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Neurobehavioral and Immunological Effects of Prenatal Cocaine Exposure in Rat

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SOBRIAN, S. K., L. E. BURTON, N. L. ROBINSON, W. K. ASHE, H. JAMES, D. L. STOKES AND L. M. TURNER. *Neurobehavioral and immunological effects of prenatal cocaine exposure in rat.* PHARMACOL BIOCHEM BEHAV **35**(3) 617–629, 1990.—Time-pregnant Sprague-Dawley rats were injected subcutaneously with 20 mg/kg of cocaine HCl or 0.9% saline daily from gestation days 15 through 21. Maternal plasma levels of approximately 720 ng/ml of cocaine did not alter maternal weight gain during treatment, duration of pregnancy, any of the litter variables or several indices of maternal behavior. Offsprings' body weight from birth to 30 days of age and physical maturation were not generally affected by prenatal cocaine exposure. While the development of surface righting, cliff avoidance, and the startle response was accelerated in cocaine-exposed offspring, acquisition of a preference for a social odor was unaltered. Prenatal cocaine also attenuated the locomotor response of the offspring to d-amphetamine and cocaine at PND 15; at PND 30 both of these catecholaminergic agonists increased activity in prenatal saline and prenatal cocaine offspring. However, the difference in plasma levels of cocaine at PND 30 suggests a possible down-regulation of adrenergic receptors following prenatal cocaine exposure. Decreased thymus/body weight ratios and splenomegaly were observed in prenatal cocaine animals at 55 days of age. Although complete neutralization of herpes simplex virus-type 1 was not observed, sera from *prenatal* cocaine offspring showed an increased rate of appearance of cytopathic effect, while sera from animals given cocaine *postnatally* showed a reduction in the rate at which viral infectivity was expressed in culture. These results indicate that prenatal cocaine exposure can alter neurobehavioral ontogeny and humoral immune responsitivity in the offspring.

Cocaine Prenatal exposure Serum drug levels Reflex development Olfactory associations Locomotor activity Herpes simplex virus-type 1 Humoral immunity

THE dramatic increase in cocaine use among young adults of child-bearing age has produced a rise in its use during pregnancy (9, 14, 45, 58, 65, 70). The subsequent increase in the potential number of fetuses exposed to cocaine is the cause of a growing concern for fetal and neonatal outcome. Cocaine, by increasing circulating norepinephrine levels, can produce maternal hypertension, decrease uteroplacental blood flow, stimulate uterine contractions, and produce fetal vasoconstriction (14, 44, 70). While clinical data concerning the perinatal consequences of in utero cocaine exposure are still preliminary and contradictory, decreased length of gestation (40,58), low birth weight (9, 35, 45, 65) and reduced occipitofrontal head circumference in newborns (9, 58, 65) have been consistently reported. However, intrauterine growth retardation was reported by MacGregor (44), Oro (58) but not Chasnoff (14) or Madden (45). Stillbirths, resulting from spontaneous abortion, placental hemorrhage and placental abruption, are also frequently seen in pregnant cocaine users (1, 9, 65).

Some reports suggest that cocaine has teratogenic potential in humans; birth defects of the heart (9), urinogential tract (14,16), and central nervous system (9,59), as well as major and minor craniofacial anomalies (9), have been found. Although Madden (45) explicitly states that cocaine is not teratogenic, 4 out of 8 infants studied presented with one or more of the following: transient heart murmur, sacral exostosis, capillary hemangiomata, subgaleal hematoma, and tachypnea. Moreover, neonates prenatally exposed to cocaine generally have been found to have decreased Apgar scores (13, 15, 65) and an increased incidence of Sudden Infant Death Syndrome (13, 15, 65, 70).

The consequences of cessation of cocaine in the newborn has been debated. When compared to the classical neonatal opiate withdrawal syndrome, withdrawal does not occur in cocaine-exposed infants. However, assessment of these neonates by the Brazelton Neonatal Behavioral Scale (14), the Finnegan Withdrawal Scoring Scheme (58), or a Neonatal Withdrawal Score Index (9) revealed a general pattern of increased irritability and crying, vigorous sucking, poor feeding behavior and seizures. In addition, alterations in the startle reflex (14), poor visual processing (58) and decreased organizational response to environmental stimuli (13) have been reported in newborns after prenatal cocaine exposure. This cluster of symptoms may represent a withdrawal syndrome that is characteristic of stimulants. Alternatively, they may represent a direct drug effect, as cocaine metabolites have

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been found in the urine of prenatally exposed neonates up to 7 days after birth (58).

Limited animal data generally support the clinical findings on prenatal cocaine exposure. However, the results are species and/or strain specific, dose dependent and contigent upon the gestational period of drug treatment. Maternal and fetal fatalities have been reported in rats with cocaine doses of 60 mg/kg or higher (17,29) and in mice with doses over 100 mg/kg (46). Cocaine-induced decreases in maternal weight, with or without decreases in food and water consumption, have been reported for rats (17,29). Concurrent decreases in fetal weight are seen in Sprague-Dawley but not Long-Evans rats. In mice, decreases in maternal food consumption are reflected only in decreased fetal weights (29). With respect to pregnancy outcome, rats exhibit dose-dependent increases in abruptio placentae, fetal edema and fetal hemorrhage (17,29); increased resorptions due to prenatal cocaine exposure are seen in both species (29,46). Although the incidence of teratogenesis is low, the types of defects reported in rats and some strains of mice are similar to those seen in humans: i.e., anophthalmia, urogenital defects, cogenital heart defects, exencephaly and delayed ossification of the skull (9, 14, 16, 59). In addition, cleft palate and skeletal defects have been reported in rodents (29,46).

Clinical studies have not yet been able to evaluate offspring for the long-term consequences of prenatal cocaine exposure, while the majority of animal research has focused on teratogenicity and pregnancy outcome. The possibility that prenatal cocaine alters long-term postnatal development has received little attention and was the major focus of the present study.

A second aim was to determine if prenatal cocaine alters immune function in the offspring. Several lines of evidence suggest that prenatal manipulations can alter immune function in the offspring. Previous work in our laboratory indicates that both immune status and immune reactivity of rat offspring are suppressed by prenatal stress (75,76), and many of cocaine's effects involve the adrenergic system and mimic stress-like states (34,82). Moreover, prenatal exposure of rodents to pesticides (28,81), methylmercury (80), and alcohol (51), as well as dietary deficiencies during pregnancy, have been shown to affect the immunocompetence of the offspring (12, 22, 53).

METHOD

Twenty-eight time-pregnant Sprague-Dawley rats were received on gestation day (GD) 8 (Charles River, Wilmington, MA) or GD 12 (Zivic Miller, Zelienople, PA); the morning that vaginal plugs were found was designated GD 1. Females were housed individually in polyethylene maternity cages ($44 \times 25 \times 30$ cm) under environmentally controlled conditions (0800 hr lights on, 1800 hr lights off; ambient temperature 20°–26°C) with ad lib access to Purina Rat Chow and tap water.

