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Response Perseveration in Rats Exposed to Alcohol Prenatally[1]

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RILEY, E. P., E. A. LOCHRY, N. R. SHAPIRO AND J. BALDWIN. *Response perseveration in rats exposed to alcohol prenatally*. PHARMAC. BIOCHEM. BEHAV. 10(2) 255–259, 1979.—Pregnant female rats consumed liquid diets containing either 35, 17, or 0% of the total calories as ethanol. Offspring of these females were tested for spontaneous alternation at 21 days of age and for reversal learning in a T-maze shock-escape paradigm at 20–21 days of age. In the spontaneous alternation test, rats exposed to alcohol prenatally took more trials than controls to enter the goal compartment opposite to that initially entered. In the T-maze escape study, alcohol-exposed offspring made more mistakes prior to criterion and more mistakes per trial than controls when the previously incorrect goal was made safe during reversal learning. In both studies linear dose-response functions were found. Furthermore, there was a significant tendency for the within-group variability to increase as the level of prenatal exposure increased, perhaps indicating that the incidence as well as the severity of behavioral dysfunction was dose dependent. The results are interpreted in terms of a delay in the development of a central inhibitory system.

Ethanol Prenatal Fetal Alcohol Syndrome Spontaneous alternation Discriminated escape Inhibition Rats

BEHAVIORALLY, children of chronic alcoholic women or women who drink heavily throughout pregnancy have been described as hyperactive, irritable, distractable, restless, intellectually inconsistent, aberrant in fine motor control, and mentally deficient [13, 14, 16, 22, 24, 25]. Similarly, animals exposed to alcohol *in utero* have been reported to exhibit overactivity in an open-field [3, 4, 6, 23], deficits in shuttle avoidance performance [23], poorer performance on certain operant schedules [18], impairment in T-maze learning [23], increased aggressiveness [15], and a preference for alcohol [3].

Recently, animals exposed to alcohol during gestation were found to be deficient in the acquisition of a passive avoidance response [21]. Animals whose mothers consumed liquid diets containing 32% ethanol derived calories took nearly three times the number of trials to criterion as controls in a simple passive avoidance situation. In a second study, a "prepared," conditioned aversion test was used to ascertain whether offspring of mothers who had consumed alcohol during pregnancy would inhibit responding to an illness-inducing fluid. Alcohol-exposed animals consumed more lithium chloride solution during its second presentation than did controls, and the amount consumed was a linear function of the percent ethanol derived calories consumed by the mother. On the basis of these studies it was hypothesized that animals exposed to alcohol prenatally were deficient in the ability to withhold responding and this interpretation is indeed consistent with much of the available human and animal data. The present paper presents two experiments to further investigate this interpretation.

Fifteen and 16 day old rats generally fail to alternate responses in a T-maze, while older animals usually choose the opposite side from that selected on the first trial [10]. This spontaneous alternation behavior requires the inhibition of prepotent responses which do not appear to be externally motivated. Furthermore, the emergence of this response corresponds with the development of a central cholinergic inhibitory system [10]. In the first experiment presented here, rats whose mothers consumed alcohol during pregnancy were tested to see whether or not they would show spontaneous alternation in a T-maze when no conventional rewards were used. If these animals do indeed evidence response perseveration they might also be expected to show deficits in reversal learning, making more errors prior to learning the new contingency. The second experiment, therefore, examined the acquisition and subsequent reversal learning of a T-maze escape response.

EXPERIMENT 1

METHOD

Animals

Parent animals were Long-Evans hooded rats, obtained from Blue Spruce Farms, Altamont, NY. Animals were maintained on a 12 hr light-dark cycle with free access to

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food and water except as noted. These parent animals were mated according to a procedure described by Asling [1]. Every evening females were individually placed with a male overnight until a vaginal smear taken the following morning indicated that copulation had occurred. The pregnant females were then weighed and housed in standard breeding cages until their pups were weaned at 21 days of age.

