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Interactive Effects of Prenatal Cocaine and Nicotine Exposure on Maternal Toxicity, Postnatal Development, and Behavior in the Rat

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Abstract

Two experiments were performed to investigate the interactive effects of prenatal coadministration of cocaine hydrochloride (C) and nicotine tartrate (N). Experiment I was designed to determine doses of C and N that could be coadministered without altering maternal gestational parameters and/or fetal viability. Exposure of Sprague-Dawley rats to combined high-dose C (20 mg/kg) and high-dose N (5.0 mg/kg) on gestation days 8–21 was not more toxic to dam or fetus than that of exposure to C alone. Experiment II investigated pregnancy outcome, postnatal development, and behavior of the offspring following drug exposure to either high-dose cocaine (20 mg/kg: CS), high-dose nicotine (5.0 mg/kg: NS), or both (NC) on gestation days 8–21. N was administered by osmotic minipump and C by sc injection. Saline-injected dams, fitted with saline-filled pumps (SS), and untreated dams, pair-fed (PF) to NC females, served as controls. Alterations in maternal variables were limited to a 10–15% decrease in food consumption in NC and CS groups. Pregnancy outcome and birth statistics were unaffected by prenatal treatment, as was offspring body weight during the first four postnatal weeks. However, the development of surface righting was delayed in CS pups, and only CS offspring were underresponsive to the stimulatory effects of dopamine agonists on activity and stereotypy. Behavioral responses to N challenge were similar in all groups. In addition, only CS offspring showed altered behavioral responses in a spontaneous alternation task. Treatment effects on dopamine D_1 and D_2 binding in the caudate nucleus were not observed. The combination of N and C did not exacerbate any of the behavioral changes seen in CS offspring. These results support the hypothesis that C is a behavioral teratogen in rodents, and suggest that in the present model, nicotine can mitigate some of the consequences of *in utero* exposure to cocaine.

Index Entries: Polydrug abuse; animal model; prenatal cocaine; prenatal nicotine; cocaine pharmacokinetics; maternal/fetal toxicity; offspring behavior; dopamine receptors.

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Introduction

Polydrug use among drug abusers is the norm rather than the exception. This is also true of pregnant women who use cocaine. Multiple drug use is one of the major risk factors that complicate pregnancy and pregnancy outcome in this population (Scanlon, 1991; Zuckerman and Bresnahan, 1991). Some of the drugs used with cocaine include nicotine (cigarets), alcohol, opiates, marijuana, amphetamines, and tranquilizers (Feng, 1993). Of these, nicotine is the drug most commonly combined with cocaine; it is estimated that as many as 85% of pregnant cocaine-using women also smoke cigarets (Cherukuri et al., 1988; Church et al., 1991; Zuckerman and Bresnahan, 1991; Graham et al., 1992).

Although it is difficult to differentiate individual effects because of the common use of multiple substances and other risk factors, clinical reports attribute serious morbidity to maternal cocaine use. Negative consequences include fetal growth retardation, decreased birth weight and head circumference, abnormal neurological and neurobehavioral function and maternal obstetrical complications involving placental abruption and prematurity (*see* Zuckerman and Bresnahan, 1991; Kain et al., 1992; Ellis et al., 1993 for recent reviews). At present, cocaine is also thought to be a potential physical teratogen. Rare but serious congenital abnormalities, such as exencephaly and interparietal encephalocele, hydronephrosis, urogenital defects, and distal limb deformities, have been reported (Chavez et al., 1989; Hoyme et al., 1990). The evidence that cocaine is a behavioral teratogen is more convincing; jitteriness, decreased attentiveness, irritable behavior, and subtle CNS problems are quite common among cocaine-exposed neonates (Zuckerman and Bresnahan, 1991). Moreover, two neurobehavioral syndromes, excitable and depressed, have been described in cocaine-exposed infants (Lester et al., 1991). However, many of these abnormalities are resolved by the end of the first postnatal year (Chasnoff et al., 1992).

Animal studies in which cocaine is the sole agent partially support the clinical reports (*see* Dow-Edwards, 1991 for review). As in human infants, effects are subtle, and appear to be restricted primarily to the CNS and behavior. Although a consensus about the nature of the behavioral abnormalities produced by prenatal cocaine exposure in animals is slow to emerge, certain findings are consistent across laboratories. Several laboratories have reported alterations in motor behaviors; both baseline (Hutchings et al., 1989; Smith et al., 1989; Spear et al., 1989b; Church and Overbeck, 1990b; Henderson and McMillen, 1990; Sobrian et al., 1990; Riley and Foss, 1991) and drug-induced locomotor activity (Sobrian et al., 1990; Foss and Riley, 1991b) as well as righting reflexes (Henderson and McMillen, 1990; Sobrian et al., 1990) are sensitive to *in utero* cocaine exposure. With respect to sensory systems, alterations in acoustically mediated behaviors and the abnormal processing of auditory information have been reported (Church and Overbeck, 1990a; Sobrian et al., 1990). Acquisition and/or retention deficits in some, but not all conditioning paradigms are also beginning to emerge as consistent findings in rodents (Smith et al, 1989; Spear et al, 1989c; Church and Overbeck, 1990b). It should be noted that although there appears to be nascent agreement with respect to affected systems, the direction of the change can differ with the postnatal age of the offspring, the route and dose of maternal cocaine administration, and the behavioral variable measured.

Maternal cigaret smoking during pregnancy has been linked to intrauterine growth retardation and subtle behavioral abnormalities in offspring. These include an increased incidence of attention deficit disorder and a variety of cognitive and perceptual problems (Butler and Goldstein, 1973; Naeye, 1978; Eriksson et al., 1979; Meyer and Carr, 1987). These effects have been attributed in part to the fact that smoking induces placental hypoxia through carbon monoxide exposure (Longo, 1977). This hypoxia can depress energy-dependent processes, resulting ultimately in growth stunting and

possibly neurological abnormalities (Martin and Becker, 1971; Sastry, 1991).

Until recently, clear identification of nicotine as a direct contributor to the adverse effects of smoking during pregnancy was obscured by the confounding factors of hypoxia and ischemia that are associated with smoking. However, chronic administration of nicotine *per se* to pregnant rats has been shown to reduce postnatal weight gain, increase brain weight, and increase seizure susceptibility in offspring (Fung, 1989; Britos and Orsingher, 1991). Moreover, offspring exposed to nicotine during gestation show accelerated neuromuscular development (Rose and Strand, 1991), alterations in motor behavior (Fung and Lau, 1989), cognitive deficits (Sorenson et al., 1991; Levin et al., 1993), and neurochemical alterations in catecholaminergic and cholinergic systems, as well as changes in markers of general neuronal development (Levin et al., 1993). Feminization of male rats and decreases in plasma testosterone levels have also been reported following prenatal nicotine exposure (Segarra and Strand, 1989).

Recent data from both clinical and animal studies indicate that multiple drug use involving cocaine during pregnancy can compound the adverse effects seen with use of either drug alone. The concomitant use of cocaine and heroin/methadone during pregnancy increased the probability of fetal loss resulting from spontaneous abortion and fetal death. Moreover, infants born to women with mixed dependencies involving cocaine had decreased birth weights, head circumference, length, and Apgar scores when compared to noncocaine-using drug-dependent or nondependent women (Ryan et al., 1987). The use of heroin and cocaine during pregnancy also has a synergistic effect on the withdrawal behavior of the infant (Fulroth et al., 1989).

The interactive effects of prenatal alcohol and cocaine exposure have recently been studied in animals. In rats, this drug combination increased the risks to the offspring without enhancing maternal toxicity. Exposure to both cocaine and alcohol was found to have greater effects regarding decreased birth weights, increased postnatal mortality, and delayed physical maturation in the offspring than use of either drug alone; differential effects on spontaneous motor activity were also reported (Church et al., 1991).

In contrast to alcohol and opiates, we are unaware of any studies of the interactive effects of maternal cocaine and nicotine on the conceptus. Given that each is independently a behavioral teratogen and that dual exposure is the rule rather than exception in humans, such studies are clearly a necessity. We report here a series of rodent experiments that attemped a first look at this interaction. To avoid the possible contribution of hypoxia/ischemia that is a consequence of cigaret smoking, we opted to study the effects of prenatal nicotine exposure in combination with cocaine.

In these experiments, we attempted to model known human drug-exposure patterns. Cocaine use is typically episodic. We accordingly utilized sc injections of cocaine in a single daily dose. In contrast, smokers attempt to "titrate" fixed levels of plasma nicotine throughout the day. We thus chose osmotic minipumps to maintain fixed levels of plasma nicotine. Dosages were designed to produce plasma nicotine levels comparable to those seen in humans smoking one to two packs of cigarets daily (Edwards and Warburton, 1984).

