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Differential Effects of Prenatal Cocaine and Retinoic Acid on Activity Level Throughout Day and Night

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CHURCH, M. W. AND J. P. TILAK. *Differential effects of prenatal cocaine and retinoic acid on activity level throughout day and night.* PHARMACOL BIOCHEM BEHAV **55**(4) 595–605, 1996.—Prenatal cocaine exposure is associated with disrupted state control and lowered activity levels. Prenatal retinoic acid excess also influences activity levels in laboratory rats. Activity level is usually monitored during a brief period in young offspring. The effects of these drugs on pup activity levels throughout the day is unknown. There is also little information on the long-lasting effects of these teratogens in adult animals. We compared the daily activity of rats which were prenatally exposed to cocaine or retinoic acid (RA). Appropriate control groups were also used. The offspring were evaluated for activity levels in a neophobic situation and for a 22-h period in same-sex groups of 3 littermates. As both pups and adults, the cocaine groups were hypoactive while the RA group was hyperactive when first placed into the testing cage (neophobic situation). Similarly, during the remainder of the 22-h testing period, the pup and adult cocaine animals exhibited reduced activity levels while the RA animals exhibited elevated activity levels. Thus, prenatal cocaine and retinoic acid exposures affected offspring activity levels differently, both drugs have long-lasting neurobehavioral effects that persist into adulthood, and effects are influenced by time-of-day. Strain-dependent differences and mechanisms of action are discussed.

Activity levels Basic rest-activity cycle (BRAC) Biorhythms Neophobia Prenatal cocaine Prenatal retinoic acid

COCAINE crosses the placenta and may cause a variety of adverse effects in both the mother and the offspring either by direct drug toxicity or by ischemia/hypoxia subsequent to vasoconstriction. Some maternal complications include hypertension, placental abruption, spontaneous abortions and premature delivery. Some neonatal effects include fetal edema, cerebral hemorrhages, visual and hearing abnormalities, intrauterine growth retardation, urinary tract abnormalities, ileal atresia, cardiac and craniofacial anomalies, skeletal anomalies, fetal death, and a variety of neurobehavioral effects (4,13).

Some of the chief neurobehavioral effects of prenatal cocaine exposure are disrupted "state control", low arousal, reduced motor and interactive abilities in neonates (6,7,16,42, 44,67) and toddlers (60). State control refers to the infant's ability to move appropriately through the various states of arousal in response to environmental demands. State control in cocaine-exposed infants can be poorly organized with infants spending most of their time in states that shut them off from external stimulation (6). Oro and Dixon (56) observed poor sleep-wake organization, another indicator of disrupted state control, in cocaine-exposed infants.

Reduced activity levels have also been reported in the animal literature. For example, we (10,12) and others (5,34,39–41,66,68,73) observed reduced activity levels in an open field test or similar conditions in periweanling and juvenile rats that were prenatally exposed to cocaine. Such rats also showed suppressed levels of juvenile play (82). Others have observed attenuated behavioral responses to perioral stimulation (65) and stimulant drug challenges (5,26,28,30,47,48,68,70; however, see 58) as well as decreased male sexual behavior (59).

These observations suggest that prenatal cocaine exposure usually depresses or otherwise disrupts the organization of activity levels in both human infants and young laboratory rats. The present study was conducted to extend these observations.

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First, we sought to replicate the reduced activity levels associated with the initial placement of animals in a novel environment and extend this observation by using a different rat strain. Supposedly, the placing of rats in a novel environment provides a measure of "emotionality" or neophobia which is characterized by reduced activity levels (22). Prenatal cocaine exposure seemingly enhances this neophobic response or at least reduces the amount of exploratory behavior relative to control animals (5,10,12,34,39–41,66,68,73). Our previous studies used Long–Evans rats purchased from Blue Spruce Farms (10) and from Charles River Laboratories (12). The present study used Sprague–Dawley rats. An ability to reproduce this phenomenon in several different animal models would either testify to the robustness of this effect or point to strain-dependent differences.

We also sought to evaluate the effects of prenatal cocaine exposure on activity levels throughout the day and night to see if reduced activity levels persisted over a prolonged period and to see if there was a disruption in the architecture of the basic rest-activity cycle (BRAC). Prenatal exposure to a variety of abused drugs are known to disrupt the BRAC (29,31,33,43,51,64), suggesting an adverse effect on neural mechanisms controlling biorhythms. Prenatal cocaine's adverse effects on an infant's state organization and sleep-wake cycle are suggestive of disrupted BRAC architecture. Activity levels in the rat vary as functions of the light/dark cycle with adult rats being particularly active during the first hour of darkness(37). Thus, monitoring activity levels as function of the light and dark phases of the day with special attention to the first hour of darkness would provide new information about the neurobehavioral effects of prenatal cocaine exposure.

In finding significant effects from prenatal methadone on the rest-activity cycle of rats, Hutchings et al. (33) tested the offspring in groups of three littermates. Since the added stress or social interactions of a group testing condition might enhance experimental effects, we examined animals in triads of same-sex littermates. We also sought to evaluate animals both as periweanling pups and young adults. Such information would indicate if treatment effects were transient or long-lasting.

