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Chronic Cocaine Exposure Affects Stimulus-Induced But Not Spontaneous Behavior of the Near-Term Mouse Fetus

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COPPOLA, D. M., L. C. MILLAR, C.-J. CHEN AND J. G. VANDENBERGH. *Chronic cocaine exposure affects stimulus-induced but not spontaneous behavior of the near-term mouse fetus.* PHARMACOL BIOCHEM BEHAV **58**(3) 793–799, 1997.—Pregnant female mice were injected subcutaneously with a 40-mg/kg dose of cocaine-HCl or physiological saline from day 1 through day 17 of gestation. On day 18 of gestation, dams were surgically prepared to allow the behavior of their fetuses to be observed. Spontaneous motor behavior was unaffected by cocaine exposure. Cocaine exposure potentiated motor responses of the fetuses to ammonia and to control injections of saline into the amniotic sac. Restriction of umbilical blood flow caused a specific stereotyped response in saline-injected fetuses, which is in agreement with studies of other species. This response was markedly potentiated in fetuses exposed to cocaine. The results suggest that the mouse may be a viable model for studies of the neurodevelopmental effects of gestational cocaine exposure and are discussed in relation to current models of the effects of long-term cocaine exposure on brain neurochemistry. © 1997 Elsevier Science Inc.

Cocaine Fetus Behavior Gestation Prenatal

IN the United States alone, there are an estimated 5 million regular users of cocaine and as many as 5000 new users of the drug each day (8). Cocaine has recently surpassed ethanol and opiate narcotics as the most frequent cause of drug-related emergency room admission (8). In one clinical study, as many as 15% of the women with high-risk pregnancies tested positive for cocaine sometime during their pregnancy (15). By any measure, cocaine abuse is a serious public health issue, the true magnitude of which can only be realized when one considers the potential carry-over effects to the fetus from maternal drug abuse. Although there is a growing literature dealing with the potential negative effects on the fetus of maternal cocaine abuse [reviewed in (8)], the available clinical and animal studies leave wide gaps in our knowledge. Animal studies, which can provide a measure of control over confounding variables unattainable in clinical studies, have established a litany of deleterious effects of cocaine exposure during gestation, ranging from soft-tissue and skeletal abnormalities to rather subtle behavioral alterations [reviewed in (7)].

With regard to neurodevelopmental effects of chronic cocaine exposure, investigators have shown that fetal rats exposed to cocaine display learning and memory deficits when tested postnatally (25,26). Studies of synaptic development have established that chronic cocaine exposure has marked and relatively permanent effects on forebrain function (6). More recently, clinical and animal studies of fetal cocaine exposure have focused on the behavioral state of the fetus. Studies on humans and sheep have demonstrated that behavioral state variables are altered in fetuses chronically exposed to cocaine (3,9,10). In experiments using acute animal preparations, spontaneous and evoked behaviors are altered by direct injection of cocaine into the rat fetus (16,17). Further knowledge regarding changes in fetal behavior engendered by drug exposure are not only important for predicting postnatal outcomes and planning therapeutic interventions but also may be essential for understanding the etiology of postnatal behavioral sequelae.

The goal of the present study was to determine the effects of chronic cocaine exposure during gestation on the spontaneous and evoked behavior of the near-term mouse fetus. The mouse has been used less commonly in neurodevelopmental studies of cocaine [but see (11,12)]. Studies in the mouse will provide

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important across-species comparisons and may offer a model with greater potential for genetic analysis of drug dependency.

Responses to chemosensory stimuli and umbilical cord ligature, both known to evoke marked increased motor activity in the fetal rat, were examined by using techniques for observing the behavior of the unanesthetized fetus. The results support the conclusion that chronic cocaine exposure increases the behavioral responses of the fetus to external stimuli while leaving spontaneous locomotor behavior unchanged.

METHOD

Subject Mothers

Thirty-eight, colony-born, ICR female mice, weighing 30–45 g, were used for this study. They were mated with ICR male mice and subsequently housed individually on the day a sperm plug was first observed. This day was considered as embryonic day 1 (E1). Pregnant dams were maintained at a temperature of 22–24°C, on a 14–10-h light–dark schedule (lights on at 0500) in polypropylene cages with corn cob bedding. Mouse chow and water were always available. Husbandry and experimental procedures followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

On day 1 of gestation (E1), the pregnant dams were assigned randomly to either a cocaine treatment group ($n = 17$) or a saline control group ($n = 21$). From E1 through E17 (birth usually occurs on E19), each dam was administered twice daily subcutaneous injections of either a sterile saline solution or a 20-mg/kg dose of cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) for a total daily dose of 40 mg/kg in the drug treatment group. The first daily injections were given between 800 and 830 h and the second daily injections between 1500 and 1700 h. The cocaine-HCl was dissolved in a sterile saline solution at a concentration that allowed each animal to receive a 20-mg/kg dosage in a volume of approximately 0.30 ml. The saline injections were isovolumetric to the cocaine solutions administered. Actual volumes for both cocaine-HCl and saline injections were 0.15–0.40 ml. No lesions of the skin at the sites of injection were noticed during the study.