Experimental design involved the use of either compound alone, combined exposure to nicotine and cocaine, pair-fed (PF) controls, and untreated controls. Minipumps were implanted in all but the PF controls, and were filled with saline for animals not receiving nicotine. Animals were implanted and/or cocaine injections began on d 8 of pregnancy, and continued until parturition. Drug effects were evaluated on maternal food and water intake and weight gain. Treatment effects on litter size, offspring weight, and growth were evaluated, and a separate conventional teratology study was conducted to determine whether these treatments produced morphological abnormalities (unpublished observations). Offspring were administered an extensive

behavioral test battery, and density of striatal dopamine receptors was evaluated. Results of all these evaluations are reported below.

Methods

General Procedures

Date-mated primiparous Sprague-Dawley rats were obtained from the National Center for Toxicological Research (Jefferson, AR) breeding colony (plug date = gestational d [GD] 0). Dams were housed individually in standard Plexiglas™ cages (44 × 25 × 30 cm), lined with wood chip bedding, under environmentally controlled conditions (7:00 AM lights on, 7:00 PM lights off; ambient temperature 20–23°C) with ad libitum access to food and water.

Females were matched on the basis of GD7 body weights and changes in body weight from GD0 to GD7, and assigned to the treatment groups involving daily exposure to nicotine tartrate (N) or cocaine hydrochloride (C), either alone or in combination, or to control conditions, involving saline injections and pair feeding (*see below* for details).

Doses of cocaine hydrochloride (Sigma, St. Louis, MO) were dissolved in 0.9% saline; injection volumes were 0.1 mL/100 g body wt. Injections were made subcutaneously on the back, starting near the neck, and sites were rotated to limit tissue necrosis. Mild ulceration without infection was seen in 3 of the 42 dams treated with cocaine; moderate ulceration was observed in only one animal. None of the lesions required antibiotic ointment and healed rapidly. We found that necrosis could be avoided by swabbing the injection site with 0.9% saline after drug administration. Doses of cocaine ranging from 10–100 mg/kg/d have been used in prenatal exposure experiments. Maternal and fetal toxicity and/or lethality are seen at doses of 60, 80, and 100 mg/kg; in the rat, isolated incidences of *terata* are observed with the upper two doses. All doses studied produce some neurobehavioral alterations in the offspring. However, doses of 10, 20, or 40 mg/kg do not disrupt the gestational process, but produce maternal serum levels of cocaine that are comparable to those measured in humans after recreational use of cocaine (Spear et al., 1989a; Sobrian et al., 1990). Daily doses of 10 or 20 mg/kg/d were chosen for the dose-finding study. The moderate dose of 20 mg/kg was chosen as our high dose, because this dose of cocaine has been previously used in our laboratory to produce an animal model of prenatal cocaine exposure (Sobrian et al., 1990), and the present design utilized an extended duration of exposure and the combined use of nicotine.

Nicotine (hydrogen tartrate salt: Sigma) was administered by osmotic minipump (Alzet: 2ML2) designed to deliver drug for a 14-d period. Pumps were installed on GD8. To insert the pump, a 2-mm incision was made on the shaved back of a dam, lightly anesthetized with methoxyflurane. Sterile forceps were inserted into the wound and used to make a 6-mm pocket between the skin and muscle layer into which the pump was placed. The incision was closed with three wound clips. Females were placed in a heated observation cage until recovery, which occurred within 2 min, and then returned to their home cages. Aseptic conditions were maintained throughout the surgery. Injections of cocaine or saline were first administered 2 h after surgery.

Animals in all groups were weighed daily starting at GD7, and changes in maternal body weight, food consumption, and water intake were recorded throughout gestation. The pregnancy or status of females not delivering pups was determined by light microscopic examination of the unstained uteri for implantation sites.

Experiment I: Dose Finding/Pharmacokinetics

The purpose of this study was (1) to determine the dose of nicotine that could be combined with a 10 or 20 mg/kg dose of cocaine without producing alterations in maternal gestational and birth statistics (*see below*) and/or

fetal viability, and (2) to determine if nicotine altered the maternal and/or fetal disposition of cocaine.

Doses of 2.5 and 5.0 mg/kg/d of nicotine tartrate were used. The "smoking dose" of nicotine, defined as the maximum dose of nicotine inhaled from one cigaret, is approx 0.04 mg/kg (Edwards and Warburton, 1984). In clinical studies involving drug-abusing women, use of 10 or more cigarets a day is defined as smoking behavior. In animal studies investigating the effect of prenatal nicotine exposure on offspring, daily doses ranging from 0.5–6.0 mg/kg of nicotine tartrate are most often used. Since 0.25 mg/kg of nicotine tartrate = 0.08 mg/kg nicotine free base, dosages of 2.5 and 5.0 mg/kg/d should approximate use of 20 and 40 cigarets/d, respectively.

Females in this study were assigned to one of five prenatal treatment groups:

1. LC/LN: 10 mg/kg of cocaine (low cocaine: LC) in combination with 2.5 mg/kg of nicotine (low nicotine: LN);
2. LC/HN: 10 mg/kg of cocaine (LC) in combination with 5.0 mg/kg of nicotine (high nicotine: HN);
3. HC/LN: 20 mg/kg of cocaine (high cocaine: HC) in combination with 2.5 mg/kg of nicotine (LN);
4. HC/HN: 20 mg/kg of cocaine (HC) in combination with 5.0 mg/kg of nicotine (HN); and
5. NN/HC: No nicotine (NN) in combination with 20 mg/kg of cocaine (HC)—these cocaine-only dams were implanted with saline-filled minipumps and served as comparisons.

Injections were administered daily from GD8–20/21. Each group consisted of six females. With expected delivery on GD21–22, pharmacokinetic determinations were conducted on GD20. Dams in the two LC groups that were not utilized were sacrificed on GD21 because of time constraints.

Pharmacokinetic Determination

Only the three high-dose cocaine groups (i.e., LN/HC, HN/HC, and NN/HC) were used in the pharmacokinetic portion of the experiment. On GD20, rats were dosed once with 20 mg/kg of cocaine sc supplemented with a pulse of radiolabeled cocaine (ca. 25 μCi of [–]-[4–^{3}H]cocaine [0.43 Ci/mmol]). Blood samples were collected from the tail vein immediately prior to dosing (time 0) and at 5, 15, 30, 60, and 115 min after dosing. For the 120-min determination, blood was obtained by cardiac puncture in rats anesthetized by overexposure to carbon dioxide. This time frame was chosen because peak plasma and brain levels of cocaine in rats have been reported at approx 2 h following chronic daily sc cocaine administration (Nayak et al., 1976; Spear et al., 1989a). Following laparotomy, gravid uteri were removed, weighed, and the status of each uterine implant was noted (i.e., resorption, dead fetus, or live fetus). All fetuses were removed from the uterus, individually weighed, and examined for gross external defects (Wilson, 1965). Live fetuses were decapitated, and trunk blood was obtained for assay; blood samples from pups were pooled by litter. To stabilize cocaine in plasma, 0.2-mL samples were mixed immediately after separation with 5 mg of NaF. Samples were brought to a final volume of 0.5 mL by the addition of 0.2 mL of carbonate buffer and 0.1 mL of internal standard (100 μg/mL of lidocaine), and were then frozen at –70°C until analysis for cocaine by the method of Duhart et al. (1993).

Dams in the two low-dose cocaine groups (i.e., LN/LC and HN/LC) were killed by overexposure to carbon dioxide on GD21. Uteri and fetuses were removed and examined as described above.

Experiment II: Maternal Toxicity/Postnatal Development and Behavior

This study was designed to determine the interactive effects of prenatal exposure to cocaine and nicotine on gestational variables and postnatal behavioral outcomes. Combined dose levels were determined by the results of Experiment I.

Dams were matched as previously described and assigned to one of three drug treatment

groups. From GD8–21, rats were exposed daily to either 5.0 mg/kg of nicotine and sc injections of 0.9% saline (NS), 20 mg/kg of cocaine and a saline-fed pump (CS), or both 5.0 mg/kg of nicotine and 20 mg/kg of cocaine (NC). Saline-injected dams fitted with saline-filled pumps (SS), and nonimplanted, noninjected dams, PF to NC females, served as controls. In three replications, 11 NC, 12 NS, 13 CS, 12 SS, and 8 PF dams were treated.

Females were allowed to deliver naturally and nurse their own young. Previous research in our laboratory (Sobrian et al., 1990) has shown that a 20 mg/kg dose of cocaine did not disrupt maternal behavior. Neither nest building, pups retrieval, nor nursing behavior was disrupted by any of the treatments utilized in the present experiment (data not shown). On GD22, cages were checked for births at 8:00 AM, 11:00 AM, and 2:00 PM. Within 2–4 h of parturition (designated as postnatal day [PND] 1), dams were temporarily removed, and for each litter, pups were counted, examined for gross external abnormalities, sexed, weighed, and crown-rump length measured. Pup body weights were measured every 7 d until PND28. Pups were weaned at PND22 and housed in like-sex groups of three to four.