On GD 14, females were ranked, high to low, according to body weight gain, and were alternately assigned to one of two prenatal treatment groups: Prenatal cocaine (PC) or prenatal saline (PS: control). On GD 15–21, dams were injected once daily with either 20 mg/kg of cocaine hydrochloride (Merck, Rahway, NJ) or 0.9% sodium chloride (vehicle) between 0900 and 1100 hours. Following injection, females were monitored for 90 minutes using a time-sampling technique; each animal was scanned once every 60 seconds for the presence of signs of excessive activation (i.e., locomotion, rearing, grooming) and/or toxicity (i.e., stereotypy, convulsions). Injections were made subcutaneously on the back, starting near the neck and moving in a caudal direction, and sites were rotated to limit tissue necrosis due to the vasoconstrictor action of cocaine.

The 20 mg/kg dose of cocaine was chosen for several reasons: 1) Preliminary data from our laboratory indicated that this dose did not produce maternal hyperactivity or stereotypy which might interfere with behavior preparatory to delivery. 2) Teratogenicity has not been reported in rodents at this dose (17, 29, 46). 3) Subcutaneous injection of this dose produced appreciable levels of cocaine and benzoylecgonine (BE) in the plasma and brains of both mothers and fetuses (77). 4) This dose of cocaine is not anorexigenic in the pregnant rat during the third week of gestation (52).

Gestational days 15 through 21 were chosen as the developmental window for drug administration because in the laboratory rat it is the period in which the fetus becomes increasingly refractory to gross structural malformations. Agents administered on or after GD 15 are more likely to subtly damage the CNS by interfering with the active neuronal proliferation and/or migration that is occurring in the cortex, cerebellum and hippocampus at this time. Drug treatment was stopped after injection on GD 21 because we have found that drug administration on the expected day of delivery (GD 22) is disruptive to parturition and maternal behavior.

On GD 22, cages were checked at hourly intervals for births from 0830–1630 hours. Females were allowed to deliver naturally and nurse their own young. Within 2–4 hours of parturition [designated as postnatal day 0 (PND 0)], dams were temporarily removed, and for each litter pups were counted, examined for gross physical abnormalities, sexed, weighed and crown-rump length measured. Stillborn and moribund pups were noted and litters were culled to ten, balancing for sex, to insure adequate nutritional status. Pup body weights were measured every 3 days from birth to 30 days of age. On PNDs 0, 3, 6 and 9, aggregate body weight by sex was recorded for each litter; on subsequent days, individual body weights were obtained from 2 males and 2 females from each litter. Pups were weaned at PND 30 and housed in wire mesh cages in like-sex groups of 3–4.

Maternal Behavior

Several indices of maternal behavior were monitored after recording birth statistics. Since pups were raised by their biological mothers, residual drug effects could disrupt the mother-pup interaction. Any disruption could subsequently alter immunocompetence in the offspring, which is accomplished in part by transfer of antibodies from mother to young (23,60).

A culled litter was dispersed throughout the cage containing clean bedding and the dam was placed at one end. The latency of the female to begin nest building and initiate and complete pup retrieval was measured to the nearest second with a stop watch. The number of pups remaining out of the nest at the end of 5 minutes (maximum test time) was also recorded. Thirty minutes later, dams were observed for presence in the nest and pup-directed behaviors (nursing and grooming), as well as eating, drinking and self-grooming. The number of unretrieved pups was noted and they were placed in the nest at this time.

Reflex and Physical Development

The appearance of physical features and reflex behaviors, ontogenic landmarks which reflect integration and maturation in the CNS (4, 11, 32), were monitored every third day from PND 0–24. Reflexes and physical features were scored either present or absent, and one or two male and one or two female pups (maximum of three) from each litter were tested repeatedly. Male and female pups were choosen at random from each litter, and both PC and PS groups contained an equal number of pups of each sex.

Physical features were observed on the following days: pinna elevation (PND 0–6); eruption of upper and lower incisors (PND 6–15); and dysjunction of both eyelids (PND 12–18).

Reflex testing involved:

Surface righting. Pup, placed on its back, turns over to rest in normal position with all 4 paws on the ground within 30 seconds (PND 0–9).

Negative geotaxis. Pup, placed on a 20° textured slope with head pointed down the incline, turns and crawls up the slope (PND 3–6).

Cliff avoidance. Pup, placed on edge of table top with forepaws and face over the edge, backs away from the cliff (PND 6–12).

Startle response. Pup extends head and withdraws fore- and hindlimbs into a crouching position at sound of loud sharp noise (snap of mouse trap) (PND 9–15).

Free-fall righting. Pup, dropped dorsal side down from a height of 30 cm, lands upright on all 4 paws (PND 15–24).

Olfactory behavior. Preference for social odors, an acquired rather than reflex behavior (20), was tested from PND 3–12. Testing was performed in an apparatus having a $20 \times 26 \times 10$ cm Plexiglas frame with a wire mesh bottom which allowed olfactory but no gustatory or tactile cues to pass from shavings placed in the two compartments below. One compartment contained 500 ml of natural pine shavings, the other an equal volume of the social test odor, which was produced by 3-day-old shavings from the pup's own cage.

At the beginning of the 2-minute test period, each pup was placed in the center of the apparatus, midway between the two odors. The latency of the pup to place both forepaws on one side of the midline and the time spent over the test odor were recorded with a stop watch to the nearest second. Pups failing to make a choice within 2 minutes were removed and scored 60 seconds (no choice).

Locomotor Activity

Spontaneous motor activity (SMA) was monitored in offspring from both cocaine- and saline-treated mothers at 15 and 30 days of age following IP injections of either cocaine hydrochloride (1 or 10 mg/kg) or d-amphetamine sulfate (Sigma, St. Louis, MO: 1 or 2 mg/kg). Control groups were injected IP with 0.9% sodium chloride, 0.1 ml/10 g body weight.

Following injection, offspring were placed individually in a polyethylene container ($44 \times 25 \times 30$ cm); the container was placed on top of an automated activity meter (Automex II, Columbus Instruments International Corporation, Columbus, OH) which was adjusted to record only lateral excursions. SMA was recorded automatically every 5 minutes for 30 minutes. Each offspring was tested only once.

Serum Drug Determinations

Determinations of serum benzoylecgonine, a major active metabolite of cocaine (50,89), were made in pregnant dams and individual offspring. Maternal samples, 1.0–1.5 ml, were drawn from the tail vein of lightly restrained, unanesthetized females on GD 18, 2 hours after cocaine administration. In rat, after *chronic* administration, plasma levels of cocaine and its metabolites peak between 2–4 hours postinjection (58). In offspring, trunk blood taken at birth was assayed for BE.

Immediately following locomotor activity testing on PND 30, offspring were sacrificed by decapitation and trunk blood was analyzed for BE or amphetamine. After acute cocaine administration, peak plasma levels occur within an hour in rat (58).

All blood samples were collected in nonheparinized tubes and centrifuged; serum was removed and frozen at −20°C.

Serum benzoylecgonine and serum amphetamine were both determined by the enzyme-mediated immunoassay transmission (EMIT) technique. The technique is dependent upon the binding of an antibody to an antigen which is the drug of interest. The changes in absorbency, measured spectrophotometrically at 340 nm which result from the unbound or active enzyme, are determined relative to a 300 ng/ml standard (7, 63, 64).

Herpes Simplex Virus-Type 1 (HSV-1)

Strain BK of herpes simplex virus was isolated on human foreskin (HFS-3) cells at the Howard University College of Medicine from a 21-year-old male with recurrent herpes labialis. It was confirmed as type 1 HSV as a result of its neutralization by specific rabbit anti-HSV-1 serum and by its clinical and biological properties (30,56). Virus pools, with average titers of 10^7 $TCID_{50}$/0.1 ml (tissue culture infectious doses fifty), were prepared in HFS-3 cells and stored in 1 ml aliquots at −73°C until used.

