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Differential Effects of Prenatal Exposure to Alcohol on Activity and Circling Behavior in Rats

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ZIMMERBERG, B., E. P. RILEY AND S. D. GLICK. *Differential effects of prenatal exposure to alcohol on activity and circling behavior in rats.* PHARMACOL BIOCHEM BEHAV **25**(5) 1021–1025, 1986.—The effects of prenatal exposure to alcohol on behavioral correlates of catecholaminergic function were examined in three experiments. In each experiment, subjects had one of 3 prenatal treatment histories: prenatal alcohol-exposed (35% ethanol-derived-calories, 35% EDC), nutritional control (0% ethanol-derived calories, 0% EDC) or standard control (lab chow, LC). In the first experiment, juvenile (33 to 59 days of age) female rats were tested for 16 hours overnight in standard rotometers. Subjects in the 35% EDC group were more active compared to both control groups, who did not differ from each other. However, the degree of circling (rotation) was not altered by prenatal exposure to alcohol. Since there was a positive correlation between age and rotation in the LC group, but not in the other 2 groups, adult female rats were similarly tested overnight in the rotometer. Again, circling behavior was not differentially affected by prenatal exposure to alcohol. Total overnight activity was no longer significantly greater in the adult 35% EDC group, although their initial activity was higher than that of the LC control group. In the third experiment, subjects from Experiment 2 were administered d-amphetamine (1.25 mg/kg) and tested in the rotometer for 1 hour. Both rotation and activity were increased by d-amphetamine, but there was not a significant interaction with prenatal alcohol exposure. Since rotation is a reflection of the intrinsic asymmetry of the nigrostriatal system, these results suggest that prenatal exposure to alcohol does not alter the development of this asymmetry.

Alcohol Fetal Alcohol Syndrome Rotation Activity Amphetamine

ALCOHOL has been well characterized as a behavioral teratogen, and overactivity is a prominent feature in animal models of prenatal exposure to alcohol [13]. Rats exposed to alcohol in utero are more active in an open-field [3, 4, 15], running wheel [13], and nose-poking and head-dipping chambers [17] than control animals. There are conflicting reports, however, concerning the role of catecholamine systems involved in arousal and activity in the etiology of prenatal alcohol exposure-related hyperactivity. For example, weanling-age rat pups and adult offspring exposed to alcohol prenatally were more sensitive to the stimulating effects of methylphenidate than control subjects [12,18]. In contrast, prenatal exposure to alcohol did not differentially enhance the activity-stimulating effects of d-amphetamine in 10 to 28 day old rat pups [2].

Circling behavior (rotation) has been used to assess the effects of pharmacological agents on nigrostriatal function after unilateral brain lesions [6, 16, 19]. Rotation has also been useful in characterizing an intrinsic nigrostriatal asymmetry in rats; the same drugs that elicit rotation in lesioned animals also induce rotation, at lower rates, in non-lesioned animals [8]. Non-lesioned rats will also rotate spontaneously at night when they are normally more active, and the direction of nocturnal rotation is usually the same as that following amphetamine [5].

Female rats' turning preferences have been related to asymmetries in striatal dopamine, dopamine metabolites, and dopamine-stimulated adenylate cyclase [7]. This investigation was designed to determine if prenatal exposure to alcohol would affect directional preference or strength of circling behavior, and whether the rotometer would be useful in detecting altered levels of activity in animals with prenatal alcohol exposure. In addition to monitoring circling behavior, the rotometer provides an independent measure of activity (extra quarter turns). The rotometer has formerly only been used to study circling behavior in adults. However, since the overactivity caused by prenatal exposure to alcohol is not apparent in some test situations in adults [1], juvenile-aged subjects were used in the first experiment.