Behavioral Testing

REFLEX DEVELOPMENT

The development of reflex behaviors was monitored from PND3–16. The reflexes chosen were those previously altered by prenatal exposure to cocaine (Sobrian et al., 1990) and included the following:

Surface righting: Pup, placed on its back, turns over to rest in normal position with all four paws on the ground within 30 s (PND0–9);
Cliff avoidance: Pup, placed on a table top with forepaw and face extended over the edge, backs away from the cliff (PND6–12); and
Auditory startle response: Pup extends head and withdraws fore- and hind-limbs into a crouching position at sound of a loud sharp noise (snap of a mouse trap) held approx 12 cm above and behind its head (PND9–18).

SPONTANEOUS ALTERNATION (SA)

This is a spatial task that requires a rat, on two successive unrewarded trials in a T-maze, to choose alternate arms of the maze. Testing was conducted in a black Plexiglas™ T-maze that consisted of a start box, the main alley, and two goal arms. Black plastic guillotine doors separated each compartment and hinged Plexiglas™ lids permitted separate access to each section of the maze. Details of the apparatus appear in Sobrian and Nandedkar (1986).

Each alternation test consisted of two trials. A trial consisted of placing the animal in the start box for 10 s; the guillotine door was than raised, and the rat was given 60 s in which to enter one of the goal arms with all four paws. The rat was then left in the chosen arm for 30 s, after which it was removed to a holding cage for 30 s. During this intertrial interval, the maze was wiped with a mild disinfectant to eliminate odor trails. Each animal was tested daily for alternation on PND27–29. Animals were scored as exhibiting reliable alternation (A) or perseverative (P) behavior, i.e., choice of the same goal arm on two consecutive trials. Animals refusing to leave the start box or choose an arm on the first trial were scored as "balks" (B). All offspring were given two trials each day, regardless of their behavior on the first trial.

Drug Challenge

On PND20–22, male and female offspring from each of the prenatal treatment groups were challenged with one of three drugs: cocaine (20 mg/kg), nicotine (1.0 mg/kg), or apomorphine (hydrochloride salt: RBI [Natick, MA], 1.0 mg/kg). Immediately after ip injection of the challenge dose, each rat was placed in a 45 × 45 cm square, lighted viewing box with a glass front and nontransparent sides and bottom. The floor of each box was divided into a 3 × 3 array of squares of equal size and used to measure activity of the animal. Six such boxes were arranged so that one observer, blind with respect to the prenatal treatment of the subject, could score the activity and stereotyped behavior of six rats simultaneously.

Table 1
Stereotypy Rating Scale

Score	Description
Apomorphine	
−2	Total inactivation/sleep
−1	Inactivation with occasional movement
0	Normal: locomotion, rearing, grooming
1	Activity with mild sniffing stereotypy
2	Intermittent down sniffing
3	Continuous down sniffing
Cocaine	
0	Normal: locomotion, rearing, grooming
1	Activation with intermittent sniffing
2	Intermittent in-place stereotypy with some activity
3	In-place stereotypy
3.5	Wall running interspersed with in-place stereotype
4	Continuous wall circling
Nicotine	
−2	Total inactivation/sleep
−1	Inactivation with occasional movement
0	Normal: locomotion, rearing, grooming
1	Activation, rearing, abnormal gait
2	Leaning plus abnormal gait with decreased activity
2.5	Leaning, abnormal gait and straub tail
3	Severe leaning, abnormal gait, staggering

The evaluation of stereotyped behavior was based on the scoring scale listed in Table 1. The scales for each of the challenge drugs were developed using age-matched naive rats of both sexes. Negative scores are used to denote progressive levels of inactivation; animals challenged with cocaine were not inactive during the 60-min observation period. Stereotypy was recorded every 3 min (each rat was observed for 15 s in every 3-min session) for a total of 20 observations/rat, over a total period of 60 min. Activity was measured as total squares entered by each rat during the 15-s observation period every 3 min. Unchallenged controls were not used, since our primary interest was in the differences in responses of prenatally treated offspring to the drugs.

D_1 and D_2 Dopamine Receptor Binding

Membranes for receptor binding assays were prepared from caudate nucleus tissue of animals sacrificed on PND20–22 as previously described (Scalzo et al., 1990). Brain regions were frozen on dry ice and stored at −70°C prior to receptor binding assay using the method of Ali et al. (1986).

Dopamine receptor binding was assayed using aliquots of membrane preparations incubated with [^{3}H]SCH-23390 (81.0 Ci/mmol; New England Nuclear, Boston, MA) for D_1 or [^{3}H]spiroperidol (24.2 Ci/mmol; New England Nuclear) for D_2 binding. Single-point assays were performed on aliquots of caudate membranes at a concentration of 1.0 n*M* for each ligand and 1.0 μ*M* for each unlabeled competitor. Incubations were carried out in triplicate for 20 min at 37°C in a total of 1 mL in the presence of 1 μ*M* of (+)butaclamol (Research Biochemical, Inc., Natick, MA). Total radioactivity was quantified by liquid scintillation spectrometry (Tracor Mark III, Elk Grove Village, IL). Specific binding was calculated as the difference between the amount of [^{3}H]SCH-23390 or [^{3}H]spiroperidol binding alone (total binding) and that in the presence of 1.0 μ*M* (+)butaclamol (nonspecific binding). Aliquots of membrane preparations were used for the determination of protein content by the method of Lowry et al. (1951), using bovine serum albumin (Sigma) as the standard.

Statistical Analyses

Interval and ratio scale data were initially evaluated by analyses of variance (ANOVAs). Duncan's Multiple Range Test was used for *post hoc* analyses of significant main effects

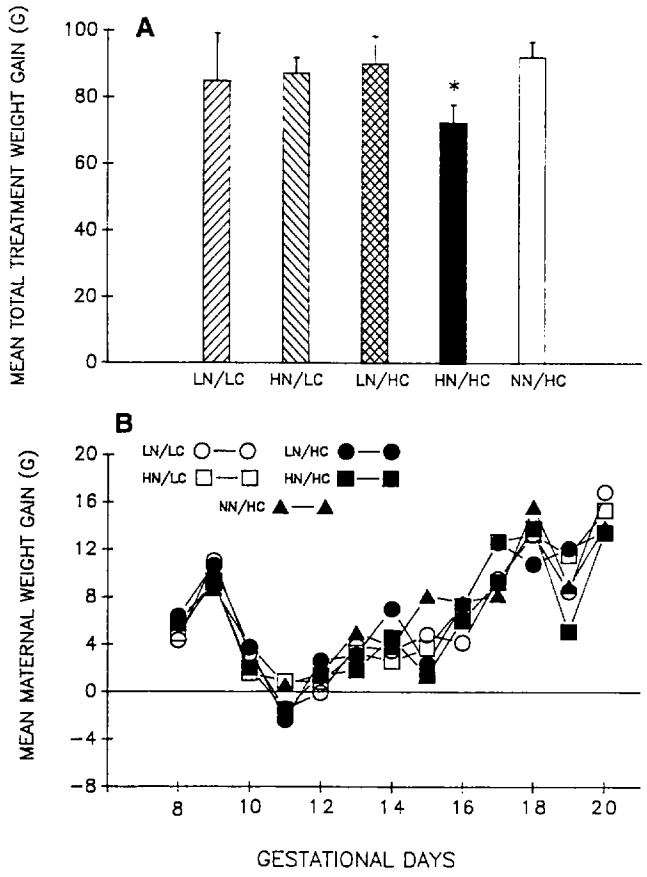

Fig. 1. Mean (±SEM) maternal weight gain during drug treatment. **(A)** Total weight gain from GD8 to 20. **(B)** Daily changes in body weight. (*) Total weight gain of HN/HC females significantly different from NN/HC females, $p < .05$.

(Kirk, 1968). Nonparametric tests (Fisher's Exact Probability Test or Chi Square Test of Independence) were used to evaluate all data that were expressed as percentages or proportions and categorical data, unless otherwise specified (Siegel, 1956). Statistical significance was assumed for probability levels of 0.05 or less (nondirectional test).

Analyses of maternal variables in Experiment I were performed only on data from either GD0–GD20 or GD8–GD20 for all five groups. Only data from pregnant females were used for analyses of gestation weight gain and food and water intake. The calculation of net weight gain accounts for the difference in gestational duration at laparotomy. In Experiment II, maternal variables were calculated on data from GD8–21, and data were confined to dams giving birth to viable litters. One-way ANOVAs were used to analyze maternal food, water, and body weight data. For analyses of variables for each day independently, data from all pregnant dams were included. For repeated measures analyses, animals with any missing data were excluded.

