

## Long-term intravenous perinatal cocaine exposure on the mortality of rat offspring

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### Abstract

To examine the effects of chronic perinatal cocaine exposure, cocaine was administered intravenously throughout pregnancy and the postpartum period to the rat. Pregnant rats were divided into five groups: nontreated (naive); normal saline control (saline); cocaine first generation (cocaine); saline in the first generation and cocaine in the second generation (Sal-2G); and cocaine in both first and second generations (Coc-2G). The rats receiving cocaine in the second generation (Sal-2G and Coc-2G) were offspring of the saline and cocaine group, respectively. All cocaine-treated groups received cocaine 2 mg/kg/day intravenously (IV), and the saline group received normal saline 0.2 ml/day IV from GD 2 to the 21st day postpartum. Mean perinatal mortality was greater in all pups exposed to cocaine in utero during gestation; Cocaine (6.4%); Sal-2G (5.6%); Coc-2G (11.4%) groups than in the noncocaine groups (3.2%, 1.3%). Weight gain, physical, and neurological developments of the offspring were not affected. It was concluded that perinatal cocaine exposure had an increased perinatal mortality even at doses approximately 10 times lower than those previously reported, which were administered by extravascular routes. These findings indicate the importance of the route of drug administration in perinatal cocaine research. © 2000 Elsevier Science Inc. All rights reserved.

*Keywords:* Perinatal cocaine exposure; Intravenous cocaine; Perinatal mortality

### 1. Introduction

Since “crack babies” became a sensational media topic during the 1980s [12], a number of adverse effects of chronic prenatal cocaine exposure on the offspring of rodents have been reported. However, whether these rodent models accurately represent the human situation has been reevaluated recently. The route of cocaine administration used in the majority of studies in rodents has been subcutaneous [2,8,10], intragastric [1,35], or intraperitoneal [7,32], none of which are commonly used by human abusers. The subcutaneous and intragastric routes require large doses of cocaine to reach plasma cocaine concentrations comparable to those attained via the intravenous route [1,2,8,10,35]. Injection via the intraperitoneal (IP) route may injure organs in the intraperitoneal cavity, and may also result in rapid absorption into the uterine mass. Consequently, doses and kinetics of cocaine via extravascular routes are not reflective of human intravenous abuse. Such large doses could theoretically lead to prolonged high plasma cocaine concentrations, causing adverse effects on fetal or neonatal rats.

The effects of cocaine given by the intravenous route in conscious rodents, using doses that are often self-administered by human cocaine abusers, has not been well documented, probably due to methodological obstacles. In the present study, cocaine, at a dose lower than that reported in previous studies, employing extravascular routes, was administered throughout pregnancy as well as the preweaning period to examine its effects on the offspring throughout the perinatal period. The objective of this study was to test whether long-term intravenous maternal administration of cocaine can produce adverse perinatal effects. Furthermore, this observation was extended to second generation of cocaine-exposed offspring.

### 2. Materials and method

The experimental protocol was approved by the Columbia University Animal Care and Use Committee. A total of 28 Sprague-Dawley female rats, 12 to 16 weeks of age at the time of pregnancy, were used in this study. All rats were purchased from a commercial breeder, and were bred in our laboratory. They were placed in a temperature-controlled room on a 12/12-h light/dark cycle, and had free access to water and food.

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### 2.1. Breeding

Nulliparous female animals were housed in pairs and were acclimated to the colony for 1 week. Thereafter, each female rat was placed with an adult male in the breeding cage. The breeding cage had an elevated stainless steel wire floor without chips to aid in the visualization of the plug. The day a plug was found was designated as gestational day 1 (GD 1). Pregnant rats were individually housed and divided into groups. Each group contained six to eight animals: nontreated (naive); receiving normal saline only (saline); cocaine only in the first generation (cocaine); saline in the first generation, then cocaine in the second generation (Sal-2G); and cocaine in the first and second generation (Coc-2G). Subjects in the second generation groups, 13 to 15 weeks at the time of breeding, were comprised of the offspring from the saline group or cocaine group. Pups remained with the mother 3 weeks postnatally while the mother continued to receive cocaine daily.

### 2.2. Implantation of the intravenous catheter

For long-term intravenous (IV) injection of cocaine or saline, a special catheter was designed using three kinds of tubing: one silicone (Baxter, McGaw Park, IL), and two polyethylene infusion tubings (PE 50, Becton Dickinson and Company, Parsippany, NJ; and Becton Dickinson Vascular Access, Sandy, UT). Silicone tubing (o.d. 1.5 mm, i.d. 0.8 mm, and length 2.5 cm) was used for the portion of the catheter that was placed into the vein. The other end of the tubing was comprised of 4.0 cm of infusion tubing covered with a rubber injection cap (PRN Adapter, Becton Dickinson Vascular Access). A small piece of polyethylene tubing was used to connect the silicone and infusion tubing. The total length of the catheter was between 8.2 to 8.4 cm and the total capacity ranged from 0.19 to 0.20 ml.