Surgical Preparation

To observe the behavior of unanesthetized fetuses, the dams were prepared surgically on E18 of gestation, 1 day after the last cocaine or control injection. The surgery, termed a chemomyelotomy [reviewed in (18)], eliminates sensation from the caudal half of the mother while avoiding the effects of general anesthesia on fetal behavior. The techniques used in the present study for producing chemomyelotomy follow those of other investigators and have been described in detail elsewhere (18). Briefly, under Metofane anesthesia, each dam was administered an injection of 10–20 μl of 100% ethyl alcohol into the spinal cord between the first and second lumbar vertebrae. This procedure produces an irreversible blockade of the spinal cord at the site of injection, paralyzing and desensitizing the hindquarters and lower abdomen. Following the chemomyelotomy, the dam was allowed to recover from the anesthesia for at least 15 min to assure that she was alert, breathing normally and completely desensitized.

Preparation of Fetuses

Following recovery from the chemomyelotomy, each dam was lightly anesthetized with Metofane to secure the dam in a supine position with rubber straps onto a metal plate. After receiving a low midline laparotomy (ca. 2.5 cm in length), the dam, now attached to the metal plate, was placed in an inclined position within a temperature-controlled (37.5 ± 0.5°C) saline bath, with the water level adjusted to be just above the midline incision. The uterus was then exteriorized, and the mother and fetuses were allowed to recover from the anesthesia and acclimate to the water bath for 15 min. Throughout the subsequent behavioral videotaping, the dams appeared calm, showing no outward signs of discomfort.

For each dam, one to four fetuses were selected one at a time for behavioral videotaping. Each fetus was prepared in one of two ways—"in-amnion" or "in-bath"—for observation of behavior. For the in-amnion preparation, the fetus, with its attached membranes, was delivered through a 7–10-mm incision in the uterine wall. Behavior was then recorded through the transparent amniotic membrane. For the in-bath preparation, the fetus was delivered into the bath through incisions in the uterus, amnion and chorion before behavioral recording proceeded. For both fetal preparations, the competence of the uterine–placental attachment and the umbilical cord were checked throughout the videotaping period. Data from fetuses that showed any signs of vascular compromise or other abnormalities were excluded from further analysis.

Behavioral Observations

The behavior of each subject fetus was videotaped at real-time speed for either 6 or 10 min between 1000 and 1400 h. Subsequently, videotapes were replayed at real-time speed and behaviors were recorded on an event recorder. Observations and recordings were done by a single observer who was blind to the maternal treatment (cocaine vs. saline) groups. Making the videotapes first instead of recording the behavior live was deemed helpful in ensuring high measures of reliability for the behavioral recording system. Reexamination of the same tape segments established reliability rates in excess of 90%. At the completion of each behavioral videotaping session, the dams and litter were killed with an overdose of Metofane, and litter counts and fetal weights were recorded.

Categories of behavior. Nine behavioral categories were used to record fetal behavior: five "basic" and four "stereotypical complex." The basic categories refer to a region of the body moved, including tail, trunk, head, hindlimbs and forelimbs. These basic categories can occur either singly or two or more simultaneously and were recorded as such. For the hindlimb and forelimb categories, bilateral and unilateral movements were not distinguished. The stereotypical complex categories are comprised of behaviors that not only involve simultaneous movement of different regions of the body but also are stereotypical with respect to the coordination of the movements. The stereotypical complex categories include body undulation (smooth continuous movement of the head, trunk, one or more limbs), body stretch (dorsal extension of the head and straightening of the trunk, lasting 1–4 s), body jerk (a sudden, sharp movement of the entire body, lasting less than 1 s) and head–limbs (a coordinated and simultaneous movement of any limb with the head). Each category was recorded as an instantaneous point event, producing frequency counts as the basis for the quantitative analysis.

Responses to external stimuli. Besides studying the spontaneous motor patterns of the fetuses in the two treatment groups, their responses to external stimuli were also compared. Near-term rat fetuses are highly responsive to chemical stimuli and show a marked and stereotyped response to um-

bilical constriction, which appears to be an ontogenetic adaptation (20,21). Therefore, as stimuli, we chose ammonium hydroxide, a potent trigeminal irritant, and umbilical cord ligature. Each fetus was randomly assigned to receive chemical stimulation or umbilical ligature.

For the chemical stimulation experiment, subjects received either an ammonium hydroxide solution or a saline solution and were observed in-amnion. Their behavior was videotaped for two consecutive 5-min periods, the first period being prior to delivery of the stimulus and the second immediately following the delivery of the stimulus. The stimuli were either 0.1 ml of 0.09% saline in distilled water or 0.1 ml of a 0.05 M ammonium hydroxide solution in saline. These solutions were introduced to the fetuses via intra-amniotic injections into the open space between the chest and chin of the fetus. A 30-gauge needle was used for the injections to minimize damage to the amniotic membrane.

