Pharmacology Biochemistry & Behavior, Vol. 12, pp. 393–401. Printed in the U.S.A.

Behavioral Effects of Prenatal Ethanol Exposure and Differential Early Experience in Rats[1]

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Received 8 June 1979

OSBORNE, G. L., W. F. CAUL AND K. FERNANDEZ. *Behavioral effects of prenatal ethanol exposure and differential early experience in rats.* PHARMAC. BIOCHEM. BEHAV. 12(3) 393–401, 1980.—Offspring of rats that were intubated with ethanol during Days 10–14 of gestation and offspring in two control groups were compared on measures of growth, viability, and performance in behavioral tasks. Influences of postnatal environment were assessed by using fostering-cross fostering procedures and by providing different postweaning housing conditions. Results were that offspring from dams treated with ethanol displayed prenatal and postnatal growth deficiency as well as increased postnatal mortality. In the open field, offspring in the Ethanol group were more active than those in the other two groups. Ethanol offspring were also more active in the Y maze and made more avoidance responses and correct discriminations. Early experience as manipulated by the fostering-cross fostering procedures and post-weaning rearing conditions had no impact on the effects of prenatal ethanol on offspring growth, viability, or behavior.

Fetal alcohol Behavioral teratology Open field Y-maze avoidance

CHILDREN with the fetal alcohol syndrome frequently display abnormalities in behavior in addition to the distinguishing facial and growth characteristics of this disorder. Behavioral disturbances observed clinically include mental deficiency, developmental delay, hyperactivity, and fine motor dysfunction (e.g., [17, 28, 29]). While the degree of physical and behavioral impairment in these children appears to be positively correlated [29], altered behavior has also been seen in the complete absence of phenotypic signs of the syndrome [19].

The role of experience during development in the etiology of behavioral effects following prenatal exposure to ethanol is not now understood. While there is no doubt that ethanol per se can produce behavioral abnormalities in offspring through damage to brain development [15], it seems reasonable to suspect that early environmental factors may help to determine the severity and persistence of these behavioral manifestations. Furthermore, environmental influences may play an even greater role in behavioral effects which occur in the absence of severe neurological damage [28]. Rearing conditions in fact have been long recognized as an important determinant of the degree of later impairment associated with certain problems of behavioral development (e.g., [18,26]).

The purpose of the present study was to assess the involvement of two types of experience during development in determining some behavioral effects of prenatal ethanol exposure in rats. The first type concerned the quality of postnatal maternal environment experienced by offspring. This was examined since ethanol intake by a pregnant female might affect her offspring through changes in her maternal care behavior or milk supply that persist postnatally [6]. Further, even if behavioral effects in offspring can be attributed to prenatal ethanol per se, it is of interest to assess the extent to which these effects can be modified by postnatal rearing with an untreated mother.

The second type of experience during development studied was the quality of rearing conditions after the offspring were weaned. This was examined since offspring reared under "enriched" living conditions might display a different degree of behavioral change following prenatal ethanol exposure than offspring reared under "impoverished" conditions. Experimental findings in another context support this view. Will *et al.* [32] observed a remarkable recovery of learning ability in rats given bilateral brain lesions early in life, but only if they had been reared under enriched conditions. Impoverished offspring continued to display a pronounced deficit in level of task performance even though the extent of initial injury for these animals was no greater than for the enriched offspring.

In the present study, offspring of females intubated with ethanol for 5 days during gestation were compared to con-

[1]Address reprint requests to: W. F. Caul. We thank Paul Schklar and Jane Boyd for their technical assistance. This research was supported in part by grants from the Distilled Spirits Council of the United States, Inc., NICHD No. 00973, and by a traineeship from the National Institute of Mental Health (Neurobiology Training Grant MH-08107-14) to G. L. Osborne.

trols on measures of activity and learning. A previous study in this laboratory that used this drug regimen and that included appropriate pair-feeding control procedures found increased activity in an open field as well as more rapid acquisition of an active avoidance response in an aversive Y maze as a function of prenatal ethanol exposure [14]. These offspring, however, were reared by their own mothers and housed in isolation after weaning. The present study sought to replicate these results and to determine whether postnatal rearing conditions might alter these behavioral effects.

METHOD

Treatment of the Mother

Nulliparous Sprague-Dawley female rats 86 days of age were purchased from Holtzman Co., Madison, WI, and housed individually in standard metal cages with free acess to lab chow and water. Lights were on in the animal rooms from 7:00 a.m. to 7:00 p.m. daily throughout the study. When the females were 100–105 days old, a male rat of the same strain was placed in each female's cage. The start of pregnancy was defined as the day that copulatory plugs were found and designated Gestation Day 0. At this time, the male was removed and the female was weighed and then left undisturbed in its home cage with free access to lab chow and water until treatment started.

Pregnant animals were matched on their Gestation Day 0 body weight and assigned to either the Ethanol, Pair-fed, or Control treatment. Assignment to groups within these triplets was random except that the Pair-fed animals had to lag behind the Ethanol animals in onset of pregnancy in order to permit appropriate pair feeding. The numbers of pregnant females assigned to the Ethanol, Pair-fed and Control treatment conditions were 62, 41, and 62, respectively. Among these Ethanol females, 53 delivered viable litters, 4 delivered dead litters, 2 failed to deliver, and 3 died during treatment. Among Pair-fed females, 40 delivered viable litters, and 1 delivered a dead litter. Among Control females, 54 delivered viable litters, 2 delivered dead litters, and 6 failed to deliver. Analyses showed that neither maternal mortality nor outcome of pregnancy was affected by treatments, $\chi^2=5.07$, $df=2$, $0.10>p>0.05$ and $\chi^2=6.77$, $df=4$, $0.20>p>0.10$, respectively.

Each of the pregnant animals was weighed on Gestation Days 9–14 and was fed powdered lab chow from Gestation Days 9–20 so that food consumption could be measured. Water was available ad lib. On Gestation Day 20, animals were weighed and then placed individually in plastic cages (45×23×15 cm). Nesting material of sugarcane waste was provided. Lab chow and water were available in the plastic cage throughout the preweaning period.