For offspring variables, the litter's datum, rather than the individual pup's datum, was used as the fundamental unit of analysis. Litter data were first analyzed with sex as an independent variable; if no significant differences were found, data were collapsed across this variable and reanalyzed. Data collected using the stereotypy rating scale were subjected to a two-way split-plot factorial ANOVA, with blocks as the repeated measure. Lord (1953) has indicated that nominal/ordinal data that conform to certain distribution characteristics can be analyzed using ANOVA without violating the assumptions of this statistic. Moreover, use of this statistical approach has appeared in recent publications (Sobrian and Nandedkar, 1986; Scalzo and Holson, 1992).

Results

Experiment I: Dose Finding and Cocaine Pharmacokinetics

Maternal Weight Gain

Daily maternal weight gains and total weight gains during drug exposure are presented in Fig. 1. Daily weight gains during the treatment period were similar in all groups. In contrast, total treatment weight gain, determined by calculating the change in weight from GD8 (the morning of treatment onset) to GD20, was significantly smaller only in females exposed to combined high-dose cocaine and high-dose nicotine (HN/HC), ($p < .01$).

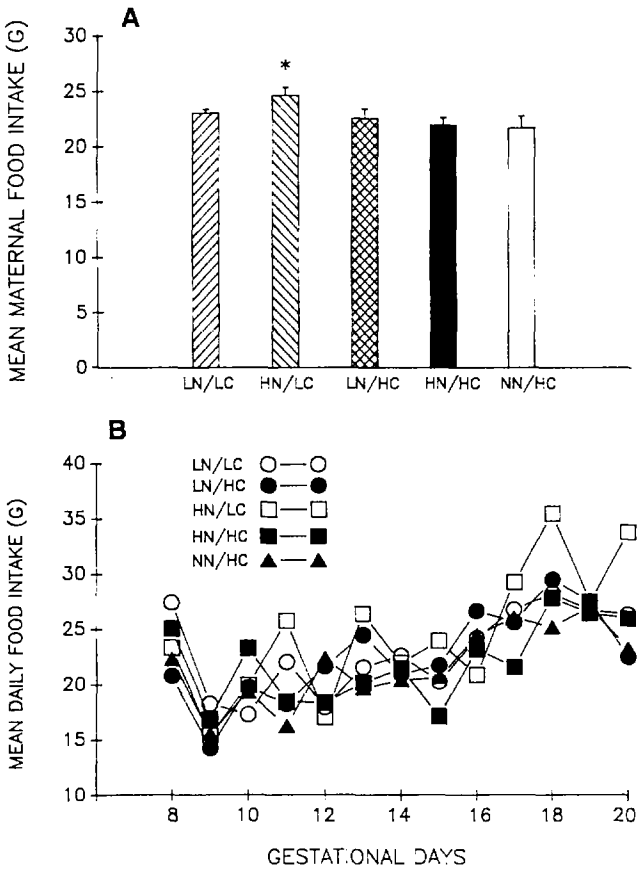

Fig. 2. Mean (±SEM) maternal grams of food eaten during drug treatment. **(A)** Mean daily food intake calculated as a function of total intake from GD8 to 20. **(B)** Maternal food intake on a daily basis. (*) Food intake of HN/LC females significantly different from NN/HC females, $p < .05$.

Maternal Food and Water Consumption

Although mean daily food consumption did not differ among the groups during the treatment period, the total amount of food consumed between GD8 and 20 was slightly, but significantly, increased in dams administered combined high-dose nicotine and low-dose cocaine (HN/LC) when compared to high-dose cocaine alone (NN/HC) ($p < .05$) (Fig. 2). The addition of high-dose nicotine to the high-dose cocaine regime (HN/HC) did not alter food intake with respect to the high-dose cocaine-only group (NN/HC). No differences were observed in either total or daily water consumption among the treatment groups (data not shown).

Maternal and Fetal Variables

Daily doses of nicotine, delivered by osmotic minipump, attained targeted levels in both low-dose (2.5 mg/kg/d) and high-dose (5.0 mg/kg/d) groups (Table 2). Prenatal administration of combined doses of nicotine and cocaine did not alter any of the maternal variables or fetal viability when compared to exposure to cocaine alone. No gross external defects were observed in offspring from any of the groups.

Cocaine Pharmacokinetics

Figure 3 shows the total serum distribution and elimination curve of radioactivity derived from [^{3}H] cocaine in pregnant rats on GD20 after sc administration of cocaine (20 mg/kg of unlabeled drug supplemented with 25 μCi/kg [^{3}H]cocaine). Doses of nicotine or saline were still being delivered to the females by osmotic pump at this time. Total radioactivity, measured in samples from tail blood, rose slowly in all groups and had not peaked at 115 min postinjection; differences in total counts were not evident at any postinjection time up to this point. Even though levels of radioactivity in the LN/HC group appeared to be somewhat higher, statistical significance was not reached owing to the relatively large variability for samples in this group. In contrast, total radioactivity, measured in cardiac samples obtained from the dams and their fetuses at 120 min postinjection was significantly different (Fig. 4); higher levels of radioactivity were evident in LN/HC dams ($p > .05$). In contrast, total counts measured in fetal samples did not differ. Comparison of the fetal/maternal ratios of [^{3}H]cocaine-derived radioactivity did not reveal any significant differences.

Experiment II: Maternal Toxicity and Postnatal Development and Behavior

The results of Experiment I indicated that the coadministration of cocaine and nicotine in the

Table 2
Maternal and Fetal Data at Time of Laparotomy (GD20–21)

Treatment	*N*	Laporatomy	Nicotine mg/kg/d	Number of live fetuses[a]	Number of resorptions[b]	Number of malformations	Weight gain, GD0–20	Net weight gain[c]
LN/LC	5	GD21	2.42 ± 2.7	12.5 ± 2.7	6 (ER)	0	127.8 ± 14.1	45.2 ± 4.7
HN/LC	5	GD21	4.85 ± 0.05	13.0 ± 0.6	3 (ER)	0	128.7 ± 17.4	51.6 ± 6.6
LN/HC	5	GD20	2.33 ± 0.05	14.0 ± 1.4	1 (ER)	1	123.0 ± 12.6	48.5 ± 5.9
HN/HC	6	GD20	4.93 ± 0.09	12.3 ± 1.3	2 (ER)	1	111.4 ± 6.20	44.2 ± 6.2
NN/HC	5	GD20	–	14.2 ± 0.2	1 (LR)	0	121.5 ± 8.2	44.3 ± 6.5

[a]No dead fetuses were found.
[b]ER = Early resorptions; LR = late resorptions.
[c]Gestational weight gain – weight of gravid uterus = net weight gain.

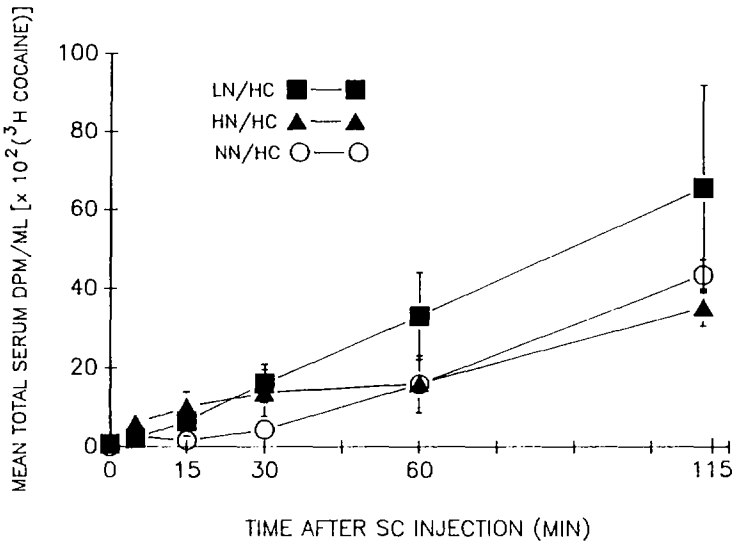

Fig. 3. Serum concentration vs time curve for total radioactivity derived from [^{3}H]cocaine, 25 μCi/kg. Samples were obtained on GD20 from pregnant rats that have been treated with high-dose cocaine, either alone (NN/HC), or in combination with low- (LN/HC) or high-dose nicotine (HN/HC) from GD8 to 20.

