

Research report

Repeated cocaine injections have no influence on tyrosine hydroxylase activity in the rat nucleus accumbens core or shell

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Abstract

Numerous reports have demonstrated augmented cocaine-evoked release of dopamine in the nucleus accumbens of rats pre-treated with cocaine. However, the extent to which repeated cocaine injections affect basal levels of dopamine is unclear. There have been reports of increases, decreases, or no change in basal levels of extracellular accumbal dopamine resulting from repeated psychostimulant administration. The present study assessed the activity of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, in the nucleus accumbens following either acute or repeated cocaine administration. The *in vivo* microdialysis technique was used to measure accumulation of the dopamine precursor DOPA following intra-accumbal administration of the DOPA decarboxylase inhibitor NSD 1015 through the microdialysis probe. This method provides an estimate of tyrosine hydroxylase activity within the nucleus accumbens. Results indicate that neither acute nor repeated cocaine administration produced any change in DOPA accumulation in either the nucleus accumbens shell or core. These data indicate that dopamine synthesis is not altered by cocaine administration.

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1. Introduction

Within the past few decades, research in the field of drugs of abuse has supported the notion that addiction to psychomotor stimulants such as cocaine is a disease of the brain [21]. Therefore, studies examining the neural mechanisms that contribute to cocaine-induced changes in the brain may lead to identifying potential therapeutics for the treatment of cocaine addiction. Several lines of evidence suggest that the mesoaccumbens dopamine system is a critical neural substrate for the rewarding properties of addictive drugs [5,8,19,37,38,44,51], and plays a role in the neuroadaptations that contribute to psychostimulant-induced behavioral

sensitization [32]. Repeated injections of cocaine in particular lead to persistent alterations in structure [27,39], neuronal activity [45,54], and gene expression [6,53] within the mesoaccumbens dopamine system.

Neurochemical modifications associated with psychostimulant-induced behavioral sensitization include changes in dopamine transmission. Microdialysis studies have demonstrated that there is a consistent enhancement of drug-evoked dopamine efflux in the nucleus accumbens of rats that display behavioral sensitization to psychostimulants (Refs. [1,15,29,35], but see also [42]). However, it is unclear if there are changes in basal levels of extracellular dopamine following repeated exposure to psychostimulants. Reports have shown increases [10,45], decreases [13,22], or no change [9,15,26,37] in basal dopamine levels in the accumbens within the first 24–48 h after cessation of repeated psychostimulant administration. There is similar confusion when basal levels of dopamine are examined after a protracted abstinence. Decreases

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[11,13,29,41] or no change [16,49] in basal extracellular dopamine are observed 5–22 days following the last of daily psychostimulant injections.

The mechanisms responsible for the psychostimulant-induced changes in dopamine neurotransmission are not completely understood, but may be a result of differential regulation of the activity of tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis [55]. One *in vitro* study demonstrated a decrease in tyrosine hydroxylase phosphorylation in the nucleus accumbens following twice daily injections of cocaine over 14 consecutive days [3], which may be due to a cocaine-induced reduction in neurofilament protein levels in the ventral tegmental area (VTA) leading to compromised axonal transport of tyrosine hydroxylase from the VTA to the nucleus accumbens [4]. However, other studies reported no change in accumbal tyrosine hydroxylase immunoreactivity or enzymatic activity following repeated cocaine administration [25,29].

Westerink et al. [50] first utilized and verified the validity of the brain microdialysis technique to determine tyrosine hydroxylase activity indirectly in the brain of the rat. DOPA decarboxylase, the enzyme that converts DOPA to dopamine, can be inhibited via administration of NSD 1015. Following perfusion of NSD 1015 through the microdialysis probe, the amount of DOPA measured in the dialysate is a reliable index of tyrosine hydroxylase activity. Systemic administration of the tyrosine hydroxylase inhibitor α -methyl-*p*-tyrosine caused a rapid decline of DOPA concentration in the dialysate, demonstrating the validity of this method [50]. Using this approach, a reduction in DOPA accumulation after perfusion of NSD 1015 through a microdialysis probe located in the nucleus accumbens of rats was demonstrated after 10 daily administrations of cocaine [7]. These data suggest a reduction in tyrosine hydroxylase activity, and thus a reduction in dopamine synthesis, in the nucleus accumbens following repeated psychostimulant administration.