METHOD

Subjects

After acclimation to the laboratory, naive, nulliparous female Long Evans hooded rats (Blue Spruce Farms, Altamont, NY) were placed individually with a male in the late afternoon, and the bedding under their cages examined for the presence of a vaginal plug the next morning (Gestational Day 1). If a plug was detected, the female was weighed, individually housed in a standard plastic breeding cage and as-

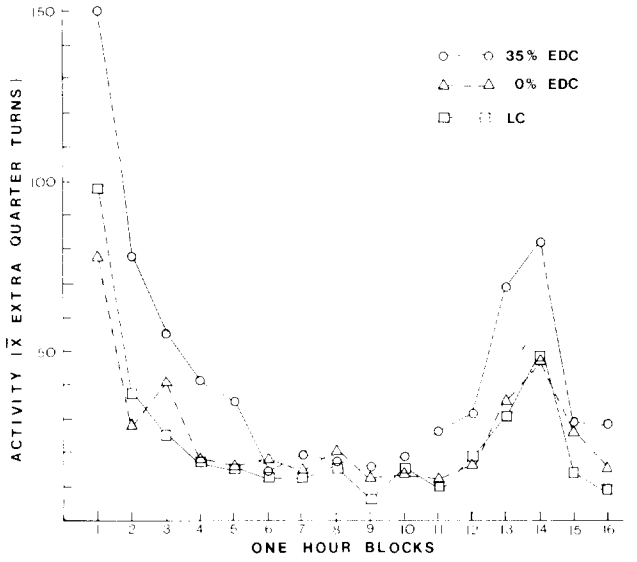

FIG. 1. Activity in juvenile female rats for 3 prenatal treatment groups (35% EDC, 0% EDC and LC) over the 16 hours of the nocturnal rotation test. Data points represent mean extra quarter turns per 15 subjects in the 35% EDC and LC groups, and per 16 subjects in the 0% EDC group.

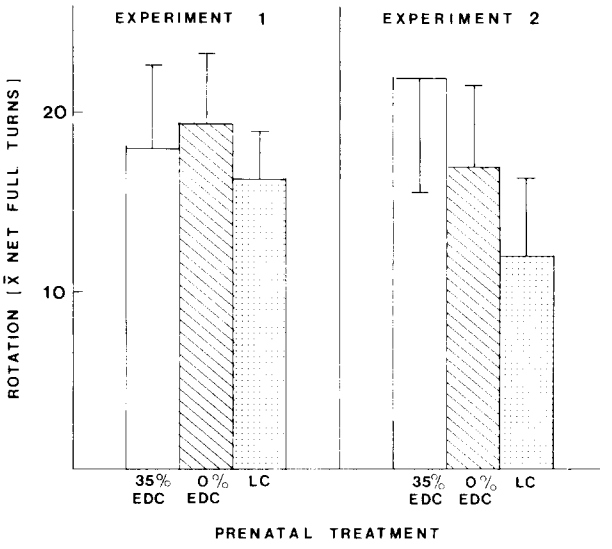

FIG. 2. Rotation in juvenile (Experiment 1) and adult (Experiment 2) female rats for 3 prenatal treatment groups (35% EDC, 0% EDC and LC) as measured by mean net full turns over 16 hours of the nocturnal rotation test. In Experiment 1, mean values ±S.E.M. represent 15–16 subjects per group; in Experiment 2 mean values ±S.E.M. represent 8 subjects per group.

signed to one of the three treatment groups. Females in the lab chow (LC) control group were provided standard lab chow and water ad lib thoughout their pregnancies. Pregnant females in the other 2 groups were treated identically to LC females from Gestational Days (GD) 1 to 5. Starting on GD 6, pregnant females in the alcohol treatment condition were given a liquid diet consisting of chocolate Sustacal (Mead Johnson), vitamin diet fortification mixture (ICN Nutritional Biochemicals), mineral supplement (ICN Nutritional Biochemicals), water and 95% ethanol. This diet provided 35% of the total caloric content as ethanol (35% ethanol-derived calories, 35% EDC). In the nutritional control group, pregnant females on GD 6 began receiving a similar liquid diet except that the ethanol was replaced isocalorically with sucrose (0% ethanol-derived calories, 0% EDC). Both diets provided 1.3 kcal/ml, and to control for caloric intake a pair-feeding procedure was utilized. Each female in the 0% EDC group was matched to one of the 35% EDC females and fed the amount consumed by that 35% EDC female, corrected for body weight, for each specific day of pregnancy. Thus, each matched pair received the same relative volume of diet (ml/kg) and hence the same number of calories on a body weight basis; the only difference being the presence or absence of alcohol.