An additional goal was to compare and contrast the effects of prenatal cocaine exposure with those of another well-defined teratogen. A number of investigators have suggested retinoids (e.g., vitamin A, retinoic acid) as appropriate "positive control" compounds for neurobehavioral teratology studies (2,78). Retinoids are of particular interest because of the critical role they play in normal development through the stimulation of Hox genes and because retinoid excess is highly teratogenic (69). There is also increased concerns about the use of retinoids to treat skin problems (acne, age spots, wrinkles), the eye disease retinitis pigmentosa, some cancers as well as its use as a nutritional supplement (69). There is some uncertainty about whether prenatal retinoid excess causes hypoactivity (2,76) or hyperactivity (2,55,77,78) in the neophobic situation. The prenatal retinoid literature also contains little or no information on adult behavior and activity levels throughout the BRAC. Thus, evaluating the long-lasting effects of a retinoid on these behavioral parameters would provide new information as well.

METHOD

All procedures described herein were approved by the Wayne State University School of Medicine's Animal Investigation Committee and were in compliance with the ethical treatment of laboratory animals as stated in NIH guidelines.

Subjects

Nulliparous female Sprague-Dawley rats (Harlan-Sprague-Dawley, Inc., Indianapolis, IN), aged 90–110 days at the time of mating, were housed in polycarbonate cages (45 × 23 × 20 cm) with a bedding of wood chips. Rooms were temperature (22±1°C) and humidity (35–40%) controlled with a timed light cycle of 12 h of light per day (0700 h to 1900 h.). Animals had ad-lib access to water and food (Teklad 10% MBD pregnancy diet).

Each female was placed individually in a breeding cage with one male. The morning on which a sperm plug was found was designated gestation day 0 (GD0). On GD0, animals were matched for weights and assigned to one of six groups. The two cocaine-treated groups (C40 and C80) received either 40 or 80 mg/kg cocaine HCl(SC 2% solution) daily from GD7-20 inclusively (term = GD22) with each daily dose split evenly and given in two treatments. The cocaine groups received their first daily treatment between 0900–1000 h and their second daily treatment between 3–4 p.m. (10,12). These dosing regimens produce respective mean (+ S.E.) peak serum benzoylecgonine levels of 2739 + 67 and 4551 + 447 ng/ml at 4 hrs post injection(15). The cocaine HCl was provided by the National Institute on Drug Abuse. The retinoic acid-treated group (RA) received 10 mg/kg all-trans-retinoic acid (Sigma Chemical Co., St. Louis, MO) suspended in corn oil (PO, 1% solution) once daily from GD7-9. Treatment was given between 0900– 1000 h. This dosing regimen was based on the literature (2,55,76-78) as well as our own pilot study which found that RA doses of 20 and 30 mg/kg resulted in high levels of embryonic mortality in Sprague-Dawley rats. Our goal was to use cocaine and retinoic acid treatments that were known to be behaviorally teratogenic but that caused little or no prenatal fatalities.

In addition to the three drug treatment groups, there were two control groups: an untreated control group (UTC) of pregnant dams which had ad lib access to food and water and which received no handling other than daily weighings, and a pair-fed control group (PFC) which received daily food allotments which were restricted to the average amount of food eaten by the C80 group. The PFC group also received injections of saline solution (SC, 0.85% solution) twice daily that were isovolumetric to the injections given to the C80 group. The purpose of the PFC group was to help assess the effects of undernourishment and handling stress that accompanies cocaine administration. Cocaine treatment does not result in reduced water consumption (11,14,15), so pair-watering was unnecessary. An intubation control group for comparison with the RA group was not used because of an interest in conserving financial and animal resources.

The weight of each pup was determined as soon as possible after delivery of the entire litter. The litters were then culled to ten pups (five male and five females, whenever possible). This litter of ten pups was then assigned to a surrogate mother who had given birth within 24 h of the mother in the control or treatment group. Assignment of treated pups to non-treated surrogate mothers was deemed a necessary precaution because drug exposure to the natural mother might disrupt milk production and maternal behaviors, thereby confounding the results of the experiment (21,27,36,83). Litters were weaned on day 21 and housed in same-sex groups of 2-5 littermates.

Traditional measures of maternal and fetal toxicity (i.e.,

maternal food and water consumption, weight gain, birth weight, postnatal growth, etc.) were monitored. After giving birth, all experimental dams were sacrificed by carbon dioxide inhalation, the uteri removed and stained in ammonium sulfide (10% v/v), and the implantation sites counted.

Behavioral Testing

Activity testing was conducted in an isolated test room that was temperature (21 ± 2°C) and humidity (45 + 5%) controlled. The room was illuminated by diffuse fluorescent ceiling lights controlled by a timer (lights on = 0700 h lights off = 1900 h.). Activity was monitored by a photobeam cage rack activity system (San Diego Inst., Inc.). Each photobeam system consisted of a metal frame containing three infra-red photobeams that were placed around a transparent polycarbonate animal cage (45 × 23 × 20 cm) that was recently cleaned and supplied with fresh bedding material (wood chips). Food and water were available throughout the testing period. Animals were transported from the Vivarium to the test room (same building) and kept on the transportation rack for 5-10 minutes before being removed from their home cage to the test cage. The activity testing rack held 15 cages. Cages were hidden from each other by screens so animals in one cage could not see animals in another cage. Testing began at noon and ended at 1000 h the next day, for a total of 22 h of continuous activity monitoring.

