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Postnatal stress of early weaning exacerbates behavioral outcome in prenatal alcohol-exposed juvenile rats

Betty Zimmerberg*, Heather E. Weston

Department of Psychology, Bronfman Science Center, Williams College, Williamstown, MA 01267, USA

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Abstract

Some of the behavioral deficits caused by prenatal or postnatal alcohol exposure have been demonstrated to be ameliorated by environmental manipulations such as handling or environmental enrichment. This experiment, in contrast, investigated whether behavioral deficits due to prenatal alcohol exposure could be exacerbated by a stressful experience, early weaning. Pregnant dams were given either a liquid diet with 35% of the calories derived from alcohol, a liquid diet without alcohol to control for any effects of the liquid diet administration, or ad libitum food and water. Half of each litter were weaned at 15 days of age (early weaning) and half were weaned at 21 days of age (normally weaned). Offspring were weighed, tested for activity in an open field at 18 days of age, and trained to find a hidden platform in the Morris water maze at 22–24 days of age. Alcohol-exposed subjects who were weaned early were more impaired in spatial navigation ability than any other group. Similarly, the combination of early weaning and prenatal alcohol exposure caused the slowest growth. All subjects exposed to alcohol, regardless of weaning condition, had greater latencies to find the platform than those from the two control groups. There was no synergistic effect of alcohol and stress on activity levels, but all early-weaned females were more active than normally weaned females; males did not show this effect. Thus, environmental stressors such as early weaning can compound detrimental symptoms of prenatal alcohol exposure. These results have implications for the understanding of the effects of the environment on neuronal plasticity.

Keywords: Early weaning; Prenatal alcohol exposure; Fetal alcohol syndrome; Stress maternal deprivation; Spatial navigation; Morris maze; Activity; Ethanol

1. Introduction

Exposure of a growing fetus to alcohol produces a predictable array of behavioral and growth deficiencies. The degree to which the neonate is affected at birth has been shown to vary due to several prenatal variables—dose, gestational age of exposure, duration of exposure, genetic vulnerability, etc. (Abel, 1996; Hannigan et al., 1999; West, 1986). However, the environmental experiences of the growing neonate after birth, when all exposure to the teratogen, alcohol, is finished, may also modulate the ultimate behavioral expression of brain damage experienced in utero (Hannigan and Berman, 2000). While previous experiments supporting the role of environment in prenatal alcohol-exposed offspring have centered on the ameliorative effects of "positive" experiences, this experiment reversed the question to determine whether a "negative" experience might worsen the effects of prenatal alcohol exposure, thus supporting the hypothesis of environmental influence from the opposite direction.

Various studies have tried to isolate general patterns and levels of maternal alcohol consumption and their effects on offspring's growth, activity, and cognitive abilities. For example, head circumference of alcohol-exposed neonates was found to be negatively correlated to measures of alcohol intake by the pregnant mother (Russel et al., 1991). A study by Streissguth et al. (1989) examining the relationship of prenatal alcohol exposure to IQ levels in 4-year-old children found that there was a significant negative relationship even in a relatively healthy, middle-class sample. Another study, however, did not find evidence of an inverse relationship between reports of maternal consumption and comprehensive tests of cognitive abilities administered five times during the first 5 years of life (Greene et al., 1991). These authors deduced that developmental outcomes of effects of prenatal

* Corresponding author. Tel.: +1-413-597-2446; fax: +1-413-597-2085.

E-mail address: betty.zimmerberg@williams.edu (B. Zimmerberg).

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alcohol exposure have more of a relationship to environmental influences than to the degree of prenatal insult.

Animal models, generally using rodents, are valuable for examining different patterns of maternal drinking and their effects on the fetus and its later development, and for separating and exploring direct prenatal injury by alcohol versus environmental factors. There have been several studies looking at the role of two well-established "positive" paradigms of environmental manipulations on the outcome after fetal alcohol exposure in rodents: enriched environment and early handling. Both Hannigan et al. (1993) and Wainwright et al. (1993) reported that 6 weeks of rearing in an enriched environment enabled prenatal alcohol-exposed rats and mice, respectively, to improve Morris-maze performance to the same degree as controls. Deficits in gait were also ameliorated by the enriched environmental rearing (Hannigan et al., 1993). However, in a subsequent study, prenatal alcohol-exposed rats reared for 10 weeks in an enriched environment did not demonstrate the increased CAI pyramidal cell apical and basilar spine densities seen in control rats (Berman et al., 1996).