On GD 1, the treatment animal was anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (7 mg/kg), and the surgical procedure was performed under sterile conditions. The neck and intrascapular area were shaved and disinfected with a 10% povidone-iodine solution (Betadine®, Purdue Frederick Company, Norwalk, CT). A cutdown incision was made on the ventral part of the neck, and the catheter was inserted into the right jugular vein and filled with 0.9% saline containing 50 IU/ml of heparin (Elkins-Sinn Inc., Cherry Hill, NJ). The exposed end of the catheter was tunneled subcutaneously, exteriorized on the back, and anchored to the soft tissue. The incision was then closed to cover the catheter completely. Upon completion of the surgery, the animal was placed in a cage that was warmed by a heating pad to avoid excess loss of body temperature during recovery. The sutures were removed 1 to 2 weeks after the surgery.

### 2.3. Drug administration

After a 24-h postoperative recovery period (GD 2), drug administration was performed daily in the morning, and

continued until the offspring reached weaning age (the 21st postpartum day). The animals in the cocaine groups received 2 mg/kg/day of cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO). The dose of cocaine was chosen based on results from a preliminary study in which rats receiving this dose of intravenous cocaine did not convulse, but did exhibit other subtoxic signs, such as excitement and restlessness. Cocaine was dissolved in sterile 0.9% saline to a final concentration of 2 mg/ml, and was injected over 1 min followed by a flush of heparinized saline. The saline group received 0.9% saline 0.2 ml/day. A group of nontreated animals was used as a control group (naive). The animal was observed for at least 30 min after cocaine administration.

### 2.4. Observations on the mother and litter

The body weights of all dams were measured throughout the treatment period. On the day of delivery, which was designated as postnatal day 0 (PD 0), gestational length, the total number of offspring, and the number of stillbirths were recorded for each animal. At that time, pups were weighed, examined for gross physical abnormalities, and their gender identified. The litters were then culled to 10 pups, balancing for gender whenever possible. Dams were allowed to nurse their own offspring, while continuing to receive saline or cocaine daily. The number of stillbirths and deaths occurring during the first week after a birth were recorded as perinatal mortality. An autopsy was performed for each perinatal death to determine the cause of death.

### 2.5. Physical development and reflex

The following examinations were performed by a maximum of two investigators in a quiet, temperature-controlled room. Pups were adapted for 30 min following separation from their mother. A heating pad was used to maintain body temperature if the pups were younger than 10 days of age. The offspring's body weights were measured on PD 0, 7, 14, and 21, and they were examined for the appearance of downy hair, pinna detachment, fur emergence, lower incisor eruption, ear opening, and bilateral eye opening every morning. The following neurological reflexes were tested daily from PD 1 to 14.

#### 2.5.1. Righting reflex

The newborn was placed on its back, and the latency to turn over to a position with all four paws on the ground: a maximum of 15 s was allowed to complete this task.

#### 2.5.2. Cliff avoidance

The body was placed on an edge with its forepaws and face extending over the edge: a maximum of 15 s was allotted for the pup to retreat from the edge.

#### 2.5.3. Negative geotaxis

The body was placed on a 25° incline with its head facing downward to test its ability to turn 180° within 3 min.

## 2.6. Cocaine and metabolites concentrations

On PD 0, 7, 14, or 21, a male and female pup from the cocaine-exposed groups were euthanized with isoflurane (Abbott Laboratory, North Chicago, IL) and blood samples immediately obtained by cardiac puncture for the determination of cocaine and its metabolites. Heart, liver, brain, and gastric contents (milk) were collected and frozen immediately on dry ice. All samples from the cocaine groups were treated with sodium fluoride to inhibit enzymatic hydrolysis. The blood samples were centrifuged immediately, and the plasma stored with other samples at  $-70^{\circ}\text{C}$  until the drug assays were performed.

## 2.7. Analytical method for cocaine and its metabolites

### 2.7.1. Internal standards

Deuterated internal standards for cocaine, norcocaine, ecgonine methyl ester benzoylecgonine, and ethylbenzoylecgonine (cocaethylene) were added to the plasma or tissue homogenate prior to processing. Deuterated cocaine and benzoylecgonine were purchased from Sigma Chemical Corp. (St. Louis, MO). Ethylbenzoylecgonine was purchased from Research Biochemicals Inc. (Natick, MA). Deuterated norcocaine and ecgonine methyl ester were donated by NIDA, and deuterated ethylbenzoylecgonine was synthesized in our laboratory by forming the ethyl ester of tri-deuterobenzoylecgonine. The EI mass spectra of each of these compounds showed fragmentation patterns with the expected mass unit shifts for the deuterated internal standard.