On GD 20, the liquid diets were replaced by ad lib lab chow and water and the breeding cages checked 3 times daily for births. Following parturition, pups were weighed, measured, and inspected for any obvious structural abnormalities. Litters were culled randomly to 10 offspring per litter (5 of each sex whenever possible). At 21 days of age, the pups were housed 2 per cage with a same-sexed littermate.

Maternal and Pup Data

Since a large nursery colony is continually maintained in this laboratory, the maternal and pup data are based on a larger group of 69, 65, and 75 litters from the 35% EDC, 0% EDC and LC groups, respectively, from which the test subjects were randomly selected. During pregnancy, the mean percent maternal weight gain was 35.9% for the 35% EDC group, 32.7% for the 0% EDC group, and 40.5% for the LC group. Analysis of variance (ANOVA) revealed a significant effect of Prenatal Treatment, $F(2,206)=24.47$, $p<0.001$, and subsequent analysis by Newman-Keuls tests indicated that each treatment group varied significantly from each other (p's<0.01). The average daily alcohol consumption by the 35% EDC mothers was 13.58 g/kg alcohol per day.

There were no differences among treatment groups in the number of pups born per litter. The litter mean birth weights in the 35% EDC litters were 6.0 g for males and 5.7 g for females; in the 0% EDC litters they were 6.8 g for males and 6.5 g for females, and in the LC litters they were 6.9 g for males and 6.6 g for females. An ANOVA on the mean birth weights of the pups revealed significant effects for both Prenatal Treatment, $F(2,400)=57.73$, $p<0.001$, and Sex, $F(1,400)=17.62$, $p<0.001$, with males weighing more than females. Comparisons among means (Newman-Keuls tests) indicated that 35% EDC pups weighed less than the other two groups ($p<0.01$). The 0% EDC and LC groups did not differ in mean birth weight.

Apparatus

The rotometer [5] consists of a cylindrical (30.5 cm in diameter) Plexiglas enclosure with a grid floor. The rotometer is situated in an environmentally controlled room with lights on/off at 7.00 hr/19.00 hr. A flexible wire, which harnesses the animal, is connected to a shaft which activates a photoelectric position sensing device that differentiates between incomplete and full (360°) rotations. Quarter turns (90°) and full turns in both left and right directions are separately recorded. Data are recorded at hourly intervals when

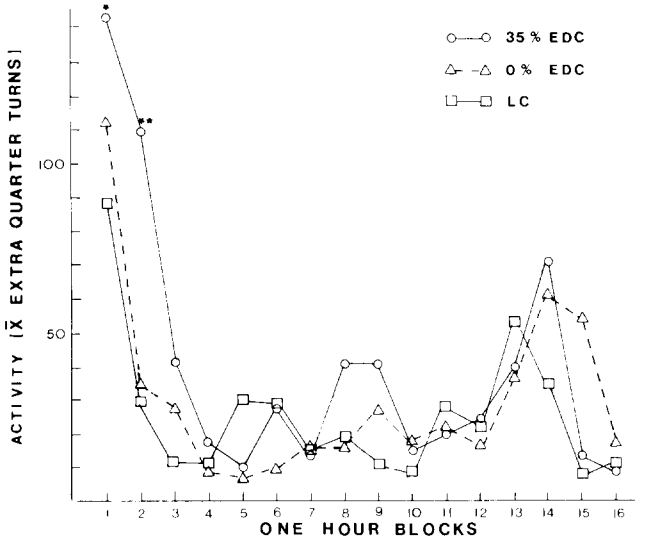

FIG. 3. Activity in adult female rats for 3 prenatal treatment groups (35% EDC, 0% EDC and LC) over the 16 hours of the nocturnal rotation test. Data points represent mean extra quarter turns per 8 subjects. *Significantly different as compared to LC group, $p<0.01$. **Significantly different as compared to LC and 0% EDC groups, $p<0.01$.