Inactivated HSV-1 was prepared by exposing virus pools to ultraviolet (UV) light at 15 cm for 15 minutes at room temperature. Complete inactivation of viral infectivity by this procedure was confirmed by assaying these pools for infectious virus on HFS-3 cells. Injection of the inactivated virus, which is antigenic but not infectious, served as the control procedure.

Titration of Virus

Virus titrations were performed using Dulbecco's phosphate-buffered saline (PBS) (27) as the diluent. Serial ten-fold dilutions were prepared and assayed for infectious virus on duplicate cultures of HFS-3 cells. These cells were grown in 35-mm plastic petri dishes with 2 ml of RPMI 1640 medium (54) supplemented with 10% fetal calf serum and antibiotics (penicillin G 100 μ/ml, streptomycin 100 μg/ml and nystatin 100 μ/ml). Viral cytopathic effect (CPE) was scored on a scale of 1+ to 4+, with 1+ indicating involvement of 25% of the cells, 2+, 50% involvement, 3+, 75% involvement, and 4+ indicating 100% of the cells showing viral CPE (69). Virus titers were calculated by the quantal method of Reed and Muench (62) and expressed as 50% tissue culture infectious doses ($TCID_{50}$)/0.1 ml.

Postnatal Cocaine Injections and Viral Immunization

At PND 34, 35, and 36 naive offspring from both prenatal cocaine (PC) and prenatal saline (PS) mothers were injected with 10 mg/kg IP of cocaine (PNC), twice daily at 0930 hr and 1630 hr; controls received injections of 0.9% saline (PNS) on a similar schedule. Four hours after the first cocaine or saline injection on PND 34, half of the offspring in each group received one IP injection of HSV-1 (0.25 ml of 10^4 $TCID_{50}$; the remainder were injected with UV-inactivated HSV-1 (INA HSV-1: 0.25 ml, IP). This design resulted in eight groups:

1. PC/PNC/HSV-1	N = 8
2. PC/PNC/INA HSV-1	N = 8
3. PC/PNS/HSV-1	N = 10
4. PC/PNS/INA HSV-1	N = 10
5. PS/PNC/HSV-1	N = 6
6. PS/PNC/INA HSV-1	N = 6
7. PS/PNS/HSV-1	N = 6
8. PS/PNS/INA HSV-1	N = 6

Each group contained an equal number of males and females, and body weights were recorded before each injection of cocaine.

Serum Antibody Titrations

Three weeks after virus injection, PND 55 rats were weighed

TABLE 1

GESTATIONAL AND BIRTH STATISTICS FOR FEMALES TREATED WITH COCAINE OR SALINE ON GD 15–21 (MEAN ± S.E.M.)

Females	GD 15–21 Weight Gain (grams)	Period of Gestation (days)	Litter Size	Serum Cocaine Levels (ng/ml)
Saline (11)	77.09 ± 5.44	22.77 ± 0.18	12.36 ± 1.33	N.D.*
Cocaine (12)	78.75 ± 5.61	22.92 ± 0.08	13.92 ± 1.14	621.42 ± 48.0

Litters	Male/Female Ratio†	Birth Weight (g) Males	Birth Weight (g) Females	Mortality PND 0	Mortality PND 7	Crown Rump Length (cm) Males	Crown Rump Length (cm) Females
Saline (11)	1:0.99	6.86 ± 0.23	6.49 ± 0.24	1	4	4.78 ± 0.11	4.69 ± 0.2
Cocaine (12)	1:1.18	6.53 ± 0.24	6.01 ± 0.32	0	2	4.64 ± 0.07	4.61 ± 0.07

*At birth, cocaine levels were not detectable in pups' sera.

†Male/female ratios were calculated from the total number of offspring in each treatment group.

and sacrificed by decapitation; sera from trunk blood were assayed for antiviral neutralizing antibodies. Whole brain, adrenals, spleen and thymus were removed from each animal, rinsed with ice-cold saline (0.9%) and immediately frozen in liquid nitrogen. Wet weights of adrenals, spleen and thymus were recorded prior to freezing. Sera and tissues were stored at −20° and −73°C, respectively, until assayed.

Assay of sera of neutralizing HSV antibody was performed utilizing the constant virus-variable serum technique (31). Serum samples were serially diluted to 1:128 by two-fold decrements in 0.5 ml of PBS. An equal volume of diluted virus containing 100 $TCID_{50}$/0.1 ml was added to each serum dilution. The mixture was shaken and incubated at 37°C for 1 hour. Duplicate cultures of HFS-3 cells containing 2 ml of complete RPMI 1640 medium were inoculated with 0.2 ml of a serum-virus mixture, incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air and read daily for viral cytopathology over a period of 6 days. The reciprocal of the highest dilution of serum at which viral cytopathic effect (CPE) was completely inhibited was designated the titer of the serum. A serum with a titer of 1:4 was considered positive. Appropriate virus control titrations and serum controls were prepared for each experiment.

Preparation of Tissues for Virus Assays

Brain and spleen were thawed and triturated with sterile sand, collected as a 10% suspension in PBS and clarified by centrifugation at 700 × g for 20 minutes in a refrigerated centrifuge. The supernatant was recentrifuged at 2000 × g for 20 minutes and stored at −73°C until assayed for virus on HFS-3 cells.

Statistical Analyses

Birth statistics (weight gain during drug treatment, length of gestation period, litter size) and maternal behaviors (latency to initiate nest building, pup retrieval) data were analyzed by a completely randomized (CR) one-factor analysis of variance (ANOVA). Two-factor ANOVAs (CRF) were employed to analyze pups' birth weights, crown-rump lengths, the number of male and female pups, and PND 30 body weights. The remaining body weights (PND 3–26) were compared with three-factor Linquist design ANOVAs (SPF), with age as the repeated variable. A similar design was used to analyze olfactory preference, latency and duration data.

The number of offspring exhibiting reflex and physical features was analyzed at each age by a chi-square test of independence. Spontaneous motor activity was analyzed by a three-factor Linquist ANOVA, with time periods as the repeated measure; prenatal treatment and postnatal drug were the other two factors. In order to include sex as a variable, the locomotor activity of PC and PS pups to each postnatal drug treatment was analyzed separately by a three-factor Linquist ANOVA, with time periods as the repeated measure.

Initial two-factor ANOVAs on serum levels of cocaine and amphetamine included gender as a dependent variable. As this variable was not significant, subsequent analyses were one-factor ANOVAs for prenatal treatment. Initial body weights, body weight changes for each time interval following HSV-1 and cocaine injections, and antibody titers were analyzed by a three-way ANOVA CRF. Organ/body weight ratios and percent viral infectivity of cells in culture were arcsin transformed before being subjected to a three-way ANOVA (38). Duncan's or Newman-Keuls' post hoc analyses were used to compare significant main effects; significant interactions were subjected to simple main effect analyses.