For the umbilical restriction experiment, the fetuses were observed in-bath while their behavior was recorded for two consecutive 3-min periods. The first period was prior to umbilical restriction (prestimulus), and the second period followed umbilical restriction (poststimulus). The umbilical restriction was achieved by ligating the umbilical cord with suture (00 Silk) approximately 1 cm from the fetal umbilicus. The suture was tied tightly enough to stop blood flow without producing obvious damage to the umbilical vessels.

The 17 dams in the cocaine treatment group had 24 fetuses in the ammonium hydroxide test group, 12 in the saline control group and 8 in the umbilical restriction test group. The 21 dams in the saline treatment (control) group had 27 fetuses exposed to ammonium hydroxide, 12 exposed to saline and 17 in the umbilical restriction test group.

Experimental Design and Data Analysis

Average fetal weight, total mass of litter and number of fetuses in a litter were compared for saline-treated and cocaine-treated dams by independent sample *t*-tests.

The length of test period, 5 min in the chemical stimulation experiment vs. 3 min in the umbilical restriction experiment, was decided on the basis of pilot studies that have demonstrated that the response to the 3-min treatment is more rapid. Because of this difference, the results of the two kinds of treatments were analyzed and are described separately. Because a given dam contributed fewer than two fetuses on average and treatments were counterbalanced within a dam, maternal effects (differences between litters) are unlikely to have contributed significantly to the results and have been ignored in the following presentation.

For chemical stimulation tests, drug treatment (cocaine or saline) is compared with type of stimulus (ammonia or saline) in a $2 \times 2 \times 2$ repeated measures analysis of variance (ANOVA) with test period (before and after stimulus delivery) as the repeated measure. A separate ANOVA was run on each behavioral variable individually and on the sum of all the different behavioral categories, termed "total behavior," and the number of different behaviors displayed, termed "behavioral complexity." ANOVA was performed by using the multivariate general linear hypothesis method within SYSTAT statistical software (version 5.1, Evanston, IL). This method accounts for the unbalanced design (unequal sample sizes) that was a random aspect of the experiment. Because there were only two levels of each factor, no post hoc tests were needed. For graphical display, the results are reported as the difference between the pretest and the posttest.

TABLE 1

MEANS ± SEM ARE SHOWN FOR THREE LITTER VARIABLES FROM SALINE (n = 25) OR COCAINE (n = 19) TREATED MOUSE DAMS. THERE WERE NO SIGNIFICANT DIFFERENCES BETWEEN THE MEAN VALUES FOR COCAINE AND SALINE TREATED GROUPS

	Saline	Cocaine
Fetal Wt. (g)	1.01 ± 0.16	0.97 ± 0.16
Litter Mass (g)	14.4 ± 4.0	14.2 ± 3.2
Litter Size	14.0 ± 2.8	14.6 ± 2.0

For umbilical restriction, drug treatment was compared in a 2×2 repeated measures ANOVA, with test period as the repeated measure. Other procedures were the same as those described for chemical stimulation.

RESULTS

Cocaine Effects on Litter Parameters

There were no significant differences in average fetus weight, total mass of litter or number of fetuses in the litter between saline-treated and cocaine-treated groups (Table 1). In each case, the values were within the normal range for this strain of mouse in our colony.

Cocaine Effects on Spontaneous Activity

Fetuses of cocaine-treated dams did not differ from saline controls in the amount of spontaneous activity recorded, as shown by the lack of significant main effects for drug in the ANOVA of the chemical stimulation experiment (5-min tests). Drug was not a significant factor when total behavior, behavioral complexity or any of the individual behaviors were used as the dependent variable. The absence of drug effects on spontaneous activity was corroborated in the tests involving umbilical cord ligature (3-min tests). Only in the case of the variable stretch was there a significant main effect for drug, which was due to a significant interaction between drug and ligature rather than to a difference in spontaneous (prestimulus) activity. Taken together, the results of both experiments provide no evidence that chronic cocaine exposure during gestation leads to a change in spontaneous motor activity. The data in Table 2 allow for a comparison of the pretest behavior of the cocaine-treated and saline-treated fetuses for both the 5-min and 3-min tests.

Effects of Chemical Stimulation

The results of chemical stimulation (Fig. 1) were consistent with several studies on rats, suggesting that chemical stimulation of the orofacial region leads to an increase in behavioral output in the near-term fetus (21–23). Injecting an ammonia solution led to a significant increase in total behavior [$F(1, 69) = 11.7$, $p < 0.001$] and behavioral complexity [$F(1, 69) = 8.0$, $p < 0.006$]. The results of the analyses for the individual behaviors demonstrate that the response was rather nonspecific because ammonia caused a significant increase in the movement of most body regions including the variables forelimbs, hindlimbs, head–limbs, and body. Stretch behavior, one of the complex movement categories, also was significantly affected. For

TABLE 2

MEANS ± SEM ARE PROVIDED FOR TWO COMPOSITE MEASURES OF BEHAVIOR, TOTAL BEHAVIOR AND BEHAVIOR COMPLEXITY, FOR COCAINE TREATED AND SALINE TREATED FETUSES. ONLY THE DATA FROM THE PRETEST, BEFORE ANY STIMULUS WAS INTRODUCED, ARE INCLUDED. DATA FROM THE TWO STIMULUS CONDITIONS AMMONIA AND SALINE HAVE BEEN COMBINED (5 MIN TESTS). AS JUDGED BY ANOVA (SEE TEXT) COCAINE DID NOT CAUSE A SIGNIFICANT CHANGE IN SPONTANEOUS BEHAVIORAL ACTIVITY IN EITHER THE 3 MIN OR 5 MIN TESTS.

