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Cocaine In Utero Enhances the Behavioral Response to Cocaine in Adult Rats

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PERIS, J., M. COLEMAN-HARDEE AND W. J. MILLARD. *Cocaine in utero enhances the behavioral response to cocaine in adult rats.* PHARMACOL BIOCHEM BEHAV **42**(3) 509–515, 1992.—The effects of cocaine exposure in utero on cocaine-induced behaviors and dopamine (DA) transmission in the nigrostriatal and mesolimbic pathways were measured in adult rats. Pregnant rats received either saline or cocaine (1 or 3 mg/kg, IV) daily throughout gestation. When offspring were 3 months of age, locomotor and stereotypic behaviors were rated after an injection of either saline or cocaine (10 mg/kg, IP). Cocaine in utero increased the response to cocaine in adult offspring and increased basal locomotion in female offspring. Cocaine in utero increased amphetamine-stimulated release in female offspring but decreased release in males. On the other hand, male rats that had received cocaine in utero exhibited greater basal tritium release. One injection of cocaine increased amphetamine-stimulated [^{3}H]DA release from striatal slices of male rats but not female rats. Neither cocaine in utero nor in vivo affected D_2 DA receptor binding in striatum nor nucleus accumbens. Thus, cocaine in utero behaviorally sensitized animals to subsequent cocaine exposure and increased [^{3}H]DA release from nigrostriatal endings, but the relationship of these two variables depended upon gender.

Cocaine In utero Dopamine Striatum Stereotypy Locomotion

COCAINE use has increased significantly in young women in their child-bearing years and unfortunately as many as 17% of these women continue to use cocaine during pregnancy (14,25). The effects of cocaine use during pregnancy include shorter gestational periods and offspring with decreased birth weights, increased incidence of urogenital malformations, and observable behavioral deficits (5–7,29). These findings have been reproduced in laboratory animals such that fetal exposure of at least 60 mg/kg/day decreases birth weight and increases physical malformations (8,13,26) while lower doses decrease learned behaviors and increase spontaneous locomotor activity up to 30 days postnatally (15,38,39). Similarly, exposure to cocaine in rats during postnatal days 1–10 (a time period comparable to third trimester exposure in humans) results in a slight increase in basal locomotion but a twofold increase in amphetamine-induced locomotion (20). Thus, in utero exposure to low doses of cocaine appears to increase locomotor activity, especially in response to another stimulant, and this effect may persist into adulthood. These data suggest that cocaine in utero may affect the adult response to cocaine although this has not yet been measured.

Cocaine exposure in adult rats increases the degree of locomotion and stereotypy induced by subsequent cocaine administration and this sensitization is long lasting [see (41)]. The mesolimbic and nigrostriatal dopaminergic pathways in the brain are thought to mediate the behavioral effects of cocaine (9) and increased dopamine (DA) transmission in these pathways has been proposed as a mechanism for cocaine sensitization. More specifically, cocaine sensitization results in enhanced striatal DA release (1,22,32–35) and postsynaptic D_1 and D_2 DA receptor responsiveness in nucleus accumbens (16,17,32). If the behavior response to cocaine in adult rats is sensitized by in utero exposure to cocaine, then it is also possible that DA transmission in nigrostriatal or mesolimbic pathways may be enhanced after cocaine exposure in utero.

The purpose of the work reported here was to test whether in utero cocaine can result in sensitization either behaviorally or neurochemically in adult offspring. Specifically, we asked whether the initial behavioral response to cocaine as an adult was affected by in utero exposure to cocaine and whether nigrostriatal and mesolimbic DA terminals were affected by in utero exposure to cocaine. We measured locomotor activity and stereotypy, [^{3}H]DA release from striatum, and D_2 DA receptor binding in striatum and nucleus accumbens both after a saline or cocaine in vivo challenge in adult rats that had been treated in utero with either saline or one of two doses of cocaine.

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METHOD

Animals

Gravid female Sprague-Dawley rats (day 1; sperm positive) from Charles River Animal Laboratories (Wilmington, MA) were implanted with intraatrial catheters as described previously (27). Prior to surgical procedures, animals were anesthetized with methoxyflurane (Metophane). A Silastic (Dow Corning Corp., Midland, MI) catheter was inserted through the right jugular vein and positioned in the right atrium. The free end was passed subcutaneously to the top of the skull, where it was secured with jewelers' screws and dental cement. Long-acting bicillin (20,000 U, IM) was administered after each procedure to minimize postsurgical infections and then animals were maintained on warming pads until full recovery from anesthesia. Rats were housed in the UF Animal Care Facility with a 12 L:12 D cycle and allowed rat chow and water ad lib. Animals were observed at all times for signs of discomfort or weight loss.