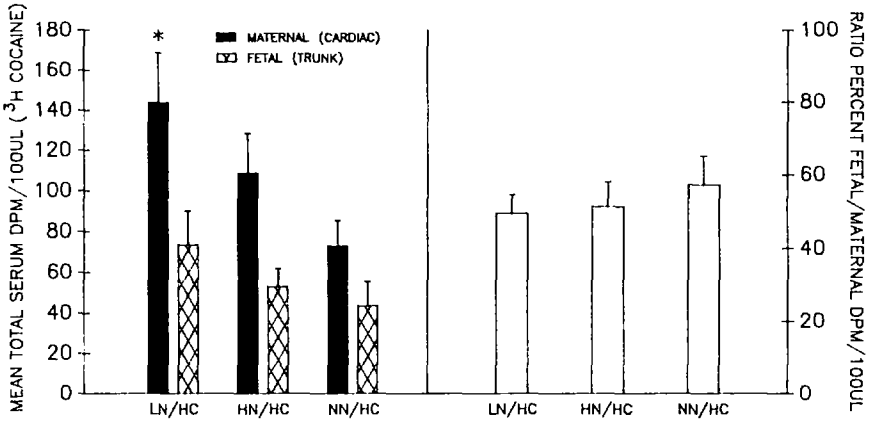

Fig. 4. Fetal and maternal serum concentrations of total radioactivity derived from [^{3}H]cocaine at 120 min postinjection **(Left)**, and the fetal/maternal ratio percent **(Right)**. (*) Levels of total radioactivity at 120 min were significantly higher in LN/HC than in NN/HC dams, $p < .05$.

highest doses used was not overtly toxic to dams or fetuses, nor was it more toxic than the high dose of cocaine alone. Therefore, in Experiment II, the high dose of cocaine and the high of nicotine were used alone and in combination.

Maternal Weight Gain

Total weight gains during drug treatment (Table 3), although reduced in NC, CS, and PF females, did not differ significantly from SS controls. However, differences in daily maternal weight gains were observed among the groups during the first week of drug exposure (Fig. 5). On GD8 and 10, weight gains of PF females were significantly smaller than those of SS, NS, and CS dams ($p < .02$ and .006, respectively), and of all four groups on GD11 ($p < .007$). On GD13, there was a rebound in

Table 3
Gestational and Birth Statistics for Females Treated with Cocaine and/or Nicotine on GD8–21 and Controls (Means ± SEM)

Females	N	GD8–21 weight gain, g	Period of gestation, d	Litter size	Mean daily dose of nicotine, mg/kg
NC	11	93.34 ± 3.37	22.27 ± 0.10	14.36 ± 0.62	4.69 ± 0.06
NS	11	101.54 ± 4.17	22.32 ± 0.08	13.45 ± 0.62	4.55 ± 0.06
CS	12	92.44 ± 3.80	22.29 ± 0.07	11.50 ± 0.82	
PF	7	88.84 ± 6.80	22.14 ± 0.09	12.86 ± 1.06	
SS	11	104.78 ± 11.58	20.52 ± 1.55	12.18 ± 1.67	

	Number		Birth weight, g		Crown rump length, cm	
Litters	Males	Females	Males[a]	Females	Males	Females
NC	7.5 ± 0.4	6.9 ± 0.5	6.29 ± 0.17	5.87 ± 0.18	4.54 ± 0.04	4.43 ± 0.05
NS	6.2 ± 0.5	7.1 ± 0.5	6.67 ± 0.15	6.22 ± 0.14	4.60 ± 0.04	4.43 ± 0.03
CS	5.4 ± 0.5	5.5 ± 0.7	6.61 ± 0.11	6.28 ± 0.17	4.63 ± 0.06	4.54 ± 0.05
PF	6.4 ± 0.7	6.2 ± 0.9	6.51 ± 0.17	6.06 ± 0.17	4.70 ± 0.07	4.58 ± 0.09
SS	5.8 ± 0.8	7.1 ± 0.9	6.10 ± 0.21	5.92 ± 0.18	4.70 ± 0.24	4.46 ± 0.07

[a]Significantly different from female counterparts, $p < .01$.

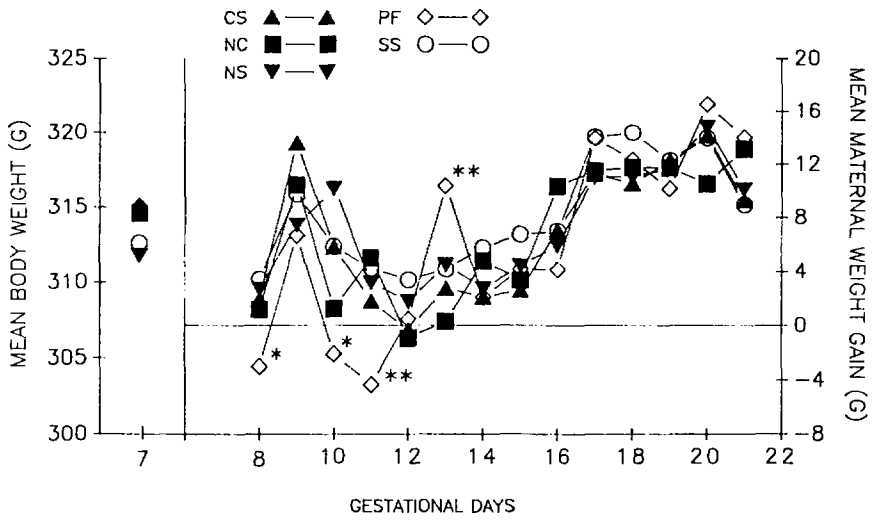

Fig. 5. Mean body weight before and daily body weight change during drug treatment. (*) PF dams significantly different from SS, NS, and CS dams, $p < .02$ (GD8); $p < .006$ (GD10). (**) PF significantly different from all four groups, $p < .007$ (GD11); $p < .001$ (GD13).

weight gain; PF females exhibited a significantly larger increase than the other four groups ($p < .001$).

Maternal Food and Water Consumption

Even though PF females were pair-fed to NC females, their food intake data were included in the ANOVA because some animals in this group did not consume all of the food given to them. Total and daily food consumption (Fig. 6) was altered by gestational exposure to cocaine, either alone (CS) or in combination with nicotine (NC). In both of these groups, total food consumed was signifi-

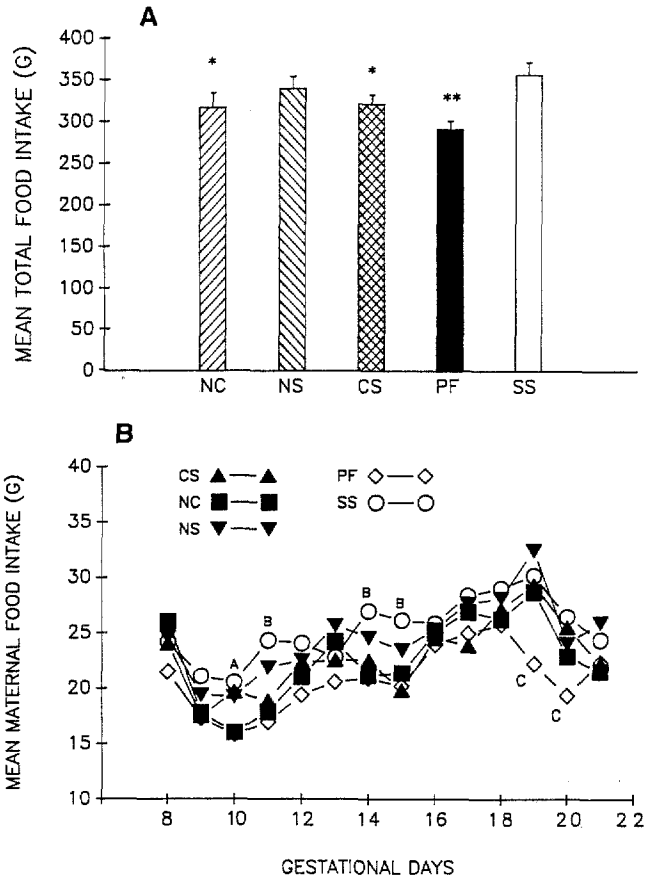

Fig. 6. Mean (±SEM) maternal total food intake **(A)** and mean daily food intake **(B)** during drug treatment. (*) NC and CS significantly different from NS and SS dams, $p < .05$. (**) PF significantly different from other four groups, $p < .05$. (A) NC and PF significantly different from SS, CS, and NS, $p < .05$. (B) NC, CS, and PF significantly different from SS and NS, $p < .008$ (GD11); $p < .004$ (GD14); $p < .001$ (GD15). **(C)** PF dams significantly different from other four groups, $p < .001$ (GD19); $p < .02$ (GD20).

cantly less than that in SS controls ($p < .05$). Food consumption in the PF groups was significantly less than in the other four groups ($p < .05$).

A similar pattern was seen when daily food intake was analyzed. Significant decreases in daily consumption first appeared in NC and PF females on GD10 ($p < .008$), and were again evident on PND11 ($p < .001$), PND14 ($p < .004$), and PND15 ($p < .001$). On the latter 3 d, food consumption was also significantly less in the CS group when compared to SS and/or NS females. Food intake was reduced in PF females on GD19 and 20 ($p < .001$ and .02, respectively). No differences were observed in either total or daily water intake among the groups (data not shown).