These studies of tyrosine hydroxylase levels and activity all lacked a distinction between subregions of the nucleus accumbens. Histochemical studies have revealed two major subregions of the nucleus accumbens, the core and shell [52]. Differential anatomical connectivity between the core and the shell indicates that each subregion has a specific function. It has been suggested that the shell primarily modulates limbic input, while the core receives information about motor processes [2,12,52]. Recent studies indicate that cocaine administration produces different neurochemical changes in the core and shell of the nucleus accumbens. Extracellular glutamate levels were elevated in the core of cocaine-pretreated rats following a challenge injection of cocaine [17,21], as well as during the reinstatement of drug-seeking behavior in animals trained to self-administer cocaine [23]. In contrast, there is a preferential increase in dopamine in the shell associated with cocaine-induced behavioral sensitization [31] or a single intravenous injection of cocaine [35]. In addition, cocaine-pretreated rats

displayed behavioral sensitization when a challenge injection of amphetamine [31] or cocaine [56] was administered directly into the shell, but not the core of the nucleus accumbens. Taken together, these studies indicate that cocaine administration results in enhanced dopamine transmission specifically in the shell subregion of the nucleus accumbens following a cocaine challenge.

The present experiments were designed to investigate the hypothesis that enhanced tyrosine hydroxylase activity in the nucleus accumbens shell alters dopamine synthesis following repeated injections of cocaine. NSD 1015 was perfused directly into the shell or core and tyrosine hydroxylase activity was estimated as a function of DOPA accumulation following either acute or repeated cocaine administration.

2. Materials and methods

2.1. Surgery

All experimental protocols were consistent with guidelines issued by the National Institutes of Health and were approved by Boston University School of Medicine's Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (251–275 g; Taconic Farms, Germantown, NY) were anesthetized with 80 mg/kg ketamine HCl and 12 mg/kg xylazine HCl (*i.m.*) and mounted in a stereotaxic apparatus. A stainless steel microdialysis guide cannula (13 mm, 20 gauge; Plastics One, Roanoke, VA) was implanted unilaterally 1 mm dorsal to the shell (+1 A/P, +1.0 M/L, –5.5 D/V) or core (+1 A/P, +2.5 M/L, –5.5 D/V) of the left nucleus accumbens relative to bregma [30]. The guide cannula was cemented in place by affixing dental acrylic to three stainless steel screws secured in the skull. Following surgery, animals were allowed to recover for 3–5 days prior to the start of the experiment.

2.2. Drug treatment

After recovering from surgery, animals were subjected to either an acute or repeated injection regimen (see Table 1). The acute group received one intraperitoneal injection of

Table 1
Drug treatment schedule

	A single injection, microdialysis 24 h later	Seven daily injections, microdialysis 24 h later	Seven daily injections, microdialysis 2 weeks later
Treatment	Cocaine	Cocaine	Cocaine
Brain region	Core (6) Shell (5)	Core (4) Shell (4)	Core (4) Shell (4)
Treatment	Saline	Saline	Saline
Brain region	Core (4) Shell (5)	Core (5) Shell (5)	Core (6) Shell (4)

Numbers in parentheses denotes the number of animals in each group.

either saline or 15 mg/kg cocaine (dissolved in 0.9% saline; National Institute on Drug Abuse, Rockville, MD) in the home cage 24 h prior to the start of the microdialysis experiment. The repeated group received seven consecutive daily injections of either saline or 15 mg/kg cocaine in the home cage. The last daily injection was 24 h prior to the start of the microdialysis experiment. Rats in the late withdrawal group received seven consecutive daily injections of either saline or 15 mg/kg cocaine in the home cage. The last daily injection was 14 days prior to the start of the microdialysis experiment. During the first week of withdrawal, the microdialysis guide cannula was implanted to insure that all animals across treatment groups had the headmount for 8–10 days.