Animals in the Ethanol and Pair-fed group were intubated with the appropriate solution on Gestation Days 10–14 at 9:00 a.m. and 9:00 p.m. The ethanol solution consisted of ethanol (31.67% v/v) in distilled water. The sucrose solution consisted of sucrose in distilled water and was equal in calories per unit volume to the ethanol solution. The drug dosage was 8 g ethanol/kg/day, with half this amount given in the morning and half given at night. A previous study using this procedure [14] found that the mean blood ethanol level for this dose was 238.9 mg% as determined from blood samples of pregnant females (n=5) taken 1 hr after each treatment on Gestation Days 10, 12, and 14.

On Gestation Days 10–20, the caloric intake of each Pair-fed animal was equated to that of its Ethanol partner. This was accomplished by matching the volume and hence calories of the fluids that were intubated as well as by matching the amount of powdered lab chow consumed. Control animals had free access to food and water throughout gestation and were not intubated.

While both food consumption and body weights for the females that delivered viable litters in the three treatment groups were equivalent on Gestation Day 9 ($F<1$ in each case), the treatment on Days 10–14 produced a marked reduction in food intake and body weight in Ethanol and their associated Pair-fed partners relative to slightly increased food consumption and body weights for the ad lib Control animals. Although caloric intake was identical for Ethanol and Pair-fed animals, females in the Ethanol group weighed less than those in the Pair-fed group by Gestation Day 14, $F(1,91)=5.7$, $p<0.05$. Following termination of the treatment, Ethanol animals resumed eating at baseline levels and gained weight as did their Pair-fed partners. On Day 20, the mean weight of Control females (401 g) was significantly greater than the weights of Ethanol (344 g) and Pair-fed (355 g) animals, $F(1,105)=137.8$, $p<0.001$ and $F(1,92)=94.9$, $p<0.001$, respectively. Pair-fed females were also heavier than Ethanol animals $F(1,91)=4.6$, $p<0.05$. Even though the Pair-fed and Ethanol treatments were equated in caloric value, unequal female body weights resulted as has been previously observed by Abel and Dintcheff [3].

Treatment of the Offspring

The plastic cages were checked at approximately 9:00 a.m., 1:00 p.m., and 6:00 p.m. daily for the presence of newborn pups. The day of birth was designated Day 0 of offspring age. Between 6 and 14 hours after newborns were first discovered, the mother was removed from the cage and the litter was examined. The following information was recorded: (a) number of live male and female pups; (b) weight of each live pup; (c) number of dead pups; and (d) any unusual aspects of each pup's appearance.

Litter size was adjusted to 8, with 4 males and 4 females retained when possible. Extra pups from large litters were marked and added to small litters of the same treatment condition to obtain the target litter size. Data on growth, viability, and behavior of these extra pups were not collected. Depending upon which mothers delivered at approximately the same time, litters were either fostered, cross fostered, or not fostered following the assessment of newborns. Litters were not disturbed following assessment of newborns and the fostering procedures until weaning. The groups of offspring resulting from these procedures are listed in Table 1.

Offspring were counted, weighed, and ear punched for identification as they were weaned at 28 days of age. Thereafter until 60 days of age, one-half of the males and one-half of the females in each litter were housed individually in standard metal cages, and the other half were housed in groups of like sex. These housing conditions were analogous to the "impoverished" and "enriched" conditions described by Will *et al.* [32]. The number of animals in each group cage was 8–14. Whenever possible, the groups consisted of offspring from the same treatment condition. Group cages were 62.5 cm on each side and 55.0 cm high, and had wire mesh walls and floors. The wall supports and tops were made of wood and painted gray. Sets of 5 "toys" consisting of wood blocks, glass jars, and cans of various sizes were rotated

TABLE 1

EXPERIMENTAL DESIGN AND NUMBER OF ANIMALS TESTED

Birth	Preweaning		Post-weaning	Number tested	
	Group	Description		Males	Females
Ethanol offspring	EO/EM	Ethanol offspring reared by their own ethanol mother	Isolated	12	17
			Grouped	12	16
	EO/dEM	Ethanol offspring reared by a different ethanol mother	Isolated	14	13
			Grouped	16	15
	EO/CM	Ethanol offspring reared by a control mother	Isolated	10	13
			Grouped	14	11
Pair-fed offspring	PO/PM	Pair-fed offspring reared by their own pair-fed mother	Isolated	12	13
			Grouped	12	13
	PO/dPM	Pair-fed offspring reared by a different pair-fed mother	Isolated	13	13
			Grouped	13	13
	PO/CM	Pair-fed offspring reared by a control mother	Isolated	12	12
			Grouped	12	12
Control offspring	CO/CM	Control offspring reared by their own control mother	Isolated	13	13
			Grouped	14	14
	CO/dCM	Control offspring reared by a different control mother	Isolated	11	12
			Grouped	11	11
	CO/EM	Control offspring reared by an ethanol mother	Isolated	14	14
			Grouped	15	14
	CO/PM	Control offspring reared by a pair-fed mother	Isolated	12	13
			Grouped	13	12

among the group cages every 7 days. Lab chow and water were available ad lib to all offspring throughout the study.

Behavioral Tests

Offspring were weighed at 60 days of age. At this time, group-housed animals were placed in pairs of like sex in standard metal cages in order to facilitate the identification of subjects during behavioral testing while maintaining the social grouping of these animals. Isolate-housed offspring remained in their individual cages after age 60 days and throughout the period of behavioral testing that commenced at 63 days of age.

The numbers of subjects run in the behavioral tests are listed in Table 1. One isolated-housed male, one isolated-housed female, one group-housed male, and one group-housed female were run from each litter having offspring in these categories. No substitutions were made in cases where a litter did not yield the appropriate four animals. Thus, each litter contributed no more than four animals to the overall pool of subjects for the study, and no more than one animal per cell as listed in the final columns on Table 1. The identity of individual animals with regard to prenatal treatment was unknown by the experimenter during behavioral testing.