In a variant of the enriched environment paradigm, postnatal alcohol-exposed and control rats were given 10 days of training on an obstacle course (Klintsova et al., 1997). Alcohol-exposed adult offspring were found to improve their performance on this course as well as gastronomy controls, although not as much as normally reared controls. This training had been previously shown to increase the number of synapses in cerebellar Purkinje cells (Black et al., 1990). The alcohol group did have comparable increases in this measure to both control groups, although the relative density of the Purkinje cell layer was decreased. In a subsequent study, postnatal alcohol-exposed and control groups given 20 days of obstacle course training were found to be equally improved on several tests of coordination and balance (Klintsova et al., 1998). Subjects given rehabilitation training also had similar increases in the number of parallel fiber synapses per cerebellar Purkinje cell (Klintsova et al., 2000).

In a series of studies looking at how early handling changed the outcome after prenatal alcohol exposure, Weinberg's laboratory demonstrated that this paradigm can have varied effects. Three minutes of handling treatment on Postnatal Days (PNs) 2 through 15 reversed alcohol-related deficits in growth and some aspects of altered thermoregulatory and corticosterone responses to stress or acute alcohol administration in adult offspring (Weinberg et al., 1995). However, similar neonatal treatment did not attenuate deficits in feedback inhibition of the HPA axis in alcohol-exposed adult offspring (Gabriel et al., 2000). In a subsequent study, handling ameliorated prenatal alcohol-related postweaning growth deficits, but did not alter prenatal alcohol-related alterations in saccharin consumption. Inhibition of consumption after an aversive experience, however, was increased by postnatal handling in the control groups, but not in the prenatal alcohol group (Gabriel and Weinberg, 2001). The early experience of handling also did not attenuate deficits associated with prenatal alcohol exposure on spatial navigation in the Morris water maze in adult or aged subjects (Gabriel et al., 2002). In contrast, 20 min of handling on PNs 2 through 21 was found to ameliorate a spatial learning deficit due to prenatal alcohol exposure in another laboratory: only the nonhandled, alcohol-exposed adult offspring took significantly more trials to learn a reversal task in a T-maze (Lee and Rabe, 1999). In this experiment, the simpler task, initial acquisition of the position, was not affected by prenatal diet or early handling.

Two behavioral measures that are often used to assess the effects of teratogens in a rodent model are spatial navigation in the Morris water maze and locomotor activity in an open field. Prenatal and postnatal alcohol exposure causes reliable deficits in both measures (Blanchard et al., 1987; Goodlett et al., 1987). After Bond and Diguisto's original finding that prenatal alcohol exposure caused hyperactivity, increases in activity have been noted in several rodent models (Bond and Diguisto, 1976; Riley, 1990). This hyperactivity seems to disappear as rats mature, but a stressor can re-elicit hyperactivity. In this experiment, we used both of these measures, spatial navigation and activity, to determine the modulating effects of early weaning as an environmental stressor.

Early weaning has been used as the model for maternal deprivation stress. One study using this paradigm found that early weaning produced higher activity levels on PN 25 than normally weaned pups, but only when the pups were isolated from littermates (Fahlke et al., 1997). In addition, adult subjects with normal weaning consumed more ethanol compared to subjects with early weaning. These results are in contrast to a previous study in which early-weaned rats consumed more alcohol than did normal-weaned controls when tested as adults (Rockman et al., 1987). The pattern of play behavior, although not the frequency, was also altered by early weaning (Janus, 1987). Disrupted play behavior was also reported by Brunelli et al. (1989). However, the combined effects of prenatal alcohol exposure and early weaning have not yet been examined. In this experiment, this combination was investigated for its effects on growth, spatial navigation, and activity to determine whether an environmental manipulation can modulate a pharmacological challenge. Juveniles were tested because these prenatal alcohol-related sequelae are most apparent at this age.

2. Methods

2.1. Subjects

Subjects were bred in this laboratory from female and male Long–Evans hooded rats (Harlan Sprague Dawley, Indianapolis, IN). Pregnant females, determined by the presence of a vaginal plug, were individually housed in plastic cages ($45 \times 25 \times 15$ cm) in an isolated nursery on a 0700 to 1900 h light–dark cycle with the temperature

maintained at 23 °C. Females in the standard control group had continuous access to standard lab chow (LC) and water throughout their pregnancies. Pregnant females in the other two groups were treated identically to LC females on gestational days (GDs) 1 to 5. Starting on GD 6, pregnant females in the alcohol liquid diet condition were given a liquid diet containing 6.7% v/v ethanol (BioServ Liquid Rat Diet F1265, BioServ, Frenchtown, NJ). This diet provided 35% of the total caloric content through ethanol. In the pair-fed control group, pregnant females on GD 6 began receiving a similar liquid diet (BioServ Liquid Rat Diet F1264) except that the ethanol was replaced isocalorically with maltrose–dextrin (0% ethanol-derived calories, 0% EDC). A pair-feeding procedure was utilized to control for caloric intake. Each female in the 0% EDC group received the average volume of diet consumed by a 35% EDC female on a milliliter per kilogram body weight basis for that day of pregnancy; this amount was derived from a decade of archived alcohol diet intake data. Diets were presented at 1700 h. On GD 20, the liquid diets were replaced by continuous access to LC and water and the breeding cages were checked three times daily for births.

