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Cocaine Disrupts Pup-Induced Maternal Behavior in Juvenile and Adult Rats

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Abstract

Impaired onset of maternal behavior in first generation rat dams was previously correlated with rearing by cocaine-treated dams and prenatal cocaine exposure. Pup-induced maternal behavior in non-lactating rats has not been examined with regard to cocaine exposure and rearing conditions. First generation male and female juveniles and young adult males reared by cocaine-treated or control dams and prenatally exposed to either cocaine or control conditions were tested for pup-induced maternal behavior at postnatal days 28 and 60. We now report disruptions in pup-induced maternal behavior in both 28 and 60 day old first generation offspring attributable to rearing condition and prenatal cocaine exposure.

Keywords

Pup-Induced Maternal Behavior; Rats; Cocaine; Rearing Environment; Prenatal Cocaine Exposure

1. Introduction

Maternal cocaine abuse during pregnancy has been correlated with generally poor maternal behavior in humans, including higher levels of maternal neglect and poor maternal-infant bonding [15,49,51]. It is probable that drug-induced maternal neglect has detrimental effects on the future social and parental behavior of adult children, especially given that many of these children are also prenatally exposed to cocaine. Previous reports using animal models found that gestational and postpartum cocaine treatment cause significant delays and disruptions in various aspects of early maternal behavior in the treated rat dam [16,17,20,23,25,39,50,55] and

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that rearing condition as well as prenatal cocaine exposure has detrimental effects on the onset of maternal behavior in next generation female offspring [17].

Pup-induced maternal behavior, historically called pup sensitization or concaveation [29,40, 45] is a procedure where both virgin female and male rats may be induced to express parental behavior towards newborn pups through continuous exposure over a period of time. Following multi-day exposure to young (1–5 days old) pups, male and virgin female rats have been shown to exhibit maternal behavior, including licking, touching, retrieval, nestbuilding, and sometimes in older rats, standing over or lying on pups in a semi-nursing posture [9,40,42, 45,46]. These studies have provided interesting clues concerning possible mechanisms underlying specific aspects of maternal behavior and possible links to performance in general social interaction situations. The validity of pup-induced maternal behavior as a modified model of maternal behavior is supported by the overlap in brain regions and pathways implicated in both normal and induced maternal behavior [34,35].

It is important to note that adult male and female pup-induced maternal behavior seems to be largely mediated by the same pathways as normal maternal behavior during lactation, but this is not the case in juveniles between 21–28 days of age [24]. Though the intact medial preoptic area (MPOA) is essential for postpartum and pup-induced maternal behavior in adult rats [13,41,43], large lesions of the MPOA in adolescents impair retrieval and nest-building behaviors only, rather than all elements of maternal behavior and gender does not seem to play as important a role [24]. Whereas adult females typically exhibit pup-induced maternal behavior faster than adult males, the reverse is true in juveniles, with males typically taking fewer days to exhibit pup-induced maternal behavior compared to females and juveniles of both sexes generally perform it several days faster than adults [2–4,14,23,30,45,54].

Various rodent studies have reported numerous effects of prenatal exposure to cocaine on subsequent adolescent and adult play, social, and aggressive behaviors [23,38,52,53]. To our knowledge, no prior study has examined the differential effects of prenatal cocaine exposure and rearing condition on next generation offspring pup-induced maternal behavior, although we have recently reported differences in postpartum maternal behavior following prenatal cocaine exposure and altered rearing conditions [17]. Since pup-induced maternal behavior has postural and behavioral components similar to play solicitation and social behavior [3, 28] it seems reasonable to suggest that cocaine-induced deficits found in those behaviors may subsequently impact pup-induced maternal behavior as well in cocaine-exposed offspring.

The aim of the present study was to assess the effects of prenatal exposure condition (cocaine or control) and/or rearing condition (reared by cocaine-treated or control dam) on the induction of pup-induced maternal behavior in non-lactating offspring as juveniles and adults. We hypothesized that cocaine-exposed and cocaine-reared offspring would display less pup-induced maternal behavior than control offspring and that there would be no significant sex differences in juvenile behavior.