RESULTS

Maternal Toxicity and Birth Statistics

Subcutaneous injections of a 20 mg/kg dose of cocaine once daily during the final week of gestation resulted in maternal serum levels of 621.42 ± 48.0 ng/ml of benzoylecgonine. At birth, 24–48 hours after the last maternal injection, serum levels were not detectable in individual offspring. Mild tissue necrosis, without infection, and subsequent hair loss developed at injection sites 72 hours after cocaine administration. Time-sampling of females' behavior revealed that cocaine produced an increase in the duration of activation. PC females exhibited locomotor activity for approximately 60 minutes following cocaine injection; in controls, locomotor activity was seen for 5–10 minutes following injections of saline. Neither stereotypy nor convulsions were seen in PC animals; food and water intake were comparable with controls after 60 minutes. Weight gain during the last third of gestation and

TABLE 2

MATERNAL BEHAVIOR OF FEMALES TREATED WITH COCAINE OR SALINE ON GD 15–21 (MEAN ± S.E.M.)

	Prenatal Saline	Prenatal Cocaine
Initial Test (PND 0)		
Latency* (sec) to begin		
Nest building	177.54 ± 30.35 (3)	112.08 ± 15.67 (0)
Pup retrieval	191.73 ± 32.55 (4)	225.00 ± 29.09 (6)
Time† (sec) to complete retrieval	266.36 ± 20.43	287.33 ± 8.37
Number of mothers not completing retrieval	6/11	9/12
Number of pups out of nest	4.64 ± 1.50	7.33 ± 1.59
30 Minutes later		
In nest	100%	83.3%
Nursing	81.8%	83.3%
Grooming pups	0%	0%
Self-grooming	0%	0%
No. of litters with pups out of nest	2	3

*Includes maximum time (300 sec) of mothers (number in parentheses) that did not begin nest building and/or pup retrieval.

†Includes maximum time (300 sec) of mothers that did not retrieve entire litter.

the length of the gestational period (Table 1) were not significantly different.

Postpartum litter data also appear in Table 1. Litter size, crown-rump length at birth and the male/female offspring ratio were unaffected by cocaine exposure on GD 15–21. Physical abnormalities, determined by gross inspection of skull, limbs and snout, were not observed in offspring at birth and did not develop subsequently; one-week pup mortality was not significantly different between the groups.

Maternal Behavior

When females were tested within 4 hours of recording births, there were no significant differences between the maternal behavior (Table 2) of cocaine- and saline-treated mothers with respect to latencies to initiate nest building, $F(1,21)=3.86$, $p<0.05$, and retrieve pups, $F(1,21)=0.58$, $p<0.05$, or the time to retrieve all pups, $F(1,21)=0.96$, $p<0.05$. Comparison of the number of successful retrievals among the groups with chi-square revealed no significant difference, $\chi^2(1)=0.96$, $p>0.05$. Maternal behavior, 30 minutes later, was similar in both groups of females.

Postnatal Body Weights

Postnatal body weights (Fig. 1), which served as a measure of the general health of the pups, did not differ at birth. Body weights increased significantly in offspring from both treatment groups between birth and 30 days of age; only at PND 9 were PC

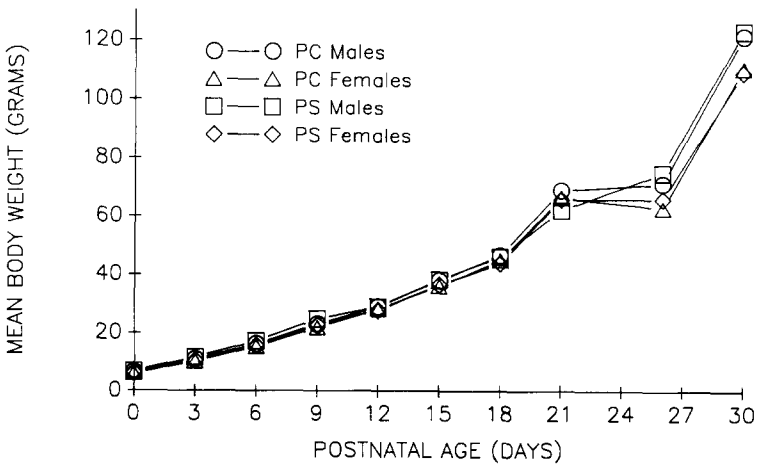

FIG. 1. Preweaning body weights of offspring of females treated on GD 15–21 with either cocaine or saline. Values shown are means of 14 males and 14 females for each point.

TABLE 3

DEVELOPMENT OF REFLEXES AND PHYSICAL FEATURES IN PRENATAL COCAINE (PC) AND PRENATAL SALINE (PS) OFFSPRING

		Postnatal Age (days)							
	(n)	3	6	9	12	15	18	21	24
Physical Development									
Pinna elevation	PS (28)	25	28						
	PC (28)	22	28						
Incisor eruption	PS (28)		0	1	25	28			
	PC (28)		0	1	26	28			
Eye opening	PS (28)				0	27	28		
	PC (28)				0	28	28		
Behavioral Development									
Negative geotaxis	PS (20)	3	17	20					
	PC (28)	11	24	28					
Surface righting	PS (20)	15	20						
	PC (28)	28*	28						
Olfactory behavior	PS (20)	19	20	20	20				
(social odor)	PC (28)	26	28	28	28				
Cliff avoidance	PS (20)		13	19	20				
	PC (28)		25*	27	28				
Startle response	PS (28)			0	13	24	28		
	PC (28)			0	23*	27	28		
Free-fall righting	PS (28)					0	6	26	28
	PC (28)					0	9	27	28

Values listed are the number of pups exhibiting response at each age.
*Significantly different from prenatal saline offspring, $p<0.01$.

offspring significantly smaller than PS controls, $F(1,20)=8.126$, $p<0.01$. The sexual dimorphism in body weight (males larger than females), first observed at PND 3, continued during the preweaning period in both prenatal treatment groups.

Physical Features and Reflex Development

The number of PC and PS offspring exhibiting each of the indices of physical development did not differ at any age tested (Table 3). However, the maturation of surface righting, cliff avoidance behavior and the acoustic startle response was accelerated by prenatal cocaine exposure; significantly more PC offspring exhibited these responses at PND 3, $\chi^2(1)=7.81$, $p<0.01$, PND 6, $\chi^2(1)=9.09$, $p<0.01$, and PND 12, $\chi^2(1)=7.74$, $p<0.01$, respectively.

Each prenatal treatment group contained an equal number of male and female pups. Overt maturational differences between the sexes could have influenced the developmental changes noted between PC and PS offspring for surface righting and cliff avoidance in that the PC group had 4 additional animals from each sex. However, inspection of these data by sex did not support this hypothesis. Of the 5 prenatal saline pups not exhibiting surface righting on PND 3, 2 were female and 3 were male. The sex of the pups not showing cliff avoidance on PND 6 was as follows: prenatal saline—4 males and 3 females; prenatal cocaine—1 male and 2 females. In testing for startle response, PS and PC groups each had 14 males and 14 females. However, more males from both prenatal treatment conditions exhibited an absence of this reflex on PND 12 than their female counterparts. Of the 15 PS pups not showing the response, 9 were male and 6 were female; in the PC group, 4 were male and 1 was female. At PND 12, there was a sexually dimorphic difference in body weights in offspring from both PC and PS mothers; however, the fact that males were heavier than females would not appear to account for their delayed development of the startle response.