	Total Behavior		Behavioral Complexity	
Period	Saline	Cocaine	Saline	Cocaine
5 min	32.4 ± 9.7	35.4 ± 9.7	4.3 ± 0.6	4.7 ± 0.6
3 min	18.1 ± 3.5	15.7 ± 6.1	4.2 ± 0.5	3.5 ± 0.7

5-min tests: N = 38 for saline, 35 for cocaine. 3 min tests: n = 17 for saline, 8 for cocaine.

the subjects of saline-treated mothers, saline injection into the amnion did not lead to a significant change in behavior (Fig. 1).

Examination of the ANOVA results for the interaction between drug and test period provide evidence for an effect of drug on behavioral responses. The significant effect of ammonia stimulation on both forelimb [$F(1, 69) = 4.1$, $p < 0.05$] and hindlimb [$F(1, 69) = 4.7$, $p < 0.03$] movement was potentiated in the cocaine-treated subjects (Fig. 1). However, there was an unexpected result that complicates the interpretation of the analysis. It is clear from Fig. 1 and the ANOVA results that the saline stimulus also increased forelimb and hindlimb movements for the cocaine-treated but not for the saline-treated subjects. This increase in the response to saline and ammonia in the groups that had received cocaine injections was manifested in the lack of a significant three-way interaction between stimulus, test period and drug treatment. There were no other significant main effects or significant interactions involving the factor drug.

Effects of Umbilical Cord Ligature

Umbilical cord ligature caused a highly significant and specific response from the fetuses (Fig. 2). Forelimb movements