Subjects were observed once per day in an open field at age 63 and 64 days. The apparatus was a wooden enclosure painted black measuring 80.0 cm on each side and 41.5 cm high. The field was divided into 16 squares of equal size by lines drawn on the floor. A 7.5 W light bulb was suspended 108 cm above the center of the field. White noise was provided to mask sounds from the outside corridor and the recording equipment. Each animal was transported individually to the test room. The subject was removed from the carrying cage by the base of the tail and placed in the start square. For the next 5 min, the number of times the animal stood up on its rear legs (rears) and the number of squares entered (ambulation) were recorded.

At age 65 days, Y-maze active avoidance training began. This task requires that an animal run into the lighted arm of a symmetrical Y maze within a specified time period to avoid electric shock. The animal must therefore learn to run both at the correct time, i.e., within 10 sec, and to the correct place, i.e., the one arm of the maze that is lighted. The interpretive value of including the brightness discrimination problem as part of the avoidance task has repeatedly been demonstrated in studies which have addressed the issue of identifying the contribution of associative and nonassociative factors that underly active avoidance performance (e.g., [7, 13, 22, 27]).

Three automated symmetrical Y mazes were used which have been described by Caul and Barrett [13]. Each arm of a maze was 27.9 cm long, 17.8 cm wide, and 19.5 cm high and had sides and a top made of black Plexiglas. The floor was made of parallel stainless steel rods 0.3 cm in diameter spaced 1.9 cm apart and was wired to record the animal's activity level during an intertrial interval and to deliver shock to the animal's feet. A 27 volt DC lamp was centered in the end wall of each arm and, when turned on, identified that

arm as safe. Photocells monitored the animal's movement from one arm of the maze to another. Footshock (1.5 mA, 60 Hz AC) was delivered through a scrambler and regulated by an auto-transformer. Relatively constant shock was provided by putting a fixed resistor (270 kΩ) in series with the animal. The mazes were isolated by plywood partitions in a darkened room provided with white noise.

Twenty trials on each of five consecutive days were presented with a constant 30-sec intertrial interval. In the Y maze a trial consisted of switching the stimulus light in random order to one of the dark arms. Entry into the lighted arm within 10 sec successfully avoided shock. Failure to enter the lighted arm within the 10-sec period resulted in shock onset after which escape responses were possible. Shock remained on in the previously safe arm and the incorrect arm as well as in the center triangular choice area until the animal entered the lighted safe arm. If, during the intertrial interval, the animal left the safe arm and broke the photo beam at the entrance of either of the dark arms, shock was initiated in the dark arms and in the center section and remained on until the animal returned to the safe arm. The following response measures were recorded during each Y-maze session: (a) avoidances—number of trials on which the animals successfully avoided shock by entering the lighted safe arm within the 10 sec CS-US interval; (b) correct discriminations—number of trials on which the animal's initial movement out of the previously safe arm was an entry into the newly lighted safe arm; and (c) intertrial activity—a numerical index of how much movement occurred within the lighted safe arm during the 30 sec interval between trials.

Data Analysis

The analyses of variance for each measure of offspring growth and behavior proceeded in the following manner. First, the relative performance of Ethanol, Pair-fed, and Control offspring that had been either fostered or not fostered at birth was analyzed. This initial set of analyses was intended to assess main effects of the Prenatal Treatment Condition variable plus any interactions involving this variable with the other between- or within-subjects factors present in the design. Using the group designations defined in Table 1, the subject pool for these analyses thus consisted of EO/EM and EO/dEM, PO/PM, and PO/dPM, and CO/CM and CO/dCM. If significant effects involving the prenatal treatment variable were detected in these analyses, further analyses were done comparing offspring that had been cross fostered at birth and offspring of the same prenatal treatment condition that had been fostered. These analyses were intended to reveal whether the treatment condition of the postnatal rearing mother influenced the performance of offspring on the various dependent measures. The groups of offspring for these comparisons consisted of EO/dEM vs EO/CM, PO/dPM vs PO/CM, and CO/dCM vs CO/EM vs CO/PM.

RESULTS

Offspring Vital Measures

Litters born to Ethanol (n=53), Pair-fed (n=40), and Control (n=54) mothers averaged 11–12 pups. There were no differences among these groups either in mean liter size, $F(2,144)=2.41$, $p=0.092$ or in the mean numbers of males or females that were born, $F(2,144)=2.47$, $p=0.086$ and $F<1$, respectively. The only obvious malformation seen among all offspring was that of missing hind legs in one Pair-fed pup. The appearance of this defect suggested that it was congenital and not due to partial cannibalization by the mother.

For the analyses of offspring birthweights, mean weight of male pups and mean weight of female pups were computed for each litter. In the Ethanol group, 1 of the 53 litters contained no females. In the Pair-fed group, 1 of the 40 litters contained no males. All 54 litters of the Control group contained both males and females. The analysis of variance which included the Treatment and Sex factors revealed that differences in birth weights did occur among treatment groups, $F(2,286)=122.45$, $p<0.001$. Newborn Ethanol pups weighed less than Pair-fed newborns, $F(1,180)=95.63$, $p<0.001$, who in turn weighed less than Controls, $F(1,183)=18.36$, $p<0.001$. Mean birth weight per litter of male offspring from Ethanol, Pair-fed, and Control mothers was 5.9 g, 6.8 g, and 7.1 g, respectively. Weight of female offspring in these groups was 5.3 g, 5.8 g, and 5.9 g, respectively. While males from the three groups weighed more than females, $F(1,286)=26.61$, $p<0.001$, the Treatment×Sex interaction was nonsignificant, $F<1$. This routine sex difference in body weight persisted throughout the study, age 28: $F(1,592)=55.7$, $p<0.001$; age 60: $F(1,560)=1889$, $p<0.001$, but as was true with the newborn assessment data this difference never interacted with the prenatal treatments ($F<1$ at each age).