Animals were tested at two ages: first as periweanling pups (aged 22 days) and then again as young adults (aged 70-90 days). All animals tested as adults had been previously tested as pups. The experimental design was not entirely crossed, however, in that some animals tested as pups were not available for testing as adults because of postnatal mortality. Three same-sex littermates were placed in a testing cage. The testing of the treatment groups was randomized so that litters from several treatment groups were tested on any given day. Females were tested at random phases of the estrous cycle.

Each breaking of a photobeam constituted an activity count. Activity counts were tallied and analyzed by 15-min epochs, except during the neophobic situation (i.e., a 45-min period when animals were first placed in the activity testing cages). During the neophobic situation, activity counts were tallied by 5-min epochs to enhance resolution of activity patterns.

Data Analyses

For the maternal and offspring maturational variables, the data were first analyzed by 1-way ANOVAs for treatment groups, followed by Student-Newman-Keuls multiple range tests to determine which treatment groups differed significantly from each other. For each offspring variable, the litter's datum was the unit of measure, not the individual pup's datum, except in calculating percent mortality and anophthalmia. These exceptions ameliorated data distortions that occurred when only one or two litters had high morbidity rates.

Except where mentioned, the offsprings activity data were first analyzed by 3-way analyses of variances (ANOVAs) with treatment groups and sex as a between-subjects factors and with the time epoch as a repeated measure. Sex was treated as a between-subjects factor instead of a within-subjects factor because the former is more conservative and because brothers and sisters are genetically different. The sex factor was not significant in any of these ANOVAs, so data were collapsed across gender to form 2-way (treatment-by-time) ANOVAs. The degrees of freedom were then adjusted to reflect the number of litters tested. When a 2-way ANOVA indicated a significant effect, post-hoc tests (simple effects tests) were used to determine which treatment groups differed significantly. Statistical significance was assumed for probability levels of 0.05 or less for predicted results (e.g., hypoactivity in the C40 and C80 groups and hyperactivity in the RA group since these are the most commonly reported results) while a more conservative probability level of 0.025 was used for all other comparisons. For the repeated measure, the conservative Greenhouse-Geisser probability level was used.

RESULTS

Maternal Variables

The effects of the treatment conditions on the maternal variables are displayed in Table 1. There were no mortalities among the treated pregnant females. Compared to the UTC group, the dams in the PFC, C40 and C80 groups had significant reductions in maternal weight gain and food consumption during the treatment period of GD7-20. Maternal water consumption was greater in the RA and C80 groups than the PFC group. When adjusting fluid intake to account for injected solutions in addition to voluntary water consumption, only the C80 showed greater maternal fluid intake than the PFC group.

Offspring Variables

The offspring variables are given in Table 2. The RA group had a significantly higher rate of prenatal mortalities than the UTC, PFC and C40 groups. The C80 group had a slightly increased prenatal mortality rate which did not differ significantly from any group. The C80 and PFC groups had significantly smaller birth weights than the other groups. In terms of physical maturation, pinna (external ear) detachment was delayed in the PFC and C80 groups. Fur emergence was delayed in the C80 group. Ear opening (the receding of the skin flap separating the middle and external ear) was delayed in the C80 group. There was no significant group difference in the mean age of eye opening. There was a high postnatal mortality rate in the RA group (assessed between birth and day 21). Two of the 16 RA litters eventually had no survivors. The UTC group also had an unusually high postnatal mortality rate. This was because one UTC litter was cannibalized by the surrogate mother. In terms of congenital defects, 7 of the 113 surviving RA offspring had either unilateral (5 pups) or bilateral (2 pups) anophthalmia. These 7 anophthalmic offspring were concentrated in 3 litters. There were 3 runts in the RA group, each from different litters.

Table 3 displays the postnatal weight gains of the male offspring. Males in the C80 group were significantly underweight from birth up to and including postnatal day (PD) 168. They were still smaller than the UTC males on PD196, but not significantly. The males in the PFC and RA groups were also underweight compared to the UTC group. These differences were statistically significant primarily in adulthood (PD112 through PD168). Group differences in the female offspring weight data (not shown) were highly similar.

Offspring Activity Levels

Figure 1 compares the all-day activity patterns of the UTC pups and adults. Periweanling pups typically had activity patterns that were independent of the light/dark cycle while adult animals showed higher activity levels during the dark phase,

TABLE 1

EFFECTS OF TREATMENTS ON MATERNAL VARIABLES (MEAN ± SE)

Variable	Group					Probability Statistics
	UTC	PFC	RA	C40	C80	
No. pregnancies	15	13	16	12	13	
Mortality rate (%)	0	0	0	0	0	
Weight gain (g)	115	91[a]	106	89[a]	77[a]	$F(4, 59) = 7.69$
from GD7–20	(5)	(4)	(6)	(7)	(5)	$p < 0.001$
Food consumption (g)	259	197[a]	253	219[a]	197[a]	$F(4, 63) = 37.17$
from GD7–20	(4)	(10)	(5)	(5)	(5)	$p < 0.001$
Water consumption (ml)	664	592	671[a]	662	704[b]	$F(4, 63) = 2.66$
from GD7–20	(27)	(21)	(23)	(23)	(29)	$p < 0.05$
Adjusted fluid intake	664	600	672	666	713[b]	$F(4, 61) = 2.58$
(ml) from GD7–20	(27)	(21)	(23)	(23)	(29)	$p < 0.05$