Pups were left undisturbed on the day births were noted. Litters were culled to 12 pups on the day following their birth (PN 0), leaving six males and six females when possible. Half of the pups from each litter were weaned "early " on PN 15, and the other half were weaned at the typical age on PN 21, equating sex when possible. All pups were ear-punched according to their sex and their number within a given condition and weighed on PN 15. After either age at weaning, subjects were placed in a standard hanging cage with two to three siblings and water and LC were continually available. Subjects were weighed again on 18, 21, and 24 days of age.

To minimize litter effects, at most two males and two females were randomly selected from the two weaning conditions from any one litter for behavioral testing. Different subjects were used for the two behavioral measures. All procedures were approved by the Institutional Animal Care and Use Committee and are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Activity testing

On PN 18, subjects were removed from the colony and brought to an adjacent room maintained at 23 °C. After being weighed, subjects were placed individually into an activity boxes (44 × 44 × 14 cm) with optical beams running along each axis at 5-cm intervals. A Macintosh computer recorded the number of times each subject crossed the beams. While testing, lights were dimmed. Open-field activity was measured for a total of 15 min, timed in three 5-min intervals. All testing was conducted between 1300 and 1500 h. Only one or two subjects of each sex were taken from any one litter (total $n=88$).

2.3. Spatial navigation (Morris water maze)

On PN 22 to PN 24, subjects were brought individually to an adjacent testing room maintained at 23 °C A subject was placed into a circular (72 cm diameter) galvanized tub filled with water made opaque with nontoxic white Crayola powder paint. The tank was divided into four quadrants (not visible to subject) and a small platform was placed into one quadrant (the "goal" quadrant) 1 cm below the surface of the water. The animal was placed, facing outward, into one of the other three quadrants (the "start" quadrant) and was allowed to swim until it had found the platform or until a 120-s ceiling was reached. The rat was removed from the tank approximately 5 s after it reached the platform and was placed in a plastic cage on a heating pad maintained at 32 °C for a 1-min interval. This procedure was repeated for three more trials each day. The platform was placed into a different "goal" quadrant for different animals but remained the same for each subject for all of its trials. All testing was conducted between 1300 and 1500 h. Only one or two subjects of each sex were taken from any one litter (total $n=105$).

2.4. Data analysis

This experiment was a $2 \times 2 \times 3$ design with sex, weaning condition, and prenatal diet as between-factor measures. Within-factor repeated measures included day for weight, time for activity, and day and trial for the Morris water maze. Data were analyzed using an ANOVA multivariate analysis. Significant main effects were analyzed by Fisher's LSD tests and significant interactions were analyzed with post hoc multiple means comparison tests (significance is indicated when $P<.05$). Simple regression analysis was conducted to compare body weight and activity or latency.

3. Results

3.1. Developmental data

Subjects were drawn from a total of 28 litters: 9 alcohol and 9 LC control and 10 pair-fed dams. The mean daily alcohol consumption by the alcohol dams was 16.81 ± 0.49 g/kg/day. Mean birth weights were 5.81 ± 0.17, 6.48 ± 0.38, and 7.42 ± 0.15 g for the alcohol, pair-fed, and LC control pups, respectively. Birth weights differed significantly by prenatal treatment [$F(2,25)=6.03$, $P=.007$]; alcohol pups weighed significantly less than the pair-fed pups, but not the control pups (approached significance at $P=.08$). Control and pair-fed pups did not differ.

3.2. Growth

All main factors had significant effects on growth (Fig. 1). Body weight differed significantly by sex [$F(1,279)=15.58$,