2.3. *In vivo* microdialysis and DOPA analysis

The dialysis probes were a modification of those described by Jolly and Vezina [14], with 3.0 mm of active dialysis membrane. The dialysis membrane had a molecular weight cutoff of 18,000 Da. The probes were inserted through the guide cannulae into the nucleus accumbens approximately 12–16 h before the experiment. Microdialysis buffer (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, 0.2 mM ascorbate, 5.0 mM glucose, pH to 7.4) was perfused through the probe overnight at a rate of 0.2 µl/min via a syringe pump. The flow rate was increased to 2.0 µl/min for 1 h before baseline samples were collected. Baseline samples were collected every 20 min over 100 min before 100 mM NSD 1015 (dissolved in microdialysis buffer; Sigma RBI, Natick, MA) was perfused through the microdialysis probe. Samples were collected every 20 min over the next 200 min and were subsequently stored at –80 °C until DOPA concentrations were measured with HPLC-EC.

Samples were thawed and placed in an autosampler (Waters, Milford, MA) connected to an HPLC system with electrochemical detection. The DOPA was separated using a C₁₈ microbore reverse phase column (ESA, Bedford, MA) and oxidized at 200 mV using an amperometric detector (ESA). The mobile phase consisted of 0.1 M sodium acetate, 20 mM citrate, 1 mM 1-octanesulfonic acid, and 0.1 mM disodium EDTA, with a pH of 2.8. The peaks were integrated using either Millennium 32 Chromatography Manager software (Waters, Milford, MA) or EZ Chrom Elite software (Scientific Software, Pleasanton, CA). All data were converted to DOPA concentration by comparison to an external standard (100 nM DOPA).

2.4. Histology

Upon completion of the experiments, the animals were overdosed with sodium pentobarbital (100 mg/kg, i.p.), and were perfused intracardially with 0.9% saline followed by 10% formalin. The brain was removed and 6–10 coronal sections (100 µm) were taken at the level of the anterior commissure with a Vibratome (Technical Products Interna-

tional, St. Louis, MO). The sections were mounted on gelatin-coated slides and stained with Cresyl violet [20]. An individual who was unaware of the rats' neurochemical response determined the accuracy of all probe and cannula placements. Animals whose cannulae were not within the core or the shell were removed from the analysis.

2.5. Statistical analysis

Separate mixed factors ANOVAs were performed in order to analyze the data from the acute, repeated/early, and repeated/late groups. The between groups measure was drug treatment, while the within groups measure was time.

3. Results

3.1. Location of microdialysis sites in the nucleus accumbens shell and core

The schematic brain sections depicted in Fig. 1 are from the atlas of Paxinos and Watson [30]. The lines represent the placement of the microdialysis probes within the nucleus accumbens shell (left) and core (right) from the experiments presented in Figs. 2–4. The shell subregion was identified as the more ventromedial aspect of the nucleus accumbens, while the core was more dorsolateral and bordering the caudate putamen. The numbers on each section indicate millimeters from bregma. Of the 72 animals used in this study (12 groups, $n=6$ in each), data from 16 were not included in the analysis due to incorrect probe placement. The sections also were checked closely for neurotoxicity induced by the microdialysis probe, but this did not result in the exclusion of any data from the analysis.

3.2. Acute cocaine administration has no effect on DOPA accumulation in the nucleus accumbens shell or core

The data summarized in Fig. 2 indicate that a single injection of cocaine had no effect on DOPA accumulation relative to saline-treated controls in the nucleus accumbens shell or core 24 h following the injection. The data from each region were analyzed separately with a mixed factors ANOVA. The between-subjects factor was drug treatment (either saline or cocaine), while the within-subjects factor was time. The analysis of the data in Fig. 2a revealed that in the shell there was no significant main effect of treatment [$F(1,8)=1.301$, $p<0.287$]. There was a significant main effect of time [$F(9,72)=17.138$, $p<0.0001$], but no significant interaction between drug treatment and time [$F(9,72)=1.040$, $p<0.418$]. The analysis of the data in Fig. 2b revealed that in the core there was only a significant main effect of time [$F(9,72)=22.016$, $p<0.0001$]. There was neither a significant main effect of treatment [$F(1,8)=0.066$, $p<0.836$], nor a significant interaction between time and treatment [$F(9,72)=0.500$, $p<0.870$].