Maternal Toxicity and Pregnancy Outcome

Gestational and birth statistics are listed in Table 3. Mean daily doses of nicotine, delivered by osmotic minipump, did not differ from the targeted dose of 5.0 mg/kg/d. Treatment weight gain and length of the gestational period were not significantly different among the groups. The number of females delivering viable litters, litter size, the number of male and female pups, and offspring body weight and crown-rump length at birth were unaffected by any of the prenatal manipulations. However, male offspring in each group were significantly heavier than their female counterparts at birth ($p < .01$).

Postnatal Development

Postnatal body weight over the first 4 wk of age did not differ among the groups (data not shown). The number of offspring in each group exhibiting a particular reflex behavior appears in Table 4. Each prenatal treatment group contained an equal number of male and female pups; inspection of the data by sex was not performed. The maturation of surface righting was delayed in pups exposed prenatally to cocaine alone; significantly fewer CS pups exhibited this behavior on PND3 ($p < .01$).

Drug Challenge

Locomotor activity and stereotyped behavior of offspring from all treatment groups in response to challenge doses of cocaine, nicotine, or apomorphine are presented in Figs. 7 and 8, respectively. A challenge dose of nicotine to NS or NC offspring did not induce a significant alteration in either stereotyped behavior or locomotor activity when compared to either SS, PF, or CS pups. Drug challenge with cocaine did not modify stereotypies in either of the two groups prenatally exposed to

Table 4
Development of Reflexes in Offspring Prenatally Exposed to Cocaine and/or Nicotine

Reflex	Treatment, *n*		Postnatal age, d						
			3	4	6	7	12	13	14–16
Surface righting	NC	(16)[a]	14 (4.6 ± 1.1)[b]	16 (8.5 ± 6.5)					
	NS	(18)	13 (7.3 ± 1.3)	18 (2.3 ± 0.4)					
	CS	(12)	8[c] (7.9 ± 1.6)	12 (2.1 ± 0.7)					
	SS	(14)	13 (5.1 ± 1.1)	14 (2.0)					
	PF	(10)	8 (6.1 ± 1.8)	10 (1.8 ± 0.3)					
Cliff avoidance	NC	(20)			16	20			
	NS	(22)			16	22			
	CS	(20)			15	20			
	SS	(22)			17	22			
	PF	(14)			9	14			
Startle response	NC	(20)					13	15	20
	NS	(20)					14	19	20
	CS	(16)					12	14	16
	SS	(20)					12	20	20
	PF	(14)					12	13	14

[a]Total number of pups tested. One male and one female pup from a litter were tested repeatedly for the development of reflex behavior, which was scored either present or absent. Values listed are number of animals exhibiting response at each age.
[b]Latency (s) ± SEM for righting reflex.
[c]Significantly different from saline controls (SS), $p = .01$.

cocaine (CS and NC) in comparison to PF, SS, or NS offspring. In contrast, the locomotor activity response to the challenge dose of cocaine in CS pups was significantly diminished ($p < .04$) during the initial observation period, when compared to NC and NS offspring (Fig. 7, middle panel).

Following challenge with the dopamine receptor agonist, apomorphine, locomotor activity (Fig. 7, bottom panel) was significantly reduced in CS offspring at several observation points during the first 30 min postchallenge when compared to NC, NS, and SS offspring ($p < .02$–003). In contrast, at the 30 min time point, NC and NS offspring were more active than SS pups ($p < .004$). A similar profile was seen with respect to stereotyped behavior following drug challenge with apomorphine (Fig. 8, bottom panel). Stereotypy was significantly reduced ($p < .05$ to $.001$) in CS offspring during the first 15 min following drug challenge in comparison to NC, NS, and SS groups.

SA

Alternation rates for offspring in each of the five treatment groups are presented in Fig. 9. Data were not analyzed with sex as an independent variable, but each group contained an equal number of males and females. Reliable alternation was not observed in any of the groups on any of the days tested. On PND27, the majority of NC, NS, PF, and SS offspring either refused to leave the start box or did not choose a goal box, behaviors referred to collectively as "balking." However, the number of

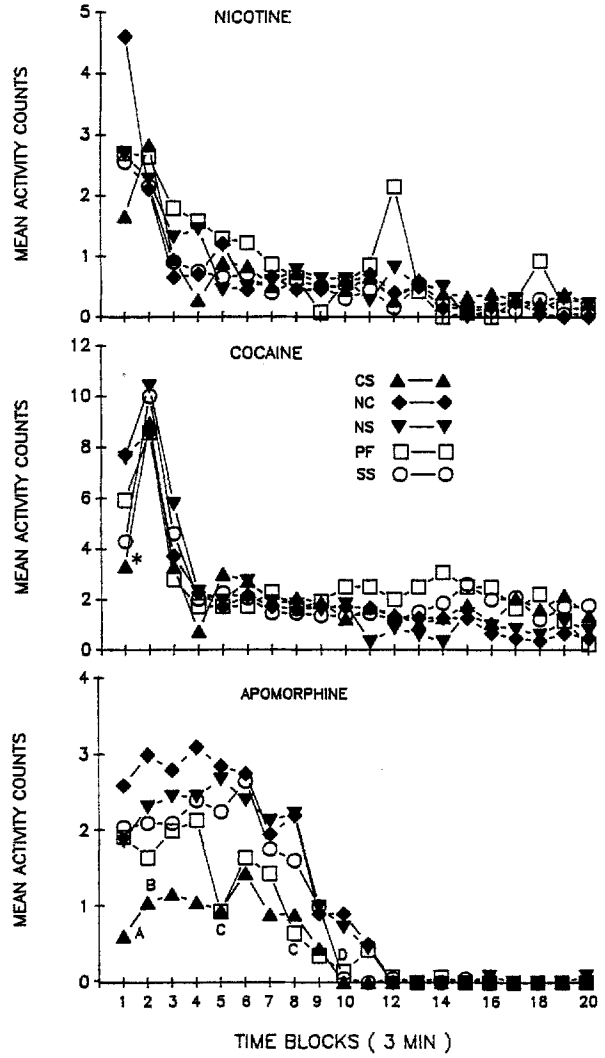

Fig. 7. Effect of prenatal exposure (GD8–21) to cocaine or nicotine, either alone or in combination on locomotor activity during a 60-min test period following an ip challenge dose of either 1.0 mg/kg nicotine **(Top)** 20.0 mg/kg cocaine **(Middle)**, or 1.0 mg/kg apomorphine **(Bottom)**. Data shown are group means for each 3-min time block. (*) CS significantly different from NC and NS, $p < .04$. **(A)** CS significantly different from other four groups, $p < .01$. **(B)** CS significantly different from NC, NS, and SS offspring, $p < .004$. **(C)** CS and PF significantly different from NC, NS, and SS, $p < .003$ (time block [TB] 5); $p < .02$ (TB 8). **(D)** CS, SS, and PF significantly different from NC pups, $p < .004$.

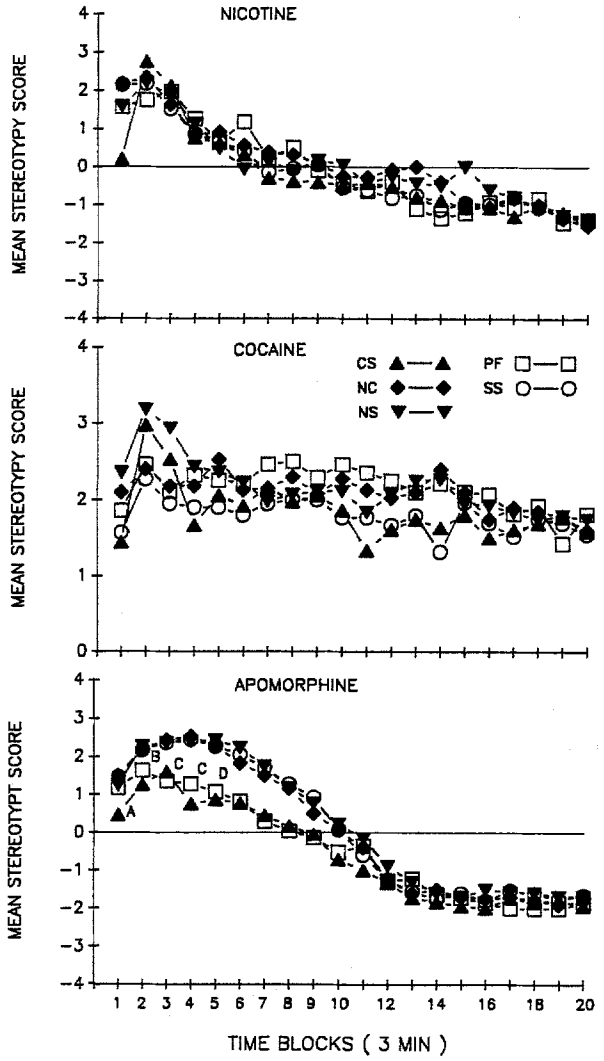

Fig. 8. Effect of prenatal exposure (GD8–21) to cocaine or nicotine, either alone or in combination on stereotyped behavior during a 60-min test period following an ip challenge dose of either 1.0 mg/kg nicotine **(Top)**, 20.0 mg/kg cocaine **(Middle)**, or 1.0 mg/kg apomorphine **(Bottom)**. Data shown are group means for each 3-min time block. **(A)** CS significantly different from other four groups, $p < .009$. **(B)** CS significantly different from NS, NC, and SS offspring, $p < .01$. **(C)** CS and PF significantly different from NS, NC, and SS pups, $p < .03$ (TB 3); $p < .004$ (TB 4). **(D)** CS and PF significantly different from NS group, $p < .02$.