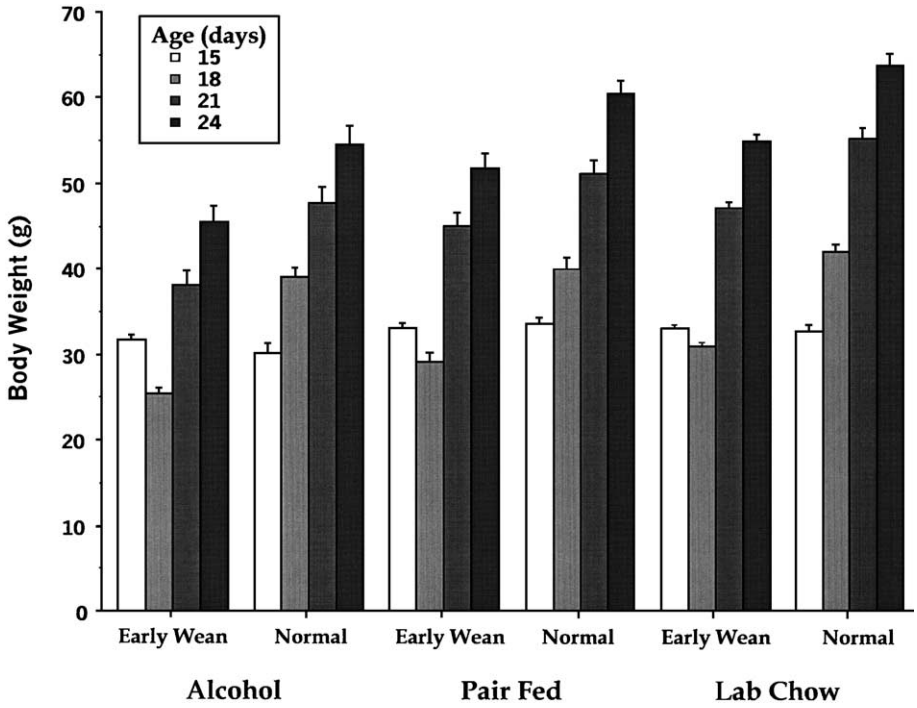

Fig. 1. Mean body weight (g) (±S.E.M.) from 15 to 24 days of age for three prenatal treatment groups (alcohol, pair-fed, and control) who were weaned at either 15 (early) or 21 (normal) days of age.

P=.0002], males weighed more than females, and by weaning condition [F(1,279)=84.95, P=.0001], normally weaned subjects weighed more than early weaned. There was an interaction between prenatal diet and weaning condition across days [F(6,279)=2.87, P=.04]. On PN 15, prior to any experimentation, only prenatal diet had a significant effect on weight. Alcohol diet subjects weighed less than pair-fed or LC subjects, who did not differ from each other. Over the next 9 days, the rate of growth of the early-weaned alcohol group compared to the two early-weaned control groups was less that that of the normally weaned alcohol group compared to its control groups. By PN 24, the normally weaned alcohol group had gained as much weight as the pair-fed, and now weighed only significantly less than LC group, while the early-weaned alcohol group still weighed less than both early-weaned control groups. Across all days combined, prenatal diet also had a main effect on body weight [F(2,279)=23.66, P=.0001]; all three groups differed.

3.3. Activity

Eighty-eight subjects were tested for open-field activity, n's=7–8 per sex per prenatal diet per weaning condition (Fig. 2). Weaning condition had a significant main effect on levels of activity [F(1,152)=8.06, P=.005]. Early-weaned rats were significantly more active than normally weaned rats. Neither sex nor prenatal diet had any main effects on activity. A significant interaction was found between sex and weaning condition [F(1,152)=7.02, P=.01]. Among males, early-weaned and normally weaned subjects did not differ significantly, while early-weaned females were significantly more active than normally weaned females. There was a significant negative relationship between body weight and total activity counts [r=−.224, F(1,86)=4.50, P=.03]. However, when males and females were analyzed separately, the relationship was still significant for females [r=−.473, F(1,38)=9.52, P=.004], but there was no relationship between weight and activity for males [r=.004, F(1,45)=0.001, P=.98].

3.4. Spatial navigation

A total of 105 subjects were tested in the Morris water maze for spatial navigation learning, n's=8–11 per sex per prenatal diet per weaning condition (Fig. 3). There was a significant interaction between prenatal diet and weaning condition [F(2,558)=3.86, P=.024]. Among subjects who had been prenatally exposed to alcohol, early-weaned rats took longer to find the platform than did normally weaned rats. In contrast, there was no significant difference between early-weaned and normally weaned rats in either the pair-fed or LC control groups.

There was an additional significant interaction of weaning condition on day of testing [F(2,558)=5.21, P=.006]; early and normally weaned subjects differed on Days 1 and 2 of testing, but not on Day 3. Day also interacted significantly with prenatal diet [F(4,558)=10.79, P=.0001]; alcohol-exposed and pair-fed subjects differed on all 3 days while the LC subjects only differed on Day 1 compared to Day 2. All subject groups exhibited learning to find the