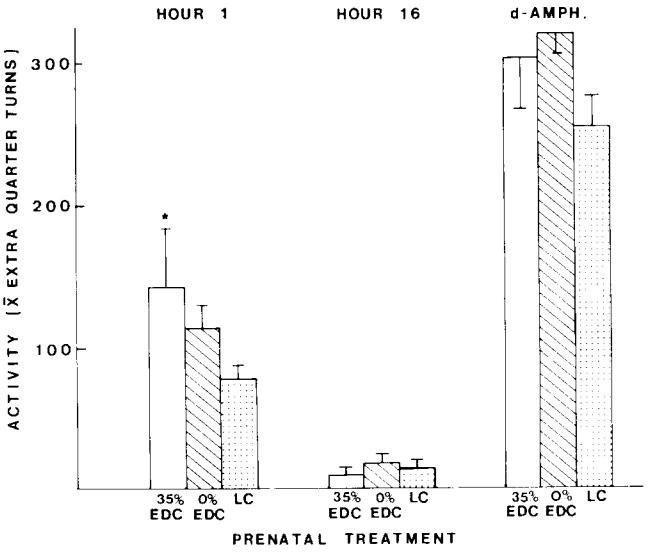

FIG. 4. Activity in adult female rats for 3 prenatal treatment groups (35% EDC, 0% EDC and LC) during the first and last hours of the nocturnal rotation test and during the subsequent hour after administration of d-amphetamine. Values represent mean extra quarter turns ±S.E.M. for 7–8 subjects per group. *Significantly different as compared to LC group, $p<0.01$.

assessing nocturnal rotation over 16 hours, and recorded at 5 minute intervals when determining the time course of drug effects over 1 hour. Rotation (full turns) in the dominant direction minus rotations in the opposite direction are referred to as "net rotations." Extra quarter turns are 90° turns not included in full turns, and are considered as a measure of general activity. Extra quarter turns in the rotometer have been found to be correlated with activity as measured in photocell activity boxes [9]. During nocturnal testing, an attached drinking tube provides water and food pellets are scattered on the floor.

EXPERIMENT 1

Procedure

In this first experiment, 16 female juvenile rats from each prenatal treatment group (35% EDC, 0% EDC and LC) were selected; each litter was represented by only 1 subject. The mean age at testing for the 35% EDC, 0% EDC and LC prenatal treatment groups were 47 days (range of 33–59 days), 49 days (33–59 days) and 47 days (42–57 days), respectively. Subjects were tested overnight in the rotometer from 16.30 to 08.30 hr the next morning. One subject in the 35% EDC group and 1 subject in the LC group escaped from the harnesses overnight and were excluded from the data analysis.

RESULTS

Effects of Prenatal Treatment on the Activity Measure

The mean hourly session extra quarter turns for the 16 hour nocturnal test are shown in Fig. 1. An analysis of variance (ANOVA) of the effect of prenatal history on overnight activity, as measured by extra quarter turns, revealed a significant effect of Prenatal Treatment, $F(2,43)=4.63$, $p<0.02$. Mean total extra quarter turns were 721, 398 and 399 for the 35% EDC, 0% EDC and LC groups, respectively. Subsequent comparisons among means (Newman-Keuls tests) indicated that the 35% EDC group were more active than the 0% EDC group or the LC group ($p<0.01$), who did not differ from each other. Although there was a significant Block effect, $F(15,645)=19.4$, $p<0.001$, there was no Block × Prenatal Treatment interaction.

Effect of Prenatal Treatment on the Rotation Measure

Mean nocturnal net rotation, the difference between full turns to the preferred direction minus full turns to the nonpreferred direction, for each prenatal treatment group, is shown in Fig. 2. An ANOVA of this measure indicated that there were no significant effects of prenatal treatment, nor were there any interactions between Block (1 hour sessions) and Prenatal Treatment. Similarly, prenatal history also did not affect the other measure of rotation, percent preference towards the preferred side.

Since rotation has previously been measured only in adults, and the net rotations in the control groups appeared to be lower than that generally found with other strains, the relationship between age and net rotation was examined by linear regressions. There was a significant relationship between age and net rotation, $r=+.623$, $t(13)=2.98$, $p=0.01$, in the LC control group. However, there were no significant relationships between age and net rotations in either the 0% EDC group or the 35% EDC group. In addition, age did not have any relationship to the other dependent variable, extra quarter turns.