The acquisition of a preference for a complex social odor was not altered in offspring prenatally exposed to cocaine; pups in both groups exhibited the expected developmental pattern (20,41). Latencies to make an initial choice significantly decreased between PND 6 and PND 12 [PC: 11.86 ± 2.21 vs. 9.18 ± 2.17 sec, respectively; PS: 18.10 ± 3.04 vs. 7.75 ± 1.72 sec, respectively; $F(2,88)=8.32$, $p<0.01$]. The amount of time spent over home-cage shavings increased between PND 6 and PND 9 [PC: 87.05 ± 4.93 vs. 93.40 ± 5.66 sec, respectively; PS: 80.65 ± 8.34 vs. 96.20 ± 6.04 sec, respectively; $F(2,88)=3.143$, $p<0.05$].

Spontaneous Motor Activity (SMA) and Serum Drug Levels

In utero cocaine exposure altered the offsprings' postnatal response to stimulant drugs at 15 but not 30 days of age. At PND 15 (Fig. 2, top 3 panels), d-amphetamine (1 mg/kg) produced a transient increase in SMA during the 5-minute period immediately

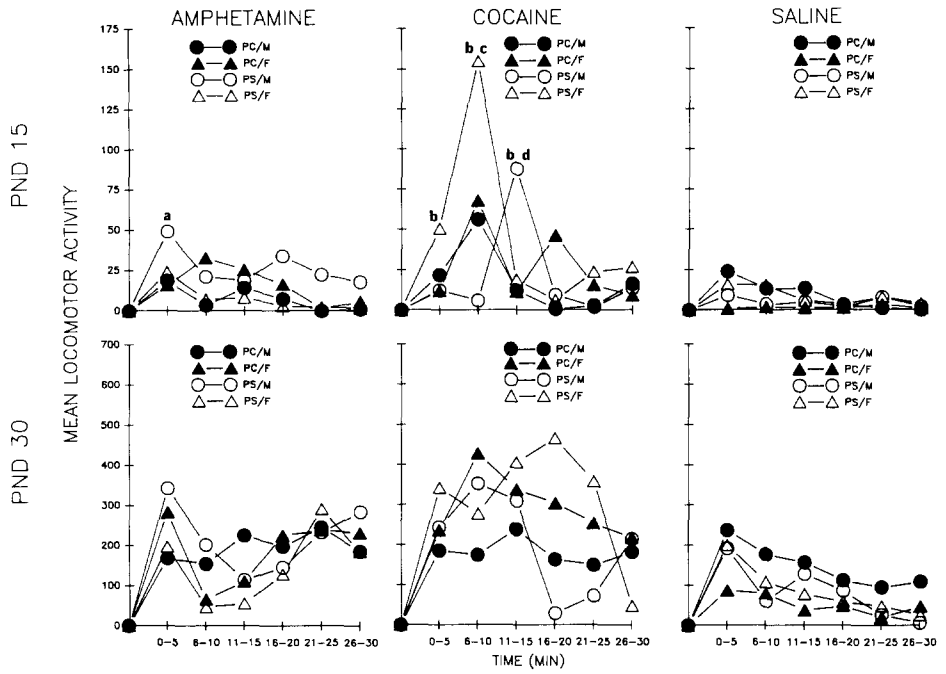

FIG. 2. Stimulant-induced locomotor activity was measure on PND 15 and PND 30 for 30 minutes following IP injection of either cocaine (PND 15 and 30: 10 mg/kg) d-amphetamine (PND 15: 1 mg/kg; PND 30: 2 mg/kg) or saline in PC and PS offspring. Values shown are means of 6 animals for each point. Each animal was tested only once. [a]PS/M + PS/F significantly different from PC/M + PC/F, $p<0.01$. [b]PS/M + PS/F significantly different from PC/M + PC/F, $p<0.01$. [c]PS/F significantly different from PC/F, $p<0.01$. [d]PS/M significantly different from PC/M, $p<0.05$.

after injection in prenatal saline pups ($mean_{males\ +\ females} = 37.56 \pm 8.46$); offspring exposed prenatally to cocaine did not exhibit this increase ($mean_{males\ +\ females} = 18.33 \pm 6.17$) [time × prenatal treatment interaction: $F(1,20) = 10.46$, $p<0.01$]. During the remaining 25 minutes, SMA was similar in all offspring. The changes in SMA seen following cocaine (10 mg/kg) represented a three-way interaction [prenatal treatment × sex × time interval: $F(5,100) = 3.357$, $p<0.05$]. Cocaine increased SMA in prenatal saline offspring during the first 15 minutes following injection (PS: mean = 55.93 ± 13.34; PC: mean = 27.30 ± 6.34). This increase was significantly larger in PS females (mean = 75.62 ± 21.16) than their male counterparts (mean = 36.24 ± 16.27) during this period. Prenatal exposure to cocaine attenuated this increase in locomotor activity; the attenuation was significantly greater in PC females (mean = 31.22 ± 8.94) than in PC males (mean = 23.39 ± 8.67). The locomotor response of the PC and PS offspring to saline injections was not significantly different.

At PND 30, prenatal cocaine exposure did not influence drug-induced motor responses (Fig. 2, bottom 3 panels). The 2 mg/kg dose of d-amphetamine increased locomotor activity in both PC and PS offspring, and produced equivalent serum amphetamine levels in both groups (PS: 135.0 ± 58.3 ng/ml; PC: 170.0 ± 50.7 ng/ml). Cocaine (10 mg/kg) also enhanced SMA in both prenatal treatment groups. However, in contrast to PND 15, activity was increased throughout the 30-minute test period. Despite similar behavioral responses in PS and PC pups, serum levels of BE were significantly higher in offspring prenatally exposed to cocaine [PC: 513.33 ± 22.01 ng/ml; PS: 360.00 ± 73.59 ng/ml; $t(10) = 1.99$, $p<0.05$]. The 1 mg/kg dose of cocaine did not produce a significant change in activity in any group when compared to saline-injected controls (data not shown).

Cocaine Injections and HSV-1 Immunization

Body weights. Body weights (Fig. 3) of PC and PS offspring were not significantly different prior to the start of cocaine/HSV-1 injections at 34 days of age; in both prenatal treatment groups, males were heavier than females, $F(1,56) = 15.12$, $p<0.001$.

Animals in all 8 groups lost weight after the first series of injections (Fig. 3, T_1). Overall, PC offspring lost significantly less weight than PS offspring, regardless of the postnatal treatments, $F(1,52) = 4.864$, $p<0.05$. However, in prenatal cocaine groups, offspring treated postnatally with cocaine and inactivated HSV-1 had the largest weight loss; in the PS groups, offspring that received cocaine and HSV-1 postnatally lost the most weight, $F(1,52) = 4.327$, $p<0.05$.

At T_2, 16 hr after the second cocaine or saline injection, body weights rebounded in all groups. Postnatal cocaine treatment was not a significant variable. However, the significant prenatal tretment by HSV-1 interaction indicated that PS offspring given HSV-1 had a larger increase in body weight than PC offspring given the virus; weight increases in PC and PS offspring exposed to inactivated virus were the same, $F(1,52) = 4.313$, $p<0.05$.

Significant changes in body weight were not observed at T_3 or T_4. Between the 5th and 6th injections (Fig. 3, T_5), animals treated postnatally with cocaine were bled from the tail vein 2 hours after cocaine injection. Serum cocaine levels determined at this time were not significantly different among prenatal cocaine and prenatal saline groups. Significant weight loss was restricted to groups that were bled (i.e., 1, 2, 5 and 6), $F(1,52) = 84.90$,

TABLE 4

ORGAN/BODY WEIGHT RATIOS OF PRENATAL COCAINE AND PRENATAL SALINE OFFSPRING TREATED POSTNATALLY WITH COCAINE AND HERPES SIMPLEX VIRUS-TYPE 1 (MEAN ± S.E.M.)