2. Methods

2.1 Subjects

2.1.1 Treatment Dams—Following a two week habituation period, virgin female (200 grams) Sprague-Dawley rats (Charles River, Raleigh, NC) were placed singly with males on a breeding rack until the day a sperm plug was found, designated as gestation day (GD) 0. Gravid females were randomly assigned to one of five treatment or control groups and singly housed and maintained on a 12:12 reverse light cycle (lights off at 0900) for 7 days. They were then transferred to a room with a regular light cycle (lights on at 0700) for the remainder of

the experiment, a procedure that generally results in the majority of dams delivering their litters during daylight hours [31].

Dam treatment/control groups included: chronic cocaine (CC), intermittent cocaine (IC), chronic saline (CS), intermittent saline (IS), and untreated (UN) dams. Chronic cocaine and CS dams received subcutaneous (sc) injections twice daily throughout gestation (GD 1–20) on alternating flanks, of 15 mg/kg cocaine HCL (dose calculated as the free base; Sigma Chemical Company, St. Louis, MO) dissolved in 0.9% normal saline (total volume 2 ml/kg), or normal saline (0.9%) respectively, at approximately 0800 and 1600. Intermittent cocaine-treated dams received the same dose and volume of cocaine as the CC dams, except that their injections only occurred on two consecutive days, every five days during gestation (GD 2, 3, 8, 9, 14, 15, 20) and on the same respective days during the postpartum period. Intermittent saline-treated dams received normal saline (0.9%) on the same injection schedule as the IC dams. The intermittent schedule was modeled after a previous study examining behavioral effects of prenatal cocaine exposure on offspring [19] and is designed to model intermittent usage patterns in humans. The IC treatment regimen was employed in addition to the CC treatment as previous research indicated differences in maternal behavior following either acute or intermittent cocaine treatment in dams [20,23,25,50,55] accompanied by differences in oxytocin (OT) system dynamics following the different treatment regimens [7,22]. UN dams were weighed and handled daily, but received no drug treatment. All treatment groups had free access to water and food (rat chow), except the CS treated dams, who were pair-fed to CC dams in order to control for the anorectic effects of cocaine, as previously described [17]. The IC and IS dams were given 50 grams of chow daily on injection days so that each group had equal amounts of food, and food consumption was measured for both groups on those days.

2.1.2 Cross-fostering—On the day of parturition, pups were removed from each dam, weighed, counted, and their gender determined before being culled to a litter of four males and four females. Total litter numbers for each group designation ranged from 12–16 generally, with a higher number of UNUN litters (28) listed in Table 1, as extra UN dams and pups were needed to provide pups for other experiments although they were not all included in this study. Litters were either returned to their natural mothers or fostered to dams from a different treatment or control group having delivered as closely as possible to the same time (usually within several hours and matched across groups for delivery time). The fostering procedure resulted in twenty five dam/offspring group combinations (see Table 1). Cross-fostering allowed for the independent assessment of the effects of prenatal drug exposure and the effects of rearing conditions (or the combination of these conditions, see Table 5) on pup-induced maternal behavior in offspring. In order to achieve sample sizes large enough within each of the 25 groups for assessment with parametric statistics, this study required four years to complete with hundreds of offspring born each spring. Each year, the same treatment and testing procedures were repeated in a new group of dams and their offspring which, while a practical necessity, also introduced some variability resulting from year of testing. This variability induced by year of testing was randomly spread across groups and did not influence group differences differentially over the four years.

On postnatal day (PND) 21, litters were weaned and housed in same sex litters of 4 males and 4 females for later testing. All procedures were conducted under federal and institutional animal care and use committee guidelines for humane treatment of laboratory subjects.