EXPERIMENT 2

Experiment 1 indicated that the prenatal exposure to alcohol was associated with increased activity, but was not associated with a differential effect on rotation. However, the overactivity related to prenatal alcohol exposure has

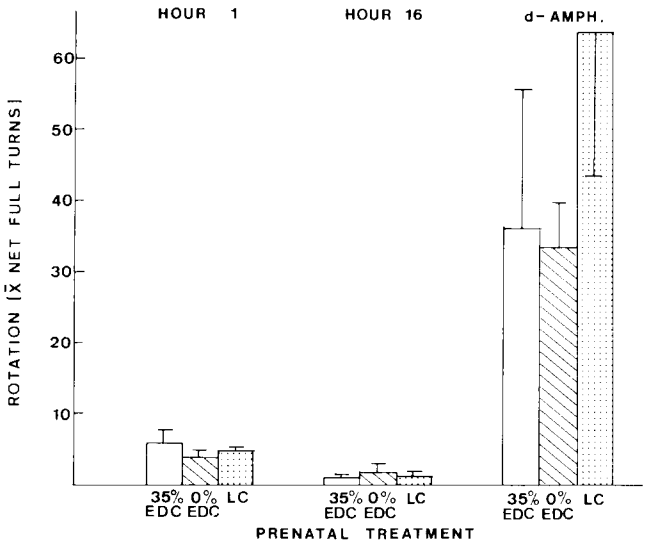

FIG. 5. Rotation in adult female rats for 3 prenatal treatment groups (35% EDC, 0% EDC and LC) during the first and last hours of the nocturnal rotation test and during the subsequent hour after administration of d-amphetamine. Values represent mean net full turns ±S.E.M. for 7–8 subjects per group.

generally not been observed when offspring are tested after 70 days of age [1]. Furthermore, age only appeared to influence the degree of rotation in the LC control group, suggesting an interaction between nutritional variables and rotation. This next experiment, therefore, had two purposes: first, to determine if the overactivity after prenatal alcohol exposure in the juvenile subjects was still present in adult females, and to determine if prenatal treatment would differentially affect rotation in an older age group.

Subjects and Procedure

Eight naive, adult female subjects from each of the 3 prenatal treatment groups (35% EDC, 0% EDC and LC) were randomly selected, with each litter represented by only 1 subject. At the time of testing, the mean ages for the 35% EDC, the 0% EDC and the LC groups were 105, 104, and 105 days, respectively. Their mean weights were 264, 261 and 261 g, respectively. As in the first experiment, subjects were tested overnight for 16 hours in the rotometer, and extra quarter turns and net rotations recorded for each 1 hour session.

RESULTS

Effect of Prenatal Treatment on the Activity Measure

The mean hourly session extra quarter turns for the nocturnal test are shown in Fig. 3. An ANOVA revealed a significant Block × Prenatal Treatment interaction, $F(30,315)=1.74$, $p<0.02$. Subsequent decomposition of this interaction by Simple Main Effects indicated significant effects of prenatal treatment in the first 2 hours of testing. In both of these blocks, the mean activity of the 35% EDC groups was significantly greater than that of the LC group (Newman-Keuls' tests, $p<0.01$). Only in the second block, however, was the mean activity significantly greater than the 0% EDC group (Newman-Keuls test, $p<0.01$). Over the total 16 hours, mean extra quarter turns for the 35% EDC, 0% EDC and LC groups were 633.5, 499.6 and 423.8, respectively; there was no Main Effect of Prenatal Treatment.

Effect of Prenatal Treatment on the Rotation Measure

Figure 2 shows the mean net rotations for each prenatal treatment group for the total overnight test. There were no differences in net rotation or directional preferences between prenatal treatment groups, nor was there any Block × Prenatal Treatment interaction.

EXPERIMENT 3

The results of the second experiment indicated that overactivity in the rotometer after prenatal alcohol exposure is less apparent in adult animals than in juvenile animals, consistent with studies of activity using other apparati [1]. The nocturnal test, however, did detect an initial activity increase in adults. Testing adult animals compared to the younger age group did not reveal any significant effect of prenatal treatment on rotation.