	Body Weight (g)	Spleen*	Thymus*	Adrenals*
Prenatal Cocaine	245.78 ± 9.63	0.2327 ± 0.0063†	0.2301 ± 0.0071†	0.0196 ± 0.0009
Postnatal cocaine				
HSV-1 (8)	234.75 ± 21.72	0.2394 ± 0.0159	0.2164 ± 0.0183	0.0183 ± 0.0018
INA/HSV-1 (8)	240.25 ± 20.38	0.2444 ± 0.0107	0.2534 ± 0.0121	0.0199 ± 0.0018
Postnatal saline				
HSV-1 (10)	251.30 ± 19.29	0.2162 ± 0.0106	0.2120 ± 0.0076	0.0225 ± 0.0020
INA/HSV-1 (10)	253.50 ± 18.61	0.2349 ± 0.0156	0.2328 ± 0.0139	0.0175 ± 0.0019
Prenatal Saline	241.83 ± 11.42	0.2099 ± 0.0063	0.2525 ± 0.0067	0.0203 ± 0.0010
Postnatal cocaine				
HSV-1 (6)	238.83 ± 28.00	0.2125 ± 0.0107	0.2436 ± 0.0110	0.0221 ± 0.0023
INA/HSV-1 (6)	251.83 ± 26.53	0.2074 ± 0.0088	0.2583 ± 0.0206	0.0209 ± 0.0021
Postnatal saline				
HSV-1 (6)	241.67 ± 15.29	0.1931 ± 0.0014	0.2688 ± 0.0085	0.0198 ± 0.0157
INA/HSV-1 (6)	233.60 ± 25.79	0.2287 ± 0.0161	0.2439 ± 0.0123	0.0180 ± 0.0022

*Organ weight/body weight × 100.
†Significantly different from prenatal saline values, $p<0.01$ (spleen), $p<0.05$ (thymus).

$p<0.001$, and was similar in all 4 groups.

Organ/Body Weight Ratios

Only prenatal cocaine exposure significantly altered organ/body weight ratios (Table 4). Splenomegaly was observed in PC offspring irrespective of postnatal exposure to cocaine or HSV-1, $F(1,51)=7.201$, $p<0.01$. In contrast, thymus/body weight ratios were significantly smaller in PC rats when compared to PS

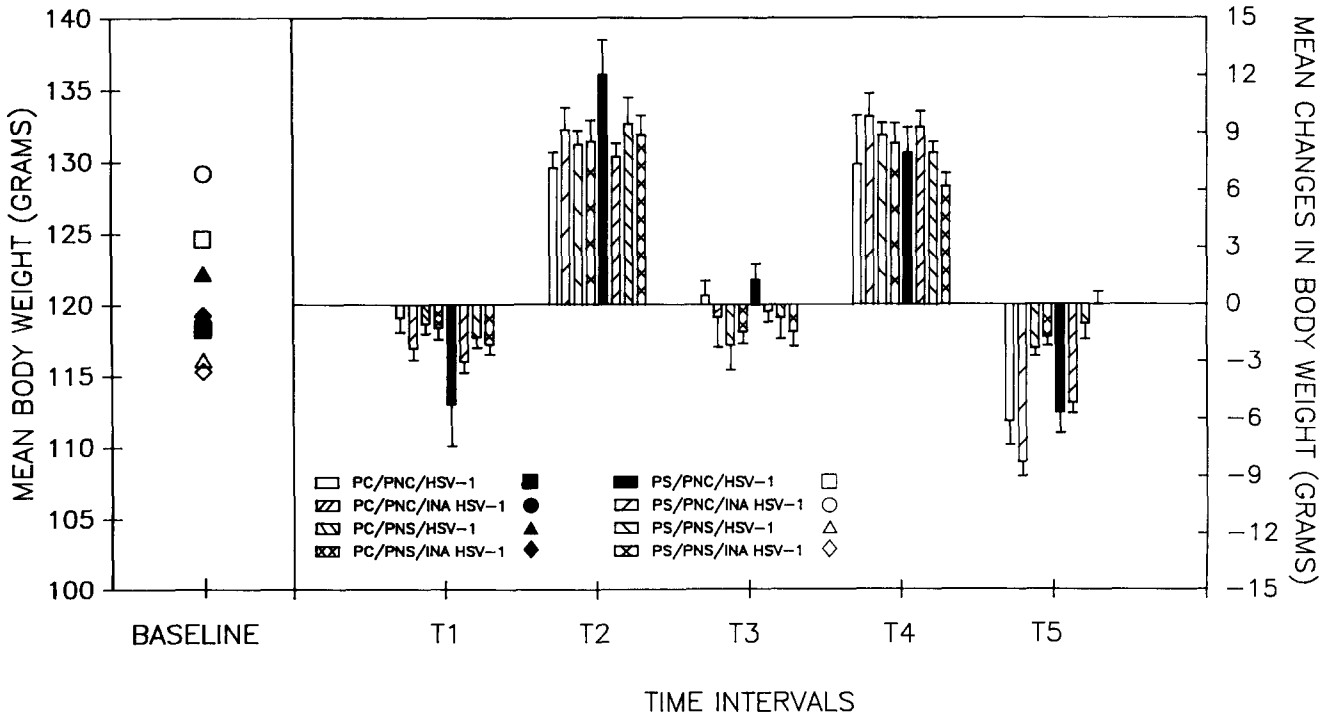

FIG. 3. Body weight and body weight changes during postnatal cocaine and HSV-1 injections. Values shown are mean ± S.E.M. Baseline: Mean body weights at PND 34, prior to any injection. T1: Time between 1st and 2nd cocaine (10 mg/kg, IP) injection (8 hr). Infectious HSV-1 or inactivated HSV-1 were injected 4 hr after 1st cocaine injection. T2: Time between 2nd and 3rd cocaine injection (16 hr). T3: Time between 3rd and 4th cocaine injection (8 hr). T4: Time between 4th and 5th cocaine injection (16 hr). T5: Time between 5th and 6th cocaine injection (8 hr). Sera were collected 2 hr after the 5th cocaine injection.

TABLE 5

PERCENT CELLS IN CULTURE INFECTED WITH HSV-1 AT 1:4 SERUM DILUTION (MEAN ± S.E.M.)