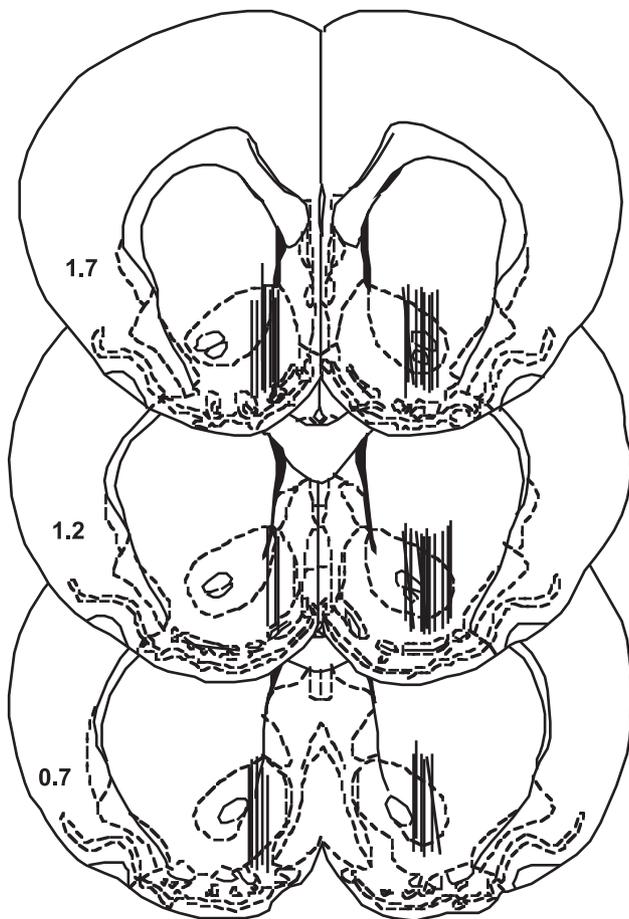


Fig. 1. Location of microdialysis probes in the nucleus accumbens. The lines represent the placement of the tips of the microdialysis probe in the shell (left) or the core (right), and the numbers are millimeters from bregma according to the atlas of Paxinos and Watson [30].

3.3. Repeated cocaine administration has no effect on DOPA accumulation in the nucleus accumbens shell or core

The data summarized in Fig. 3 indicate that seven daily injections of cocaine did not produce any effect on DOPA accumulation relative to saline-treated controls in the nucleus accumbens shell or core 24 h following the last injection. The data were analyzed separately with a mixed factors ANOVA. The between-subjects factor was drug treatment (either saline or cocaine), while the within-subjects factor was time. The analysis of the data in Fig. 3a revealed that in the shell there was a significant main effect of time [$F(9,63)=24.345$, $p<0.0001$]. There were no significant main effects of treatment [$F(1,7)=0.002$, $p<0.969$], and no significant interaction between drug treatment and time [$F(9,63)=0.340$, $p<0.958$]. Fig. 3b is similar such that in the core there was a significant main effect of time [$F(9,63)=13.656$, $p<0.0001$], but no significant main effect of treatment [$F(1,7)=0.005$, $p<0.945$], and no significant interaction between drug treatment and time [$F(9,63)=1.842$, $p<0.078$].

The data summarized in Fig. 4 indicate that seven daily injections of cocaine also did not produce any effect on DOPA accumulation relative to saline-treated controls in the nucleus accumbens shell or core 14 days following the last injection. The data were analyzed in the same manner as above. The analysis of the data in Fig. 4a revealed that in the shell there was a significant main effect of time [$F(9,45)=7.009$, $p<0.0001$], no significant main effect of treatment [$F(1,5)=0.002$, $p<0.967$], and no interaction between treatment and time [$F(9,45)=0.483$, $p<0.878$] 2 weeks after the last of the daily injections. Fig. 4b is similar such that in the core there was a significant main effect of time [$F(9,72)=16.938$, $p<0.0001$], no significant main effect of treatment [$F(1,8)=0.300$, $p<0.599$], and no interaction between the two [$F(9,72)=0.886$, $p<0.542$].