2.2 Behavioral Testing Procedures

2.2.1 Subjects and Test Procedures: Juveniles One male and one female pup from each separate treatment and control litter was randomly chosen for testing and were single housed on PND 27 in a standard rat cage that became their new home cage and which was also

used for testing. On the evening preceding the first testing day (PND 27), 10 strips of paper towel were placed in each cage as nest-building material. The following day (PND 28) both the pup-induced maternal behavior subjects and stimulus pups were taken from the animal colony room to a separate testing room and testing began between 0730 and 1200. Surrogate females provided fresh stimulus pups for testing (all between the ages of PND 1–5 as this is viewed as preferable [47]) such that on each test day a test subject was provided with three randomly chosen and well nourished (milk bands apparent) combinations of male and female pups who were kept with a lactating dam overnight.

After a two minute habituation without pups each subject was observed over a 15 minute period after the introduction of stimulus pups using an interval observation and scoring procedure where they were scored every five minutes for at least a single occurrence of any of the following behaviors: **retrieval of 1, 2, and 3 pups** (subject carries the pups in its mouth from the front to rear of cage); **group pups** (subject groups pups together); **lick pups** (subject licks a pup); and **stand over pup** (subject stands over or lies on top of at least 2 pups). Only the occurrence (not latency, duration or total frequency) of a behavior was noted during each of the 3 five minute epochs.

In order to reduce the risk of infanticide on the first day of testing, only one pup was placed in the cage for the first five minute epoch. If the subject had not attacked the pup after five minutes, two more pups were added and the session continued for an additional 10 minutes. Thereafter test sessions began with all three pups placed in the front of the cage at the start of testing. If pups were attacked at any point, testing was stopped, pups were immediately removed, a kill session was scored for that test subject and the subject was returned to the animal colony room until the following test day, when the same procedure would be repeated. Any subject that attacked a pup on any two days was removed from the study and their data was excluded from the sensitization analyses.

The criteria for the adequate performance of pup-induced maternal behavior in juveniles included retrieval and grouping of all three pups within the 15 minute period on two consecutive days, within seven days of testing. Cage bedding was not changed during the week of testing, as nest quality was scored on test days four and seven (new nesting paper was placed with the rat on the night before the day that the nest was scored). Nest quality was assessed according to the system described by Numan and Callahan [36] as follows: **0 = no nest; 1 = poor nest, no walls, no paper used; 2 = fair nest, flat, but all paper used; 3 = good nest, low walls, all paper used; and 4 = excellent nest, high walls, all paper used.**

After completion of a test session, the subject and their stimulus pups were returned to the animal colony room where the pups remained with the subject overnight. The following morning, the stimulus pups were removed from the subject's cage and returned to surrogate dams for a minimum of 24 hours. Subjects were brought to the testing room each day, and placed in the testing chamber with three new stimulus pups. Subjects continued testing until criteria was met or their time limit was reached (7 days for juveniles).

2.2.2 Subjects and Test Procedures: Adult Males On PND 59, a single male was randomly selected from each treatment and control litter, and singly housed for adult pup-induced maternal behavior testing beginning on PND 60. Ten strips of paper towel were placed in the cage as nest-building material. No females were tested for pup-induced maternal behavior in adulthood, because they were used for testing postpartum maternal behavior [17] and therefore none were available for pup-induced maternal behavior testing at this age. Testing began between 0730 and 1200 the following day (PND 60). Testing procedures and conditions were the same as for juvenile offspring, except that the criteria for success were different for adult pup-induced maternal behavior. The criteria for adult males included not only retrieval