	Hours After Inoculation				
	48	72	96	120	Hours to 100% Viral Infectivity
Prental Cocaine					
1) PNC/HSV-1	28.12 ± 7.37‡	51.56 ± 7.99‡	92.187 ± 4.05†	100.00	105.00 ± 4.39†
2) PNC/INA HSV-1	51.56 ± 2.83*	75.00 ± 4.09*§	96.87 ± 2.04§	100.00	102.00 ± 3.93
3) PNS/HSV-1	48.21 ± 5.74	78.57 ± 7.07†	100.00†	100.00	89.14 ± 4.43†
4) PNS/INA HSV-1	43.06 ± 3.67*	70.83 ± 5.10*	95.83 ± 2.94	100.00	98.64 ± 4.81
Prenatal Saline					
5) PNC/HSV-1	33.33 ± 4.17‡	62.50 ± 3.23‡	95.83 ± 4.17	100.00	100.00 ± 3.98
6) PNC/INA HSV-1	27.50 ± 7.29	50.00 ± 3.95§	92.50 ± 5.00§	100.00	105.60 ± 5.88
7) PNS/HSV-1	34.38 ± 4.63	46.87 ± 8.27	90.62 ± 4.63	100.00	108.00 ± 5.37
8) PNS/INA HSV-1	35.00 ± 6.12	80.00 ± 6.37	100.00	100.00	91.20 ± 4.80

PNC = Postnatal cocaine.
PNS = Postnatal saline.
*Groups 2 + 4 significantly different from groups 6 + 8, $p<0.05$.
†Groups 1 + 3 significantly different from groups 5 + 7, $p<0.05$.
‡Groups 1 + 5 significantly different from groups 3 + 7, $p<0.05$.
§Groups 2 + 6 significantly different from groups 4 + 8, $p<0.05$.

controls, $F(1,51)=4.804$, $p<0.05$. Neither body weight at sacrifice nor adrenal/body weight ratios were altered by prenatal or postnatal treatments.

Antibody Titers

Sera were analyzed for anti-HSV-1 neutralizing antibody 3 weeks after a single IP injection of HSV-1. Antibody was not detected at any serum dilution between 1:4 and 1:128, as complete inhibition of viral infectivity was not observed for sera from any treatment groups after a 5-day incubation period.

To determine whether sera from animals exposed prenatally and/or postnatally to cocaine would inhibit HSV-1 expression in culture, plates were read for viral cytopathology at 48, 72, 96 and 120 hours after inoculation with a serum-virus mixture. Statistical analyses of the percent of infected cells in the cultures (Table 5) revealed significant three-way interactions (prenatal treatment × postnatal treatment × virus exposure) at each time point [48 hours: $F(1,45)=5.25$, $p<0.05$; 72 hours: $F(1,45)=18.55$, $p<0.001$; 96 hours: $F(1,45)=5.14$, $p<0.05$]. At 48, 72 and 96 hours postinoculation, sera from prenatal cocaine animals showed less inhibition of cytopathic effect than sera from prenatal saline animals, as evidenced by the increased percent of infected cells in prenatal cocaine cultures. However, at 48 and 72 hours, this increased viral expression was seen only in cultures inoculated with serum from PC animals injected with inactivated HSV-I; at 72 and 96 hours, this increase also appeared in cultures containing sera from PC animals injected with infectious virus.

In contrast, sera from animals exposed to cocaine postnatally, prior to and during exposure to HSV-1, responded to the antigenic stimulation of the virus. At 48, 72 and 96 hours, there was a decrease in the percent of infected cells when compared with the percent in cultures inoculated with a serum-virus mixture from animals injected postnatally with saline. This decrease in viral infectivity was again a function of virus activity; at 48 and 72 hours, serum from animals exposed to infectious HSV-1 exhibited this inhibition. At 72 and 96 hours, inhibition occurred in cultures inoculated with virus serum mixture from animals exposed to inactivated HSV-1. In addition, the time at which 100% of the cells in culture showed viral cytopathology was accelerated in cultures inoculated with serum-virus mixture from prenatal cocaine animals exposed to infectious but not inactivated virus, $F(1,45)=6.654$, $p<0.05$.

Clinical symptoms of HSV-1 infection, such as muscle weakness, tremor, or paralysis (20), were not observed and HSV-1 was not isolated from either brain or spleen of any of the animals injected with infectious virus.

DISCUSSION

Conflicting data in the clinical literature on the effects of prenatal cocaine exposure may result from variations in prenatal care and in life styles due to socioeconomic variables, as well as differences in duration, frequency and combinations of multiple drug use (8). Control of these confounding variables, which is necessary to determine the consequence of in utero cocaine exposure per se on the mother and infant, can be readily accomplished using an appropriate animal model. The present data suggest that subcutaneous administration of cocaine to the pregnant rat is a reasonable animal model for investigating human drug abuse during pregnancy. Local vasoconstriction at the site of subcutaneous injection did not prevent cocaine from entering the maternal circulation; extrapolation from recently published data (77) indicates that serum benzoylecgonine levels observed in this study would result from serum cocaine levels of 720 ng/ml, values comparable to those reported in humans after recreational use (19,55).

Dose-related increases in both fetal and maternal plasma levels of cocaine and benzoylecogonine have been reported after subcutaneous administration of 10, 20 or 40 mg/kg of cocaine to pregnant rats; moreover, BE levels are higher in fetal than in maternal brain 2 hours following cocaine injection (77). Since BE has potent stimulatory activity when administered centrally (50,89), it may be the compound responsible for the long-term consequences of prenatal cocaine exposure. In the present study, BE was not detected in the sera of pups at birth, which may reflect the fact that cocaine administration was terminated 24–48 hours prior to parturition. However, following prenatal cocaine exposure in

human neonates, drug metabolites are present in the urine during the first postnatal week (58).

The subcutaneous route of administering cocaine did not disrupt the gestational process, retard the growth of the offspring, or interfere with maternal behavior. Moreover, the trend ($p<0.07$) was for PC dams to begin nest building sooner than their control counterparts and for a larger number to complete the process within the 5-minute test period. The presence of normal maternal behavior underscores the lack of maternal toxicity observed with the present model of prenatal cocaine administration and supports the hypothesis that the observed changes in immune function in the offspring (see below) were mediated by prenatal drug exposure rather than a disruption in postnatal antibody transfer (23,60). In contrast to reports for pregnant rats given higher doses of cocaine (17,29), decreases in maternal and newborn weights were not observed nor were teratogenic insults. At birth, cocaine-exposed pups did not exhibit any of the withdrawal symptoms previously reported in the offspring of pregnant rats treated with morphine sulfate throughout gestation, i.e., hyperactivity, hypersensitivity to tactile stimulation and lack of suckling behavior (72,74).

Offsprings' body weights at birth and throughout the preweaning period were generally not affected by prenatal cocaine exposure, nor was physical maturation, a finding consistent with several recent reports from other laboratories (71,78). However, prenatal exposure to cocaine during the last third of pregnancy accelerated the development of several reflex behaviors and attenuated the offsprings' locomotor response to CNS stimulant drugs. Since these changes occurred during the second postnatal week, they are probably the result of changes induced by prenatal cocaine exposure rather than drug withdrawal phenomena.

The accelerated appearance of the startle reflex observed in offspring of cocaine dams in the present study is in general agreement with clinical reports (14,18) that cocaine infants are more responsive to auditory stimuli and show a greater startle response than drug-free infants. Early development of the righting reflex and cliff avoidance were also seen in cocaine-exposed rat pups. As similar effects on behavioral development have also been reported in offspring of gestationally stressed dams (2,73), the present results may reflect cocaine's ability to activate adrenergic systems and produce physiological responses that resemble those evoked by stress (34). In contrast, exposure of the pregnant rat to a 40 mg/kg dose of cocaine from gestation days 8–20 (78), or 10 mg/kg of the drug on GD 4 through GD 18 (71), did not alter reflex maturation in the offspring. These differences highlight the necessity of parametric manipulation of dose, length of exposure, as well as developmental window, in order to definitively determine the neurobehavioral effects of in utero cocaine.