[a]Different from UTC group.
[b]Different from PFC group.
UTC = untreated control.
PFC = pair-fed control.
RA = retinoic acid.
C40 = 40 mg/kg cocaine daily.
C80 = 80 mg/kg cocaine daily.

particularly during the first hour of darkness. These general BRAC patterns were exhibited by all treatment groups. The analyses of activity counts throughout the 22-h recording period indicated no significant phase-shifting or disruption of BRAC rhythmicity. There were, however, significant group effects on activity levels for both the pup and adult data, due primarily to the RA group showing more activity than most other groups (see Table 4). There were also significant interactions between the group and time variables, the nature of which is described below in relation to predetermined phases of the daily light/dark cycle.

(1) Neophobic situation (12 p.m. to 12:45 p.m.). As men-

TABLE 2

EFFECTS OF TREATMENTS ON OFFSPRING VARIABLES (MEAN ± SE)

Variable	Group					Probability Statistics
	UTC	PFC	RA	C40	C80	
No. litters	14	13	14	12	13	
No. implantation sites/dam	14.2	13.8	13.4	12.1	14.9	$F(4, 61) = 1.55$
	(0.6)	(0.4)	(1.0)	(1.2)	(0.7)	N.S.
No. live	12.2	12.8	9.4	10.8	12.1	$F(4, 61) = 2.13$
offspring/litter	(0.8)	(2.7)	(1.0)	(1.2)	(0.9)	N.S.
No. prenatal mortalities	2.0	1.4	4.2[c]	1.9	2.7	$F(4, 61) = 3.95$
	(0.4)	(0.4)	(0.8)	(0.5)	(0.6)	$p < 0.01$
Birth weight (g)	6.2	5.8[a]	6.1	6.4	5.4[a]	$F(4, 60) = 7.40$
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	$p < 0.001$
Pinna detachment (day)	2.2	2.5[a]	2.1	2.1	2.7[a]	$F(4, 124) = 7.93$
	(0.0)	(0.1)	(0.1)	(0.1)	(0.2)	$p < 0.001$
Fur emergence (day)	8.0	8.0	8.0	8.0	8.1[c]	$F(4, 120) = 4.34$
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	$p < 0.01$
Ear opening (day)	13.2	13.4	13.5	13.3	14.0[c]	$F(4, 121) = 8.98$
	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	$p < 0.001$
Eye opening (day)	15.6	15.7	15.4	15.8	15.8	$F(4, 121) = 1.59$
	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	N.S.
Postnatal mortality (%)	8.0[b]	1.6	9.6[b]	3.8	4.0	X^2 (4) = 10.56
						$p = 0.032$
Anophthalmia (%)	0.0	0.0	6.2[c]	0.0	0.0	X^2 (4) = 29.53
						$p < 0.001$

[a]Different from UTC, PFC and C40 groups.
[b]Different from UTC, C40 and RA groups.
[c]Different from all other groups.
N.S. = not significant.

TABLE 3

POSTNATAL WEIGHTS (GRAMS) OF MALE OFFSPRING (MEAN ± SE)

Variable	Group					Probability Statistics
	UTC	PFC	RA	C40	C80	
No. Litters	14	13	14	12	13	
Day 0 (Birth)	6.4	6.0[a]	6.3	6.6	5.5[b]	$F(4, 57) = 7.00$
	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	$p < 0.001$
Day 7	14.8	14.3[a]	14.5	15.8	12.2[b]	$F(4, 58) = 7.14$
	(0.4)	(0.5)	(0.5)	(0.3)	(0.5)	$p < 0.001$
Day 14	29.7	28.3	29.6	31.7	26.6[a]	$F(4, 58) = 3.42$
	(0.8)	(1.1)	(1.0)	(0.7)	(1.1)	$p < 0.02$
Day 21	46.0	44.5[a]	45.8	47.8	41.5	$F(4, 58) = 2.12$
	(1.1)	(1.5)	(1.7)	(1.5)	(2.0)	N.S.
Day 28	80.6	75.7[a]	77.1	83.2	72.2[a]	$F(4, 58) = 3.13$
	(1.5)	(2.5)	(2.9)	(0.8)	(3.1)	$p < 0.05$
Day 56	277	271	266	279	258[a]	$F(4, 58) = 2.72$
	(4)	(4)	(7)	(3)	(6)	$p < 0.05$
Day 84	375	353	357	366	345[a]	$F(4, 58) = 3.25$
	(6)	(5)	(8)	(6)	(7)	$p < 0.01$
Day 112	436	407[c]	403[c]	422	398[c]	$F(4, 56) = 4.41$
	(8)	(7)	(8)	(7)	(7)	$p < 0.01$
Day 140	465	433[c]	428[c]	452	425[c]	$F(4, 56) = 4.52$
	(7)	(9)	(9)	(7)	(8)	$p = 0.01$
Day 168	482	448[c]	442[c]	469	446[c]	$F(4, 56) = 4.21$
	(8)	(10)	(9)	(7)	(7)	$p < 0.01$
Day 196	500	458	458	476	468	$F(4, 55) = 2.34$
	(11)	(11)	(10)	(10)	(16)	N.S.