Rats were administered cocaine (Sigma Chemical Corp., St. Louis, MO) starting on the sixth day after surgery via the IV cannula twice daily (at 0900 and 1800 h) in two equal doses of 0.5 and 1.5 mg/kg for a combined daily cocaine exposure of 1 and 3 mg/kg/day. Each dose of cocaine was contained in an injection volume of 1 ml/kg body wt of sterile saline. Control animals received an equal volume of saline. These injection regimens and doses of cocaine were chosen based upon pilot studies in which they were shown to stimulate locomotor activity without inducing anorexia, severe stereotypy, or convulsions. Daily saline or cocaine exposure was continued until parturition. At this time, animals were cross-fostered to cocaine-naive mothers to eliminate the effects of cocaine on postnurturing. Body weights of pups were monitored weekly during the first 3 months of life and on the day of the in vivo drug challenge.

Behavioral Measurements

At 3 months of age, offspring were divided according to gender and the dose of cocaine received in utero, thereby resulting in six groups (F0, F1, F3, M0, M1, M3). Each group was randomly divided in two; one half received one injection of cocaine (10 mg/kg, IP) and the other half received saline (in vivo treatment). Thus, data were collected from 12 different treatment groups as follows: M0S, M1S, M3S, M0C, M1C, M3C, F0S, F1S, F3S, F0C, F1C, and F3C. For example, M0C signified male offspring injected with cocaine that had received saline in utero and F1S signified female offspring injected with saline that had received 1 mg/kg cocaine in utero.

The behavioral effects of the in vivo treatment were assessed after injection. Locomotor and stereotyped behaviors were rated concurrently by an observer, who was blind to the drug treatment, for 10 s periods every 2.5 min beginning 10 min prior to and continuing until 50 min after injection. The behavioral chambers were cylinders of quarter-inch wire mesh, 1 ft. in diameter and 15 in. high. The following stereotypy rating scale (12) was used: (0) no gnawing or locomotor movements, with or without grooming (normal quiet behavior); (1) walking around the cage, sniffing over the grid (normal exploratory behavior); (2) licking the wires and pushing their nose through the grid, more rapid locomotor movement, no grooming; (3) moving around with biting, gnawing at the wires, rearing (definite repetitive movements); and (4) restricted locomotion to a small area with intense gnawing on the wires or head-bobbing.

For statistical purposes, the ratings from the last 50 min were summed to give each subject one behavioral score for each day of testing. Data from these experiments were analyzed by the nonparametric Kruskal-Wallis test to compare more than two groups and the Mann-Whitney *U*-test for follow-up comparisons on two groups. In addition, data were subjected to three-factor analysis of variance (ANOVA) (as described below) since this test provides considerable power in assessing interactions between two or more variables. Animals were killed 24 h after the in vivo drug challenge and half of each brain was used for release assays while the other half was used for binding studies.

[^{3}H]DA Release From Striatal Slices

The method used for measuring [^{3}H]DA release from superfused striatal slices has been described previously (34). One side of striatum was dissected and sliced (0.4-mm thick) with a Brinkmann (Westbury, NY) tissue chopper and the slices were incubated in Krebs' solution (pH = 7.4) saturated with 95% O_2/5% CO_2 for 30 min at 35°C. The composition of the Krebs' buffer was (in mM): NaCl, 118; glucose, 11.1; $NaHCO_3$, 25; KCl, 4.7; NaH_2PO_4, 1.0; $MgCl_2$, 1.2; $CaCl_2$, 1.3; EDTA, 0.004; ascorbate, 0.11. To label the stores of DA, slices were incubated for an additional 30 min in 4 ml fresh buffer containing 0.1 μM [^{3}H]DA (35 Ci/mmol; 3,4-[7-^{3}H]dihydroxyphenylethylamine; New England Nuclear, Newton, MA). Rinsed, prelabeled slices were placed into separate glass chambers maintained at 33°C and perfused with oxygenated Krebs' buffer at a rate of 1 ml/min. The superfusate was collected at 5-min intervals beginning 50 min after the start of superfusion. At 70 min, slices were superfused for 2.5 min with 2 μM *d*-amphetamine (Sigma). At 115 min, slices were exposed to 5-Hz electric pulses (20 mA, 2-ms duration) for 60 s. Radioactivity in both superfusate and solubilized tissue was determined by liquid scintillation counting. Neither DA uptake blockers nor monoamine oxidase inhibitors were included in the superfusion buffer; therefore, the tritium collected after amphetamine stimulation should have been comprised of [^{3}H]DA; whereas that evoked by electrical stimulation should have been comprised of predominately [^{3}H]dihydroxyphenylacetic acid (DOPAC) (30).

The amount of tritium released in each fraction was expressed as a percentage of total tritium present in each slice at the time of sample collection. Spontaneous release was averaged from the two 5-min fractions preceding each stimulus plus the first fraction after each stimulus in which release returned to prestimulation levels. The evoked tritium release was calculated by subtracting spontaneous release from the tritium efflux collected in each fraction beginning immediately after the stimulus and continuing until the efflux equaled spontaneous. Each release assay contained at least two slices from both a saline- and cocaine-injected animal of the same gender and in utero treatment condition. N indicates the number of animals per group. Three-factor ANOVAs with between-group variables of in utero treatment (0, 1, 3 mg/kg, IP), in vivo treatment (S, C), and gender (M, F) were used for statistical analysis in conjunction with Neuman-Keuls follow-up comparisons when appropriate.