### 2.7.2. Extraction procedure

Deuterated standards were added to plasma or tissue homogenate before processing. To 0.1–0.5 ml of plasma or tissue homogenate 1 ml 5% sulfosalicylic acid was added, and the precipitated proteins were then removed by centrifugation. The clear supernatant was made alkaline with 1 M carbonate buffer and extracted with 8 ml 1.5% isoamyl alcohol in heptane. The organic layer was then back extracted with 1.2 ml of 0.1 N HCl. (The aqueous layer was kept for more polar metabolite extraction.) The sample was mixed, centrifuged, and aspirated. The aqueous layer was then made basic with 1 M carbonate buffer, and extracted with 100  $\mu\text{l}$  of 15% isoamyl alcohol in toluene. The organic phase was removed, taken to dryness via vacuum centrifuge, and then derivatized at  $80^{\circ}\text{C}$  for 30 min with pentafluoropropionic anhydride (20  $\mu\text{l}$  in 100  $\mu\text{l}$  ethyl acetate). The organic layer was then again taken to dryness in a vacuum centrifuge (Savant Instruments, Farmingdale, NY).

### 2.7.3. Benzoylecgonine and ecgonine methyl ester extraction

The aqueous extract remaining following the initial extraction of cocaine and ethylbenzoylecgonine was transferred to another tube to which 5 ml of a solution of 20% ethanol in chloroform was added. After mixing and centrifugation, the upper layer was aspirated and then the organic layer (lower layer) transferred to a disposable tube. This tube was dried down in the Savant vacuum centrifuge. The

sample was then derivatized using four parts pentafluoropropionic anhydride and one part trifluoroethanol solution via incubation in a heating block at  $80^{\circ}$  for 30 min. The tube was then dried down in the vacuum centrifuge. Benzene (50  $\mu\text{l}$ ) was added, the sample vortexed, and the benzene transferred to the same vial in which the cocaine and ethylbenzoylecgonine were concentrated. This vial was then used for injection into the system.

The GC/MS system was comprised of a capillary column, 15 meters HP 1 crosslinked methyl silicone. The MS was operated in the PCI mode using a mixture of 5% ammonia in methane as the ionizing gas and monitoring ions for each of the compounds. These were ecgonine methyl ester 346(M+1) 349(M+1); benzoylecgonine 372(M+1), 375(M+1); cocaine 304(M+1), 307(M+1); ethylbenzoylecgonine 318(M+1), 321(M+1); norcocaine 453(M+1), 458(M+1), where the second ion monitored is the deuterated internal standard. In addition, secondary ions were monitored to confirm specific structures. The oven temperature was programmed using a cold trapping procedure where injection took place at a column temperature of  $80^{\circ}\text{C}$  and the program was  $80^{\circ}\text{C}/\text{min}$  then  $30^{\circ}\text{C}/\text{min}$  to  $280^{\circ}\text{C}$ —end run.

Depending upon the nature of the study, the standard curves encompassed 0–500 ng/ml or g up to 10  $\mu\text{g}/\text{ml}$  or g in the acute toxicity studies. In the latter case, larger amounts of internal standard were added. Standard curves for each of these compounds were linear throughout the entire range, with low intercepts and correlation coefficients of 0.999+. The intrainter coefficients of variation for these compounds was <6% across the entire range of the samples tested, and we found the procedure to be highly sensitive and reliable (LDQ 1 ng/ml). The advantage of the GC/MS simultaneous ion monitoring with specificity confirmation using secondary fragments cannot be overemphasized. Quality control (QC) samples at low, medium, and high concentrations of cocaine and all metabolites were analyzed with each analytical run.

## 2.8. Statistical analysis

Two-way analyses of variances (ANOVA) were employed for analysis of maternal and offspring weight gain, length of gestation, and litter size, and for measures of physical development and neurological reflexes as well as for stillbirth and neonatal mortality. All data are expressed as mean  $\pm$  SE. A *p*-value of less than 0.05 was considered statistically significant. In all analyses, the litter is the unit of observation. For example, mean birth weight and percent neonatal mortality were calculated for each litter. The sample in each analysis was, therefore, the number of litters rather than the number of pups.

## 3. Results

### 3.1. Maternal data

The intravenous catheter remained patent throughout the experimental period. None of the animals developed infection or leakage around the suture. The dams in the cocaine

groups did not develop convulsions, but did exhibit excitement, manifested as sniffing or circling following the drug administration. These behavioral changes ceased within 30 min. The degree of behavioral alteration after cocaine administration remained unchanged throughout the treatment period.

The mean ( $\pm$  SE) maternal body weight in each group on GD 1 was as follows: naive,  $265 \pm 15$  g; saline,  $254 \pm 15$  g; cocaine,  $278 \pm 5$  g; Sal-2G,  $336 \pm 15$  g; and Coc-2G,  $305 \pm 16$  g. The percent change in body weight from GD 1 is depicted in Fig. 1. Dams in all three cocaine groups gained significantly less body weight by GD 7 ( $p < 0.01$ ) than did noncocaine-treated groups. These differences were still present but diminished at GD 21, and repeated measures of ANOVA indicated weight gain differences between groups over the total 21 day gestational period were not statistically significant.