and grouping of all pups, but also standing over at least 2 pups during the 15 minute period on two consecutive days, within a ten day period. When an adult subject met criteria on a given day, the following day's session was videotaped using a Panasonic VHS (AG188U) recorder with low light sensitivity and allowed to continue for 30 minutes, instead of the usual 15 minutes. If the subject failed to meet criteria again within the first 15 minutes of taping, the videotape was discarded and the session was terminated (as success required meeting criteria on two consecutive days). Adult subjects continued testing until criteria was met or their time limit was reached (10 days). The video cameras were always in the test room and have an almost undetectable noise level at the distance from which taping occurred relative to the cages. Recorded sensitization sessions for subjects who met criteria were later analyzed for frequency, duration, and latency of the following 8 behaviors: **touch/sniff pups** (subject touches the pups with its nose or front paws or sniffs them); **retrieve pup** (subject carries pup in its mouth from front to rear of cage); **group pups** (subject puts pups together in a group); **lick pup** (subject licks a pup); **nest-build** (subject manipulates or moves the paper strips with its mouth or paws); **stand over pup** (subject stands over or lies on top of at least 2 pups); **rear-sniff** (subject rears on hind legs and sniffs the cage or air); and '**other**' (any behavior other than those designated above).

Video taped sessions were scored by two independent observers blind to treatment condition with inter-and intra-reliability set at 95% or better concurrence for frequency and latency, and 80% or better for duration of behaviors displayed by the subject, using a computer program that calculated the frequency, duration, and latency of all relevant behaviors. Behaviors not displayed by the subject were assigned a frequency and duration of zero and the highest possible latency (1800 seconds for the 30-minute test).

2.3 Statistical Analyses

Data recorded for analysis for all groups included: gestational data, the number of days to criteria, number of attack animals (that attacked twice) per group, gender, prenatal exposure, and rearing conditions, nest quality scores, and successful completion rates. Subjects that killed pups on two days were excluded from comparisons of testing and completion rates and kill percentages were compared separately for groups. The percentage of subjects from each prenatal exposure condition and rearing condition to successfully meet criteria for pup-induced maternal behavior at PND 28 and 60 were compared using a one-tailed two-sample Z-test of proportion (Stata v8.2, StataCorp LP, College Station, Texas). Group comparisons included the chronic treatment and control groups (CC, CS, UN); the intermittent treatment and control groups (IC, IS, UN); and the combined non-fostered (same rearing and prenatal condition) comparison groups (CCCC, ICIC combined versus ISIS, CSCS combined versus UNUN). Gestational data was analyzed using an Analysis of Variance (ANOVA) followed by Tukey HSD post hoc tests where appropriate when overall significant differences were found. Behavioral data acquired from taped sessions following PND 60 testing were compared using a two factor Analyses of Variance (rearing condition × prenatal exposure) for between groups differences based on success or failure to meet criteria. Statistical significance was set at less than or equal to the $p \leq .05$ level.

3. Results

3.1 Treatment Dam Gestational Variables

Gestational and litter variables are presented in Table 2. There were significant effects of dam treatment on gestational weight gain [$F(4,354)=20.95$, $p \leq .01$] and litter birth weight [$F(4,360)=2.75$, $p \leq .03$]. CC- and CS-treated dams gained less weight than UN dams ($p \leq .01$), while IC dams gained less weight than both UN ($p \leq .01$) and IS dams ($p \leq .05$). Lower birth weight litters in the CC- and IC-treated dams were probably the result of cocaine treatment; however both

CC and CS also had slightly fewer pups although there were no significant differences between groups in the average weight per pup. There were also no significant differences between groups on weight at breeding, number of gestation days, food consumption between intermittent dam groups, pup number, pup gender ratio, or culled litter weight.

3.2 Real Time Observer Coded Behavior

3.2.1 PND 28 Pup-Induced Maternal Behavior—As table 3 indicates, there was a significant effect of rearing condition on the proportion of animals reaching criteria, such that a lower proportion of offspring reared by IC-treated dams met criteria than offspring reared by untreated dams ($z=3.0$; $p\leq.01$), but there were no effects of prenatal exposure condition or gender on success rates. Nest quality also did not differ between groups. Similar group scores were found for the mean number of days to reach criteria and there were no differences in the percentage of kills between treatment and control offspring. All animals that killed a stimulus pup initially went on to kill a second time eventually and were thus excluded from analyses.