[^{3}H]Spiperone Binding to D_2 DA Receptors

Tissue for binding assays was frozen immediately in powdered dry ice and stored at −80°C until sectioning. Sagittal tissue sections were cut (30 μm) using a cryostat at −20°C to

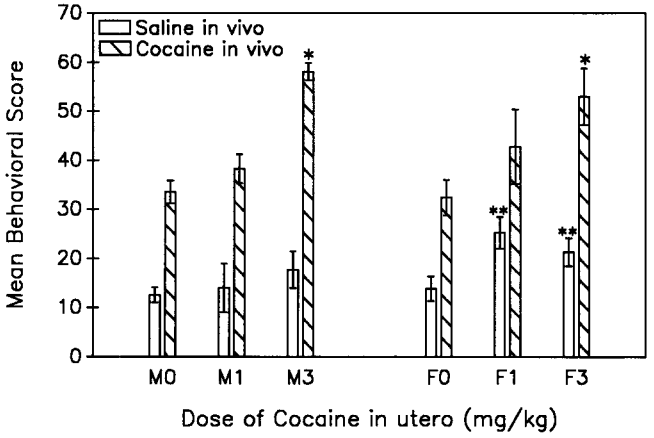

FIG. 1. In utero exposure to cocaine increased the behavioral response to cocaine as an adult. Stereotypic and locomotor behavioral scores were summed for 50 min after an injection of cocaine (10 mg/kg, IP) or saline (in vivo treatment) in adult male (M0, M1, M3) and adult female (F0, F1, F3) rats exposed to different doses of cocaine in utero. In vivo cocaine increased behavior in all rats but had a greater effect in rats receiving 3 mg/kg in utero cocaine. n = 3-8 as indicated in Table 1. *$p < 0.05$ compared to either Group M0C or F0C; **$p < 0.05$ compared to Group F0S.

include substantia nigra, striatum, and nucleus accumbens (31). Duplicate sections were thaw-mounted onto gelatin-coated slides and stored at −20°C for up to 5 days. Quantitative autoradiographic analysis of specific binding of [^{3}H]spiperone (Amersham Corp., Arlington Heights, IL) has been described previously (32). Thawed sections were incubated at 37°C for 100 min in 20 mM Hepes buffer containing 154 mM NaCl and 0.8 pM [^{3}H]spiperone. Binding in the presence of 200 μM racemic sulpiride (Sigma) was used as a measure of nonspecific binding; thus, specific binding represented only D_2 DA sites and not D_1 DA or serotonin receptors. The sections were washed for 40 min in ice-cold buffer and dipped in deionized water before drying and apposing, along with plastic tritium standards (Amersham), to tritium-sensitive film.

The illuminated image of each autoradiogram was collected by a Nikon camera and digitized using the Turnkey system (Imaging Research Inc., Toronto). The standards included on each piece of film were fit with a Michaelis-Menton function to generate a standard curve for transforming the digitized gray values into concentrations (fmol/mm^2) of radioligand bound. Areas of interest of each digitized image were defined and the amount of binding in these areas was determined. Specific binding was derived by subtracting nonspecific binding from total binding. Specific binding was compared across treatment groups using ANOVA as described for release assays.

RESULTS

General

Body weight (monitored weekly) increased in all offspring from 21 days postnatal to 90 days of age (data not shown). There was no difference in weight due to in utero cocaine treatment at any time nor was there a difference due to gender before 38 days postnatally. At this point, male rats began to gain weight faster than female rats (data not shown). There was a gender × days interaction, $F(7, 546) = 500$, $p < 0.001$, supporting this observation. There was also no difference in the body weights of animals assigned to the saline or cocaine in vivo treatment groups. There was no observed delay in the onset of puberty in female rats as monitored by vaginal opening nor was there any alteration in estrous cyclicity as determined by daily vaginal lavage when measured for a 2-week period at 60 days of age.

Locomotor and Stereotyped Behavior

The behavior of adult male and female rats was rated in response to a saline or cocaine challenge injection (Fig. 1). Behavioral scores were greater after cocaine injection compared to after saline injection due to an increase in locomotor activity with mild stereotypy (Fig. 1). The effects of cocaine in vivo were significant both when analyzed using ANOVA, $F(1, 52) = 185$, $p < 0.001$, and Kruskal-Wallis, $H(11) = 55$, $p < 0.001$, with subsequent Mann-Whitney U comparisons between saline and cocaine treatment ($U \leq 3$ for each of the six comparisons, $p < 0.05$). There was no effect of gender on the response to saline or cocaine in animals that had received saline in utero.