### 3.2. Offspring data

Mean gestational length, litter size, number of stillbirths, birth weight, and perinatal mortality are summarized in Table 1. There were no significant differences in litter size and gender ratio between groups, nor gender differences in birth weight within any group. Stillbirth and perinatal mortality due to cocaine were significantly greater in all cocaine groups than in the noncocaine groups ( $p < 0.05$ ). Perinatal mortality and stillbirth were higher (11.4%) in Coc-2G than any other groups. The cause of each stillbirth was not clearly evident at autopsy. All eight of the neonatal deaths occurred within a 5-day postnatal period, but necropsies could not be performed because the bodies were cannibalized.

Upon gross examination on PD 0, no anomalies were observed. One female offspring in the cocaine group was

found to have hypoplasia of the right cerebral hemisphere after she was sacrificed for tissue sampling on PD 14; however, her physical and neurological development was no different from that of other litter mates.

#### 3.2.1. Physical development

There were no differences in birth weight among groups (Table 1), and postnatal weight gain in each group was similar throughout the observation period (Fig. 1). The mean ages (days) for the appearance of major physical maturational milestones including pinna detachment, the appearance of downy hair, fur emergence, lower incisor eruption, ear opening, and bilateral eye opening are summarized in Table 2. The mean day of postnatal development when the pups achieved each maturational milestone was similar in all groups.

#### 3.2.2. Neurological reflexes

Figure 2 demonstrates the number of offspring that were able to perform each reflex within the allotted time period. By 7 days, 92.3% of the offspring (36 pups of total 39) in the naive, 86.8% (46 of 53) in the saline, 97.5% (39 of 40) in the cocaine, 100% (24 of 24) in the Sal-2G, and 100% (46 of 46) in the Coc-2G were able to assume the upright position in the righting reflex test. For the cliff-avoidance test, more than 50% of the offspring on PD 1 elicited swift responses by backing away from the cliff, and the majority of pups in all groups were able to demonstrate this response early in the first week. The results of the negative geotaxis test were similar in all groups as well; 97.4% of offspring (38 pups of total 39) in the naive, 84.9% (45 of 53) in the saline, 100% (43 of 43) in the cocaine, 95.8% (23 of 24) in the Sal-2G, and 90.9% (40 of 44) in the Coc-2G were able to turn  $180^\circ$  on the slope by 7 days of age. Mean latencies for

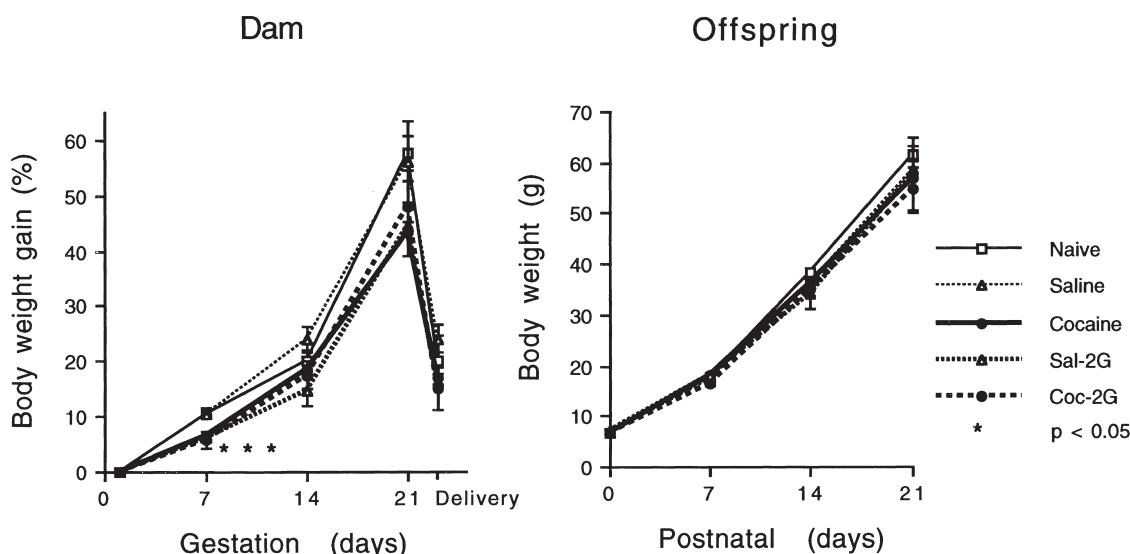


Fig. 1. Changes in maternal weight gain (%) from the GD 1 throughout pregnancy (left) and average weight gain in offspring (g) during postnatal period (right). \*Significantly different from the values in naive group.