Mortality of offspring was observed in 28 of the 53 Ethanol litters, 7 of the 40 Pair-fed litters, and 10 of the 54 Control litters as assessed at weaning. Among EO/EM, 25.7% died prior to age 28 as compared to only 3.1% of PO/PM and 2.0% of CO/CM. Mortality was greater in Ethanol offspring compared to the Pair-feds, $\chi^2(1)=19.97$, $p<0.001$ and Controls, $\chi^2(1)=23.13$, $p<0.001$. The later two groups did not differ on this measure (Fisher's exact test $p=0.486$). Cross fostering failed to offset the decreased viability of Ethanol offspring as evidenced by the nonsignificant comparison of EO/EM, EO/dEM, and EO/CM, $\chi^2(2)=1.34$, $p>0.50$, and did not adversely affect Control offspring as shown by the nonsignificant comparison of CO/CM, CO/dCM, and CO/EM, $\chi^2(2)=2.67$, $0.30>p>0.20$. There was no significant sex difference in viability among EO/EM, $\chi^2(1)=2.8$, $0.10>p>0.05$.

Body weights of offspring at weaning were reliably affected by prenatal treatment, $F(2,592)=9.72$, $p<0.001$. Ethanol fostered and nonfostered offspring (mean=82.6 g) weighed less than Control fostered and nonfostered offspring (mean=85.3 g), $F(1,395)=4.61$, $p<0.05$, who in turn weighed less than Pair-fed fostered and nonfostered pups (mean=88.5 g), $F(1,382)=4.74$, $p<0.05$. Cross fostering failed to offset the reduced weights of Ethanol offspring since EO/CM with a mean of 75.7 g were not heavier than EO/dEM with a mean of 82.2 g. Further, cross fostering to an Ethanol mother did not reduce weights of Control offspring since CO/EM with a mean of 87.1 g were not lighter than CO/dCM with a mean of 82.1 g.

Unlike survival rates from birth through weaning, viability of offspring from weaning through age 60 days was unaffected by the prenatal treatment, $\chi^2(2)=5.42$, $0.10>p>0.05$. In fact, only two of 104 EO/EM died and no PO/PM or CO/CM died from Day 28 to Day 60.

The primary analysis of body weight at 60 days of age revealed a significant effect of prenatal treatment, $F(2,560)=48.61$, $p<0.001$. EO/EM and EO/dEM with a mean weight of 224.6 g, weighed less than PO/PM and PO/dPM with a mean weight of 244.7 g $F(1,385)=70.768$, $p<0.001$ and also less

than CO/CM and CO/dCM with a mean weight of 246.4 g, $F(1,372)=79.667$, $p<0.001$, while the latter two treatment groups did not differ significantly, $F<1$. Cross fostering failed to offset the reduced weights of Ethanol offspring in that EO/CM with a mean of 216.4 g were not heavier than EO/dEM with a mean weight of 225.9 g. Likewise, the enriched environment did not increase weights of Ethanol offspring since EO/CM and EO/dEM that were housed after weaning in the group cages had a mean weight of 217.0 g, which was not greater than the weight of EO/CM and EO/dEM that were housed in isolation whose mean weight was 225.3 g. The comparison of EO/CM and EO/dEM further revealed that the combination of having had a Control mother during the preweaning period plus living in an enriched environment from age 28–60 days also failed to increase weights of Ethanol offspring as evidenced by the nonsignificant Fostering Procedure×Housing Condition interaction ($F<1$).

Offspring Behavior

Figure 1 shows the rearing and ambulation data from the open field for Ethanol fostered and nonfostered offspring, Pair-fed fostered and nonfostered offspring and Control fostered and nonfostered animals.

In the analysis of the rearing measure, significant Treatment, $F(2,292)=7.64$, $p<0.001$, Days $F(1,289)=427.11$, $p<0.001$, Sex $F(1,292)=45.82$, $p<0.001$, and Treatment×Days $F(2,289)=3.30$, $p<0.05$ factors resulted. Subsequent comparisons showed that on Day 1, Ethanol offspring reared with the same frequency as Pair-fed offspring, $F<1$, while both of these prenatal treatment groups reared more than Control offspring $F(1,96)=9.16$, $p<0.01$ and $F(1,184)=6.61$, $p<0.05$, respectively. On Day 2, however, the Ethanol offspring reared more than both Pair-fed $F(1,201)=9.34$, $p<0.01$ and Control offpsring $F(1,197)=14.02$, $p<0.001$, while the latter two groups did not differ, $F<1$. Although females reared more than males, the elevated levels of rearing produced by prenatal ethanol occurred in both male and female offspring, Treatment×Sex, $F<1$. Further, the treatment effect was present in offspring housed in groups as well as in those housed in isolation. Neither the main effect of Housing Condition nor the Treatment×Housing Condition interaction was significant, $F<1$ in each case.

Just as the enriched environment did not return the amount of rearing by Ethanol offspring to Control levels, cross fostering also failed to offset the effect of prenatal ethanol exposure on this behavior. The comparison of EO/CM with EO/dEM showed that the two groups were equivalent in rearing, $F<1$. Further, this analysis demonstrated that the combination of enriched environment plus having a Control mother during the preweaning period was ineffective in reducing the elevated levels of rearing by Ethanol offspring since the Fostering Procedure×Housing Condition interaction was non-significant, $F<1$.

Cross-fostered Control offspring did, however, differ from fostered Controls in rearing. Both CO/EM and CO/PM had a greater number of rears than CO/dCM, $F(1,94)=5.16$, $p<0.05$ and $F(1,87)=17.306$, $p<0.001$, respectively, although this effect of Fostering Procedure $F(2,140)=8.44$, $p<0.001$ did not interact with Days $F<1$ or with Housing Condition, $F(2,140)=1.06$, $p=0.352$. Apparently these differences resulted from an atypical, low level of rearing by CO/dCM rather than from any facilitating influence of being cross fostered to an Ethanol or Pair-fed surrogate mother. CO/dCM

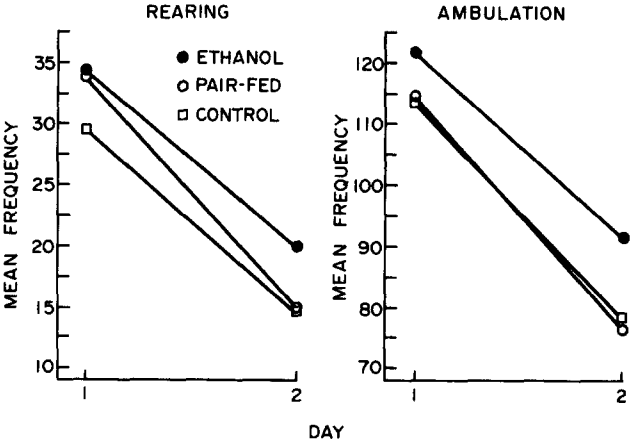

FIG. 1. Mean number of rears and mean ambulation in the open field for fostered and nonfostered offspring combined in the three prenatal treatment groups.

also reared less than CO/CM, $F(1,91)=6.38$, $p<0.05$, while CO/EM, CO/PM, and CO/CM were equivalent on this measure, $F(2,149)=2.57$, $p=0.078$.