The effects of in utero cocaine exposure on behavior were dependent upon in vivo drug treatment. After a cocaine injection, behavioral scores were greatest in animals that had received 3.0 mg/kg cocaine in utero (Fig. 1) due to increased stereotypy compared to animals receiving saline in utero. Mann-Whitney comparisons were significant between Groups M0C and M3C, $U = 0$, $p < 0.001$, and Groups F0C and F3C, $U = 0$, $p < 0.001$, but not with Groups M1C or F1C. After a saline injection, both doses of cocaine in utero increased locomotor behavior, especially in female offspring (Fig. 1). Mann-Whitney comparisons were significant between Groups F0S and F1S, $U = 1$, $p < 0.005$, and between Groups F0S and F3S, $U = 3$, $p < 0.05$, but not between Groups M0S, M1S, or M3S.

When these same data were subjected to ANOVA, similar

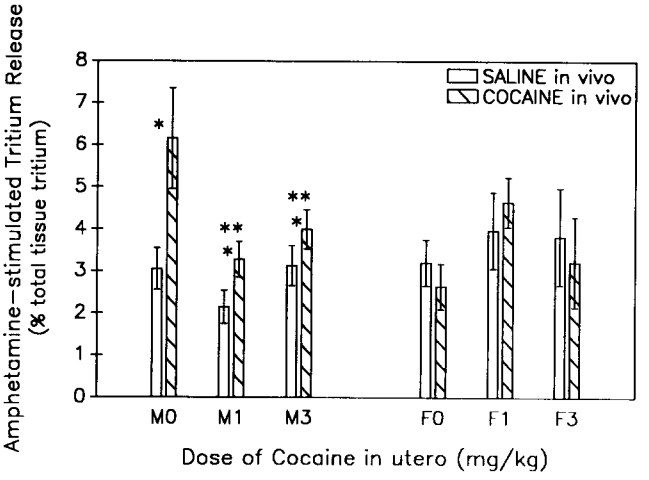

FIG. 2. Cocaine in utero enhanced amphetamine-stimulated [^{3}H]DA release from striatum of female but not male rats. Male rats (M0, M1, M3) or female rats (F0, F1, F3) were exposed to cocaine or saline in utero and then received a cocaine or saline injection as an adult. Cocaine in vivo increased amphetamine-stimulated release in male rats but this effect was diminished by cocaine in utero. Triplicate slices from each rat were exposed to 2 μM amphetamine for 2.5 min. Shown are mean values ± SEM for n = 3-8 per group. *$p < 0.05$ compared to respective saline-in vivo group; **$p < 0.05$ compared to Group M0 or F0 (combined across in vivo variable).

results were found. There was a significant in vivo × in utero interaction, $F(2, 52) = 7.0, p < 0.005$, in addition to a main effect of cocaine in utero, $F(2, 52) = 18.9, p < 0.001$, as well as the main effect of cocaine in vivo already discussed above. When the interaction was broken down according to in vivo treatment, there was a significant in utero effect in both cocaine-injected, $F(2, 26) = 19.3, p < 0.01$, and saline-injected rats, $F(2, 26) = 3.3, p < 0.05$. There was also a significant effect of gender in saline-injected animals, $F(1, 26) = 5.1, p < 0.05$.

[^{3}H]DA Release

Previously, we found that amphetamine-stimulated release of [^{3}H]DA from striatal slices was increased from 1 day to 2 weeks after rats received a single cocaine injection (34). These results were replicated in male rats only: In vivo cocaine enhanced amphetamine-induced [^{3}H]DA release in male rats killed 1 day later but had no effect in female rats. These data were supported by a significant in vivo main effect, $F(1, 50) = 4.0, p < 0.05$, and a significant gender × in vivo interaction, $F(1, 50) = 5.98, p < 0.05$. The interaction was due to a significant effect of in vivo treatment in males, $F(1, 30) = 10.8, p < 0.05$, but not females.

On the other hand, cocaine exposure in utero tended to decrease release overall in male rats and increase release overall in female rats (Fig. 2), although there was a large degree of variability in these data. ANOVA revealed a significant interaction of gender × in utero treatment, $F(2, 50) = 6.12$, $p < 0.005$, that was due to a significant decrease in males, $F(2, 30) = 4.5, p < 0.005$, but only a trend for an increase in females, $F(2, 20) = 2.2, p < 0.1$.

Electrically-stimulated tritium release was also measured in each treatment group 1 day after the challenge injection (Fig. 3). Electrically stimulated release was decreased in all animals that had received 1 mg/kg cocaine in utero compared to the saline or 3-mg/kg treatment groups. There was a main effect of in utero treatment, $F(2, 50) = 4.5, p < 0.05$. There were no effects involving in vivo cocaine or gender on electrically stimulated release.