[a]Different from C40 only.
[b]Different from all groups..
[c]Different from UTC groups.

tioned earlier, a rat's initial activity level after being placed in a novel environment provides a measure of emotionality or fearfulness. Data from this "neophobic" situation were first analyzed by a 2 × 5 × 9 ANOVA for the age, group and time parameters with age and time as repeated measures. Age was included in this analysis because our previous studies indicated a dissipation of cocaine-induced neophobia with aging (10,12). Of the 66 litters tested as pups, 60 were tested as adults, resulting in slightly different sample sizes when the age groups were analyzed separately or together.

Results are presented in Table 5. There was a significant effect for time, reflecting a progressive decline in activity levels over the 45-min period: $F(8, 276) = 62.12, p < 0.001$. Contrary to expectations, there was no significant age effect: $F(1, 55) = 0.31$, $p = 0.58$. There was a significant group effect: $F(4, 55) = 7.64, p < 0.01$. Post-hoc analyses indicated that the C40 group had significantly lower activity levels than the UTC, PFC and RA groups; and the C80 group had significantly lower activity levels than the UTC and RA groups, but not the PFC group. In contrast, the RA group had significantly higher activity levels than all other groups. The UTC and PFC groups did not differ significantly from each other; and the C40 and C80 groups did not differ. There were no significant interactions between the age, group and time factors. The lack of interactions suggested that periweanling and adult activity patterns in the neophobic situation were highly similar. This is illustrated by the comparison of the periweanling and adult data in Fig. 2 and in Table 5.

(2) Afternoon light phase (12 p.m. to 7 p.m.). The ANOVA of the pup data indicated a significant group difference in activity level during the afternoon light phase with the RA group being significantly more active than the UTC, PFC, C40 and C80 groups (see Table 6).

The ANOVA of the adult data also indicated a significant group difference with the PFC group being more active than all other groups (see Table 6). Visual inspection of the PFC data indicated that this group had normal activity levels during the first 4-5 h of the afternoon; but when the other groups showed declining activity late in the afternoon, the PFC groups activity level remained steady.

(3) First hour of darkness (7 p.m. to 8 p.m.). The analysis of pup data indicated no significant group difference in activity levels during the first hour of darkness (see Table 6).

The analysis of the adult data showed a significant group difference with the C40 group being less active than all other groups (see Table 6). Adult rats are crepuscular, showing increased nocturnal activity particularly during the first hour of darkness. Visual inspection of the data in Table 6 shows that the adult rats exhibited their highest activity levels during the first hour of darkness but that this effect was absent in the pups. This was consistent with the activity patterns illustrated in Fig. 1.

(4) Evening dark phase (1900 h. to 0700 h.). The ANOVA of the pup data indicated no significant group differences in activity level when the period of darkness was considered in its entirety (see Table 6).

The analysis of the adult data showed a significant group difference with the RA group being more active than all other groups (see Table 6).

(5) Morning light phase (0900 h. to 1000 h). The ANOVA

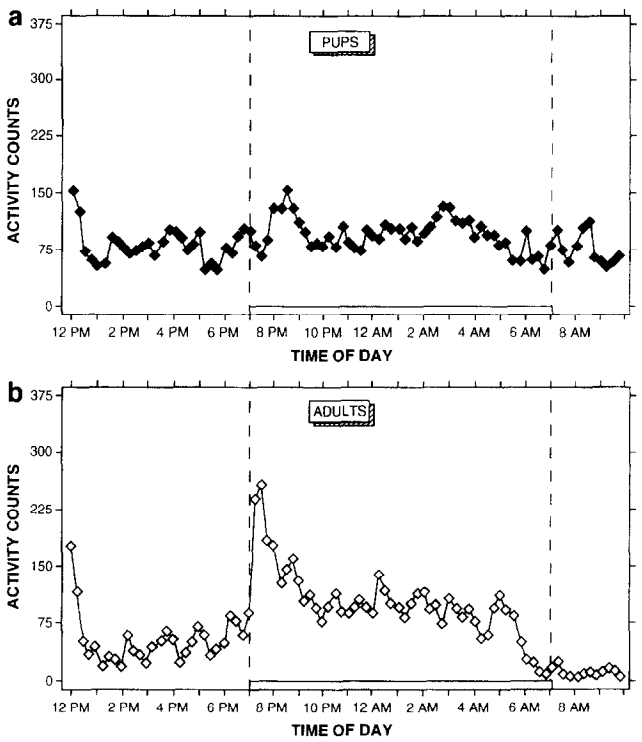

FIG. 1. (a)All-day (22-h) activity patterns for periweanling pups and adult rats (b) in the untreated control group. Pup activity levels were largely independent of the light/dark cycle while adult activity levels were higher during the dark phase. The shaded bar indicates the dark phase (0700 h. to 1900 h). Activity counts are group means tallied by 15-min epochs. Standard errors for the pup and adult means ranged from 3 to 10 and 3 to 12 counts, respectively.

of the pup data during the morning light phase indicated a significant group difference in activity levels with the PFC and C80 groups being significantly less active than the UTC and RA groups (see Table 6).