The right panel of Fig. 1 displays the mean ambulation score of fostered and nonfostered offspring over the two days of testing. A significant main effect for Treatment was found, $F(2,292)=4.36$, $p<0.05$ indicating that Ethanol offspring entered more squares than either Pair-fed or Control offspring, $F(1,201)=6.69$, $p<0.01$ and $F(1,198)=6.37$, $p<0.05$, respectively. The latter two groups did not differ on this measure, $F<1$. Other findings were that male offspring entered fewer squares on Day 2 of testing relative to Day 1, $F(1,292)=62.06$, $p<0.001$ and $F(1,291)=186.04$, $p<0.001$, respectively. However, neither the Treatment×Sex nor the Treatment×Days interaction was significant, $F<1$ in each case. Further, neither the postweaning enriched environment nor having had a Control mother during the preweaning period, nor a combination of these two factors was effective in returning the elevated levels of ambulation by Ethanol offspring to Control levels. In the original analysis, the Treatment×Housing Condition interaction was not significant, $F<1$. The comparison of EO/CM with EO/dEM showed that these two groups were equal in ambulation, $F(1,198)=1.64$, $p=0.20$ and that Fostering Procedure did not interact with Housing Condition or Days, $F<1$ in each case. Likewise for Control offspring, no differences were found in ambulation among CO/EM, CO/PM, and CO/dCM, $F(2,140)=1.38$, $p=0.254$, and neither interaction of Fostering Procedure with Housing Condition or Days was significant, $F(2,140)=2.05$, $p=0.130$ and $F(2,140)=2.47$, $p=0.087$, respectively.

Measures of offspring behavior in the aversive Y maze are displayed in Figs. 2–4. As suggested by differences in group performance seen in Fig. 2, the Treatment effect revealed in the analysis of avoidance responding, $F(2,292)=5.37$, $p<0.01$ is attributable to the higher level of avoidance responding by Ethanol offspring relative to either Pair-fed or Control offspring. EO/EM and EO/dEM made more avoidances relative to PO/PM and PO/dPM, $F(1,201)=7.98$, $p<0.01$ and CO/CM and CO/dCM, $F(1,198)=8.65$, $p<0.01$, while the latter two groups did not differ, $F<1$. Further, this elevated level of responding in Ethanol offspring was not affected by the enriched environ-

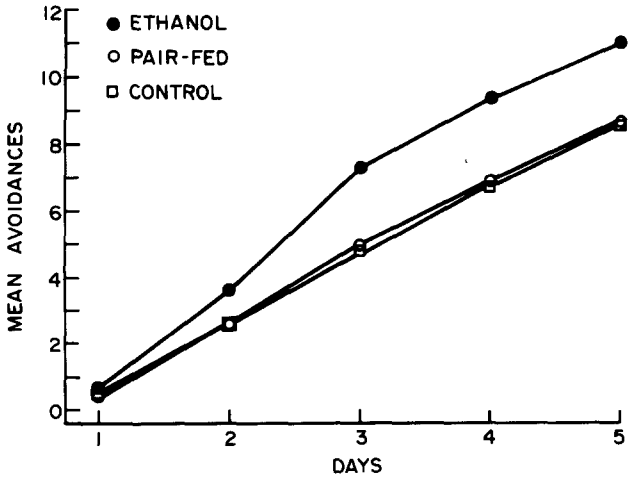

FIG. 2. Mean number of avoidances made in the Y maze by fostered and nonfostered offspring combined in the three prenatal treatment groups.

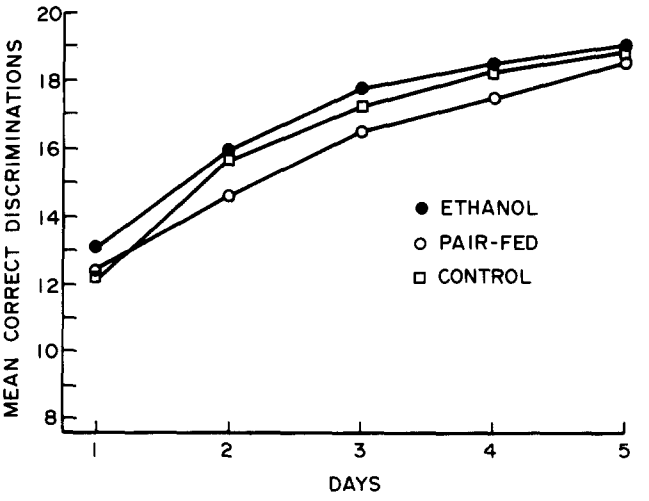

FIG. 3. Mean number of correct discriminations in the Y maze by fostered and nonfostered offspring combined in the three prenatal treatment groups.

ment condition since the Treatment×Housing Condition interaction was nonsignificant, $F(2,292)=1.01$, $p=0.37$. Likewise, cross fostering of Ethanol offspring did not alter the number of avoidances made by EO/CM relative to EO/dEM, $F<1$. This analysis also indicated that the combined effects of enriched environment plus having had a Control mother during the preweaning period were insufficient in returning avoidance responding of Ethanol offspring to Control levels (Fostering Procedures×Housing Condition, $F<1$). Cross fostering of Control offspring similarly had no effect on avoidance as shown in the comparison of CO/EM, CO/PM, and CO/dCM, $F<1$.