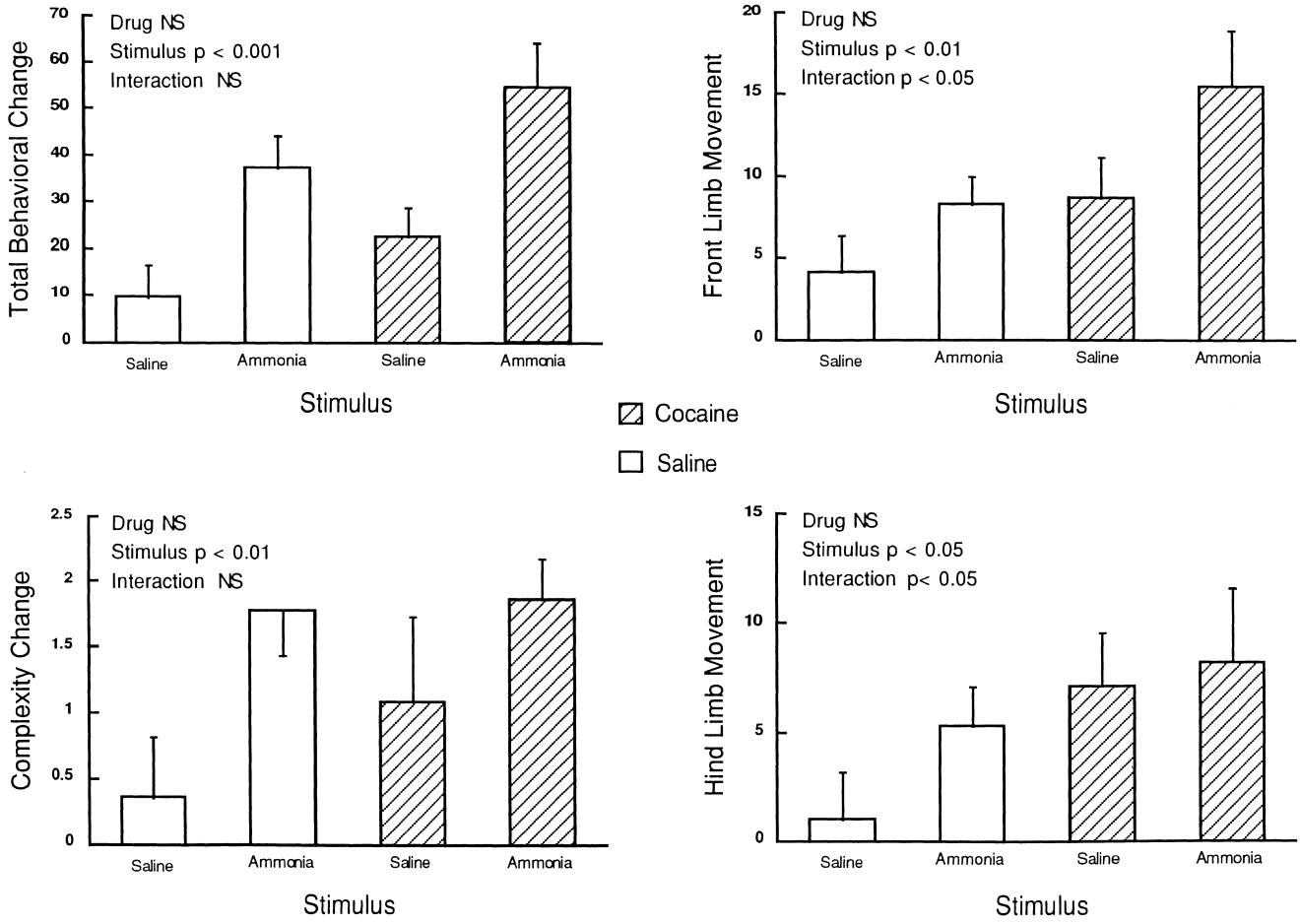

FIG. 1. Columns represent the means and vertical lines represent the SEM for the differences between the behavioral counts in the 5 min prior to stimulus delivery (pretest) and after stimulus delivery (posttest). Significance levels are derived from the repeated measures ANOVA ($df = 1, 69$). The interaction is for Drug × Period (pretest vs. posttest). None of the three-way interactions among stimulus, drug and period were significant. NS = nonsignificant ($p > 0.05$).

[$F(1, 23) = 11.9$, $p < 0.002$] and stretch movements [$F(1, 23) = 28.3$, $p < 0.0001$] increased in frequency after ligature, which also resulted in significant changes in total behavior [$F(1, 23) = 19.8$, $p < 0.0001$] and behavioral complexity [$F(1, 23) = 6.2$, $p < 0.0001$]. None of the other behaviors were significantly affected by umbilical cord ligature. Drug treatment did not significantly affect spontaneous activity, as shown by the lack of a drug main effect for most variables. However, for the stretch behavior, there was a highly significant [$F(1, 23) = 8.1$, $p < 0.0009$] main effect for drug that must be interpreted in light of a highly significant interaction between drug treatment and test period [$F(1, 23) = 8.9$, $p < 0.007$]. It is clear from a comparison of prestimulus period activity and poststimulus period activity that cocaine markedly potentiated the stretch response to umbilical cord ligature, whereas the spontaneous rate of the stretch behavior was unaffected (Fig. 2). There was also a significant interaction of drug treatment and test period for the complex variable head–limbs [$F(1, 23) = 7.3$, $p < 0.01$]. Examination of the individual means for drug treatment and test period reveals that the significant interaction of these factors was due to a decline in complex behavior after ligature in the saline group and a nonsignificant increase in complex behavior in the cocaine group. This last result is hard to reconcile with the other results because it is the only instance in which a stimulus resulted in a decrease in behavioral rate, a situation that was not mirrored in the individual behaviors, forelimb and head movement, of which this complex category is derived.

DISCUSSION

In agreement with most previous results of chronic gestational cocaine exposure (5,24), spontaneous behavior of the mouse fetus was not affected by cocaine exposure in the present study. In contrast, acute cocaine exposure causes a marked increase in rat fetal activity (17). The single dosage tested in the current study, 40 mg/kg/day delivered in two equal units, was chosen based on results from other rodent experiments (1,4,5,11,25). A similar daily dosage, injected intraperitoneally throughout gestation, failed to increase mortality,