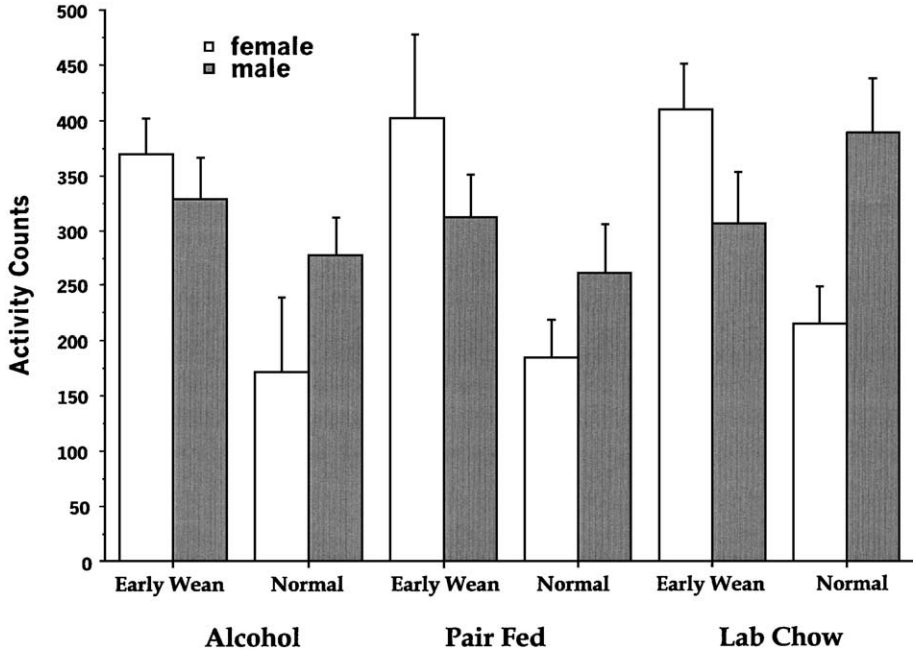

Fig. 2. Mean activity counts (±S.E.M.) for males and females at 18 days of age for three prenatal treatment groups (alcohol, pair-fed, and control) who were weaned at either 15 (early) or 21 (normal) days of age.

platform across both days and trials—there was a significant main effect for day [$F(2,558)=223.99$, $P=.0001$], and for trials [$F(3,558)=65.76$, $P=.0001$].

There were also main effects of prenatal diet [$F(2,558)=16.27$, $P=.0001$], and weaning condition [$F(1,558)=18.49$, $P=.0001$]. Overall, alcohol subjects took significantly longer to find the platform than LC and pair-fed subjects, but the two control groups did not differ from each other. Similarly, all subjects that weaned early had longer latencies than all normally weaned subjects. There was no main effect of sex nor were there any interactions of any other factors with sex. There was no significant relationship between body weight on Day 24 and latency to find the platform.

4. Discussion

The results of our study indicated that in two of the measures, spatial navigation and body weight, a negative early experience worsened the outcome of prenatal alcohol

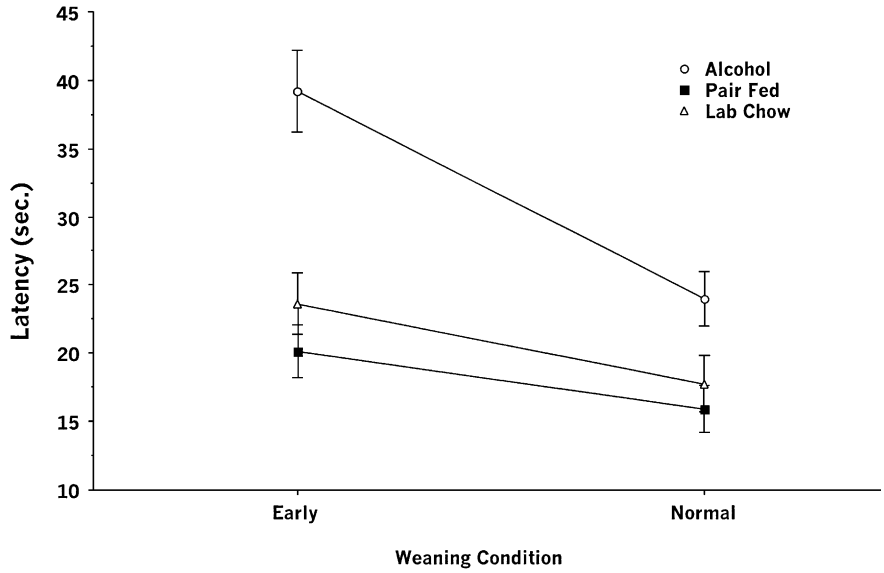

Fig. 3. Mean latencies (s) to reach a hidden platform (±S.E.M.) averaged over 3 days of testing for three prenatal treatment groups (alcohol, pair-fed, and control) who were weaned at either 15 (early) or 21 (normal) days of age.

exposure. Subjects who were both early weaned and exposed to alcohol manifested the greatest latencies to reaching the hidden platform and the lowest body weights. There was no significant interaction, however, between prenatal diet and weaning condition on activity. These results suggest that there may be some selectivity in which alcohol-induced deficits are susceptible to modulating effects of the environment. This selectivity may be due to either the task (e.g., learning vs. spontaneous activity), or to some other variable such as age of testing or a maternal-derived factor.