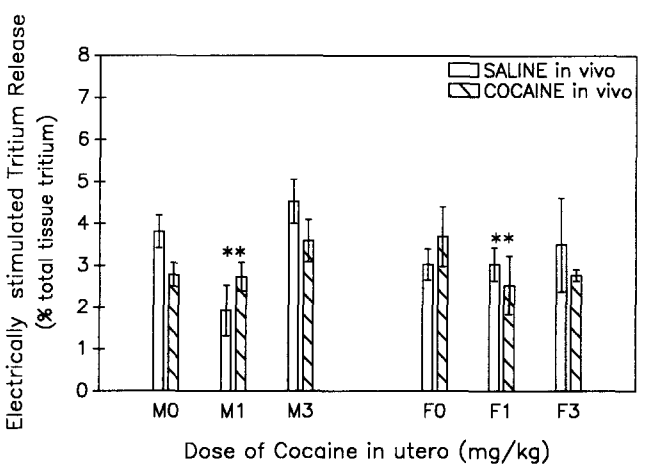

FIG. 3. In utero exposure to 1 mg/kg cocaine decreased electrically stimulated [^{3}H]DA release from striatum. Male rats (M0, M1, M3) or female rats (F0, F1, F3) were exposed to cocaine or saline in utero and then received a cocaine or saline injection as an adult. Triplicate slices from each rat were exposed to 5-Hz electrical pulses for 1 min. Shown are mean values ± SEM for $n = 3$–8 per group. $^{**}p < 0.05$ compared to Group M0 or F0 (combined across in vivo variable).

TABLE 1

THE EFFECT OF COCAINE TREATMENT (IN VIVO VS. IN UTERO) ON BASAL TRITIUM RELEASE AND TOTAL TISSUE TRITIUM LEVELS IN STRIATAL SLICES

Groups	n	Basal Release (% total tissue tritium)	Total Tissue Tritium (dpms × 1000)
M0S	7	1.9 ± 0.1	98.5 ± 10.2
M0C	6	2.0 ± 0.1	98.2 ± 13.7
F0S	6	2.0 ± 0.1	120.0 ± 24.3
F0C	5	1.8 ± 0.1	114.4 ± 6.9
M1S	4	1.8 ± 0.2	108.0 ± 20.6
M1C	4	1.9 ± 0.3	113.0 ± 38.8
F1S	4	2.0 ± 0.1	94.3 ± 11.0
F1C	5	2.1 ± 0.1	82.0 ± 12.2
M3S	7	2.3 ± 0.1*	118.5 ± 11.8
M3C	8	2.2 ± 0.1*	115.1 ± 14.0
F3S	3	1.8 ± 0.2	102.1 ± 8.4
F3C	3	1.9 ± 0.1	104.9 ± 17.2

Basal tritium release was measured before amphetamine stimulation. Total tissue tritium was the sum of released tritium plus that remaining in the tissue at the end of the experiment. Group abbreviations are defined in the text. The values shown are means ± SEM

*$p < 0.05$.

Basal tritium release was slightly increased in male rats that had received 3 mg/kg cocaine in utero regardless of in vivo treatment (Table 1). These data were supported by a gender × in utero interaction, $F(2, 50) = 5.68, p < 0.01$, that was followed up by a significant effect in males, $F(2, 30) = 4.5$, $p < 0.05$, but not females. The amount of radioactivity present in each slice at the start of superfusion did not differ due to gender nor due to treatment with cocaine in utero or in vivo (Table 1).

[^{3}H]Spiperone Binding to D_2 DA Receptors

Specific binding of [^{3}H]spiperone to D_2 DA receptors was densest in striatum, nucleus accumbens, and olfactory tubercle. Although it appeared as if binding in striatum was greater in females than in males by about 20% (Fig. 4), this effect only approached significance, $F(1, 46) = 3.01, p < 0.08$. There were no significant effects on binding in any of these regions due to direct effects or interactions of any of the treatment variables (Fig. 4).

DISCUSSION

In utero exposure to moderate doses of cocaine enhanced the behavioral response to cocaine in both adult males and females. Previously, in utero exposure to lower doses of cocaine increases spontaneous locomotion in offspring at early postnatal ages (10) with some evidence for longer-lasting effects in offspring 30 days or older (15). The effects of cocaine exposure in utero on the response to cocaine as an adult have not been previously studied. Thus, these data provide evidence that adults may be behaviorally sensitized to cocaine by prior cocaine exposure in utero.

In addition, cocaine in utero increased locomotor activity after a saline injection in female but not in male rats. These data support previous reports of increases in non-drug-induced locomotion after in utero stimulant exposure (15,21). However, there has been no previous evidence to support the