Nocturnal rotation is a reflection of the intrinsic asymmetry of the nigrostriatal system; this asymmetry is enhanced by the administration of d-amphetamine. The next experiment was designed to determine if d-amphetamine-induced rotation would reveal any underlying differential effect of prenatal exposure to alcohol that was not apparent in the nocturnal test. Furthermore, since d-amphetamine also increases activity in the rotometer, this experiment might detect an interaction between prenatal treatment and d-amphetamine-induced activity. Methylphenidate, a piperidine derivative of amphetamine that is a similarly-acting behavioral stimulant, has been found to produce greater enhancement of activity in open field tests in rats prenatally exposed to alcohol compared to control groups [12,18]. However, a recent study of the effects of d-amphetamine on open field activity in alcohol-exposed offspring did not find any altered sensitivity to d-amphetamine [2].

Procedure

The subjects in Experiment 2 were injected with 1.25 mg/kg of d-amphetamine, IP, at the end of the nocturnal rotation test. This dose induces maximal rotation in female rats [8]. They were then returned to the rotometers for 1 hour, with quarter turns and net rotations rocorded every 5 minutes. One subject in the 0% EDC group escaped from the harness and was therefore not included in the data analysis.

RESULTS

Effect of Prenatal Treatment on the Activity Measure

The effect of d-amphetamine administration on the mean number of extra quarter turns in each prenatal treatment group, compared to both the first and last hour of the nocturnal test, is shown in Fig. 4. d-Amphetamine increased the activity in all groups compared to both the first hour, $F(1,20)=49.29$, $p<0.001$, and the last hour, $F(1,20)=148.82$, $p<0.001$. However, there was no interaction with Prenatal Treatment on d-amphetamine-induced increases in activity.

Effect of Prenatal Treatment on the Rotation Measure

Figure 5 shows the mean net rotations for each prenatal

treatment group for the first and last hours of the nocturnal test, and for the hour after d-amphetamine administration that followed the nocturnal test. d-Amphetamine-induced rotations were significantly greater than those in first hour, $F(1,20)=14.90$, $p=0.001$, and those in the last hour, $F(1,20)=17.63$, $p<0.001$. Rotation was also greater in the 1 hour d-amphetamine test than in the total 16 hours of overnight testing, $F(1,20)=7.55$, $p<0.02$.

DISCUSSION

The results of these experiments suggest that the overactivity caused by prenatal exposure to alcohol in the rat may persist later in its life cycle than generally determined in open field tests. Furthermore, this is the first use of the rotometer in behavioral teratology, and the nocturnal rotation test appears to be a sensitive method for detecting different levels of activity in groups with distinct prenatal histories.

In contrast to altered activity levels, particularly in the juvenile age group, rotation was not affected by prenatal exposure to alcohol. This was the case for both nocturnal and d-amphetamine-induced rotation. The differential alteration of activity levels compared to the lack of effect on circling behavior suggests that any altered catecholaminergic function following in utero exposure to alcohol would be more likely to involve the mesolimbic rather than the nigrostriatal system [10].

The role of catecholaminergic neuronal systems in the regulation or modulation of hyperactivity following prenatal alcohol exposure is still in question. This study, in agreement with Bond [2], finds no evidence of altered sensitivity to d-amphetamine in the group exposed to alcohol in utero. However, these results appear to conflict with those of Ulug and Riley [18] and Means and colleagues [12]; both of these studies report an increased response to methylphenidate in subjects prenatally exposed to alcohol. In the later study [12], as in this investigation, adult subjects were tested, although the subjects' sex and method of measuring activity were different.

Both amphetamine and methylphenidate release catecholamines from neuronal pathways. However, their mechanisms are not identical; it is thought that while amphetamine preferentially releases neurotransmitter from a newly synthesized pool, methylphenidate preferentially releases catecholamines from a storage pool [14]. Prenatal exposure to alcohol may alter the metabolism of catecholamines such that the relative size or availability of these pools is changed.

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