This differential susceptibility in different measures can also be seen in studies of the effects of handling on ameliorating the effects of prenatal alcohol exposure, in which some outcomes are improved and some are not (Gabriel and Weinberg, 2001; Gabriel et al., 2000, 2002; Lee and Rabe, 1999; Weinberg et al., 1995). From one perspective, handling can be viewed as maternal separation, since the pups are taken away from the dam. Results from different laboratories are hard to compare, since the time of "handling" (separation from dam) can be 5 or 20 min. Also, in some laboratories, the subject is actually touched, while in others, no contact is provided during the time away from the dam. These procedural differences might also explain why handling did not always have a beneficial effect on alcohol deficits. Interestingly, in one study, handling actually increased the latency to find the platform in the prenatal alcohol-exposed subjects compared to controls (Gabriel et al., 2002); perhaps this early maternal separation was actually a stressor that exacerbated the alcohol deficit like early weaning in the present study. Handling also did not cause the predicted improvement in prenatal alcohol-exposed subjects in a conditioned task aversion task, indeed, again, an exacerbation was reported (Gabriel and Weinberg, 2001). In a preliminary report, 2 min of daily handling on PN 2 to PN 15 was found to increase the latency to find the platform in 28-day-old rats who were exposed to alcohol in utero, while it decreased the latency in controls (Hannigan et al., 2001). Thus, handling may even be experienced as stressful by alcohol-exposed subjects when it is processed as a "positive" manipulation by nonexposed subjects.

One concern we had was that the undernutrition experienced by the combined alcohol and early-weaning subjects, as seen in body weight, was responsible for any differences in behavioral measures. There was no relationship between body weight and latency in the Morris water maze. Also, on the last day of testing, the normally weaned alcohol subjects did not weigh less than the pair-fed group, but did have slower latencies. These analyses suggest that the weight differences did not confound the spatial navigation results. In the activity measure, there were no effects of prenatal diet to confound. While body weight was negatively correlated with activity counts (that is, smaller subjects were more active), that negative relationship was due only to females. Arguing against a confound in the activity results is that both males and females of all prenatal treatment groups who were early weaned weighed less, while only the early-weaned females were more active. Another confound could have resulted from more than one subject being tested from a litter; this confound was hopefully minimized by using at most only two males and two females of any one litter for behavioral testing.

Early weaning itself had a deleterious effect on all three measures—spatial navigation, growth, and activity. Early-weaned rats took significantly longer to learn the Morris water maze, weighed less, and were hyperactive relative to normally weaned controls, regardless of prenatal diet or sex. The activity results are consistent with the previous study showing that early weaning resulted in higher activity levels on PN 25 compared to normally weaning (Fahlke et al., 1997). However, in that study, only males were tested, while in this study, the effects of early weaning on activity were seen in the female subjects; males did not differ by weaning condition. The age difference in these two studies may also be important, since we did see differences in spatial navigation in both males and females at a more similar age to the subjects in Fahlke's study. In addition, Brunelli et al.'s (1989) study on early weaning also showed a greater effect on disruption of play behavior in females versus males. Similar to our study, West and Michael (1988) reported that females had a greater increase in activity than males in response to either handling or amphetamine. In adult rats, females are more likely to respond to novel situations with an increase in activity, while males more often display anxiety (Fernandes et al., 1999). Interestingly, female minks had a greater response to early weaning than male minks when tested for stereotypical behaviors (Jeppesen et al., 2000). In two previous studies, we also found an effect of early stress only in females. Females with a history of maternal separation in the first week of life groomed more in response to a novel stress as adults, while males did not show this effect (Zimmerberg et al., 1999). Prenatal alcohol-exposed female adult offspring with a history of maternal separation on PN 2–7 had greater concentrations of the neurosteroid allopregnanolone in the prefrontal cortex and the hippocampus after a cold water swim test than did alcohol/nonseparated females as well as the other control groups, while males did not show any differences among prenatal or postnatal groups (Zimmerberg and Brown, 1998). It is possible that some organizational effect of early sex hormone exposure sensitizes females to stress when tested at 18 days of age for activity, or there may be a sexually dimorphic neural substrate or sex difference in HPA axis response. Further study will be necessary to elucidate the source of this sex difference in response to early weaning.

This is the first demonstration of an effect of early weaning on a learned task. Early-weaned subjects took longer to find the platform on both Days 1 and 2. However, by Day 3, the early-weaned subjects had latencies no different from normally weaned subjects. It would be interesting to test performance on other learned tasks as

well as to find out if Morris maze performance was impaired in older subjects.