3.2.2 PND 60 Pup-Induced Maternal Behavior—As indicated in Table 4, a significantly lower proportion of male offspring reared by CC-treated dams met criteria compared to CS-reared offspring ($z=2.3$, $p\leq.02$), and although there was a lower proportion of IC-exposed males that were successful, means were not significantly different from UN- or IS-exposed males (kills were not included in the criterion comparison and animals that killed were not included as test subjects). At PND 60 there were no effects of prenatal exposure on criteria success rates, nest quality (average score of 2), number of days to meet criteria, or percent that killed stimulus pups.

3.2.3 Non-Cross-fostered Offspring—Table 5 indicates results for combined rearing and prenatal exposure conditions (pups were not cross-fostered). Statistics include both separate and combined group means with both cocaine offspring groups (ICIC, CCCC) compared to control groups (UNUN, ISIS, CSCS). There were no differences in criteria success between combined CCCC and ICIC groups at PND 28 compared to combined control CSCS, ISIS, or UNUN groups reared by their own dam (Table 5). Interestingly, fewer PND 60 male offspring in ICIC and CCCC groups combined met criteria compared to combined control groups CSCS, ISIS ($z=2.65$; $p\leq.01$), and UNUN males ($z=1.67$; $p\leq.05$).

3.3 Videotaped Behavior

At PND 60, there was a significant main effect of rearing condition on the duration of touch sniff [$F(4,16)=2.18$, $p\leq0.04$]. CC reared males who met criteria touched pups for a shorter time period (duration) than CS reared males that met criteria ($p\leq0.02$). There were no other significant group differences.

4. Discussion

Although we found group differences in successful pup-induced maternal behavior, they were not entirely as predicted. We predicted that fewer pups would meet criteria if they were exposed prenatally to cocaine or if they were reared by a cocaine-treated dam than pups with saline or no exposure that were reared by saline-treated or untreated dams. Our findings suggest that prenatal cocaine exposure alone has little effect on pup-induced maternal behavior in the late juvenile period with no sex-related effects in PND 28 offspring. Expectations of differences resulting from prenatal exposure to cocaine in the PND 28 offspring were based on data showing that prenatal cocaine exposure has been associated with deficits in play behavior and play solicitation in young juvenile offspring [4,52,53]. Since these behaviors have components similar to those involved in pup retrieval and social interaction, it is interesting that there was no translation of disruption of pup-induced maternal behavior resulting from prenatal exposure

in our study. Overall, success rates for all groups were lower than those previously reported in the non-drug paradigms of maternal behavior literature, which may primarily be a reflection of the age we began testing and our selected criteria for success. Many previous reports of pup-induced maternal behavior in juveniles tested at earlier ages (less than PND 25). It has recently been reported that testing begun after PND 27 can result in a sharp drop in the interaction of juveniles with pups and indeed that PND 30 rats sometimes have lower interaction rates, almost identical to post-pubertal (PND 60) rats [24,37]. Our PND 28 baseline rates were already somewhat low, probably accounting for our lower percentages of success in all groups. The experimental differences in our study, which are consistent across ages, can best be considered in light of our baseline values rather than values based on the bulk of previous literature which did not involve drugs or cross-fostered groups.

There were significant effects of rearing condition, regardless of prenatal exposure experience, in IC-reared PND 28 offspring who exhibited lower rates of pup-induced maternal behavior than controls. These findings are interesting given that IC-treated rearing dams continued to receive injections on schedule (see methods) during the postpartum period, so pups reared by these dams had intermittent postnatal exposure to cocaine through the milk of the dam until weaning. In addition, IC-rearing dams also displayed significantly altered maternal behavior whenever cocaine was present in their system during the postnatal period as previously reported [17], which could have negatively impacted the offspring reared by these dams.