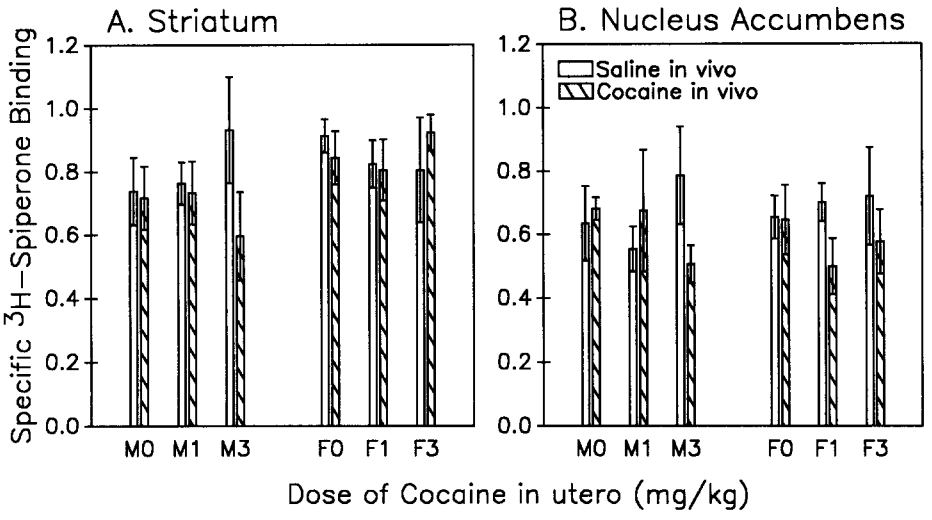

FIG. 4. In utero exposure to cocaine did not affect D_2 DA receptor binding in striatum or nucleus accumbens. Specific binding of 0.8 nM[^{3}H]spiperone in the nigrostriatal (panel A) and mesolimbic (panel B) terminal regions. Shown are mean values ± SEM. n = 4–8 for each group.

gender-specific nature of our findings. On the other hand, we found no difference in the initial response to cocaine or in behavioral sensitization caused by in utero cocaine due to offspring gender. This is in opposition to findings that gender and ovarian steroids are important determinants of stimulant-induced behaviors and the degree of cocaine and amphetamine sensitization in adults [see (36,41)]. The lack of gender specificity in the current study may be due to the behavioral measurements employed since previous studies measured stimulant-induced rotations after unilateral striatal lesions. It should also be kept in mind that variability in circulating levels of estrogen and progesterone during the estrous cycle can affect locomotor behavior (18,19) and stimulant-enhanced behaviors (2,4,33). Thus, it is possible that if the data had been categorized according to the phase of estrus at the time of testing the differences between the present findings and previous studies might be reconciled.

The mechanism by which cocaine exposure in utero enhances locomotion and stereotypy in adults has also not been previously studied. It has been hypothesized that the amount of DA present at the nigrostriatal and mesolimbic terminals in the fetus determines the number of DA receptors that will develop and therefore determines postsynaptic responsiveness and subsequent behavior responsiveness to agonists. In support of this hypothesis, fetal exposure to haloperidol or α-methyl-para-tyrosine decreases apomorphine-induced rotations and the number of D_2 DA receptors in the striatum of adult offspring (37). Thus, one might expect that cocaine in utero would increase DA levels in the fetal synapses and therefore increase postsynaptic DA receptors. However, in the present study neither cocaine in vivo nor in utero affected D_2 DA receptor binding in nucleus accumbens or in striatum. Similarly, previous findings failed to find an effect of fetal exposure to cocaine on D_1 DA receptor binding in striatum or nucleus accumbens (11). On the other hand, these data are consistent with the lack of persistent effects of cocaine exposure in adults on the number or affinity of D_1 or D_2 DA receptors in striatum or nucleus accumbens (32). Instead, the functional state of these receptor subtypes appears to be affected in adults exposed to cocaine (16,17,32). Similarly, the effect of cocaine in utero on the function of these receptors should be studied.

A second hypothesis explains behavioral sensitization caused by previous cocaine via a persistent increase in DA release from striatal and accumbal endings (41). In the present study, amphetamine-induced release was increased 30–50% by both 1 and 3 mg/kg cocaine in utero in adult female rats. Accordingly, this enhanced release could explain the augmentation of cocaine-mediated behaviors, as well as the increase in behaviors after a saline injection that was observed in female rats. Again, the large degree of variability in the release data from female offspring may have obscured some differences due to treatments. This variability could have been due to differences in circulating ovarian steroid levels due to estrous cyclicity at the time of tissue collection or injection. Ovarian steroids can directly affect striatal DA release and receptors (40) and can also affect the response of the nigrostriatal DA system to stimulants (2,3,32). Thus, it is possible that if this study were repeated using ovariectomized and steroid-replaced animals more subtle interactions of cocaine in utero and in vivo on striatal DA release may be revealed.

In male rats, the behavioral response to cocaine was not as closely related to amphetamine-stimulated [^{3}H]DA release from striatum as in female rats. As seen previously, one injection of a moderate dose of cocaine in vivo increased amphetamine-stimulated [^{3}H]DA release from striatum in adult male rats (34,35), which may be related to the development of behavioral sensitization to one injection of cocaine (24). Along these lines, one would have expected a large enhancement in striatal DA release from males that had received cocaine both in utero and in vivo since this treatment produced the greatest increase in the behavioral response to cocaine. Instead, cocaine in utero decreased amphetamine-stimulated release in male rats overall but especially in those that had received cocaine in vivo. On the other hand, basal tritium efflux was increased in male rats exposed to 3 mg/kg cocaine in utero regardless of cocaine treatment in vivo. These data would support an elevation of behaviors mediated by nigrostriatal DA transmission, such as that seen in Group M3C. However, one might have also expected a concomitant increase in basal locomotion in Group M3S and this effect was only slight.