Table 1  
Litter variables (mean  $\pm$  SE)

Group	Naive	Saline	Cocaine	Sal-2G	Coc-2G
Gestational length (days)	23 $\pm$ 0.1	23 $\pm$ 0.1	23 $\pm$ 0.2	23 $\pm$ 0.0	23 $\pm$ 0.0
Litter size	13 $\pm$ 0.1	11 $\pm$ 1.0	11 $\pm$ 1.7	15 $\pm$ 0.7	13 $\pm$ 0.4
Stillbirths (%)	2.3	0.0	4.8 <sup>a</sup>	3.8 <sup>a</sup>	8.1 <sup>a</sup>
Birth weight (g)	6.9 $\pm$ 0.1	6.8 $\pm$ 0.1	6.9 $\pm$ 0.2	7.1 $\pm$ 0.4	6.6 $\pm$ 0.2
Perinatal mortality (%)	3.2	1.3	6.4 <sup>a</sup>	5.6 <sup>a</sup>	11.4 <sup>a</sup>

<sup>a</sup>Significantly greater than the noncocaine groups.

the righting and negative geotaxis on each postnatal day are shown in Fig. 3. There were no significant differences between groups. As only a few offspring were able to demonstrate this response before PD 4, the latencies of negative geotaxis varied at the beginning. In general, the saline group tended to have a longer latency and the naive group had a shorter latency, but these differences did not reach significant differences because of the high variability.

### 3.2.3. Drug concentrations

Blood sampling from pups was performed in the morning immediately after the separation from the dam, approximately 24 h after the previous maternal injection. Only one sample from the Sal-2G group had detectable cocaine concentrations in plasma on PD 14, but the concentration level, 10 ng/ml, was very low. On the other hand, cocaine's metabolites, including ecgonine methyl ester (EME) and benzoylecgonine (BE), were detected in plasma in all cocaine groups (Fig. 4). On PD 0, 50% of the samples (four samples of a total of eight) revealed cocaine metabolites in the cocaine, 33% (2 of 6) in the Sal-2G, and 70% (7 of 10) in the Coc-2G group. None of the metabolites were detectable, thereafter, in the cocaine group, but they were detected in all 4 postnatal days in the Sal-2G group. In the Coc-2G group, 20% of the samples obtained on PD 7, and 10% on PD 14 had metabolites, but none did on PD 21. These concentrations did not reach statistical significance because of their high variability.

In the removed pup's organs, cocaine and the metabolites were more frequently detected in the 2G groups than in the cocaine group. In the heart, no drug concentrations were found on any postnatal day in the cocaine group, whereas 50% of samples (three samples of a total of six) obtained from the Sal-2G group on PD 0 and 67% (4 of 6) on PD 21

had detectable cocaine metabolites; 40% (4 of 10) of samples obtained from the Coc-2G group on PD 0, and 10% (1 of 10) on both PD 7 and PD 14 had detectable cocaine (Table 3). In the liver, cocaine metabolites were found 38% of samples (3 of 8) in the cocaine, 67% (4 of 6) in the Sal-2G, and 70% (7 of 10) in the Coc-2G group on PD 0. However, only 0 to 35% of samples were detected for drug concentrations after PD 7 in all groups. Cocaine and/or metabolites were present only on PD 0 in all cocaine-exposed groups in the brain. The cocaine concentration in milk (pup's gastric content) was assumed to be detectable, because this was the only route of cocaine intake for offspring. However, measurable drug concentration was detected only from one sample (25%) on PD 0 but not on PD 7 and PD 14 in the cocaine group, and 33% (2 of 6) on PD 7, 17% (2 of 6) on PD 14 in the Sal-2G group. In contrast, in the Coc-2G group, 75% of samples on PD 0, 80% on PD 7, and 22% on PD 14 revealed low concentrations of cocaine, EME, and BE (Table 3). Samples of gastric contents from PD 21 were not obtained because offspring had already started consuming their food instead of milk.

## 4. Discussion

We have developed a method for the long-term drug administration in conscious rat in which a specially designed catheter was implanted intravenously that remained adequately patent for at least 6 weeks. This model is simple, reliable, and nearly stress free, which is a similar technique that has been described by others [16].

The results using this method indicate that daily intravenous administration of a low dose of cocaine during pregnancy produced higher perinatal mortality than has been reported when extravascular routes of administration have

Table 2  
Postnatal physical development in offspring (mean days  $\pm$  SE)

	Naive	Saline	Cocaine	Sal-2G	Coc-2G
Pinna detachment	2.6 $\pm$ 0.2	2.9 $\pm$ 0.2	3.0 $\pm$ 0.3	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0
Downy hair	2.9 $\pm$ 0.1	2.9 $\pm$ 0.2	2.8 $\pm$ 0.3	3.3 $\pm$ 0.3	2.8 $\pm$ 0.2
Fur emergence	6.4 $\pm$ 0.4	7.0 $\pm$ 0.2	7.0 $\pm$ 0.0	6.7 $\pm$ 0.3	6.7 $\pm$ 0.2
Incisor eruption	10.2 $\pm$ 0.4	10.0 $\pm$ 0.2	10.0 $\pm$ 0.0	10.3 $\pm$ 0.3	10.2 $\pm$ 0.2
Ear opening	12.6 $\pm$ 0.2	12.4 $\pm$ 0.2	12.2 $\pm$ 0.2	12.5 $\pm$ 0.5	12.3 $\pm$ 0.2
Eye opening	14.8 $\pm$ 0.2	14.9 $\pm$ 0.3	14.3 $\pm$ 0.2	14.5 $\pm$ 0.5	14.7 $\pm$ 0.2