An analysis comparing the effects on DOPA accumulation resulting from acute cocaine administration, one day of

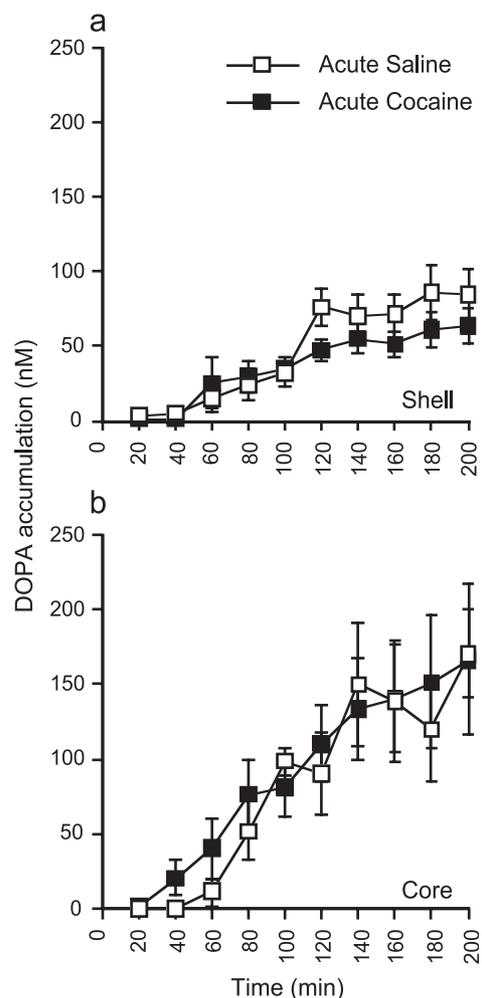


Fig. 2. Acute cocaine administration has no effect on DOPA accumulation in the nucleus accumbens (a) shell or (b) core. NSD1015 was perfused through the microdialysis probe and samples were collected every 20 min over 200 min in order to assess the concentration of extracellular DOPA. The data are presented as mean (\pm S.E.M.). There were five rats in the acute saline shell group, five rats in the acute cocaine shell group, four rats in the acute saline core group, and six rats in the acute cocaine core group.

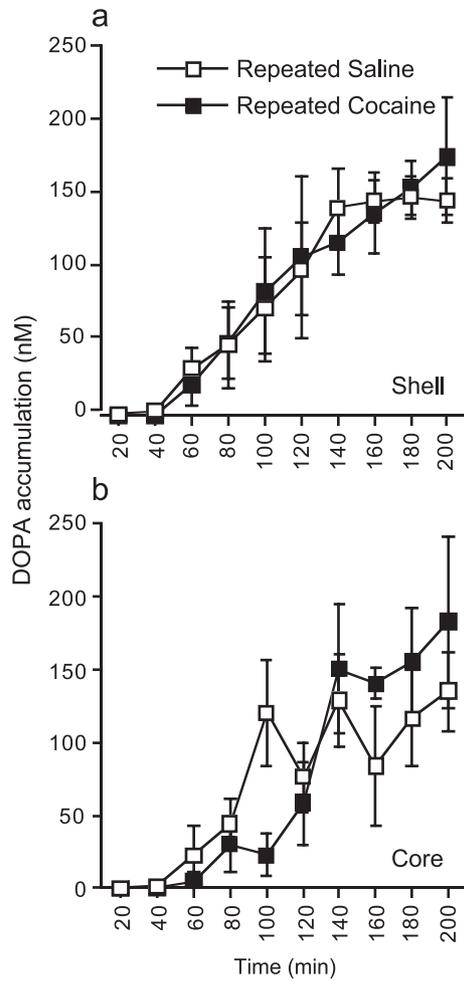


Fig. 3. Repeated cocaine administration has no effect on DOPA accumulation in the nucleus accumbens 24 h after the last of seven daily injections. NSD1015 was perfused through the microdialysis probe into the (a) shell or (b) core, and samples were collected every 20 min over 200 min in order to assess the concentration of extracellular DOPA. The data are presented as mean (\pm S.E.M.). There were five rats in the repeated saline shell group, four rats in the repeated cocaine shell group, five rats in the repeated saline core group, and four rats in the repeated cocaine core group.