Mean correct discriminations for offspring in the Y maze are presented in Fig. 3. A Treatment effect was detected in the analysis of these data, $F(2,292)=14.48$, $p<0.001$ that reflects the fact that Ethanol offspring made more correct discriminations than did Controls, who in turn were superior to Pair-feds on this measure. Additional analyses showed that EO/EM and EO/dEM were significantly different in number of correct discriminations from CO/CM and CO/dCM, $F(1,201)=28.28$, $p<0.001$ and that these Controls were significantly different from PO/PM and PO/dPM, $F(1,185)=7.12$, $p=0.008$. The fact that the Treatment×Housing Condition interaction in the original analysis was nonsignificant, $F<1$, indicates that the enriched environment did not alter the effects of the prenatal treatments. Correct discriminations of Ethanol offspring were unaffected by cross fostering since EO/CM and EO/dEM did not differ significantly on this measure, $F(1,98)=2.92$, $p=0.087$. This analysis also indicates that the combined effects of enriched environment plus cross fostering failed to affect the elevated discrimination performance of Ethanol offspring, as indicated by the nonsignificant Fostering Procedure×Housing Condition interaction, $F<1$. In addition, cross fostering did not change the number of correct discriminations made by the other two prenatal treatment groups. PO/CM and PO/dPM did not differ in correct discriminations, $F(1,92)=2.88$, $p=0.089$ nor did CO/EM, CO/PM, and CO/dCM, $F(2,140)=1.60$, $p=0.205$. As with Ethanol offspring, Fostering Procedure did not interact with Housing Condition in either Pair-fed or Control offspring, $F<1$ in each case.

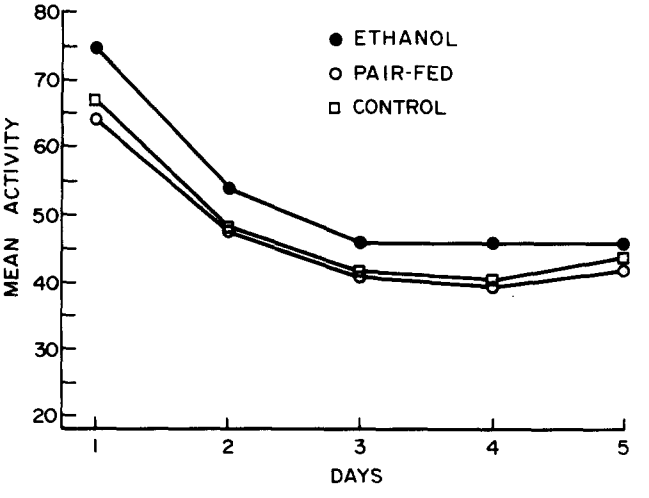

FIG. 4. Mean intertrial activity in the Y maze of fostered and nonfostered offspring combined in the three prenatal treatment groups.

Intertrial activity of fostered and nonfostered offspring is displayed in Fig. 4. The significant Treatment effect, $F(2,292)=6.86$, $p<0.001$ can be readily seen in the figure. Ethanol offspring stood out as being more active between trials than Pair-feds and Controls, $F(1,201)=13.32$, $p<0.001$ and $F(1,198)=6.74$, $p<0.01$, respectively, while the latter two groups did not differ on this measure, $F<1$. As was the case for avoidances and correct discriminations in the Y maze and for rears and ambulations in the open field, the elevated intertrial activity for Ethanol offspring was reduced neither by the enriched environment nor by having had a Control mother during the preweaning period, nor by a combination of these two factors. The Treatment×Housing Condition interaction in the original analysis was not significant, $F<1$. A comparison of EO/CM and EO/dEM showed that the two groups were equivalent on this measure, $F(1,98)=2.61$, $p=0.106$ and that the Fostering Procedure×Housing Condition interaction was nonsignificant,

$F<1$. Intertrial activity of Pair-fed and Control offspring was also unaffected by cross fostering. PO/CM and PO/dPM were equal in activity, $F<1$, and Fostering Procedure did not interact with Housing Condition in this analysis, $F(1,92)=3.67, p=0.055$. Similarly, no differences were found among CO/EM, CO/PM, and CO/dCM on this measure, $F(2,140)=1.13$, $p=0.327$ and the Fostering Procedure $\times$Housing Condition interaction was nonsignificant, $F<1$.

DISCUSSION

The ethanol treatment did not adversely affect the number of viable litters delivered at term or the number of viable male and female pups that were born per litter, and did not produce any readily apparent birth defects. The only clear influence on outcome of pregnancy of the 8 g/kg ethanol prenatal treatment was lowered birth weights of offspring. This effect was also found in the previous study of Caul *et al.* [14] for their 8 g group, but was not seen for the groups receiving lower dosage levels. Lowered birth weights as well as decreased litter size and increased fetal death, stillbirths, morbidity, and malformations are commonly reported in the literature on prenatal ethanol [23].

Viability and body weights of Ethanol offspring as assessed at weaning were below that of Pair-fed and Control offspring. It is interesting to note that while Pair-fed offspring had lower weights than Controls at birth, this order was reversed at weaning indicating catch-up growth in the former group. Pair-feds and Controls did not differ in viability. While no further mortality occurred after weaning through the period of the behavioral testing, Ethanol offspring showed no evidence of catch-up growth during this time and continued to weigh less than Pair-feds and Controls, while the latter two groups by age 60 were equivalent in weights. Reduced postnatal viability and growth of Ethanol offspring as occurred in the present study have also been reported by others [21,31].

Behavioral findings of the present study were that Ethanol offspring were more active than Pair-fed and Control offspring both in the open field on measures of rearing and ambulation as well as in the Y maze between trials. Ethanol offspring also made more avoidance responses and correct discriminations in the Y maze than either other group. Elevated levels of activity for offspring in the open field following prenatal ethanol exposure have also been reported by Branchey and Friedhoff [12], Bond and Di Giusto [8,9] and Caul *et al.* [14]. The performance of Ethanol offspring in the Y maze likewise is consistent with the findings of Caul *et al.* [14] as well as the shuttlebox avoidance results of Auroux and Dehaupas [5]. The increase in Y-maze avoidance responding seems compatible with the impaired passive avoidance performance seen by Riley *et al.* [24] but stands in contrast to the negative results of Abel [1] for one-way active avoidance acquisition. However, Abel used very low dosage levels in his study (1 and 2 g/kg) which may have resulted in the failure to find drug effects on avoidance performance. Caul *et al.* [14] included a 2 g ethanol group in their study and in fact also found no increase in active avoidance responding by these offspring.

