Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1059–1064, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Effects of Prenatal Exposure to Cocaine on the Rest–Activity Cycle of the Preweanling Rat

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Received 23 March 1992

ZMITROVICH, A. C., D. E. HUTCHINGS, D. L. DOW-EDWARDS, D. MALOWANY AND S. CHURCH. *Effects of prenatal exposure to cocaine on the rest-activity cycle of the preweanling rat.* PHARMACOL BIOCHEM BEHAV 43(4) 1059–1064, 1992.—Either 45 or 60 mg/kg cocaine HCl was administered from days 8–22 of gestation. Pair-fed and nontreated groups served as controls and all treated and control litters were fostered at birth to untreated dams. To examine whether cocaine produces effects on the rest-activity cycle of the offspring, groups of three littermates from each of the treated and control groups were tested for an 8-h observation period on electronic activity monitors at 22 days of age. Neither activity level nor the rest-activity pattern were affected by cocaine. These findings are discussed in relation to previous studies of cannabis and methadone effects on the rest-activity measure.

Cocaine HCl Prenatal Rats Rest-activity cycle Developmental toxicity

IN previous work from our laboratory, we reported that rats prenatally exposed to cocaine showed a transitory increase in activity at 3 weeks of age (12). The present study was conducted to extend this behavioral observation by measuring the rest-activity cycle. This is carried out using a technique developed in our laboratory that provides a sensitive measure of altered activity levels following prenatal exposure to other abuse and abuse-related compounds (11,13,14). For this, groups of dams were administered one of two doses of cocaine HCl in the last 2 weeks of pregnancy. One control group was administered vehicle and pair fed and watered to the highest dose and another was nontreated. All litters were surrogate fostered at birth and the rest-activity cycle of treated and control offspring determined at 22 days of age.

METHOD

Animals and Timing of Pregnancy

Individual nulliparous Wistar females weighing 200–224 g (Hilltop Lab Animals, Inc., Scottsdale, PA) were paired with males of the same strain in hanging wire cages. The pans beneath the cages were examined in the early afternoon for the presence of sperm plugs. The day a plug is found is designated day 1 of gestation (G1). Gravid dams were then randomly assigned to either one of two dose-level cocaine groups, to the nontreated (NT) control group, or assigned by weight to the pair-fed (PF) control group. All animals were housed in standard Plexiglas cages on wood chips. All dams except those in the pair-fed condition had continuous access to Purina Lab Chow and water. Lights automatically came on at 0600 h and went off at 1800 h.

Drug Administration and Control Groups

Beginning on G8 and continuing daily through G22, either 45 or 60 mg/kg (COC45, COC60) of cocaine HCl (Sigma Chemical Co., St. Louis, MO) dissolved in sterile water was administered by gastric intubation in a volume corresponding to 5 ml/kg body weight. This route was selected because it was found to produce dose-related plasma levels in the dam (6) without producing gastrointestinal lesions (7). These dose levels were selected on the basis of previous work (12) that determined that 60 mg/kg is well below the ED_{50} for producing seizures. Previously, 30 mg/kg was used as the low dose and although producing effects in dams it produced no observable effects in offspring. For the present study, the lowest dose was thus increased to 45 mg/kg. One control group (PF) received the vehicle and was pair fed to the food and water intake of the COC60 group. A nontreated control group (NT) was left undisturbed throughout pregnancy.

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Pair Feeding

Pair feeding to the COC60 group for both food and water intake was carried out using a yoked design. On gestation days 7–22, food and water intake was measured daily for each dam in the COC60 group. Food was placed in stainless steel hanging food dispensers and water in 8-oz bottles. Food and water containers were weighed daily and consumption was recorded.

Each COC60 dam was yoked to a pair-fed control dam matched by body weight (±5 g). On days 7–22 of gestation, each pair-fed dam was given access to the same amount of food and water consumed by their yoked drug-treated dam on the same gestation day. To minimize spillage, rat chow was placed in conical ceramic food dishes that sat on the cage floor.