Prenatal drug exposure condition had no significant effects on successful completion of the task in PND 60 males (Table 4) but fewer males reared by CC-treated dams, regardless of their prenatal exposure condition, met criteria than CS-reared males. The fact that rearing by CC treated dams altered adult rates of pup-induced maternal behavior likely indicates an effect of the amount or quality of maternal behavior received by the male on their subsequent behavior. Interestingly, rearing by IC treated dams, which did impact juveniles, did **not by itself** affect success rates in PND 60 males. Recent articles examining rearing effects resulting from differentially responsive dams on next generation offspring maternal behavior indicate an important role for quality and degree of maternal influence on subsequent generations [5,6, 10,11,17]. The most dramatic effects at PND 60 were found in success rates for the cocaine-exposed pups (CC and IC) reared by their own natural dams (see Table 5). These findings in part support our hypotheses that prenatal cocaine exposure when combined with rearing by cocaine-treated dams significantly disrupts pup-induced maternal behavior in PND 60 males. None of the ICIC offspring and only a small percentage of the CCCC offspring met criteria at PND 60 compared to control exposed offspring reared by their natural dams. Rearing by a cocaine-treated dam following prenatal exposure to cocaine seems to have an additive negative effect on relative success rates. These findings were limited by the fact that **when subjects that killed pups were eliminated,** only three ICIC males were available and able to complete testing.

The adult rates of successful pup-induced maternal behavior were more similar to the juvenile rates than expected [47] but again given our later juvenile testing period, it is probably not as surprising to find similarities in overall success rates between animals at PND 28 and PND 60 males. The success criterion for adults was more stringent as well so comparisons between juvenile and adult rates are tentative at best. We chose to test PND 60 males at an age that has not been extensively studied with regard to pup-induced maternal behavior in young adults [24] but is an age with strong evidence of various social and behavioral differences following cocaine exposure or rearing by cocaine treated dams [8,17–19,21,33]. Had we chosen ages and paradigms more typical of the general postpartum maternal behavior literature, perhaps we would have seen different effects. We also did not test PND 60 females because they were already selected for use on other tasks. In retrospect, it would have perhaps been better to have videotaped all sessions (instead of just the day when they met criteria) rather than to use interval

scoring as group comparisons of latency and duration measures could have proven interesting in the final analysis. We would then have been able to discern more detailed information for behaviors such as lick, which we now only have a yes or no score recorded. Given the large number of animals tested, we felt initially that the number of tapes required for analyses were prohibitive and thus the interval scores were used. Future studies should examine drug effects on this behavior in both males and females at even younger ages more typically reported in the literature for comparative value with previous reports.

As indicated in the initial report on the maternal behavior of the dams of these offspring [17] the complex research design involved in intergenerational studies have necessary limitations for which we made every effort to control, and which are detailed in a previous report [17]. The cross-fostering of large groups of animals requires intensive observation and control to optimize the time of foster placement, and there are disagreements as to possible effects of fostering alone [10,27]. The CS and IS control groups were quite similar to each other and generally to UN control groups although the CSCS group had the highest success rates as adults. If CS exposure or rearing by CS dams alone was sufficient to have an effect on these pups we would have also expected to see main effects of rearing or exposure, but we did not. Any behavioral differences between saline treated and UN dams probably resulted from the stress of the pair feeding procedure or injections, as previously suggested [26,44,48]. Regardless of these limitations, these findings are interesting additions to the animal literature on cocaine exposure and rearing effects on subsequent behavior in both genders.

The mechanisms and pathways involved in pup-induced maternal behavior are not clearly understood and although behavioral postures are somewhat similar in appearance to lactating maternal behavior, non-lactating and postpartum maternal behavior are clearly very different in many aspects, including endocrine patterns and magnitude of response to pups. Future studies will focus on mechanistic aspects of this behavior, including involvement of the oxytocin system, which is integral to the onset of postpartum maternal behavior and has been indicated as a mediator of cocaine's effects on lactating maternal behavior in rat dams.