withdrawal after repeated cocaine administration, or 14 days of withdrawal after repeated cocaine administration, indicated that in the shell there was a significant main effect of treatment [$F(5,20)=3.090$, $p<0.032$], a significant main effect of time [$F(9,180)=43.487$, $p<0.0001$], and an interaction between these factors [$F(45,180)=2.319$, $p<0.0001$]. However, the post hoc analysis (Fisher's LSD) revealed differences not only in the cocaine–cocaine comparisons between drug treatments, but there were also saline–saline differences (data not shown). While it is unclear why these differences were observed, the 24-h and 2-week groups were done at different times of the year, when different environmental factors may have prevailed. However scarce, there are reports that tyrosine hydroxylase activity varies according to an annual/seasonal cycle [9,36]. For this reason, the focus is on the cocaine–saline differences. The same analysis indicated that in the core while

there was no significant main effect of drug treatment [$F(5,23)=0.350$, $p<0.877$], there was a significant main effect of time [$F(9,207)=50.455$, $p<0.0001$]. There was no significant drug \times time interaction [$F(45,207)=1.006$, $p<0.470$].

A separate mixed factors ANOVA comparing the effects of repeated cocaine and one day of withdrawal to repeated cocaine and 14 days of withdrawal on DOPA accumulation indicated that in the shell there was no significant main effect of treatment [$F(3,12)=2.268$, $p<0.133$]. However, there was a significant main effect of time [$F(9,108)=26.663$, $p<0.0001$], and a significant interaction between treatment and time [$F(27,108)=2.156$, $p<0.003$]. Post hoc analyses revealed significant differences between the 24-h and 2-week groups in the cocaine-treated group of rats at the 140-, 160-, 180-, 200-, and 220-min time points. There were also differences between the two groups in the saline-treated rats, making the data difficult to interpret (data not

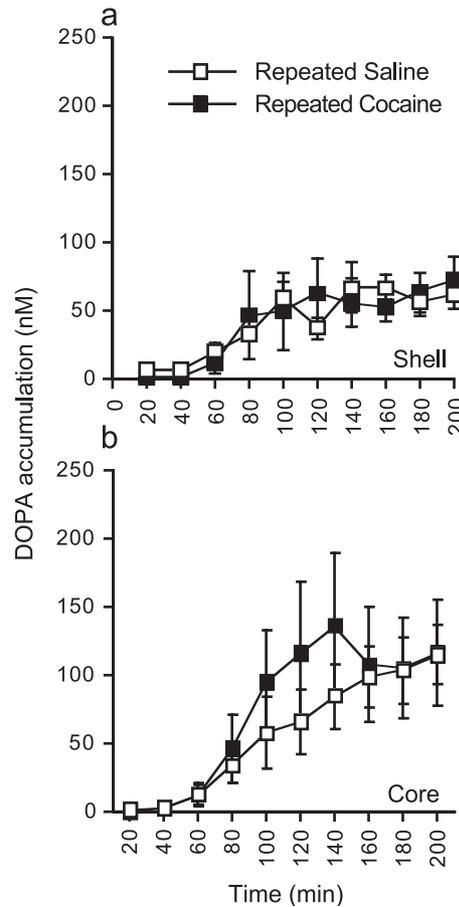


Fig. 4. Repeated cocaine administration has no effect on DOPA accumulation in the nucleus accumbens 2 weeks after the last of seven daily injections. NSD1015 was perfused through the microdialysis probe into the (a) shell or (b) core, and samples were collected every 20 min for 200 min in order to assess the concentration of extracellular DOPA. The data are presented as mean (\pm S.E.M.). There were four rats in the repeated saline shell group, four rats in the repeated cocaine shell group, six rats in the repeated saline core group, and four rats in the repeated cocaine core group.

shown). The statistical analysis further revealed that in the core there was a significant main effect of time [$F(9,135)=29.373$, $p<0.0001$], but there was neither a significant main effect of treatment [$F(3,15)=0.200$, $p<0.895$] nor was there a significant interaction between these two factors [$F(27,135)=1.329$, $p<0.148$], when comparing the effects of repeated cocaine and 1 day of withdrawal to repeated cocaine and 14 days of withdrawal on DOPA accumulation.