Bond and Di Giusto [10,11], however, report fewer shuttlebox avoidance responses in offspring of Wistar rats that were provided with a liquid diet containing 35% ethanol-derived calories throughout gestation compared to offspring from mothers that had lab chow provided during gestation. Further, Abel [2] intubated Long-Evans rats with 4 g/kg or 6 g/kg ethanol throughout gestation and found that their offspring made fewer shuttlebox avoidance responses than did offspring from intubated pair-fed control mothers. While it is not possible to ignore strain, treatment and other procedural differences that exist among these studies, it is interesting to note that those reporting decreased avoidance performance have tested animals at 112 and 150 days of age while those reporting enhanced avoidance performance have tested animals at 65–69 days of age. It may be that the behavioral effects of prenatal exposure to ethanol change is a function of offspring age. In fact, Bond and Di Giusto [9] report that prenatally exposed offspring are more active in an open field at 28 and 56 days of age but not at 112 days of age relative to offspring from mothers provided with lab chow during gestation.

The major new finding of the present study was that prenatal ethanol effects on offspring growth, viability, and behavior appear quite stable regardless of differential early experience. Ethanol offspring reared by a dam without previous exposure to the drug and that lived in an enriched environment after weaning exhibited no fewer effects than did Ethanol offspring reared by dams exposed to the drug and that lived in an impoverished postweaning environment. These data are in agreement with recent clinical observations [30] which failed to find consistent changes in intellectual functioning of children with the fetal alcohol syndrome following improvement in the quality of their early environment.

The present study thus clearly confirms and extends the experimental literature documenting altered growth, viability, and behavior of offspring following prenatal exposure to ethanol. However, the question of whether these effects are attributable to prenatal ethanol per se or rather to artifactual aspects of the drug treatment must be carefully considered. A list of such variables possibly confounded with prenatal ethanol administration would include prenatal stress associated with intubation, prenatal undernutrition due to reduced food intake by the mothers, impaired postnatal maternal function, selective mortality of offspring, reduced litter size prior to weaning, and reduced body size of offspring.

Prenatal stress and undernutrition have been shown to affect offspring behavior in other contexts [4,20]. In the present study offspring of mothers that were placebo intubated and pair-fed to Ethanol mothers during gestation made more rears on Day 1 in the open field and fewer correct discriminations in the Y maze than did Control offspring. However, Ethanol offspring differed in behavior from these Pair fed offspring as well as from Controls. It is especially important to note that, in terms of the number of correct discriminations made, Ethanol offspring performed better than Controls whereas Pair-fed offspring performed more poorly on this measure than both other treatment groups. There is thus no indication that prenatal intubation stress or undernutrition due to depressed maternal food intake produced the behavioral changes seen in Ethanol offspring or that these variables interacted in an additive fashion with effects of prenatal ethanol. The failure to find reduced viability or body weights in Pair-fed offspring relative to Controls at either weaning or testing lends support to this view.

The possibility that the ethanol treatment impaired postnatal maternal function and thereby altered the behavior of offspring [6] is unlikely given the absence of effects on Control offspring due to cross fostering to Ethanol mothers. Control offspring reared by Ethanol mothers displayed

neither reduced viability or body weights nor the behavioral changes seen in Ethanol offspring. These findings corroborate the conclusion of Abel and Dintcheff [3] who cross-fostered all offspring at birth to nontreated surrogate mothers.

Since more Ethanol than Pair-fed or Control offpsring died in the experiment, it could be argued that the differences in behavior of the Ethanol group represented the peculiar characteristics of the survivors rather than actual changes in behavior of individual offspring. A related possibility is that, since the mortality observed among Ethanol offspring occurred prior to weaning, reduced litter size led to a condition of overnutrition in the survivors and thereby increased performance of these offspring in the behavioral tasks. Varied litter size has in fact been found to alter behavior of offspring in certain experimental situations [20]. However, the fact that the 6 g Ethanol group in the previous study by Caul *et al.* [14] exhibited increased activity and Y-maze avoidance performance without concomitant decline in viability seems to negate both of these arguments. Further, the extreme differences in litter size that are necessary for behavioral changes to occur in offspring [16] suggest that this variable was not important in producing the present results.

In addition to experiencing reduced postnatal viability, Ethanol offspring in the present study also weighed less than those in the other two treatment groups. Variable body size may thus have interacted with the specific characteristics of the task (e.g., the dimensions of the open field or the distance between grids in the Y maze) to produce group differences in behavior. Again, however, the findings of Caul *et al.* [14] do not support this argument since offspring in their 6 g Ethanol group did not weigh less than those in their Pair-fed or Control groups. In summary then, the altered behavior of Ethanol offspring in the present study does not appear to have resulted from artifactual effects of drug administration but rather is most likely attributable to the action of prenatal Ethanol per se.

Given the fact that the results of the present study with its complete fostering-cross fostering design replicate and extend the open field and Y maze results of a previous study [14], and that such results are clearly attributable to prenatal ethanol, it now becomes important to specify the mechanisms by which such behavioral change occurs. The findings of previous investigations of open field and avoidance behavior and the results presented here with regard to open field, Y-maze avoidance and Y-maze activity measures, suggest that offspring exposed to ethanol prenatally are generally more active or reactive in the testing environments used. Consideration of how this elevated activity level interacts with the task demands of various testing situations yields the prediction of reduced passive avoidance efficiency in prenatally exposed offspring. Riley *et al.* [24] have reported data consistent with this prediction.

This view, however, does not easily account for the effects of prenatal ethanol exposure on taste aversion [24], spontaneous alternation [25], T-maze escape reversal learning [25], and the present result of enhanced brightness discrimination in the Y maze. Many of these findings fit nicely with the view of Riley *et al.* [24,25] that offspring exposed to ethanol display impaired ability to inhibit responding. Research should now focus on elaborating the mechanisms, both in terms of appropriate behavioral analysis and in terms of biochemical correlates, through which prenatal ethanol exposure yields marked changes in behavior.