The development of dynamic postural reflex adjustments in the rat involve the regulation of three peripheral systems: the vestibular, extraceptive (tactile) and proprioceptive (4). Vestibular reactions (e.g., surface righting) depend largely on brainstem structures, which are the first to mature (36), whereas placing reactions (e.g., cliff avoidance) depend upon cerebral maturation (3). Noradrenergic fibers extensively innervate the brainstem and neocortex; dopaminergic fibers are found in the latter as well. If behavioral measures can serve to index brain development, the present results suggest that prenatal exposure to cocaine may accelerate anatomical and/or neurochemical maturation in the CNS.

Evidence presented in several recent reviews (25, 37, 42, 43, 68, 86) generally supports the hypothesis that behavioral alterations resulting from prenatal exposure to neuroactive drugs have underlying biochemical causes which can be traced to disruptions in the maturation and/or function of the CNS. Alterations have been reported in parameters that range from binding sites for specific drugs, brain amino acids and amino acid neurotransmitter precursors, monoamine levels and metabolism, receptor kinetics for catecholamine and serotonin binding sites to cellular maturation and metabolism and the anatomical development and distribution of cell types, synapses and dendritic spines. In general, imbalances in neurotransmitters during ontogeny, whether increases or decreases, are thought to be deleterious to the anatomical, neurochemical and functional maturation of the brain, because of their trophic function during development (10, 39, 48, 49), although results to the contrary have been reported (37).

Several studies suggest that these alterations represent temporal shifts in developmental processes rather than aberrations in specific systems. Chronic prenatal exposure to nicotine (69), methadone (68) or alcohol (85) delays neuronal cellular maturation and retards synaptic development. In contrast, the presence of diazepam (66) or the atypical antidepressant, nomifensine (24), during prenatal development can stimulate the development of their respective binding sites.

Rat pups acquire a preference for home-cage odor during exposure to their dam and siblings in the nest; this preference is first demonstrable at PND 3–4 and is a learned response (20). In the present study, attraction to this social odor was unaffected by prenatal cocaine exposure. Spear *et al.* (78) have recently reported that prenatal cocaine-exposed offspring showed marked impairment on a simple conditioning task which required learning the association between an odor and milk at PND 7. However, the lemon scent used as the positive conditioned stimulus in their experiment is a botanical odor which, upon initial exposure, elicits strong aversive responses in rat pups as young 3 days of age (20). Therefore, prenatal cocaine may have altered the ability of the pups to adaptively associate naturally aversive botanical odors with a biologically significant unconditioned stimulus (i.e., milk). The ethological relevance of the social odor used in the present study may protect responses involving it from disruption by prenatal manipulations.

Postnatal challenge with monoaminergic agonists was conducted to determine if prenatal cocaine exposure would alter the sensitivity of the offspring with respect to drug-induced response. The elimination of the locomotor increase to d-amphetamine in PC offspring at 15 but not 30 days of age suggests a transient tolerance or desensitization to this agonist. Transient alterations in drug-mediated responses to amphetamine have been reported for several other behaviors following gestational cocaine exposure (52,79). However, behavioral and biochemical data following cocaine challenge to PC offspring at PND 15 and 30 suggest a desensitization which appears to be more long term. No evidence of sensitization to cocaine challenge following prenatal exposure was seen at either PND 15 or 30. In addition, the smaller decreases in body weight in PC offspring after the first postnatal exposure to cocaine at PND 34 were also suggestive of a tolerance to the drug's anorexic effects.

This diminished sensitivity to cocaine and d-amphetamine could result from altered development of receptor mechanisms by in utero cocaine. At the molecular level, cocaine inhibits presynaptic reuptake of NE and DA, increasing circulating catecholamine levels (33). Agonist-induced down-regulation of adrenergic receptors, accompanied by desensitization of receptor-mediated responses, has been characterized in brain and peripheral tissue (84). Prenatal exposure to psychoactive substances such as opiates, ethanol and amphetamine, has resulted in a significant decrease in alpha$_1$ and beta-adrenergic receptor binding sites in human placenta (61). More recently, prenatal cocaine use has been associated with the down-regulation of beta-adrenergic, mu-opiate and delta-opiate receptors in human placenta (87).

Because of the transplacental transfer of cocaine, elevations in fetal catecholamines may cause a similar down-regulation of developing adrenergic receptor system in the fetal CNS (61,87),

and disruption of receptor-mediated signals by cocaine could alter brain development (61). Animal experiments, which report long-term elevations in brain catecholamines (47) and serotonin (83) following in utero exposure to amphetamines, further support the hypothesis that receptor down-regulation may be responsible for the altered drug sensitivity following prenatal cocaine exposure.

The second aim of these experiments was to evaluate the immunocompetence of offspring prenatally exposed to cocaine following reexposure to the drug as young adults. Previous work in other laboratories indicates that exposure of adult mice to cocaine suppressed both the splenic plaque-forming cell (PFC) assay to sheep red blood cells (humoral immunity) and the delayed type hypersensitivity response to dinitrofluorobenzene (cellular immunity) (88). In contrast, initial exposure to high doses of cocaine enhanced antibody production to a T-dependent antigen and the PFC response in adult mice (34), and reversed the heroin-induced depression of T-cells to form E-rosettes (cell-mediated immunity) (26). Susceptibility to infection by *Streptococcus pneumoniae* and resistance to solid tumors were not altered by cocaine injection (34). Since cocaine's effects on immune function are dependent upon dose, sex and duration of treatment, differences in these parameters may account for these divergent results.

In the present study, prenatal cocaine decreased thymus/body weight ratios but increased spleen-body weight ratios. Although these ratios are not functional measures of immunity, they may reflect a long-term alteration in components of the immune system, such that cellular function may be diminished while humoral responses may be enhanced by in utero cocaine exposure.

However, splenomegaly in PC offspring was not indicative of enhanced humoral immunity. Neutralizing anti-HSV-I antibodies were not detected in sera from any treatment group, despite previous reports that administration of cocaine prior to and during the inductive phase of the immune response enhances antibody production (34). Moreover, previous results from our laboratory (75) indicate that in rat, a single IP injection of infectious HSV-1, in a dose similar to that employed in the present study, produced anti-HSV-1 antibody titers as great as 1:64, and even higher titers (i.e., 1:128) in prenatally stressed offspring. The efficacy of a rat model is made more apparent by the fact that titers of this magnitude are produced in rabbits only after multiple injections of infectious HSV-1 (5).

Although complete neutralization of HSV-1 was not observed, both prenatal and postnatal cocaine exposure altered viral infectivity. Sera from prenatal cocaine offspring exhibited an increased rate in the appearance of CPE in comparison with sera from prenatal controls. In contrast, sera from animals given cocaine postnatally showed a reduction in the rate at which viral infectivity was expressed in culture, when compared with sera from postnatal saline animals. The time course of these effects with respect to virus activity was as expected, given that inactivated HSV-1 is cleared more rapidly in vivo than infectious virus (6). Therefore, alterations in the immune system induced by prenatal and/or postnatal cocaine may be subtle and difficult to detect with traditional immunological measures.

In summary, prenatal exposure to cocaine during the last third of gestation in rat 1) can enhance development of several indices of postnatal neurobehavioral ontogeny in the absence of maternal toxicity or gross teratogenicity in the rat; 2) may produce a down-regulation of dopaminergic and/or adrenergic receptors resulting in a pharmacodynamic desensitization in offspring; and 3) causes subtle alterations in humoral immunity.

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