4. Discussion

The present studies employed the technique of *in vivo* microdialysis in order to measure extracellular levels of DOPA. Recent studies indicate that DOPA is released in a Ca^{2+} -dependent manner, and may fulfill the criteria for being a neurotransmitter [21,24,25]. NSD-induced DOPA accumulation, an indirect measure of tyrosine hydroxylase activity, was examined in the nucleus accumbens following cocaine administration. The present results indicate that acute cocaine administration has no effect on DOPA accumulation in the nucleus accumbens shell or core. Further, repeated cocaine administration has no effect on DOPA accumulation in either subterritory at either the early or late withdrawal time point. Together, these data suggest that basal levels of dopamine synthesis are unchanged by a single administration of cocaine or daily injections of cocaine.

The present data are inconsistent with the previous report of Brock et al. [7], which demonstrated a 50% reduction in basal DOPA accumulation in the nucleus accumbens of rats 24 h after being treated daily with cocaine for 10 consecutive days. Other data demonstrated that three daily systemic injections of cocaine lowered the amount of DOPA accumulation in the nucleus accumbens [18], while twice-daily injections of cocaine over 14 days decreased the enzymatic activity of accumbal tyrosine hydroxylase [3]. Several other studies have reported no cocaine-induced changes in tyrosine hydroxylase protein levels or enzymatic activity in the nucleus accumbens [14,25,29]. However, a more detailed immunohistochemical study showed that 2 days after cessation of cocaine treatment (twice daily injections over five consecutive days) there was a decrease in tyrosine hydroxylase immunoreactivity in the core, while 14 days after cessation of treatment there was an increase in immunoreactivity in the shell [46]. This distinction is important because there is a greater enhancement of dopamine release in the shell of animals repeatedly treated with psychostimulants [31].

The reason for the discrepancy between those experimental data and the present data could be due to several differences between the experimental designs. The present experiments utilized a shorter injection regimen than that described in the report by Brock et al. [7], but a longer regimen than that of Kalivas et al. [18]. In the experiments described by Kalivas et al. [18], NSD 1015 was adminis-

tered systemically rather than locally. Furthermore, DOPA accumulation was measured at a single time point, whereas DOPA accumulation was monitored over time (200 min) with microdialysis in the current studies. The remainder of the cited reports demonstrated cocaine-induced reductions in either tyrosine hydroxylase immunoreactivity [46] or phosphorylation state [3] in the nucleus accumbens and did not involve administration of NSD 1015.

While an acute psychostimulant injection produces an increase in extracellular dopamine in the nucleus accumbens, repeated psychostimulant administration causes an enhancement of this neurochemical effect when a challenge injection is given [15,17,47]. It is unclear whether increases in basal levels of extracellular dopamine underlie this phenomenon of sensitized dopamine release, because challenge injections were not administered in studies reporting no change [10,16,29,41,49] or a decrease [11,13,22,29,41] in basal levels of extracellular dopamine in the accumbens following repeated cocaine injections. The present data demonstrate merely that neither acute nor repeated cocaine administration has an effect on basal rates of dopamine synthesis, suggesting that repeated psychostimulant injections have no effect on basal levels of extracellular dopamine in the nucleus accumbens.

Alterations in mesoaccumbens dopamine transmission play a critical role in both the initiation and expression of behavioral sensitization to cocaine. For example, a cocaine challenge injection results in augmented dopamine transmission in the nucleus accumbens shell of rats pretreated with repeated cocaine injections [1,15,30,36]. The present work, which used NSD 1015-induced DOPA accumulation as an indirect measure of tyrosine hydroxylase activity, showed that neither acute nor repeated cocaine injections altered tyrosine hydroxylase activity within the nucleus accumbens core or shell, which indicates that repeated cocaine does not alter dopamine synthesis in either subregion of the nucleus accumbens.

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