Fostering

The strain of rat used here consistently litters between 0700 and 1300 h, thus permitting parturition to be observed and fostering to be carried out within 1–5 h. The number of live births, sex, and birthweights were recorded on the day of birth, postnatal day (PND) 0. Stilllbirths, as well as pups that died between postnatal PND 0–5, constituted "perinatal mortality." Litters were culled when necessary to 10 pups and those containing less than 8 pups were sexed and weighed but excluded from further testing. When culling, the sex ratio was kept as equal as possible; otherwise, culled pups were randomly selected. Treated and control dams were sacrificed to determine the number of implantation sites. All treated and control litters were surrogate fostered to an untreated dam that had given birth within 48 h.

Equipment

Activity testing is carried out in a shielded test room continuously illuminated with diffuse fluorescent ceiling fixtures and maintained at a temperature of 22–24°C. A six-channel electronic activity monitor with six remote sensors, each measuring 25.4 × 48.3 × 10.2 cm (Stoelting Co., Chicago, IL) is used for behavioral testing. The operating principle of the sensors has been previously described (11). The sensors are stacked 40.6 cm apart in a vertical metal rack with the metal shelving acting as an insulator between the respective sensor fields. The threshold reset time is placed in the "normal" mode and the activity level (i.e., sensitivity) control set at 15. This setting is selected so that neither breathing movements nor the myoclonic twitching and fine distal movements that accompany REM sleep produced counts. Activity counts are collected at 60-s intervals using an IBM XT PC.

Activity Testing

Offspring from each of the NT (n = 11), PF (n = 7), COC45 (n = 10), and COC60 (n = 10) litters were tested on PND 22. On the day of testing, each litter was removed from its foster dam and divided into triads of littermates. Whenever possible, each triad was composed of either two males and one female or two females and one male. Each triad was then placed in a standard 48.3 × 26.7 × 20.3-cm polycarbonate cage on approximately 1.5 cm of fresh wood chips. The cage was then placed on one of the six monitors and recording began for a test period of 8 h (480 min). Neither food nor water was provided during testing and offspring were tested only once. Testing was carried out from approximately 0900–1700 h.

TABLE 1

MATERNAL AND OFFSPRING EFFECTS
(Mean ± SEM)

	NT	PF	COC45	COC60
Total pregnant	11	8	11	11
Deaths	0	1	1	1
Litters evaluated, toxicity	11	7	10	10
Litters evaluated, growth and behavioral effects	11	7	10	10
Maternal weight gain (g)	184.3 ± 5.5 (n = 9)*	148.6 ± 11.2†	146.2 ± 4.5†	154.7 ± 7.7†
Implantation sites	15.5 ± 0.5	15.7 ± 0.8	15.4 ± 0.5	16.3 ± 0.6
% Resorption	4.5 ± 1.5	3.6 ± 1.8	3.5 ± 2.3	8.0 ± 2.8
% Perinatal mortality	1.6 ± 0.8	0.0 ± 0.0	2.7 ± 1.5	4.2 ± 1.6
% Total offspring mortality	6.2 ± 2.0	3.6 ± 1.8	6.2 ± 3.3	12.2 ± 3.6
% Live births				
Male	40.5 ± 5.1	42.7 ± 4.8	50.8 ± 5.3	47.1 ± 3.1
Female	59.5 ± 5.1	57.3 ± 4.8	49.2 ± 5.3	52.9 ± 3.1
Litter size	14.6 ± .4	15.1 ± .8	14.7 ± .7	14.6 ± .6
Birthweight				
Male‡	7.2 ± .2	6.9 ± .2	6.6 ± .2	6.8 ± .1
Female	6.2 ± .2	6.3 ± .3	6.3 ± .2§	6.2 ± .2§

*Decreased ns resulted from missing data.
†$p < 0.005$, significantly different from NT.
‡$p < 0.002$, significantly different from females.
§$p < 0.02$, significantly different from NT.