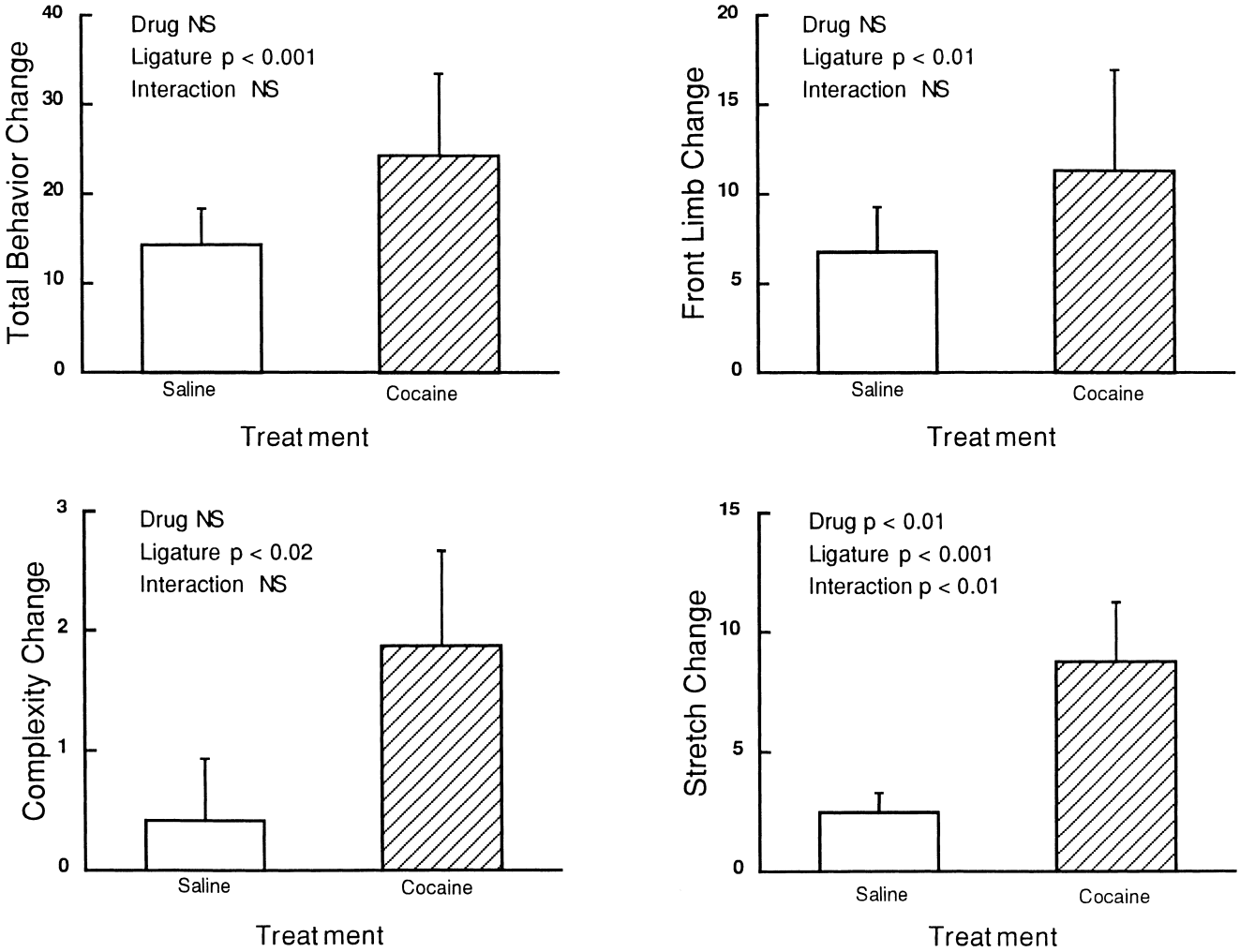

FIG. 2. Columns represent the means and vertical lines represent the SEM for the differences between the behavioral counts in the 3 min prior to (pretest) and after (posttest) umbilical ligation. Significance levels are derived from the repeated measures ANOVA ($df = 1, 23$). The interaction is for Drug × Period. NS = nonsignificant ($p > 0.05$).

soft-tissue malformations or weight changes in full-term mouse fetuses (1,4). However, the same dosage as used in the present study caused a reduction in weight gain through E14 in mouse embryos (11). In rats, daily subcutaneous injections of 40 mg/kg cocaine during gestation have produced neurobehavioral alterations in some studies (25) but not in others (5). Therefore, the dose used in the present study is likely below the level of toxicity and above the threshold for neurobehavioral effects, at least by some measures. The absence of toxic effects at the 40-mg/kg dosage is supported in the present study by the lack of cocaine effects on the survival and growth of fetuses. This lack of overt toxicity accords with the results of several other studies of rodents [e.g., (12,24,26), but see (11)].

The responses of the mouse fetus to chemical stimulation are in agreement with previous studies in rats (20,22,23). Rat pups exposed to high concentrations of chemical stimuli respond with a transient increase in frequency and complexity of motor activity. Although we did not study the time course of response, it is clear from our results that the mouse fetus also increases activity in response to chemical stimulation. In 5 of the 9 individual behavior categories, there was a significant increase when ammonia was delivered, resulting in a significant increase in total activity and behavioral complexity (number of different behaviors displayed). Cocaine, although having no influence on spontaneous motor activity, potentiated the fetal response to chemical stimulation in a specific but unexpected way. Both forelimb and hindlimb movements, behaviors that make up a large percentage of overall behavior in the mouse fetus, were significantly more affected by stimulation in cocaine-treated fetuses. Surprisingly, this result was true for both saline and ammonia injections, suggesting that saline was not an undetectable or neutral stimulus. Significant responses to saline were only manifest in cocaine-treated animals (Fig. 1). The response evoking features of saline, which was meant to be a control stimulation, are unknown. The disturbance of the fetal membranes and amniotic fluid caused by the injection may have triggered the response. Alternatively, the difference in temperature or chemical makeup of the saline solution vs. amniotic fluid may have been the basis of the response. Whatever triggered the response in the cocaine group, the saline control group did not respond when saline was the stimulus. Cocaine exposure also potentiated the response to ammonia; however, the potentiation was not significantly greater than that for saline, as shown by a lack of a three-way interaction in the ANOVA among stimulus, drug and period. Taken together, these results suggest that gestational cocaine exposure creates heightened sensitivity of the fetus to somatosensory and chemosensory stimuli.