Since amphetamine releases DA via reversal of the DA transporter (23,30), this change may represent enhanced synaptic transporter function. Similarly, basal DA release may

also represent reversal of the DA transporter due to the calcium-independent, reserpine-insensitive characteristics of this efflux (32). Thus, the current findings of enhanced spontaneous and amphetamine-stimulated DA release after cocaine in utero may actually reflect changes in the function of the DA transporter. On the other hand, total tissue tritium, another indicator of transporter function, was not affected by either cocaine treatment or gender. These results may be reconciled with studies measuring specific sodium-dependent DA uptake or [^{3}H]nomifensine binding to the DA transporter. Similarly, the effects of cocaine in utero on parameters of uptake and release in nucleus accumbens should also be measured since release from accumbens is enhanced in cocaine-sensitized adults (28).

In summary, in utero cocaine increased the behavioral response to cocaine equally in female and male adult offspring and also increased basal locomotion in adult females. While changes in amphetamine-stimulated striatal [^{3}H]DA release could account for some of the behavioral effects of cocaine in utero in females, it was not related to the degree of behavioral sensitization in males. Instead, basal tritium release was enhanced in these rats. The apparent increase in calcium-independent release caused by cocaine in utero may indicate a change in storage pools of DA similar to that suggested as an explanation for the increase in striatal DA release frequently observed following repeated cocaine or amphetamine administration (41). It will be of interest to measure whether behavioral sensitization to chronic cocaine exposure as an adult is affected by in utero exposure to cocaine. Similarly, increases in nigrostriatal and mesolimbic DA transmission caused by cocaine sensitization as an adult (32) may also be affected by in utero exposure to cocaine.

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REFERENCES

1. Akimoto, K.; Hamamura, T.; Otsuki, S. Subchronic cocaine treatment enhances cocaine-induced dopamine efflux studied by in vivo intracerebral dialysis. Brain Res. 490:339–344; 1989.
2. Becker, J. B.; Cha, J.-H. Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. Behav. Brain Res. 35:117–125; 1989.
3. Becker, J. B.; Ramirez, V. D. Sex differences in the amphetamine-stimulated release of catecholamines from rat striatal tissue in vitro. Brain Res. 204:361–372; 1981.
4. Becker, J. B.; Robinson, T. E.; Lorenz, K. A. Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. Eur. J. Pharmacol. 80:65–72; 1982.
5. Chasnoff, I. R.; Chisum, G. M.; Kaplan, W. E. Maternal cocaine use and genitourinary tract malformations. Teratology 37:201–204; 1988.
6. Chasnoff, I. J.; Griffith, D. R.; MacGregor, S.; Dirkes, K.; Burns, K. A. Temporal patterns of cocaine use in pregnancy. JAMA 261:1741–1744; 1989.
7. Cherukuri, R.; Minkoff, H.; Feldman, J.; Parekh, A.; Glass, L. A cohort study of alkaloidal cocaine ("crack") in pregnancy. Obstet. Gynecol. 72:147–151; 1988.
8. Church, M. W.; Dintcheff, B. A.; Gessner, P. K. Dose-dependent consequences of cocaine on pregnancy outcome in the Long-Evans rat. Neurotoxicol. Teratol. 10:51–58; 1988.
9. Costal, B.; Naylor, R. J. Mesolimbic and extrapyramidal sites for the mediation of stereotyped behavior patterns and hyperactivity by amphetamine and apomorphine in the rat. Adv. Behav. Biol. 21:47–76; 1977.
10. Dow-Edwards, D. L. Cocaine effects on fetal development: A comparison of clinical and animal research findings. Neurotoxicol. Teratol. 13:347–352; 1991.
11. Dow-Edwards, D. L.; Freed, L. A.; Fico, T. A. Structural and functional effects of prenatal cocaine exposure in adult rat brain. Dev. Brain Res. 57:263–268; 1990.
12. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia 10: 316–323; 1967.
13. Fantel, A. G.; MacPhail, B. J. The teratogenicity of cocaine. Teratology 26:17–19; 1982.
14. Frank, D. A.; Zuckerman, B. S.; Amaro, H.; Aboagye, K.; Bauchner, H.; Cabral, H.; Fried, L.; Hingson, R.; Kayne, H.; Levenson, S. M.; Parker, S.; Reece, H.; Vinci, R. Cocaine use during pregnancy: Prevalence and correlates. Pediatrics 82:888–895; 1988.
15. Henderson, M. G.; McMillan, B. A. Effects of prenatal exposure to cocaine or related drugs on rat developmental and neurological indices. Brain Res. Bull. 24:207–212; 1990.
16. Henry, D. J.; Greene, M. A.; White, F. J. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Repeated administration. J. Pharmacol. Exp. Ther. 251:833–839; 1989.
17. Henry, D. J.; White, F. J. Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor sensitivity within the rat nucleus accumbens. J. Pharmacol. Exp. Ther. 258:882–890; 1991.
18. Hruska, R. E.; Silbergeld, E. K. Estrogen treatment enhances dopamine receptor sensitivity in the rat striatum. Eur. J. Pharmacol. 61:397–400; 1980.
19. Hruska, R. E.; Silbergeld, E. K. Increased dopamine receptor sensitivity after estrogen treatment using the rat rotational model. Science 208:1466–1468; 1980.
20. Hughes, H. E.; Pringle, G. F.; Scribani, L. A.; Dow-Edwards, D. L. Cocaine treatment in neonatal rats affects the adult behavioral response to amphetamine. Neurotoxicol. Teratol. 13:335–339; 1991.
21. Hutchings, D. E.; Fico, T. A.; Dow-Edwards, D. L. Prenatal cocaine: Maternal toxicity, fetal effects and locomotor activity in rat offspring. Neurotoxicol. Teratol. 11:65–69; 1989.
22. Johnson, K. M.; Snell, L. D. Sensitization to the behavioral effects of cocaine is associated with altered dopamine metabolism and release in rat brain. Soc. Neurosci. Abstr. 13:1718; 1987.
23. Liang, N. Y.; Rutledge, C. O. Comparison of the release of [^{3}H]-dopamine from isolated corpus striatum by amphetamine, fenfluramine and unlabeled dopamine. Biochem. Pharmacol. 31: 983–992; 1982.
24. Lin-Chu, G.; Robinson, T. E.; Becker, J. B. Sensitization of rotational behavior produced by a single exposure to cocaine. Pharmacol. Biochem. Behav. 22:901–903; 1985.
25. Little, B. B.; Snell, L. M.; Palmore, M. K.; Gilstrap, L. C. Cocaine use in pregnant women in a large public hospital. Am. J. Perinatol. 5:206–207; 1988.
26. Mahalik, M. P.; Gautieri, R. F.; Mann, D. E. Teratogenic poten-