The ANOVA of the adult data also indicated a significant group difference with the C40 and PFC groups showing slightly elevated activity levels (see Table 6).

DISCUSSION

The effects of the treatment conditions on the maternal variables and offspring physical maturation were consistent with the literature. For example, the reductions in maternal weight gain and food consumption observed in the cocaine and pair-feeding conditions have been reported previously (8,9,11,12). The C80 group showed a significant increase in maternal water consumption. This phenomenon has been reported previously and may reflect a cocaine-induced polydipsia and/or diuresis (11,12,14,15,57). The C80 group also exhibited reduced birthweight and postnatal weight gain and signs of delayed physical maturation (i.e., delayed pinna detachment, ear opening, fur growth) which are consistent with previous observations (8,9,11,12). The PFC group also showed reduced birthweights and some delays in physical maturation, suggesting that similar effects in the C80 group were influenced by undernutrition. The RA group had increases in prenatal and postnatal mortality and anophthalmia which are consistent with the literature (2). Prenatal exposure to cocaine, retinoic acid, and undernutrition also had differential effects on activity levels as summarized below.

When the RA group differed from controls, it was always in the direction of hyperactivity. Specifically, (a) the RA rats exhibited elevated activity levels at both ages during the neophobic situation, (b) the RA pups exhibited elevated activity during the afternoon light phase, (c) the RA adults showed elevated activity during the entire evening dark phase, (d) and both RA pups and adults were more active than all other groups when the entire light/dark cycle was considered in its entirety. This is consistent with the majority of the literature showing that increased activity is usually (2,55,77,78), but not always (2,76), caused by retinoid excess when administered during the second week of gestation in the rat. Our results extend these observations to include different phases of the light/dark cycle and show a persistance of elevated activity levels into young adulthood. Elevated activity in the open-field test is regarded as an indicator of impulsivity, decreased fearfulness and hyperactivity (17, 22).

When the cocaine-exposed groups differed from controls, it was generally in the direction of reduced activity levels. Specifically, reduced activity levels were exhibited by the C40 pup and adults during the neophobic situation. The C80 offspring were also hypoactive in the neophobic situation, but only when the pups and adult data were combined. For unknown reasons, the effect was weaker in the C80 than the C40 offspring.

As for the rest of the light/dark cycle, the C80 pups were significantly hypoactive during the morning light phase, and the C40 adults exhibited significantly reduced activity levels during the first hour of darkness. The one inconsistent result was the slight but significantly elevated activity of the adult C40 group during the morning light phase. Occasional bursts of hyperactivity during select periods of the day might be

TABLE 4

ACTIVITY COUNTS PER 15-MIN EPOCH DURING 22-HR PERIOD (MEAN ± SE)

	Group					
Condition	UTC	PFC	RA	C40	C80	Probability Statistics
(a) Pups	90	86	95[a]	92	86	$F(4, 61) = 2.56$
	(2)	(2)	(2)	(2)	(2)	$p < 0.05$
(b) Adults	77	82	95[b]	73	79	$F(4, 43) = 5.33$
	(2)	(3)	(2)	(2)	(2)	$p < 0.01$

[a] More active than PFC and C80 groups ($p < 0.01$).
[b] More active than all other groups ($p < 0.01$).

TABLE 5

ACTIVITY COUNTS PER 5-MIN EPOCH DURING THE NEOPHOBIC SITUATION (MEAN ± SE)

Condition	Group					Probability Statistics
	UTC	PFC	RA	C40	C80	
(a) Pups	38	36	47[a]	29	31	F (4, 61) = 3.61
	(2)	(2)	(3)	(2)	(2)	$p < 0.01$
(b) Adults	38	31	46[a]	22[b]	31	F (4, 55) = 5.35
	(3)	(2)	(2)	(2)	(2)	$p < 0.01$
(c) Combined	38	33	46[a]	26[c]	31[b]	F (4, 55) = 7.64
	(2)	(2)	(2)	(2)	(2)	$p < 0.01$

[a] More active than all other groups ($p < 0.01$).
[b] Less active than UTC and RA groups ($p < 0.05$ or better).
[c] Less active than UTC, PFC and RA groups ($p < 0.05$ or better).

characteristic of prenatal cocaine exposure, providing further indication of a disrupted BRAC. It is also possible that this modest burst of hyperactivity might have been anomalous and unreproducible. Nonetheless, the general trend for reduced activity levels is consistent with the majority of the prenatal cocaine literature.

Our laboratory (10,12) and others (5,34,39,66,68,70,73) have previously observed that prenatal cocaine exposure caused reduced activity levels for 15–30 min when a periweanling pup was placed in the neophobic situation. The results of the present study were consistent with these observations. This extends our initial observations made with two different lines of Long–Evans rats (10,12) to include the Sprague–Dawley rats used by the present study. One difference is that the present study saw a persistence of this effect into adulthood while the other studies saw a dissipation of this effect with maturation. Another difference is that the present study monitored groups of 3 same-sex littermates, while our previous studies monitored animals singly. Thus, this effect seems to occur in both group and isolated conditions.