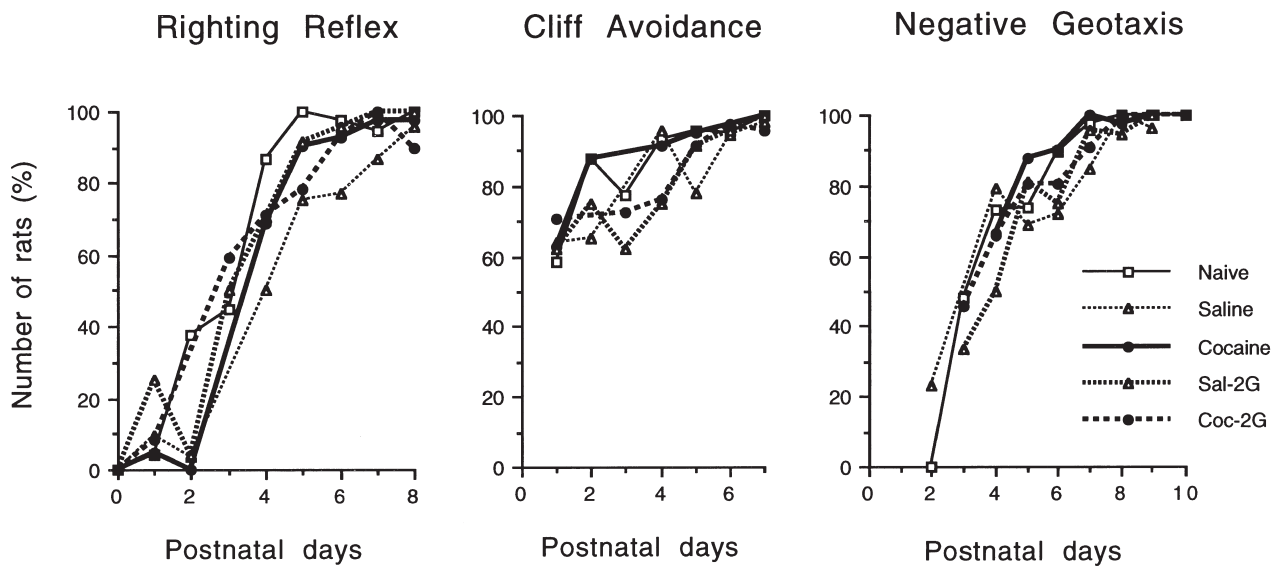


Fig. 2. The abilities of righting reflex, cliff avoidance, and negative geotaxis. Values indicate the number of offspring (%) in each group that was able to perform each reflex within the allowing period.

been used [1,2,7,8,10,32,35]. This is especially apparent in the second generation of pups whose mothers were also exposed to cocaine throughout the gestational and postnatal periods. Compared with extravascular routes of administration, the intravenous route may significantly influence the hemodynamic state of the maternal–fetal unit, even at small doses.

Rats have been commonly used as an animal model in behavioral research with cocaine. Many of the reported findings, however, have been strikingly irreproducible. Hutchings [12] speculated that this inconsistency may be related to the use of different doses. Several investigators

have found behavioral effects on the offspring only at extremely high dose ranges without measuring plasma and tissue cocaine concentrations. In most of the rodent behavioral experiments, cocaine was administered via the subcutaneous or intragastric route, neither of which approximates the pharmacokinetic characteristics following intravenous or intranasal cocaine abuse in humans. We have found that 20 mg/kg of cocaine injected either subcutaneously or intragastrically did not produce plasma cocaine concentrations that would be expected to produce toxicity in rats [19]. On the contrary, intravenous administration of a much smaller dose of cocaine, 2 mg/kg, did produce a CNS subtoxic con-