Obstructing flow of the umbilical vessels caused a stereotypical response in the mouse fetus similar to that previously described for the rat (20). Both species display dorsal extensions of the head and trunk that are rare or absent from the repertoire of nonevoked behavior. It has been convincingly argued that this response, which has been studied in a number of rodent species, is an ontological adaptation that helps return the blood flow in the umbilical vessels to normal, after the cord is collapsed by changes in fetal or maternal positioning (20). Whatever the cause of this stereotyped behavior in the mouse, responses were potentiated in the present study by cocaine exposure. The response variable stretch, consisting of the dorsal extensions already described, was performed more than three times as often on average in the cocaine group vs. the saline group after umbilical ligature (Fig. 2). Another laboratory reported that the responses of fetal rats to umbilical cord compression were diminished if they had been acutely exposed to alcohol (19). Taken together, these results suggest that this response might be more widely exploited for studying the effects of substance abuse on fetal behavior.

The present results of both experiments support the conclusion that cocaine exposure during gestation influences evoked but not spontaneous behavior in the fetus. It is interesting, in light of these data, that a recent clinical study employing ultrasound observations found a higher frequency of startle behavior in fetuses from mothers that used cocaine during gestation (9). If these findings prove widely applicable, they might require a reevaluation of the developing nervous system's sensitivity to cocaine because most studies of fetal behavior in humans and sheep have focused on nonevoked behavioral state variables (3,9,10). Two considerations mitigate our conclusions. First, our experimental design does not distinguish direct from indirect effects of cocaine exposure. The response changes reported in the present study may have been due to the vasoconstrictor effects of cocaine on the maternal and fetal cardiovascular system (indirect effect) rather than on neurotransmitter systems of the fetal nervous system (direct effect). For example, the marked increase in response to umbilical cord ligature may have been due to sensitization of the fetus to hypoxia because the vasoconstrictor effect of cocaine on the umbilical vessels is known to result in just such fetal oxygen deprivation [reviewed in (8)]. Insufficient data are available to resolve this point, but clearly further studies are warranted given our findings. A second complication arises from the dosing regimen. Specifically, the experimental design was directed at examining the neurodevelopmental effects of chronic cocaine exposure rather than the short-term influences of cocaine on behavior. To exclude the short-term influence, cocaine injections were stopped 1 day prior to behavioral testing. However, this regimen leaves untested the possibility that the behavioral alterations observed were a function of temporary withdrawal phenomena rather than of more permanent effects on neurobehavioral development. In this regard, two recent studies in rats have reported decreases in the response to tactile and chemical stimulation in fetuses after a single injection of cocaine, just the opposite of the potentiation of response reported in the present study (16,17). Also, potentiation of responses is inconsistent with at least one study of the withdrawal syndrome in rat pups, which reported a diminished responsiveness to external stimuli (2).

Some data are available that might explain the neural substrates of the behavioral alterations after gestational cocaine exposure reported herein. Cocaine, a potent inhibitor of dopamine (DA) re-uptake at presynaptic terminals, can lead to upregulation of the D_2 type of DA receptor in young rats with gestational exposure to the drug (13). This upregulation appears to occur selectively in the striatum but not in the nucleus accumbens [(14), cited in (13)], both parts of the DA pathways in the brain. The striatum is thought to mediate stereotypical behavior, whereas the nucleus accumbens may mediate more general locomotor behavior [reviewed in (13)]. These findings may explain why a stereotypical behavioral response like that observed after umbilical ligature was influenced by gestational cocaine exposure but nonevoked behavior was unaffected. However, given the complex pattern of alterations in the DA system and other transmitter systems in the brain that attend chronic cocaine exposure, this apparent convergence of evidence remains tentative pending further studies.

In a previous study, the same gestational dosage and treatment regimen as used in the present study caused a deficit in first-order conditioning in 9-day-old mice (11). Our results support the conclusion that fetal behavior is affected by gesta-

tional cocaine exposure at a dosage that is not overtly toxic to mother or fetus. However, behavioral alterations were observed in response to chemical stimulation or vascular compromise but were not found in nonevoked behavior. This finding suggests that a simple assessment of state variables, heretofore a common practice in fetal behavior studies, may be insufficient in studying the effects of maternal cocaine abuse.