REFERENCES

1. Abel, E. L. Effects of ethanol on pregnant rats and their offspring. *Psychopharmacology* **57:** 5–11, 1978.
2. Abel, E. L. Prenatal effects of alcohol on adult learning in rats. *Pharmac. Biochem. Behav.* **10:** 239–243, 1979.
3. Abel, E. L. and B. A. Dintcheff. Effects of prenatal alcohol exposure on growth and development in rats. *J. Pharmac. exp. Ther.* **207:** 916–921, 1978.
4. Archer, J. E. and D. E. Blackman. Prenatal psychological stress and offspring behavior in rats and mice. *Devl Psychobiol.* **4:** 193–248, 1971.
5. Auroux, A. and M. Dehaupas. Influence de la nutrition de la mere sur le development tardif du systeme nerveux central de la progeniture. I. Amelioration, chez le rat, de la capacite d'apprentissage de la progeniture par alcoolisation de la mere. *C. r. Séanc. Soc. Biol.* **164:** 1432–1436, 1970.
6. Baer, D. S. and D. W. Crumpacker. Effects of maternal ingestion of alcohol on maternal care and behavior of progeny in mice. *Behav. Genet.* **5:** 88–89, 1975.
7. Barrett, R. J., N. J. Leith and O. S. Ray. A behavioral and pharmacological analysis of variables mediating active-avoidance behavior in rats. *J. comp. physiol. Psychol.* **82:** 489–500, 1973.
8. Bond, N. W. and E. L. di Giusto. Effects of prenatal alcohol consumption on open-field behaviour and alcohol preference in rats. *Psychopharmacologia* **46:** 163–165, 1976.
9. Bond, N. W. and E. L. Di Giusto. Prenatal alcohol consumption and open-field behaviour in rats: Effects of age at time of testing. *Psychopharmacology* **52:** 311–312, 1977.
10. Bond, N. W. and E. L. Di Giusto. Avoidance conditioning and Hebb-Williams maze performance in rats treated prenatally with alcohol. *Psychopharmacology* **58:** 69–71, 1978.
11. Bond, N. W. and E. L. Di Giusto. Effects of prenatal alcohol consumption on shock avoidance learning in rats. *Psychol. Rep.* **41:** 1269–1270, 1977.
12. Branchey, L. and A. J. Friedhoff. Biochemical and behavioral changes in rats exposed to ethanol *in utero*. *Ann. N.Y. Acad. Sci. U.S.A.* **273:** 328–330, 1976.
13. Caul, W. F. and R. J. Barrett. Shuttle-box versus Y-maze avoidance: Value of multiple response measures in interpreting active-avoidance performance of rats. *J. comp. physiol. Psychol.* **84:** 572–578, 1973.
14. Caul, W. F., G. L. Osborne, K. Fernandez and G. I. Henderson. Open-field and Y-maze avoidance performance in rats as a function of prenatal ethanol exposure. *Addict. Behav.*, in press, 1979.
15. Clarren, S. K. and D. W. Smith. The fetal alcohol syndrome. *New Engl. J. Med.* **292:** 1063–1067, 1978.
16. Joffe, J. M. *Prenatal Determinants of Behaviour*. Oxford: Pergamon Press, 1969.
17. Jones, K. L., D. W. Smith, C. N. Ulleland and A. P. Streissguth. Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* **1:** 1267–1271, 1973.
18. Kirk, S. A. *Early Education of the Mentally Retarded*. Chicago: University of Chicago Press, 1958.
19. Landesman-Dwyer, S., L. S. Keller and A. P. Streissguth. Naturalistic observations of newborns: Effects of maternal alcohol intake. *Alcoholism: Clin. exp. Res.* **2:** 171–177, 1978.
20. Leathwood, P. Influence of early malnutrition on behavioral development and learning in rodents. In: *Early Experiences*, edited by G. Gottlieb. New York: Academic Press, 1978.
21. Martin, J. C., D. C. Martin, G. Sigman and B. Radow. Offspring survival, development, and operant performance following maternal ethanol consumption. *Devl Psychobiol.* **10:** 435–446, 1977.

22. Osborne, G. L. and W. F. Caul. Y-maze avoidance performance and activity change as a function of CS quality in rats. *Behav. Biol.* **15:** 183–192, 1975.
23. Randall, C. L. Teratogenic effects of *in utero* ethanol exposure. In: *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms,* edited by K. Blum. New York: Academic Press, 1977.
24. Riley, E. P., E. Lochry and N. R. Shapiro. Lack of response inhibition in rats prenatally exposed to alcohol. *Psychopharmacology* **62:** 47–52, 1979.
25. Riley, E. P., E. A. Lochry, N. R. Shapiro and J. Baldwin. Response perservation in rats exposed to alcohol prenatally. *Pharmac. Biochem. Behav.* **10:** 255–259, 1979.
26. Skeels, H. M. Adult status of children with contrasting early life experience. *Monogr. Soc. Res. Child Dev.* **31:** No. 2, 1966.
27. Steranka, L. R. and R. J. Barrett. Facilitation of avoidance acquisition by lesion of the median raphe nucleus: Evidence for serotonin as a mediator of shock-induced suppression. *Behav. Biol.* **11:** 205–213, 1974.
28. Streissguth, A. P. Psychologic handicaps in children with the fetal alcohol syndrome. *Ann. N.Y. Acad. Sci. U.S.A.* **273:** 140–145, 1976.
29. Streissguth, A. P., C. S. Herman and D. W. Smith. Intelligence, behavior, and dysmorphogenesis in the fetal alcohol syndrome: A report of 20 patients. *J. Pediat.* **92:** 363–367, 1978.
30. Streissguth, A. P., C. S. Herman and D. W. Smith. Stability of intelligence in the fetal alcohol syndrome: A preliminary report. *Alcoholism: Clin. exp. Res.* **2:** 165–170, 1978.
31. Tze, W. J. and M. Lee. Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats. *Nature* **257:** 479–480, 1975.
32. Will, B. E., M. R. Rosenzweig and E. L. Bennett. Effects of differential environments on recovery from neonatal brain lesions, measured by problem-solving scores and brain dimensions. *Physiol. Behav.* **16:** 603–611, 1976.