Statistical Analysis

Data analysis was carried out on an IBM XT using SYSTAT. Nonparametric tests were used to analyze maternal and offspring effects expressed as proportions. Analyses of variance (ANOVAs) were performed on normally distributed data including total maternal weight gain, implantation sites, litter size, and birth weights. Posthoc univariate tests were used when appropriate. A repeated-measures ANOVA was used for analysis of maternal weight gain and food and water intake across gestational days, as well as postnatal growth. The litter was used as the unit of analysis for body weight and the rest-activity measure.

RESULTS

Maternal and Offspring Effects

Table 1, in addition to showing maternal and offspring toxicity effects, contains the *n*s for the various phases of the

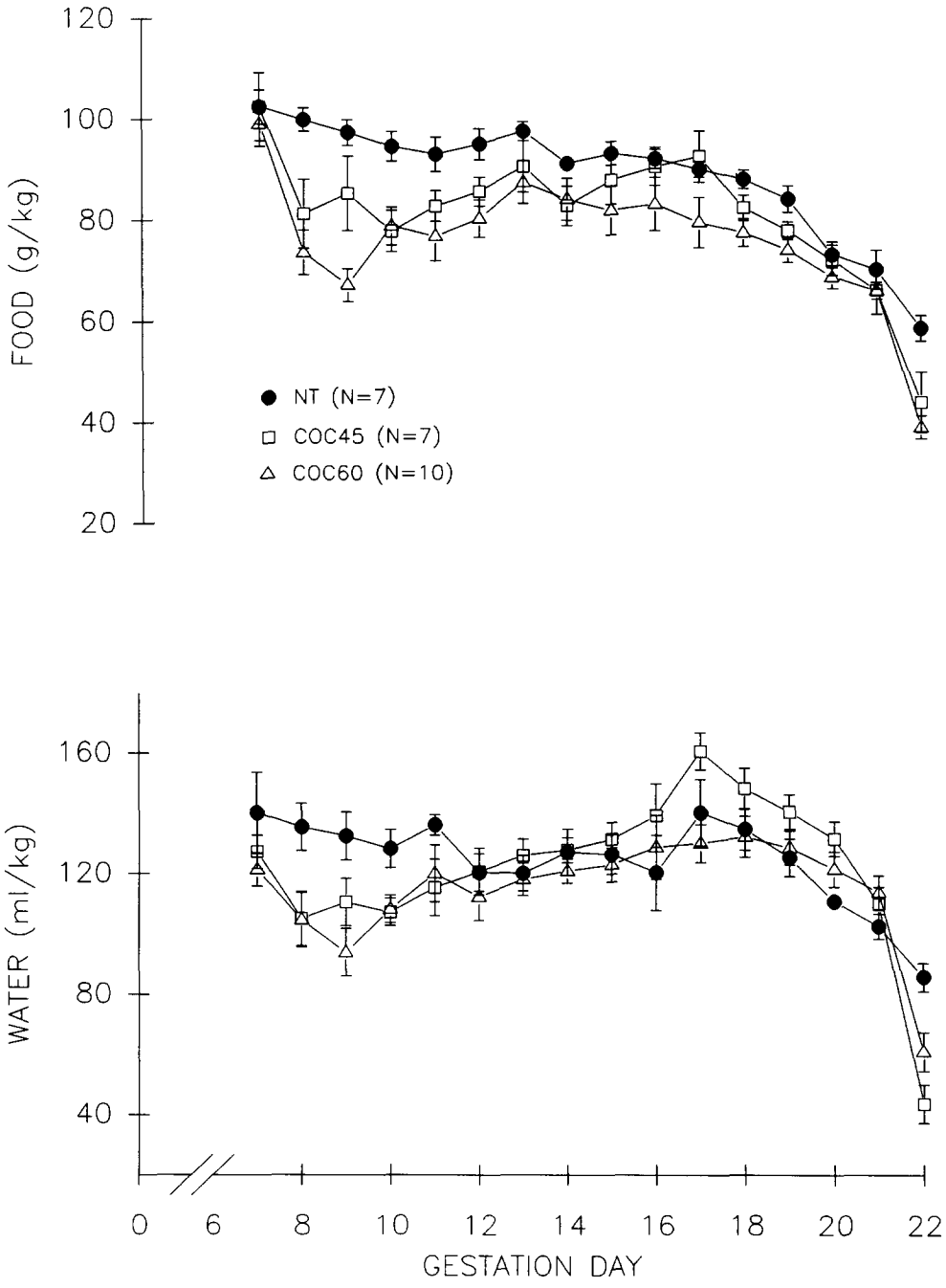

FIG. 1. Mean (±SEM) food (g/kg) and water (ml/kg) intake for nontreated and cocaine-treated dams from G7-22.

study. One dam in each of the PF, COC45, and COC60 groups died during dosing. As shown in Fig. 1, following the beginning of daily intubation cocaine-treated dams consumed less food (upper panel) and water (lower panel) but recovered to the control level by G12-13. A repeated-measures ANOVA failed to reveal a significant effect for water consumption. A repeated-measures ANOVA of food consumption showed a significant effect of treatment, $F(2, 17) = 4.79$, $p < 0.02$, as well as an interaction between treatment × gestational day, $F(30, 255) = 1.54$, $p < 0.04$. Posthoc analysis revealed a decrease in food consumption during the first few days of dosing (G8-12) in the COC60 group that recovered by G13. There was a trend toward a similar decrease in the COC45 group that was only significant on G10 and there was no difference between the two dose groups. On G22, both COC45 and COC60 dams consumed significantly less food and water than the NT.