Prenatal diet also had main effects on growth and spatial navigation. Those receiving prenatal alcohol exposure in utero weighed significantly less and were slower in learning the spatial navigation task than those whose mothers consumed a pair-fed liquid diet or LC and water during their pregnancies. Spatial navigation in the Morris water maze has been demonstrated in several laboratories to be impaired by both pre- and postnatal alcohol exposure (Blanchard et al., 1987; Goodlett et al., 1987; Kelly et al., 1988). We have also demonstrated deficits in spatial learning in adult offspring in a T-maze spatial alternation test (Zimmerberg et al., 1991). Contrary to some previous studies, prenatal alcohol did not induce hyperactivity, perhaps because this test was too short in duration to elicit the typical impairment of habituation that probably explains alcohol-related hyperactivity in an open field. For example, Bond (1985) reported hyperactivity on PN 16 and PN 22 in rats with prenatal alcohol exposure, but their activity was measured during a 30-min test period.

Although the inferences to human behavior are tenuous, these results do indicate that the environmental conditions of children born with moderate alcohol exposure should be carefully studied for their effects on outcome. Children with alcoholic mothers or mothers who drink heavily may be more likely to endure maternal deprivation. A study of mother–infant interactions at 6 months of age reported a ''suboptimal quality of interaction'' in alcohol-exposed infants that was correlated with maternal alcohol intake (Platzman et al., 1990). When studied at one year of age, alcohol-exposed infants were observed to have less secure attachment behavior towards their mothers than control infants, and attachment difficulties were found to be positively related to the amount (self-reported) of alcohol consumed by the mother during pregnancy (O'Connor et al., 1987). Mothers might become discouraged by their children's slow development and hyperactivity and may then feel frustrated and be unable to care for them. These attachment difficulties might be a factor in determining the environment of the affected child. In one study, most children diagnosed with Fetal Alcohol Syndrome (FAS) were no longer living with their biological mothers at 10 years of age (Streissguth et al., 1985), and in another study, nearly all the children with any level of documented maternal alcohol consumption were in foster care or an adoptive home at the time of follow-up (Caruso and ten Bensel, 1993).

Although children who are prenatally exposed to alcohol may recover from or compensate for the neural damage if they are in a supportive and stimulating environment, those children subjected to environmental stressors may be less likely to improve with age. The results of this study suggest that one environmental stressor, early weaning can exacerbate a cognitive deficit in alcohol-exposed juvenile rats. Further studies on the mechanisms of neuronal plasticity underlying these behavioral results in animals may help guide the development of environmental therapeutic approaches.

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References

Abel E, editor. Fetal alcohol syndrome: from mechanisms to prevention. Tarrytown: Elsevier, 1996.

Berman RF, Hannigan JH, Sperry MA, Zajac CS. Prenatal alcohol exposure eliminates the effects of environmental enrichment on hippocampal dendritic spine density. Alcohol 1996;13:209–16.

Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis in cerebellar cortex of adult rats. Proc Natl Acad Sci 1990;87:5568–72.

Blanchard BA, Riley EP, Hannigan JH. Deficits on a spatial navigation task following prenatal exposure to alcohol. Neurotoxicol Teratol 1987;9:253–8.

Bond NW. Prenatal alcohol exposure and hyperactivity in rats: effects of D-amphetamine and alpha-methyl-*p*-tyrosine. Neurobehav Toxicol Teratol 1985;7:461–7.

Bond NW, DiGuisto EI. Effects of prenatal alcohol consumption on open field behavior and alcohol preferences in rats. Psychopharmacology 1976;46:163–8.

Brunelli SA, Shindledecker RD, Hofer MA. Early experience and maternal behavior in rats. Dev Psychobiol 1989;22:295–314.

Caruso K, ten Bensel R. Fetal alcohol syndrome and fetal alcohol effects. The University of Minnesota experience. Minn Med 1993;76:25–9.

Fahlke C, Hard E, Eriksson CJ. Effects of early weaning and social isolation on subsequent alcohol intake in rats. Alcohol 1997;14:175–80.

Fernandes C, Gonzalez MI, Wilson CA, File SE. Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. Pharmacol Biochem Behav 1999; 64:731–8.

Gabriel KI, Weinberg J. Effects of prenatal ethanol exposure and postnatal handling on conditioned taste aversion. Neurotoxicol Teratol 2001;23:167–76.

Gabriel KI, Yu W, Ellis L, Weinberg J. Postnatal handling does not attenuate hypothalamic–pituitary–adrenal hyperresponsiveness after prenatal ethanol exposure. Alcohol Clin Exp Res 2000;24:1566–74.

Gabriel KI, Johnston S, Weinberg J. Prenatal ethanol exposure and spatial navigation: effects of postnatal handling and aging. Dev Psychobiol 2002;40:345–57.

Goodlett CR, Kelley SJ, West JR. Early postnatal alcohol exposure that produces high blood alcohol levels impairs development of spatial navigation learning. Psychobiology 1987;15:67–74.

Greene T, Ernhart CB, Ager J, Sokol R, Martier S, Boyd T. Prenatal alcohol exposure and cognitive development in the preschool years. Neurotoxicol Teratol 1991;13:57–68.