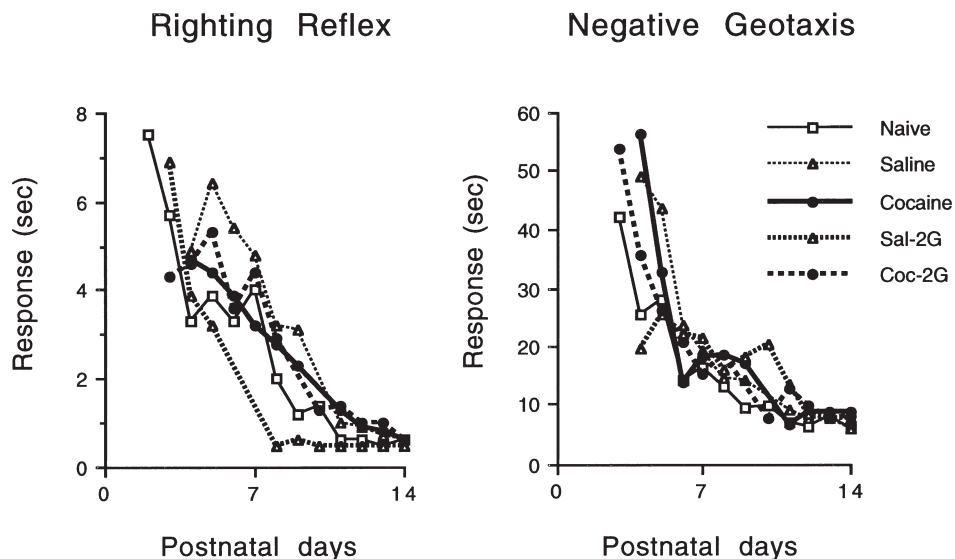
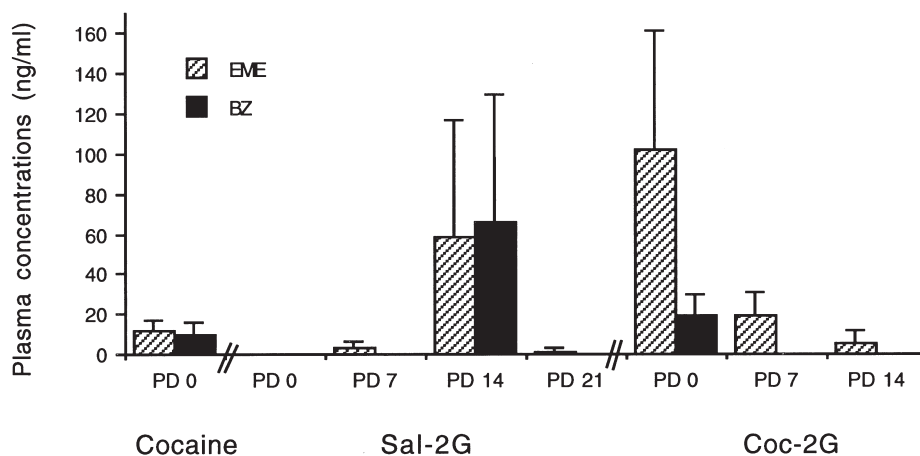


Fig. 3. Mean latencies for righting reflex and negative geotaxis.

DETECTABLE DRUG IN OFFSPRING PLASMA (%)

Age (days)	0	7	14	21
Cocaine	50	0	0	0
Sal-2G	33	17	33	33
Coc-2G	70	20	10	0

Fig. 4. Plasma concentrations of ecgonine methyl ester and benzoylecgonine on postnatal day 0, 7, 14, and 21. Columns represent mean  $\pm$  SE.

concentration in the rat [19]. After intravenous injection, a peak plasma cocaine concentration is reached immediately, followed by a rapid decline which becomes undetectable after 60–90 min [21]. In contrast, drug concentrations in the subcutaneous (20 mg/kg) route peak gradually over a 15-min period and disappear more slowly, resulting in a greater area under the time–concentration curve when compared to the intravenous route [19].

We have also previously demonstrated in the acute study, that transient increases in both systolic and diastolic pressure occur immediately after the intravenous injection of cocaine to the pregnant rat [18]. This was accompanied by decreases uterine blood flow [18] and increases uterine contractility in the rat [21]. Reduction in the uteroplacental circulation due to cocaine's strong vasoconstrictive action could cause fetal hypoxia and decreased nutrient transfer, thus leading to the high

Table 3  
Concentrations of cocaine and its metabolites in major organs (ng/g)

	Heart				Liver				Brain				Milk			
	COC	NC	EME	BE	COC	NC	EME	BE	COC	NC	EME	BE	COC	NC	EME	BE
Cocaine																
PD 0	—	—	—	—	—	—	40	9	—	—	33	9	—	—	13	—
PD 7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PD 14	—	—	—	—	80	23	8	45	—	—	—	—	—	—	—	—
PD 21	—	—	—	—	—	—	—	—	—	—	—	—	SN	SN	SN	SN
Sal-2G																
PD 0	—	—	8	35	—	—	18	7	—	—	3	—	SN	SN	SN	SN
PD 7	—	—	—	—	—	—	—	3	—	—	—	—	—	—	78	58
PD 14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	138	165
PD 21	—	—	—	7	—	—	—	3	—	—	—	—	SN	SN	SN	SN
Coc-2G																
PD 0	30	—	—	84	23	2	80	30	2	—	19	20	90	—	95	—
PD 7	8	—	—	—	—	—	4	—	—	—	—	—	23	—	51	18
PD 14	7	—	—	—	—	—	—	—	—	—	—	—	6	—	10	3
PD 21	—	—	—	—	—	—	—	—	—	—	—	—	SN	SN	SN	SN

—: Not detected. SN: Sample not taken.