On G1, treatment and control dams did not differ with

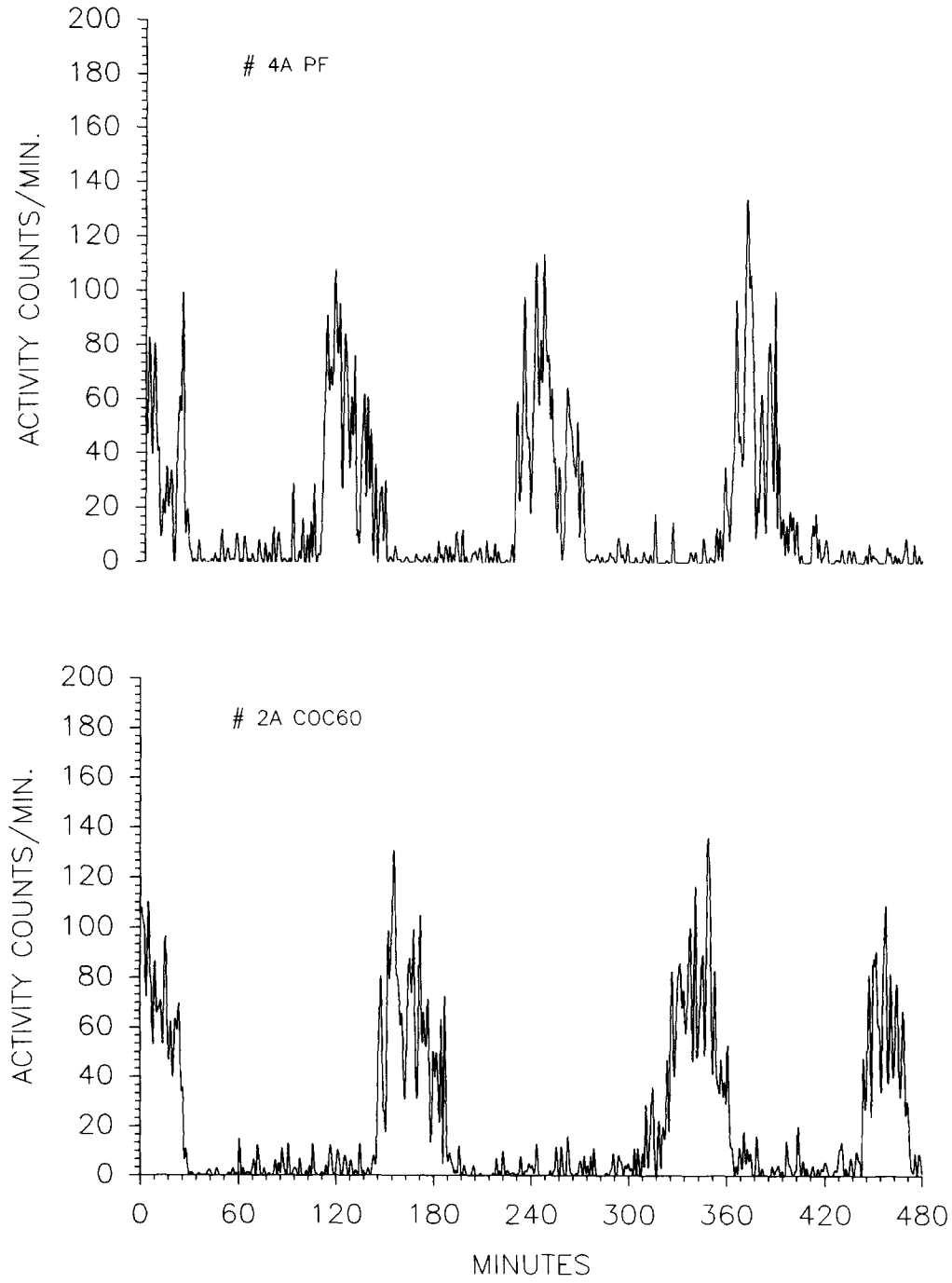

FIG. 2. Activity counts/min for pair-fed (PF) and COC60 offspring. Data in the upper and lower panels represent counts for individual triads.

respect to body weight. Table 1 shows that PF, COC45, and COC60 dams failed to gain as much body weight from conception to term compared to NT dams; a one-way ANOVA revealed a significant treatment effect, $F(3, 32) = 6.06$, $p < 0.002$. There were no treatment effects on mean implantation sites, percent resorption, percent perinatal mortality (i.e., pups either stillborn or dying soon after birth), percent total offspring mortality (i.e., percent resorption and percent perinatal mortality), or litter size. Also, percentage of live males and females did not differ significantly across groups.

Overall, as expected, male offspring weighed significantly more than female offspring at birth, $F(1, 67) = 13.02$, $p < 0.002$. There was a trend for a treatment effect on male birthweights and a significant effect of treatment on female birthweights, $F(3, 34) = 2.94$, $p < 0.05$. Posthoc analysis revealed that COC45 and COC60 females weighed significantly less than NT females but there was only a trend for lower weights among PF females. Weights of male and female offspring on PNDs 5, 10, 22, 30, 40, and 50 were not significantly different across treated and control groups.

Behavioral Effects

Activity data representative of individual triads of a PF and COC60 are shown in Fig. 2. Counts of 0–5 represent periods when the triad was huddled and either sleeping or at rest and are referred to as "sleep-rest" periods. The brief spikes that range from 6–50 are typically produced by periods of waking of one or more animals and repositioning within the group; active exploration by the group produces counts of 50 and above.

The PF and COC60 data. shown in the upper and lower panels, respectively, of Fig. 2, are similar in that they show a distinct rhythmicity; periods of active exploration lasting about 45–60 min are separated by sleep-rest periods of about 60–110 min. Mean activity counts/min for the entire test period are shown in Table 2. Although the mean activity counts were somewhat lower for the NT compared with other groups, an ANOVA failed to reveal a significant treatment effect. Another measure we applied to these data is the number of times the activity level crosses the 50-unit line during the 480-min test period (10,13). Fifty serves as a "threshold" representing a point between high and low activity from which an "activity change score" is derived. The change scores, calculated for each of the test triads and shown in Table 2, are not significantly different across groups.

