., 1992), the cellular localization has not been experimentally evaluated. Thus, it is possible that sensitization to cocaine may be associated with an alteration in the modulation of GABA transmission by D_1 receptor stimulation (see Kalivas and Stewart, 1991, for discussion). Supporting a role for the stimulation of D_1 receptors in the initiation of behavioral sensitization to psychostimulants is the finding by Stewart and Vezina (1989) that microinjection of relatively high doses of the D_1 antagonist SCH-23390 into the VTA/SN prevents behavioral sensitization produced by the systemic administration of amphetamine.

Considering the apparent importance of the stimulation of D_1 receptors in the VTA/SN in the initiation of behavioral sensitization (Stewart and Vezina, 1989), it follows that the observed increase in extracellular dopamine content in the VTA/SN of cocaine-sensitized rats is stimulating D_1 receptors. Other evidence supports a role for the VTA/SN in the initiation of behavioral sensitization to psychostimulants. The repeated microinjection of amphetamine in the VTA/SN, but not into the nucleus accumbens, results in an augmented behavioral response to a systemic challenge of amphetamine or cocaine (Hitzemann et al., 1980; Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992). Also, the changes in G-protein ADP-ribosylation produced by daily cocaine administration were specific for the VTA/SN and did not occur in the nucleus accumbens, striatum, or lateral substantia nigra (Striplin and Kalivas, 1992; but see Nestler et al., 1990, who found changes in the ventral tegmental area and nucleus accumbens, but not in the substantia nigra and striatum). Although the psychostimulants act in the VTA/SN to induce behavioral sensitization, none of the alterations in D_2 receptor function (Ackerman and White, 1990), G-proteins (Striplin and Kalivas, 1992), or extracellular dopamine content (present report) endure for 14 d. In contrast, behavioral sensitization to psychostimulants persists indefinitely (Robinson and Becker, 1986; Kalivas and Stewart, 1991). Thus, while the neurochemical and physiological alterations produced in the VTA/SN by daily cocaine may be involved in the initiation of behavioral sensitization, they are not the permanent alterations that underlie the long-term sensitized response. A better candidate for the long-term change is the augmentation in terminal field dopamine release, which has been shown *in vivo* (Robinson et al., 1988; Kalivas and Duffy, 1993), and *in vitro* (Kolta et al., 1985; Robinson and Becker, 1986) to persist for at least 21 d after discontinuing repeated psychostimulant injections. Also, long-term changes in the responsiveness of neurons in the nucleus accumbens to a D_1 agonist have been documented (Henry and White, 1991).

The augmentation in the effect of acute saline on extracellular dopamine in the VTA/SN may indicate the presence of a conditioned response. Thus, the injection procedure and administration of saline were sufficient to produce a modest elevation in extracellular dopamine and photocell counts in rats pretreated with daily cocaine. Conditioning of the motor stimulant effect of psychostimulants is well documented (Tilson and Rech, 1973; Post et al., 1981; Beninger and Hahn, 1983; Beninger and Herz, 1986; Stewart and Vezina, 1988; Weiss et al., 1989). Although not apparent in the accompanying report (Kalivas and Duffy, 1992), a similar neurochemical augmentation has been observed in the ventral striatum of cocaine-pretreated rats (Kalivas and Duffy, 1990; Fontana et al., 1991; but see Brown and Fibiger, 1991).

In summary, daily cocaine pretreatment resulted in an augmented behavioral response to an acute cocaine challenge that was associated with an increase in extracellular dopamine in the VTA/SN at 24 hr, but not 14 d after discontinuing the daily cocaine injections. Likewise, at 24 hr the basal extracellular concentration of dopamine was elevated in daily cocaine-pretreated rats. It is proposed that the augmented extracellular dopamine content in the VTA/SN may result from desensitization of D_2 receptors. Furthermore, it is hypothesized that the augmented extracellular concentration of dopamine may increase the stimulation of D_1 receptors in the ventral mesencephalon and that this sequence of events is critical for the initiation of behavioral sensitization to psychostimulants.

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