incidence of stillbirth [28,34]. Furthermore, the cardiovascular system is more sensitive to cocaine during pregnancy, which was likely due in part to the gestational increase in progesterone [24]. Therefore, it is possible that cocaine induced hemodynamic changes, which may have caused acute maternal complications such as hypertension, arrhythmia, and myocardial ischemia, adversely affecting fetal well-being [27]. Although these maternal physiological alterations caused by intravenous cocaine administration are transient, the repeated episodes of these insults could have secondary influenced the increased perinatal mortality rate observed in the present study, rather than being caused by direct effects of cocaine on perinatally exposed fetuses. Plasma concentration of cocaine following a bolus intravenous injection with the dose of 2.5 mg/kg to the pregnant rat is approximately 2000 ng/ml at the end of injection, which is decreased to 1000 ng/ml by 5 min, and to 120 ng/ml by 60 min [21]. The fetal to maternal concentration ratio of cocaine in the rat is approximately 0.3–0.4 in the extensive experiments in our laboratory [19]. Based on this ratio, an estimated fetal cocaine concentration in plasma would be 600–800 ng/ml at the end of maternal infusion of the drug, but decreased rapidly to about 300 ng/ml by 5 min and 40 ng/ml by 60 min—concentrations that are unlikely to be toxic to the fetus.

Although the slow maternal weight gain in all cocaine groups in the first week had been noted during the pregnancy, these animals gained weight sufficiently after the second week, and no significant differences were seen on GD 21. The effects of surgery performed on GD 1 did not influence weight gain because the dams in the saline group showed the same gain as did the naive group. Additionally, no effects on maternal mortality, litter size, or offspring birth weights were observed.

In the neurological behavior testing, there are several studies in which prenatal cocaine exposure has [15] and has not [4,30] altered negative geotaxis in the past. These inconsistent results may also be related to the different routes and doses of cocaine administration as well as different treatment periods. Although there were no differences between groups in three neurobehavioral tests for 14 days in our study, further tests may be necessary beyond 14 days of age to find whether cocaine exposure causes long-term adverse effects or not.

In the second generation, although the observed physical and neurological outcomes in offspring were not different from the control groups, perinatal mortality, especially the incidence of stillbirth, was increased in Coc-2G pups. The mechanism of the exaggerated effects in this group is not clear. It may be an impairment of hepatic capacity to metabolize cocaine in the second generation, or it may also be altered sensitivity to cocaine, which has been reported in adult offspring prenatally exposed to cocaine by the intravenous route [23]. In fact, prenatal cocaine exposure results in an increased affinity of striatal dopamine D<sub>2</sub> receptors [25], which may play an important role in cocaine sensitization [22]. In contrast, both reduced sensitivity to cocaine [35] and attenuated catecholaminergic activity [5,30] were ob-

served in adult offspring that received cocaine subcutaneously during the prenatal period. The inconsistency in these results regarding sensitivity to cocaine may be due to the different routes or dosages of drug administration. In addition to increased sensitivity to cocaine during pregnancy and repeated administrations of cocaine [9,13], the response to cocaine in second generation rats may be sensitized by in utero exposure to cocaine, and the effects are enhanced when they receive cocaine in their adulthood. Because we did not measure the sensitivity to cocaine, further investigations are obviously warranted.

Maternally administered cocaine and its metabolites are transferred rapidly to the fetus [14,17,20,26,31]. High drug concentrations in fetal liver and brain are reported following maternal administration of cocaine [6,17,20,29,33]. In addition, orally administered cocaine is concentrated in the breast milk intensely. After administering radioactive cocaine to lactating rats, the milk/blood concentration ratio for cocaine was as high as 12.9, with a mean value of 7.8, thus placing the suckling offspring at risk because cocaine in milk is chemically stable [33]. Consequently, the effects of cocaine in the neonate, including tachycardia, tachypnea, hypertension, irritability, or tremulousness, may develop following ingestion of milk a mother who has recently ingested cocaine [3,11,33]. In the present experiment, unlike other cocaine studies, dams were exposed to cocaine not only during pregnancy but also throughout nursing, which mimics a situation in which human mothers may continue abusing cocaine, even after giving birth. However, plasma and organ concentrations of cocaine and its metabolites in offspring were barely detectable in all cocaine groups. As these samples were obtained approximately 24 h after the previous maternal injection, it was the time when cocaine concentration was undetectable in the mother. If the offspring had ingested maternal milk close to the time of sampling, cocaine might have been detectable in plasma and/or organs. In the Coc-2G group, cocaine and metabolites were detected in their gastric content more frequently than those found in the first generation, however, the concentrations were extremely low and unlikely to cause any toxic manifestations.

In summary, perinatal cocaine exposure caused a high incidence of perinatal mortality in the rat, particularly in the second generation of animal that has been exposed to cocaine pre- and postnatally. These results were observed at doses of cocaine approximately 5–50 times lower than those previously reported, which were administered via extravascular routes. Although these effects are subtle, and may not be universal. To our knowledge, this is the first report of evidence of an abnormality in the second generation of cocaine-exposed neonates, indicating the need for further research in this area.

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