Similarly, analysis of mean total rest periods (i.e., number of mins with counts 0–5) failed to reveal a significant effect of treatment nor was an effect found for maximum number of consecutive rest periods (i.e., maximum number of consecutive minutes with counts 0–5). The one measure that did reveal a treatment effect was "maximum peak activity," defined as the highest activity count over the entire 480-min test session, $F(3, 34) = 3.40$, $p < 0.03$. Posthoc analysis showed that both COC45 and COC60 as well as PF control litters had significantly higher values than the NT.

DISCUSSION

In a previous study, we examined 30 and 60 mg/kg of prenatally administered cocaine (12), whereas here our lowest dose was 45 mg/kg. In both studies, we found that cocaine administered during the last 2 weeks of pregnancy produced a moderate decrease in food and water intake among dams. Because the maternal weight decrement seen among cocaine-treated dams was similar to that of PF, it suggests that the maternal weight effects resulted from the effect of cocaine on food and water intake. Of the three dams that died from each of the PF and drug-treated groups in the present study, all appeared to result from a problem with the intubation procedure and were not drug related. Neither of these studies found effects on resorptions or offspring mortality. But, the present study did find small but significant decreases in female birthweights for both doses of cocaine.

The rest-activity cycles measured at PND 22 in the present study are identical to control data previously published from our laboratory (11,13,14). Other animal studies of early cocaine exposure on preweanling rats have reported decrements in early associative learning (17) and a difference in response to amphetamine challenge (9). Church and Overbeck (4) reported hypoactivity at PND 20 but because the effect also occurred in pair-fed controls it may not have been drug related. In a previous study, we examined the ontogeny of motor activity over the first month of life and found that offspring prenatally exposed to 60 mg/kg cocaine were more active on PNDs 20 and 23. The present study using the rest-activity measure failed to reveal any differences in activity among cocaine-exposed offspring.

Effects on the rest-activity measure have been reported following prenatal administration of methadone (11,14). These are characterized by offspring that show fewer rest periods, disrupted rhythmicity, and more frequent changes from high to low activity. These effects parallel similar effects described among infants undergoing subacute withdrawal following prenatal exposure to either heroin or methadone. In contrast, we found that prenatal exposure to Δ^9-tetrahydrocannabinol failed to produce any effects on the rest-activity measure (13), observations that also parallel the lack of neona-

TABLE 2

REST-ACTIVITY MEASURE
(Mean ± SEM)

	NT	PF	COC45	COC60
Litters evaluated, behavioral effects	11	7	10	10
Mean activity count	17 ± 2	20 ± 1	20 ± 2	20 ± 1
Activity change score	42 ± 2	47 ± 5	48 ± 2	43 ± 2
Rest periods				
Total	270 ± 11	261 ± 11	275 ± 14	262 ± 8
Maximum number of consecutive	50 ± 6	35 ± 3	50 ± 7	41 ± 4
Maximum peak activity	120 ± 5	133 ± 3*	134 ± 2*	136 ± 4*

*$p < 0.03$, significantly different from NT.

tal withdrawal in human infants following maternal use of marijuana.

The initial clinical observations of human neonates prenatally exposed to cocaine reported poor state regulation, tremulousness, and irritability and mentioned the similarity of these symptoms to those seen among opioid-exposed infants (1,2,3). Subsequent reports, however, failed to confirm these observations and described cocaine-exposed infants as showing deficits in habituation (8) but few if any symptoms similar to those associated with opioid abstinence (15,16). Indeed, Coles et al. (5) described neonates prenatally exposed to cocaine as showing not only autonomic depression but an overall pattern of low arousal. We suggested the normal rest-activity cycle of preweanling rats may be disrupted only by opioids or perhaps the sedative-hypnotic compounds (10). The failure to observe any disruption following prenatal cocaine administration is consistent with the possible specificity of this measure to these classes of compounds. Whether or not the rest-activity measure is so specific awaits testing with a broader range of compounds. The present observations indicate, however, that cocaine is not among the compounds that disrupt this behavior.

ACKNOWLEDGEMENT

This research was supported by Grants DA05401 (A.C.Z.), DA03544 (D.E.H.), and DA04118 (D.D.E.) from the National Institute on Drug Abuse.

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