TABLE 6

ACTIVITY COUNTS PER 15-MIN EPOCH DURING DIFFERENT PHASES OF THE DAILY CYCLE (MEAN ± SE)

Condition	Group					Probability Statistics
	UTC	PFC	RA	C40	C80	
Afternoon light phase (12 pm to 7 pm):						
(a) Pups	81	76	94[a]	80	78	F (4, 60) = 4.95
	(3)	(3)	(3)	(4)	(3)	$p < 0.01$
(b) Adults	55	70[a]	59	50	54	F (4, 50) = 3.99
	(3)	(3)	(3)	(3)	(3)	$p < 0.01$
First hour of darkness (7 pm to 8 pm):						
(a) Pups	86	80	105	103	83	F (4, 60) = 1.56
	(9)	(9)	(8)	(11)	(7)	N.S.
(b) Adults	204	191	211	148[b]	197	F (4, 43) = 4.28
	(12)	(19)	(13)	(17)	(13)	$p < 0.01$
Evening dark phase (7 pm to 7 am):						
(a) Pups	98	99	99	103	96	F (4, 60) = 0.71
	(3)	(3)	(2)	(3)	(3)	N.S.
(b) Adults	106	108	136[a]	100	109	F (4, 43) = 6.39
	(3)	(4)	(3)	(3)	(3)	$p < 0.01$
Morning light phase (7 am to 10 am):						
(a) Pups	79	56[a]	80	70	62[c]	F (4, 60) = 5.07
	(5)	(4)	(4)	(5)	(4)	$p < 0.01$
(b) Adults	15	22[d]	19	24[e]	17	F (4, 43) = 2.73
	(1)	(3)	(2)	(3)	(2)	$p < 0.05$

[a] More active than all other groups ($p < 0.01$).
[b] Less active than all other groups ($p < 0.01$).
[c] Less active than UTC and RA groups ($p < 0.01$).
[d] More active than UTC ($p < 0.05$).
[e] More active than UTC and C80 groups ($p < 0.05$).
N.S. = not significant

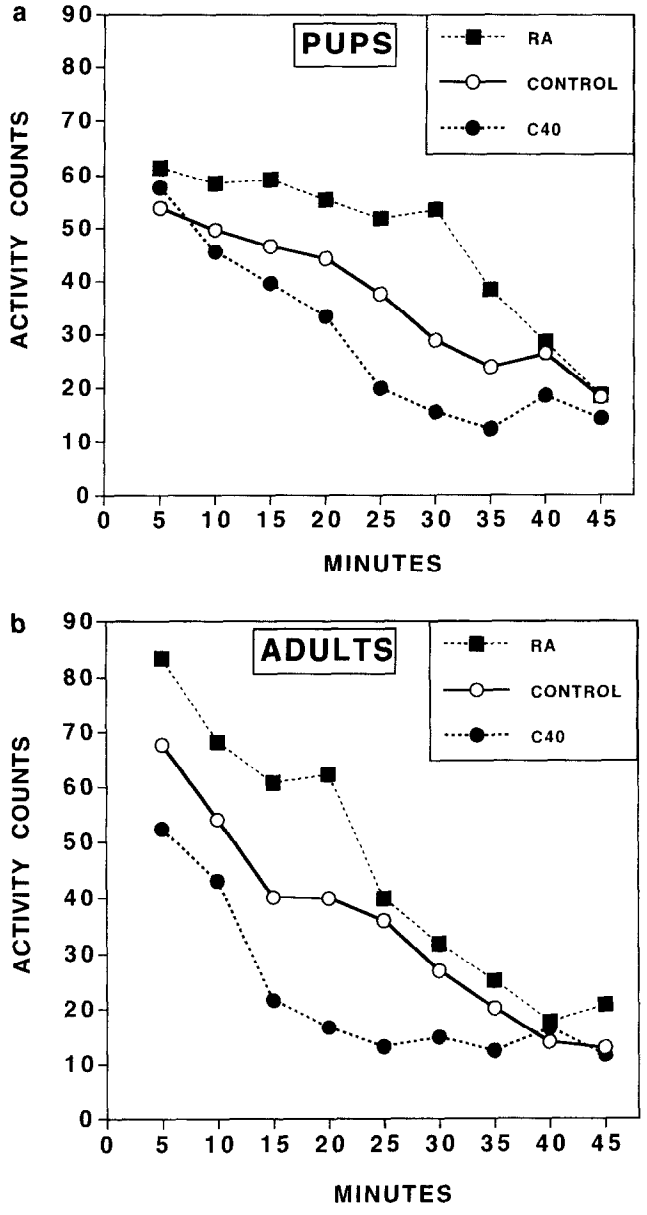

FIG. 2. Activity patterns during the neophobic situation. The periweanling pups (a) and the adults (b) showed some similar group differences. Animals in the RA group were the most active while those in the C40 group were the least active. Animals in the UTC and PFC control groups were intermediate and combined into one control group in this figure for clarity. Activity counts are group means tallied by 5-min epochs. A summary of means and standard errors for all treatment groups are presented in Table 5.