CS offspring showing balking behavior was significantly less than that in the other four groups ($p < .04$). A similar pattern was seen on PND29; offspring from both prenatal cocaine groups (i.e., CS and NC) showed reduced balking behavior ($p < .04$).

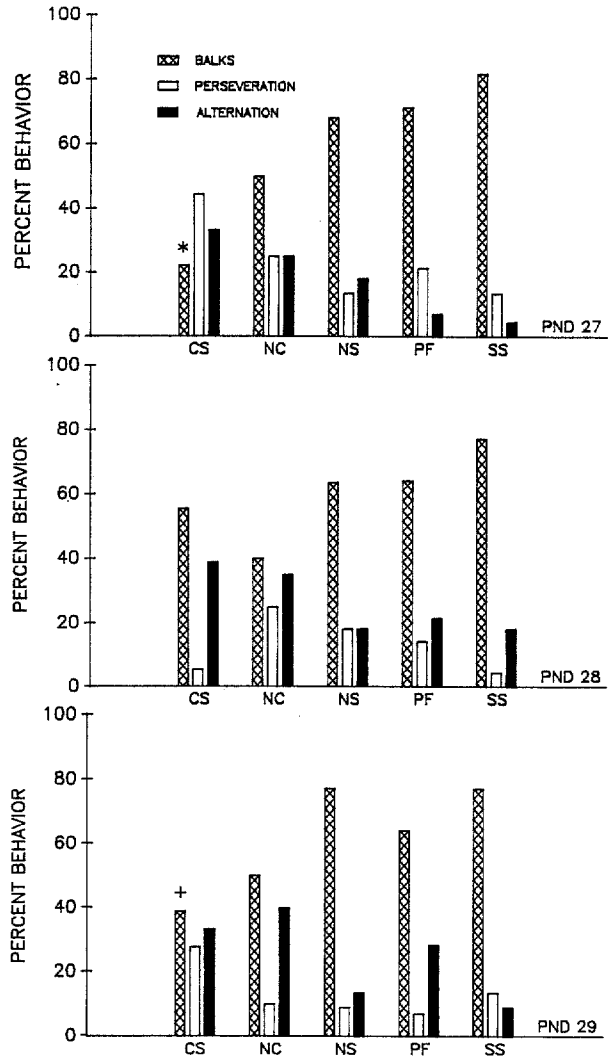

Fig. 9. Spontaneous alternation behavior as a function of prenatal exposure to cocaine and nicotine, either alone or in combination. Data shown are the percent of offspring in each group that exhibited reliable alternation (choice of different goal arms on two consecutive trials), perseverative behavior (choice of the same goal arm on two consecutive trials), or balking behavior (failure to either leave the goal box or choose a goal on one or both trials). (*) Percent balking behavior in CS groups significantly different from other four groups, $p < .04$. (+) Percent balking behavior in CS offspring significantly different from NS, PF, and SS groups, $p < .04$.

D_1 and D_2 Dopamine Receptor Binding

Prenatal exposure to cocaine and nicotine, either alone or in combination, did not alter D

Table 5
Specific Binding of Dopamine D_1 and D_2 Receptors in the Caudate Nucleus of Offspring Prenatally Exposed to Cocaine and/or Nicotine (Means ± SEM)

	Fmol bound/mg total protein	
Treatment	Females	Males
D_1 receptors		
CS	862.64 ± 55.88	876.46 ± 68.22
NC	852.92 ± 52.90	916.64 ± 60.06
NS	891.79 ± 46.77	877.32 ± 50.57
PF	940.92 ± 77.63	870.82 ± 57.91
SS	861.24 ± 53.37	896.56 ± 49.66
D_2 receptors		
CS	518.79 ± 49.93	564.36 ± 64.42
NC	523.63 ± 44.06	558.83 ± 52.23
NS	575.78 ± 42.47	525.84 ± 47.29
PE	508.49 ± 67.32	487.83 ± 28.94
SS	548.33 ± 47.31	530.70 ± 47.77

or D_2 receptors in the caudate nucleus of the offspring. Single-point studies revealed no difference in either [^{3}H]spiroperidol or [^{3}H]SCH-23390 binding in this brain region (Table 5).

Discussion

The present experiments were designed to investigate the effects of prenatal exposure to cocaine and nicotine, either alone or in combination, on maternal, fetal, and offspring parameters.

Cocaine

There appears to be a nascent agreement with respect to the effects of exposure to prenatal cocaine in rodents. The results of the present studies replicate and confirm previous findings from our and other laboratories, and may begin to provide markers for early detection and long-term consequences in affected neonates.

Exposure of female rats during the last two-thirds of pregnancy to cocaine induced mild maternal toxicity. Although pregnancy duration and outcome and litter size were not

altered, maternal food intake was decreased in both studies in dams receiving cocaine alone. This decrease occurred in the absence of a reduction in treatment weight gain.

Dose-dependent decreases in maternal food intake following gestational exposure to cocaine have been previously reported for doses of 40 mg/kg/d and above (Hutchings et al, 1989; Church et al., 1990; Heyser et al., 1992). Concomitant decreases in weight gain have been reported by some investigators (Hutchings et al., 1989; Church et al., 1990; Heyser et al., 1990, 1992), whereas others report no change in maternal body weight with similar doses (Smith et al., 1989; Spear et al., 1989b; Riley and Foss, 1991).

The effects of prenatal cocaine exposure on maternal water intake are controversial. Changes in water intake were not observed in either of the present studies. However, no change in water consumption (Heyser et al., 1992) as well as both dose-related decreases (Hutchings et al., 1989) and increases (Church et al., 1990; Church and Rauch, 1992), with or without changes in food consumption or body weight changes, has been reported. Differences in routes of drug administration, the gestational window of drug exposure, and the nature of the control group used for comparison may account, in part, for the divergent results on ingestive behaviors.

Offsprings' body weights at birth and throughout the first four postnatal weeks were generally not affected by prenatal cocaine exposure. This replicates our earlier finding (Sobrian et al., 1990) and is also in agreement with other reports in which comparable doses of cocaine have been used (Smith et al., 1989; Spear et al., 1989c). Decreases in pups' birth weight have been reported following exposure to maternal doses of 30 mg/kg/d or more (Church et al., 1990; Henderson and McMillen, 1990; Seifert and Church, 1991), but again the finding is not consistent (Hutchings et al., 1989; Spear et al., 1989b). Moreover, this initial difference has been reported to persist during the preweaning period (Church et al., 1990), whereas others report no differences at PND15 and 30 (Henderson and McMillen, 1990). These differences do not appear to be related to alterations in either maternal food intake or body weight gain during drug treatment. Although divergent results are not completely resolved by differences in the duration or window of fetal exposure, limiting cocaine administration to the last third of gestation appears to mitigate this effect on the offspring (Hutchings et al., 1989).

Prenatal exposure to cocaine alone delayed the development of the surface righting reflex; alterations in negative geotaxis and acoustic startle were not observed. Previous work in our laboratory revealed an accelerated development of all three of these reflexes; however, maternal cocaine exposure was limited to the last third of pregnancy (Sobrian et al., 1990). Delayed development of the righting reflex has been reported in offspring exposed to 30 mg/kg of cocaine on GD1–20 (Henderson and McMillen, 1990; Johns et al., 1992a).

Clinical reports suggest that cocaine infants are more responsive to auditory stimuli and show a greater startle response than drug-free infants (Chasnoff et al., 1985; Anday et al., 1989; Cohen et al., 1989). The effect of prenatal cocaine exposure on the development of the auditory responses in animals has been studied by several laboratories. Church and collaborators (Church et al., 1990; Church and Overbeck, 1991) found developmental delays in the maturation of the brainstem auditory evoked potential and permanent sensorineural hearing loss in some of the offspring. These effects dissipated with aging and required high prenatal exposure of cocaine. In contrast, Foss and Riley (1991a) found no effects on startle habituation or pre-pulse inhibition of startle in 60-d-old rats that could be attributed to prenatal cocaine exposure. These divergent results may reflect the longer treatment period employed by Church and collaborators, i.e., GD7–20 vs GD14–21, since rat strains, doses, and routes of administration of cocaine were similar.