Several conclusions may be drawn from this study, primarily that both chronic and intermittent cocaine impact success on a pup-induced maternal behavior task in juveniles and in young adult males. Secondarily, this report highlights the a role for both rearing and prenatal environment in the offspring and although significant alterations were primarily associated with rearing condition, the combination of prenatal exposure to cocaine and rearing by a natural cocaine-treated dam were most evident by adulthood. **Given the number of drug exposed children fostered early in their life, preclinical models focused on the influence of rearing environment may be particularly relevant.**

This paper completes another piece of the puzzle in the context of cocaine's effects on maternal/care-giving behavior. Whereas there are persistent, but temporally diminishing, disruptions in postpartum maternal behavior of cocaine treated rat dams, female offspring exhibit fewer, but still significant deficits in the onset of postpartum maternal behavior attributable to both prenatal exposure to cocaine or rearing by cocaine-treated dams. In the present findings, non-lactating next generation offspring reared by cocaine-treated mothers and especially those additionally exposed to cocaine prenatally were less likely to respond as caregivers to young pups than other offspring. While the ethological value of the virgin pup-sensitization model is less clear since the behavior itself is not as clearly documented in nature as postpartum maternal behavior, it provides valuable information as a model to study how the hormones of pregnancy, parturition, and the postpartum period serve to promote a given set of responses and to determine hormone dependency for a particular pattern of behaviors. While we are still determining the role of cocaine-induced oxytocin system changes in both postpartum and pup-

induced maternal behavior, it is clear that cocaine via direct treatment, prenatal exposure, or through altered rearing behavior, impacts all of these behaviors negatively. Future reports will focus on not only the biological mechanisms underlying the outcomes presented in this and previous studies, but also how both rearing and prenatal environments act separately and in combination to alter a variety of social/aggressive behaviors in offspring.

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Table 1

Experimental Groups Resulting from Cross-Fostering

		Rearing Dam Treatment				
		CC	**IC**	**CS**	**IS**	**UN**
Litter Prenatal Exposure Condition	**cc**	CCcc (13)	ICcc (13)	CScc (14)	IScc (14)	UNcc (12)
	ic	CCic (12)	Icic (15)	CSic (12)	ISic (14)	UNic (15)
	cs	CCcs (15)	ICcs (14)	CScs (13)	IScs (13)	UNcs (13)
	is	CCis (13)	ICis (13)	CSis (14)	ISis (16)	UNis (12)
	un	CCun (11)	ICun (15)	CSun (16)	ISun (19)	UNun (28)

Note. Capital letters indicate the original or parent dams' treatment; lower case letters indicate the prenatal exposure condition of first generation offspring. Group designations are as follows: chronic cocaine (CC), chronic saline (CS), intermittent cocaine (IC), intermittent saline (IS), or no treatment (UN). The total number of offspring, for each group are listed in parentheses. Group sizes varied as a result of extra breeding for testing purposes and loss of dams for various reasons during the study.

Table 2

Maternal Litter Data for Original Dam Treatment Groups

Dam Gestational Treatment	Number of Dams	Gestational Weight Gain (g)	Whole Litter Weight (g)	Number of Pups
CC	67	**134.8±2.6** ^{U}	**86.3±2.0** ^{u}	14.3±0.4
CS	71	**132.3±2.5** ^{U}	**85.3±1.9** ^{u}	13.5±0.3
UN	81	157.7±2.4	91.5±1.8	14.5±0.3
IC	70	**147.4±2.5** ^{sU}	89.6±1.9	14.4±0.3
IS	77	154.4±2.4	92.3±1.8	14.6±0.3

Note. Group designations are as follows: chronic cocaine (CC), chronic saline (CS), intermittent cocaine (IC), intermittent saline (IS), or no treatment (UN). Means with capitalized superscripts differ at $p \leq 0.01$ while lower case superscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: S(s) indicates groups significantly different from respective saline control, U(u) indicate groups significantly different from UN treated dams.