The open-field test, originally introduced by Hall (22), putatively provides a method for measuring individual differences in "emotionality." Denenberg (17) operationally defined the emotional animal as one that has low activity, resulting from an increased duration of initial "freezing." behavior and/or decreased exploratory behavior. This initial freezing or reduced exploration is a species-specific defensive reaction of rats that is displayed in certain fearful situations. When it is displayed in response to a novel environment or stimulus, it is often described as a "neophobic response." The animal will show relatively decreased exploratory behavior for a period of 15–30 min or so. Eventually, the animal habituates to the novel environment and moves about in a normal manner (17). While increased emotionality or neophobia are the common interpretations of the relative decrease in activity levels shown by treated animals in a novel environment, it is also possible that the cocaine-exposed animals were behaviorally depressed, showing decreased play or other social behaviors, experiencing low arousal or a disrupted state control, lacking the motivation to explore their environment, or engaged in nonexploratory behaviors such as grooming, resting or stereotypy.

Recent research has provided a biological mechanism for prenatal cocaine's effects on locomotor activity. The brain's dopaminergic (DA) system is involved in the expression of active behaviors such as motor and sexual behaviors (61). Thus, altered activity levels suggest an attenuated or otherwise dysfunctional DA system. Indeed, there is considerable evidence for a dysfunctional DA system in the striatum, substantia nigra, ventral tegmentum and related structures as a result of prenatal cocaine exposure (3,5,18,23,26,28,30,38,45–50,52–54,57,63,70–72,75,80,81).

Another likely site of DA dysregulation is the hypothalamic suprachiasmatic nucleus (SCN). The SCN functions as a biological clock that generates circadian rhythms (62). Even though we did not observe noteworthy fragmentation or phase shifting of the BRAC, it is still possible that some of the observed activity level changes were due to SCN dysregulation. For example, prenatal exposure to cocaine or other D1-dopamine receptor agonists can alter an offspring's circadian pattern of rest and activity by stimulating the DA system of the fetal SCN (74,79). Such a dysregulation of the SCN could result in disrupted biorhythms, irregularities in the sleep/wake cycle and timing abilities.

Although most studies report reduced activity levels in a neophobic or similar situation as a result of prenatal cocaine exposure (5,10,12,34,39–41,66,68,73), a few studies report no effects (19,20,24,84) or even agitation (24,25,32,35,57). Two studies failing to find statistically significant effects in a neophobic situation nonetheless showed strong trends for reduced activity (19,20). Both studies used very small population sizes ($n = 7$ pups and 4 litters, respectively), perhaps explaining their failure to achieve statistical significance. Some others failing to observe depressed activity may have used cocaine doses that were too low (24) or used the Wistar rat strain (32,84), a strain different from that used by other studies. Strain can be an important factor. For example, there are dramatic genotypic differences in the susceptibility to cocaine's maternal toxicity with Sprague-Dawley rats showing lesser sensitivity than Long-Evans rats (15). Thus, some strains will be less sensitive to cocaine or will be affected differently. Consistent with this fact, the Sprague-Dawley offspring in the present study showed less of an activity reduction in the neophobic situation than the Long-Evans rats in our previous studies (10,12). Also, as underscored by the present study, the manifestations of treatment effects can vary as functions of time-of-day and whether the animal is tested in a novel or familiar environment.

Another factor can be the type of activity monitored. For example, Kunko et al (40) observed that while prenatal cocaine exposure resulted in rats exhibiting less exploratory behavior, the same animals showed increased stereotypy. Thus, monitoring devices that tally both stereotypic and exploratory movements as one category (e.g., vibration monitors) would make stereotypy-prone rats appear to have normal or exces-

sive activity levels despite exhibiting reduced exploratory activity. Finally, it is also known that the timing of prenatal exposure (embryonic versus fetal), age of testing (pre- versus postweanling), prior testing experience, and being tested in groups versus alone can result in differential effects on activity levels (1).

The activity levels of the PFC group were generally not different from the UTC group. There were a few exceptions, however. That is, the adult PFC animals were relatively hyperactive during the afternoon and morning light phases. Also, the periweanling PFC pups exhibited relatively low activity levels during the morning light phase. These data suggest that prenatal undernutrition can alter activity levels during specific phases of the light/dark cycle, but the pattern of effects did not parallel those exhibited by the C80 group except for the morning light phase where both groups showed decreased activity as pups.

In summary, the present study provided new information on the contrasting effects of prenatal cocaine and retinoic acid exposure on behavior in terms of the neophobic situation, activity levels throughout the day and night, and youth versus adulthood. Prenatal cocaine exposure usually resulted in reduced activity levels and prenatal retinoic acid exposure usually resulted in elevated activity levels, indicating that these drugs have differential effects on the offspring. While these effects were sometimes strong and other times only modest, there were noteworthy consistencies in the pup and adult data during the neophobic situation, suggesting that this is a particularly reliable and sensitive test condition. The persistence of neurobehavioral effects into young adulthood indicated that prenatal exposure to either drug can have long-lasting consequences. The results also indicated that the drug-related alterations in activity levels varied as functions of the light/dark cycle and possibly caused some disruption of the basic rest/activity cycle. Further research should examine exposed children for such behavioral alterations (e.g., altered stress responsivity, poor sleep, attention deficits, low arousal, hyper-activity/hypoactivity, reduced social interactions, poor motivation).

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