tial of cocaine hydrochloride in CF-1 mice. J. Pharmacol. Sci. 69:703–706; 1980.
27. Millard, W. J.; Martin, J. B.; Audet, J.; Sagar, S. M.; Martin, J. B. Evidence that reduced growth hormone secretion observed in monosodium glutamate-treated rats is the result of a deficiency in growth hormone-releasing factor. Endocrinology 110:540–550; 1982.
28. Ng, J. P.; Hubert, G. W.; Justice, J. B. Increased stimulated release and uptake of dopamine in nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. J. Neurochem. 56:1485–1492; 1991.
29. Oro, A. S.; Dixon, S. D. Perinatal cocaine and methamphetamine exposure: Maternal and neonatal correlates. J. Pediatr. 111:571–578; 1987.
30. Parker, E. M.; Cubeddu, L. X. Effects of *d*-amphetamine and dopamine synthesis inhibitors on dopamine and acetylcholine neurotransmission in the striatum, II. Release in the presence of vesicular transmitter stores. J. Pharmacol. Exp. Ther. 237:193–203; 1986.
31. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
32. Peris, J.; Boyson, S. J.; Cass, W. A.; Curella, P.; Dwonkin, L. P.; Larson, G.; Linn, L.-H.; Yasuda, R. P.; Zahniser, N. R. Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmcol. Exp. Ther. 253: 38–44; 1990.
33. Peris, J.; Decambre, N.; Coleman-Hardee, M. L.; Simpkins, J. W. Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [^{3}H]-dopamine release. Brain Res. 566:255–264; 1991.
34. Peris, J.; Zahniser, N. R. One injection of cocaine produces a long-lasting increase in [^{3}H]-dopamine release. Pharmacol. Biochem. Behav. 27:533–535; 1987.
35. Peris, J.; Zahniser, N. R. Persistent augmented dopamine release after acute cocaine requires dopamine receptor activation. Pharmacol. Biochem. Behav. 32:71–76; 1989.
36. Robinson, T. E.; Becker, J. B. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157–198; 1986.
37. Rosengarten, H.; Friedhoff, A. J. Enduring changes in dopamine receptor cells of pups from drug administration to pregnant and nursing rats. Science 203:1133–1135; 1979.
38. Smith, R. F.; Mattran, K. M.; Kurkjian, M. F.; Kurtz, S. L. Alterations in offspring behavior induced by chronic prenatal cocaine dosing. Neurotoxicol. Teratol. 11:35–38; 1989.
39. Spear, L. P.; Kirstein, C. L.; Bell, J.; Yoottanasumpun, V.; Greenbaum, R.; O'Shea, J.; Hoffman, H.; Spear, N. E. Effects of prenatal cocaine exposure on behavior during the early postnatal period. Neurotoxicol. Teratol. 11:57–63; 1989.
40. Van Hardesveldt, C.; Joyce, J. N. Effects of estrogen on the basal ganglia. Pharmacol. Biochem. Behav. 10:1–14; 1986.
41. Zahniser, N. R.; Peris, J. Neurochemical mechanisms of cocaine-induced sensitization. In: Lakoski, J. M.; Galloway, M. P.; White, F. J., eds. Cocaine: Pharmacology, physiology and clinical strategies. Boca Raton, FL: CRC Press; 1992: 229–260.