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REFERENCES

1. Arpasi, P.; Chakraborty, J.: Effects of cocaine on fetal and postnatal development in mice. Life Sci. 52:2063–2069; 1993.
2. Barr, G. A.; Wang, S.: Behavioral effects of chronic cocaine treatment in the week-old rat pup. Eur. J. Pharmacol. 233:143–149; 1993.
3. Burchfield, D. J.; Graham, E. M.; Abrams, R. M.; Gerhardt, K. J.: Cocaine alters behavioral states in fetal sheep. Dev. Brain Res. 56:41–45; 1990.
4. Chen, C.-J.; Vandenbergh, J.: Effect of chronic cocaine on reproduction in female house mice. Pharmacol. Biochem. Behav. 48: 909–913; 1994.
5. Church, M. W.; Overbeck, G. W.: Prenatal cocaine exposure in the Long-Evans rat: II. Dose-dependent effects on offspring behavior. Neurotoxicol. Teratol. 12:335–343; 1990.
6. Dow-Edwards, D. L.: Long-term neurochemical and neurobehavioral consequences of cocaine use during pregnancy. Ann. N.Y. Acad. Sci. 562:280–289; 1989.
7. Dow-Edwards, D. L.: Cocaine effects on fetal development: A comparison of clinical and animal research findings. Neurotox. Teratol. 13:347–352; 1991.
8. Ellis, J. E.; Byrd, L. D.; Sexson, W. R.; Patterson-Barnett, C. A.: In utero exposure to cocaine: A review. South. Med. J. 86:725–731; 1993.
9. Hepper, P. G.: Human fetal behaviour and maternal cocaine use: A logitudinal study. Neurotoxicology 16:139–143; 1995.
10. Hume, R F.; O'Donnell, K. J.; Stanger, C. L.; Killam, A. P.; Gingras, J. L.: In utero cocaine exposure: Observations of fetal behavioral state may predict neonatal outcomes. Am. J. Obstet. Gynecol. 161:685–690; 1989.
11. Kosofsky, B. E.; Wilkins, A. S.; Gressens, P.; Evrard, P.: Transplacental cocaine exposure: A mouse model demonstrating neuroanatomic and behavioral abnormalities. J. Child Neurol. 9:234–241; 1994.
12. Laviola, G.; Fiore, M.; Loggi, G.; Alleva, E.: Prenatal cocaine potentiates the effects of morphine in adult mice. Neuropharmacology 33:825–831; 1994.
13. Moody, C. A.; Frambes, N. A.; Spear, L. P.: Psychopharmacological responsiveness to the dopamine agonist quinpirole in normal weanlings and in weanling offspring exposed gestationally to cocaine. Psychopharmacology 108:256–262; 1992.
14. Scalzo, F. M.; Ali, S. F.; Frambes, N. A.; Spear, L. P.: Weanling rats exposed prenatally to cocaine exhibit an increase in striatal D_2 dopamine binding associated with an increase in ligand affinity. Pharmacol. Biochem. Behav. 37:371–373; 1990.
15. Shannon, M.; Lacouture, P. G.; Roa, J.: Cocaine exposure among children sent at a pediatric hospital. Pediatrics 83:337–342; 1989.
16. Simonik, D. K.; Robinson, S. R.; Smotherman, W. P.: Central administration of cocaine produces age-dependent effects on behavior in the fetal rat. Behav. Neurosci. 108:1179–1187; 1994.
17. Simonik, D. K.; Robinson, S. R.; Smotherman, W. P.: Cocaine alters behavior in the rat fetus. Behav. Neurosci. 107:867–875; 1993.
18. Smotherman, W. P.; Richards, L. S.; Robinson, S. R.: Techniques for observing fetal behavior *in utero*: A comparison of chemomyelotomy and spinal transection. Dev. Psychobiol. 17:661–674; 1984.
19. Smotherman, W. P.; Robinson S. R.: Stereotypic behavioral response of rat fetus to acute hypoxia is altered by maternal alcohol consumption. Am. J. Obstet. Gynecol. 157:982–986; 1987.
20. Smotherman, W. P.; Robinson, S. R.: Response of the rat fetus to acute umbilical cord occlusion: An ontogenetic adaptation. Physiol. Behav. 44:131–135; 1988.
21. Smotherman, W. P.; Robinson S. R.: Behavior of rat fetuses following chemical or tactile stimulation. Behav. Neurosci. 102:24–34; 1988.
22. Smotherman, W. P.; Robinson S. R.: Rat fetuses respond to chemical stimuli in gas phase. Physiol. Behav. 47:863–868; 1989.
23. Smotherman, W. P.; Robinson S. R.: Olfactory bulb transection alters fetal behavior after chemosensory but not tactile stimulation. Dev. Brain Res. 57:175–180; 1990.
24. Sobrian, S. K.; Ali, S. F.; Slikker, W. Jr.; Holson, R. R.: Interactive effects of prenatal cocaine and nicotine exposure on maternal toxicity, postnatal development, and behavior in the rat. Mol. Neurobiol. 11:121–143; 1995.
25. Spear, L. P.; Kirstein, C. L.; Frambes, N. A.: Cocaine effects on the developing central nervous system: Behavioral, psychopharmacological, and neurochemical studies. Ann. N.Y. Acad. Sci. 562:290–307; 1989.
26. Vorhees, C. V.; Reed, T. M.; Acucc-Smith, K. D.; Schilling, M. A.; Cappon, G. D.; Fisher, J. E.; Cunfeng, P. U.: Long-term learning deficits and changes in unlearned behaviors following in utero exposure to multiple daily doses of cocaine during different exposure periods and maternal plasma cocaine concentrations. Neurotoxicol. Teratol. 17:253–264; 1995.