Table 3

Effects of Rearing Dam Treatment and Prenatal Exposure on Criteria Completion Success in Postnatal Day 28 Offspring (Both sexes)

Rearing Condition					Prenatal Exposure				
Rearing Dam Treatment	**Number Tested**	**% Meeting Criteria**	**Mean Days to Criteria**	**Kills**	**Exposure Condition**	**Number Tested**	**% Meeting Criteria**	**Mean Days to Criteria**	**Kills**
CC	106	32%	5 ± 0.3	3	CC	103	33%	5 ± 0.3	1
CS	102	32%	5 ± 0.3	1	CS	102	39%	5 ± 0.2	4
UN	113	42%	5 ± 0.2	2	UN	129	32%	5 ± 0.2	1
IS	111	32%	6 ± 0.2	1	IS	100	28%	5 ± 0.3	2
IC	104	22%*	5 ± 0.3	1	IC	102	28%	6 ± 0.3	0

Note: Group designations are as follows: Chronic cocaine (CC), chronic saline (CS), intermittent cocaine (IC), intermittent saline (IS) and untreated (UN). On postnatal day (PND) 28, fewer offspring reared by IC treated dams met criteria for sensitization than did those reared by untreated dams (*$p \leq .01$). Male and female offspring are included as there were no gender related differences. Kills designates the total number of subjects per group that killed pups.

Table 4

Effects of Rearing Dam Treatment and Prenatal Exposure Condition on Criteria Success in Postnatal Day 60 Male Offspring (excluding kills)

Rearing Condition					Prenatal Exposure				
Rearing Dam Treatment	**Number Tested**	**% Meeting Criteria**	**Mean Days to Criteria**	**Kills**	**Exposure Condition**	**Number Tested**	**% Meeting Criteria**	**Mean Days to Criteria**	**Kills**
CC	21	19%*	8 ± 3.7	17	CC	26	31%	8 ± 1.5	9
CS	25	52%	7 ± 0.6	15	CS	24	54%	8 ± 3.7	16
UN	28	32%	9 ± 4.5	9	UN	25	44%	7 ± 0.6	16
IS	22	50%	7 ± 0.7	18	IS	22	41%	8 ± 0.7	16
IC	21	42%	7 ± 1.7	12	IC	20	25%	8 ± 1.5	14

Note: Group designations are as follows: Chronic cocaine (CC), chronic saline (CS), intermittent cocaine (IC), intermittent saline (IS) and untreated (UN). On postnatal day (PND) 60, fewer offspring reared by CC treated dams met criteria for sensitization than did those reared by CS treated dams (

*
$p \leq .05$).

Table 5

Effects of Combined Rearing Dam Treatment and Prenatal Exposure Condition on Criteria Success in Offspring at PND 28 and 60

PND 28						PND 60					
Combined Groups	**Individual Groups**	**Individual Group Number Tested §**	**Individual Group % Meeting Criteria**	**Combined Group Number Tested §**	**Combined Group % Meeting Criteria**	**Combined Groups**	**Individual Groups**	**Individual Group Number Tested §**	**Individual Group % Meeting Criteria**	**Combined Group Number Tested §**	**Combined Group % Meeting Criteria**
Cocaine	CCCC	27	41%	52	27%	Cocaine	CCCC	8	13%	11	9%
	ICIC	25	12%				ICIC	3	0%		
Untreated	UNUN	38	42%	38	42%	Untreated	UNUN	10	40%	10	40%*
Control	ISIS	24	21%	48	21%	Control	ISIS	6	33%	13	62%*
	CSCS	24	30%				CSCS	7	86%		

Note: Group designations are as follows: the first two group letters indicate rearing dam treatment and the second two indicate prenatal exposure condition such that groups include; Chronic cocaine (CC), chronic saline (CS), intermittent cocaine (IC), intermittent saline (IS) and untreated (UN).

§ denotes group numbers exluding those that killed. On PND 60, significantly fewer CCCC and ICIC offspring combined met criteria than did ISIS and CSCS offspring combined (

** p≤.01) and UNUN (

* p≤.05).