Hannigan JH, Berman RF. Amelioration of fetal alcohol-related neurodevelopmental disorders in rats. Exploring pharmacological and environmental treatments. Neurotoxicol Teratol 2000;22:103–11.

Hannigan JH, Berman RF, Zajac CS. Environmental enrichment and the behavioral effects of prenatal exposure to alcohol in rats. Neurotoxicol Teratol 1993;15:261–6.

Hannigan JH, Spear LP, Spear NE, Goodlett CR, editors. Alcohol and

alcoholism: effects on brain and development. Mahwah (NJ): Lawrence Erlbaum Associates, 1999.

Hannigan JH, McMechan A, Shannon C, Gooch M, Simmons R, Najor S, Berman RF. The impact of early neonatal handling on spatial learning in young rats exposed prenatally to alcohol. Alcohol Clin Exp Res 2001;25:121A.

Janus K. Early separation of young rats from the mother and the development of play fighting. Physiol Behav 1987;39:471–6.

Jeppesen LL, Heller KE, Dalsgaard T. Effects of early weaning and housing conditions on the development of stereotypies in farmed mink. Appl Anim Behav Sci 2000;68:85–92.

Kelly SJ, Goodlett CR, Hulsether SA, West JR. Impaired spatial navigation in adult females but not adult male rats exposed to alcohol during the brain growth spurt. Behav Brain Res 1988;27:247–257.

Klintsova AY, Matthews JT, Goodlett CR, Napper RM, Greenough WT. Therapeutic motor training increases parallel fiber synapse number per Purkinje neuron in cerebellar cortex of rats given postnatal binge alcohol exposure: preliminary report. Alcohol Clin Exp Res 1997;21: 1257–63.

Klintsova AY, Cowell RM, Swain RA, Napper RM, Goodlett CR, Greenough WT. Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats: I. Behavioral results. Brain Res 1998;800:48–61.

Klintsova AY, Goodlett CR, Greenough WT. Therapeutic motor training ameliorates cerebellar effects of postnatal binge alcohol. Neurotoxicol Teratol 2000;22:125–32.

Lee MH, Rabe A. Infantile handling eliminates reversal learning deficit in rats prenatally exposed to alcohol. Alcohol 1999;18:49–53.

O'Connor MJ, Sigman M, Brill N. Disorganization of attachment in relation to maternal alcohol consumption. J Consult Clin Psychol 1987;55: 831–6.

Platzman KA, Coles CD, Smith IE. The effects of maternal drinking on mother–child interaction at six months. Alcohol Clin Exp Res 1990; 14:329.

Riley EP. The long-term effects of prenatal alcohol exposure in rats. Alcohol Clin Exp Res 1990;14:670–3.

Rockman GE, Hall A, Markert L, Galvin GB. Early weaning effects on voluntary ethanol consumption and stress responsivity in rats. Physiol Behav 1987;40:673–6.

Russel M, Czarnecki DM, Cowan R, McPherson E, Mudar PJ. Measures of maternal alcohol use as predictors of development in early child-hood. Alcohol Clin Exp Res 1991;15:991–1000.

Streissguth AP, Clarren SK, Jones KL. Natural history of the fetal alcohol syndrome: a 10-year follow-up of 11 patients. Lancet 1985;2:85–91.

Streissguth AP, Barr HM, Sampson PD, Darby BL, Martin DC. IQ at age 4 in relation to maternal alcohol use and smoking during pregnancy. Dev Psychol 1989;25:3–11.

Wainwright PE, Levesque S, Krempulec L, Bulman-Fleming B, McCutcheon D. Effects of environmental enrichment on cortical depth and Morris-maze performance in B6D2F2 mice exposed prenatally to ethanol. Neurotoxicol Teratol 1993;15:11–20.

Weinberg J, Kim CK, Yu W. Early handling can attenuate adverse effects of fetal ethanol exposure. Alcohol 1995;12:317–27.

West JR, editor. Alcohol and brain development. New York: Oxford University Press, 1986.

West CH, Michael RP. Mild stress influences sex differences in exploratory and amphetamine-enhanced activity in rats. Behav Brain Res 1988;30: 95–8.

Zimmerberg B, Brown RC. Prenatal experience and postnatal stress modulate the adult neurosteroid and catecholaminergic stress responses. Int J Dev Neurosci 1998;16:217–28.

Zimmerberg B, Sukel H, Steckler J. Spatial learning of adult rats with fetal alcohol exposure: deficits are sex-dependent. Behav Brain Res 1991;42: 49–56.

Zimmerberg B, Rackow SH, George-Friedman KP. Sex-dependent behavioral effects of the neurosteroid allopregnanolone (3α,5α-THP) in neonatal and adult rats after postnatal stress. Pharmacol Biochem Behav 1999;64:717–24.