Acute postnatal challenge with dopaminergic and cholinergic agonists was conducted to

determine if prenatal drug exposure would alter the sensitivity of the offspring with respect to drug-induced responses. Both cocaine and apomorphine challenge resulted in an attenuated increase in locomotor activity in cocaine-only offspring, suggesting a tolerance or desensitization to these dopamine agonists, induced by prenatal exposure. The duration of the effect was prolonged with apomorphine, which might be indicative of a differential effect on pre- and postsynaptic mechanisms. We previously reported a similar reduction in activity in cocaine-exposed offspring following challenge with both *d*-amphetamine and cocaine (Sobrian et al., 1990). Cocaine-induced stereotypy was unaltered by prenatal cocaine exposure. In contrast, challenge with apomorphine reduced the intensity of the stereotype response in prenatal cocaine offspring, again suggesting a subsensitive response of postsynaptic dopaminergic receptors. This diminished sensitivity could result from altered development of receptor mechanisms by *in utero* cocaine (Sobrian et al., 1990). Prenatal cocaine exposure has been associated with the downregulation of adrenergic and opiate receptors in human placenta (Wang and Schnoll, 1987).

Although no evidence of sensitization to challenge with either drug was seen for either behavior in the present study, Foss and Riley (1991b) reported that prenatal cocaine exposure increased the number of quadrants entered and rearing behavior in an open field in offspring challenged with cocaine. The age at which offspring were tested may account for the differences. In the present study, offspring were tested at 3 wk of age; in the latter study, rats were 80–86 d of age. When tested in nondrugged situations, younger cocaine offspring tend to be hypoactive in comparison to controls (Church and Overbeck, 1990b; Riley and Foss, 1991), whereas older animals tend toward hyperactivity (Foss and Riley, 1991a; Johns et al., 1992b). The differences observed following drug challenges may be an exaggeration of these age-related locomotor responses.

SA can be considered an unrewarded spatial learning task (Smith et al., 1989), and has been used to assess possible disruptive effects of prenatal drug exposure (Sobrian and Nandedkar, 1986; Smith et al., 1989). This behavior exhibits a distinct ontogenetic pattern (Douglas et al., 1973); perseverative responses dominate at PND17–20, with reliable levels of alternation first appearing between PND25 and 30. Although the number of offspring exhibiting alternation behavior increased over the 3-d test period, reliable levels of alternation, i.e., levels significantly >50%, were not seen in any of the groups on PND27–29. Offspring in all groups showed some perseveration, i.e., choice of the same goal arm of two consecutive trials; however, the majority of offspring refused either to leave the start box or enter a goal arm, a response termed "balking" or nonchoice behavior. The present results are in general agreement with those of other laboratories, which report no differences in the percent of alternation responses (Smith et al., 1989; Church and Overbeck, 1990b: Johns et al 1992b). However, changes in subtle indices, such as latency to leave the start box or enter a goal arm (Smith et al., 1989; Church and Overbeck, 1990b) or direction of turning (Church and Overbeck, 1990b), have been reported. Additionally, prenatal cocaine exposure has been shown to alter the development to spontaneous alternation. Church and Overbeck (1990b) reported no perseveration in rats 21 d of age who were exposed prenatally to cocaine, suggesting an accelerated development of this behavior.

In the present study, the frequency of nonchoice behaviors, i.e., balks, was significantly reduced in prenatal cocaine offspring on PND27 and 29, with fewer offspring showing a nonchoice response. This effect may reflect either hyperactivity or a hyperreactive response to a novel situation. Data from several laboratories do not support the first alternative; prenatal cocaine offspring do not exhibit exaggerated locomotor responses at 4 wk of age (Sobrian et al., 1990; Church and Overbeck, 1991; Riley and Foss, 1991).

Nicotine

Exposure to nicotine alone was evaluated in the second study. In contrast to the effects seen with cocaine alone, exposure to 5.0 mg/kg/d of nicotine from GD8 to 21 had no significant effects on any of the maternal or fetal variables assessed. Behavioral changes were limited to a transient increase in locomotor activity that occurred after a 30-min delay following acute drug challenge with apomorphine.

Recent work using infusions of nicotine to pregnant rats has shown that nicotine can cause growth retardation, impaired patterns of neuronal cell replication and differentiation, and alterations in several neurotransmitter systems in the CNS, as well as peripheral catecholaminergic pathways in the offspring (Lichtensteiger et al., 1988; Navarro et al., 1990a). Reports of treatment effects on gestational variables and litter size have not been consistent. Use of a continuous infusion appears to eliminate the decrease in pup birth weight reported following injection or oral administration of nicotine (Fung, 1989; Zahalka et al., 1992, 1993). Moreover, a transient weight increase during the first four postnatal week has been reported in offspring gestationally exposed to nicotine (Levin et al., 1993). Animal models that use slow infusions of nicotine that are devoid of other tobacco components and do not cause hypoxia and ischemia have also demonstrated that nicotine alters behavior (Zahalka et al., 1993). Offspring hyperactivity appears to be one of the most consistent findings (Fung, 1989), and it was also observed in the present study.

Cocaine and Nicotine

The primary purpose of the present experiments was to investigate the interactive effects of prenatal exposure on GD8–21 to cocaine (20 mg/kg) and nicotine (5.0 mg/kg) in combination on pregnancy outcome and offspring development and behavior in the rat. We were also interested in determining whether nicotine coadministration would modify maternal and/or fetal distribution of cocaine or alter dopamine receptor binding in the CNS. The results did not support our original contention that combined drug exposure would result in enhanced maternal or fetal toxicity. In contrast, the data suggest that nicotine may mitigate some of the consequences of *in utero* exposure to cocaine.

With respect to maternal variables, combining cocaine and nicotine caused a reduction in food intake. This effect was dose-dependent, occurring only in females receiving the high dose of both drugs. However, the decrease was no greater than that seen with high-dose cocaine alone, suggesting that nicotine did not contribute substantially to the anorexigenic effects of cocaine. In contrast, nicotine may provide dose-dependent protection; food intake was not altered in females exposed to low-dose nicotine and high-dose cocaine.

Despite the decrease in food intake, no interactive effects of cocaine and nicotine were observed on pregnancy outcome, gestational length, maternal mortality, litter size, or birth weights. Combined drug exposure did not alter maternal water intake in either of the studies.

With respect to behavioral variables in the offspring, again there appeared to be no enhancement of toxicity following combined exposure to cocaine and nicotine. In contrast, the data are suggestive of a protective effect; the delayed development of the righting reflex seen in offspring exposed to cocaine alone was ameliorated in the NC offspring. Moreover, the subsensitivity exhibited by the CS offspring in response to acute drug challenge with cocaine and nicotine was not seen in this group.

The enhanced toxicity seen when cocaine and alcohol are combined prenatally (Church et al., 1991) has been tentatively attributed to increased peak blood levels or decreased elimination of each drug. The facts that cigaret smoking-induced alteration in drug absorption, distribution, metabolism, excretion, and effectiveness is well documented (Miller, 1990) and both nicotine and cocaine can depress active transport by the human placental villi

and transplacental amino acid transport (Sastry, 1991) support the premise that combined exposure to these drugs could alter pharmacokinetic parameters. The data from the present experiment indicated that there is a transient increase in maternal levels of cocaine; however, since this increase was not related to increased maternal or fetal toxicity, the suggestion is that, for cocaine, pharmacodynamics rather than pharmacokinetic changes may be responsible for the observed effects.

Since the mechanism of action of both nicotine and cocaine involve dopaminergic systems, it was possible that any interactive effects might be manifested through multiple effects on dopaminergic functioning. Offspring prenatally exposed to nicotine show an increased ability of the striatal tissue to synthesize DA (Fung, 1989); decreases in the number of DA binding sites, but an increase in affinity of DA receptors in the striatum have also been reported (Fung and Lau, 1989). An increase in D_2 receptor binding associated with an increase in affinity was found in the caudate offspring prenatally exposed to cocaine (Scalzo et al., 1990); however, there were no changes in striatal D_2 receptors or D_1 receptors in either tissue. Increased labeling of DA terminals in the limbic and neocortical region has also been reported (Clow et al., 1991). In the present study, no alterations were found in either D_1 or D_2 receptor binding in the caudate or striatum of offspring exposed to cocaine or nicotine either alone or in combination. This suggests that D, or D_2 receptors are not involved in the observed behavioral alterations. The effects may be mediated through adrenergic, serotonergic, or opiate receptors, which can be altered by prenatal exposure to either cocaine or nicotine (Navarro et al., 1990b; Clow et al., 1991; Henderson et al., 1991; King et al., 1991).

In summary, the results of the present experiment do not support the premise that combined exposure to nicotine and cocaine results in enhanced maternal or fetal toxicity. In contrast, they suggest that nicotine can mitigate some of the consequences of *in utero* exposure to cocaine in this model. The mechanism of this effect is currently unknown.

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