On Days 5–20 of pregnancy seven females were given free access to liquid diets containing 35% ethanol derived calories (EDC). Six females were allowed access to a 17% EDC diet and six to a 0% EDC diet. The 17% and 0% diets were isocaloric to the 35% diet (1 Kcal/ml) supplying sucrose, instead of, or in conjunction with, ethanol. The composition of the liquid diets is presented in Table 1 and consisted of chocolate Nutrament (Mead Johnson, Inc.), Vitamin Diet Fortification Mixture (ICN Nutritional Biochemicals), 95% ethanol and/or sucrose. A pairfeeding procedure was employed to control for daily caloric intake. Mothers in the 35% group were allowed free access to their diets but females in the other two groups had their intakes restricted. Each animal in the 17 and 0% groups was matched to a 35% animal, who had free access to its diet, and was fed the amount consumed by this 35% animal on a ml/kg/day basis. This procedure required that the 35% animal, within a matched group, be at a more advanced stage of pregnancy than the 17 and 0% animals in that group. This usually amounted to only one or two days difference. Thus, each animal in a matched group received the same volume of diet, and since the diets were isocaloric, the same number of calories, on a body weight basis. For any given day of pregnancy only the dose of alcohol varied. The liquid diets were replaced by free access to lab chow and water on Day 20 of pregnancy. In order to better assess any effects the liquid diets might have, six pregnant females who had free access to lab chow and water throughout the study were included. All females were weighed on Days 1, 5, 10 and 15 of pregnancy and blood alcohol levels determined on Days 10 and 15 [17].

After the liquid diets were replaced, cages were checked three times daily for offspring. As soon as possible after birth the pups were weighed, inspected for any obvious anomalies, and the litter was culled to 10 pups. At 5, 10, and 15 days of age the pups were again weighed and examined.

Male offspring from each of the four maternal diet conditions were randomly selected as subjects for the following study, with the restriction that each litter have adequate representation. There were 20 animals per group with the exception of the 17% group, which contained 19 animals. Animals were 21 days of age and housed with their mothers and littermates at time of testing.

Apparatus

Testing was conducted in a T-maze with a 60 cm alleyway leading to two arms, each 37 cm in length. The alleyway was 18 cm wide, 35 cm high, and was painted flat black. The start box, 21 cm long, and each goal box, 19 cm long, were separated from the rest of the maze by guillotine doors to prevent retracing. The floor of the maze was covered with heavy brown paper which was changed after each animal was tested. The T-maze was located in a darkened room, illuminated by a 25-W red light suspended 42 cm above the floor of the maze at the choice point.

Procedure

Each animal was removed from its home cage and placed in a holding cage adjacent to the maze for 30 sec. The pup was then transferred to the start box and faced away from the door. Five seconds later the door was raised and a timer started. After the animal had entered the alleyway, the start box door was lowered to prevent reentry. The subject was allowed to explore the T-maze freely until it entered either goal box with all four feet. At this time the appropriate guillotine door was lowered, timing stopped, and the animal retained in the goal box for 60 sec. Following another 30 sec period which the animal spent in the holding cage, another identical trial was given. This procedure continued until the opposite goal from that selected on the first trial was entered by the animal. Response latency and the goal chosen were recorded for each trial. Infrequently, an animal would not leave the start box or would remain in the alleyway, short of the choice point. After an animal had remained in the start box for 5 min, the animal was gently prodded out into the alleyway. However, all trials were terminated after 10 min if neither goal had been selected.

TABLE 1
FORMULATION OF LIQUID DIETS (100 ML)

	Percent Ethanol Derived Calories		
	0	17	35
Nutrament (ml)	64.07	64.07	64.07
Vitamins (g)	0.24	0.24	0.24
95% EtOH (ml)	0.00	3.33	6.67
Sucrose (g)	8.75	4.37	0.00
Water*			

*Added to other ingredients to make a solution with a total volume of 100 ml.

RESULTS

Mothers in the 35% group consumed an average (± SE) of 12.96 (± .37) g ethanol per kg body weight while those in the 17% group consumed 6.66 (± .17) g/kg. However, no obvious withdrawal reactions were noted in either group. No differences between the groups consuming liquid diets were found for maternal weight gain during pregnancy, gestation length, or litter size. However, prenatal alcohol exposure did influence the pups' body weights from birth through 15 days of age. Offspring of mothers who consumed the 35% diet were significantly lighter than the 0% controls. All other comparisons failed to approach significance as did the comparisons between animals from the 0% and lab chow control groups. Some of these data are presented in more detail elsewhere [21].

The average number of trials before alternation is presented in Fig. 1. These data represent means of litter averages and all analyses were performed on these litter averages so as not to bias the results in case there was a high degree of intralitter correlation. It is apparent that there is a direct relationship between the mean number of trials required to alternate and the amount of ethanol derived calories consumed by the mother. An analysis of these data indicated a significant effect of maternal diet, $F(2,16)=16.95$, $p<0.01$. Moreover, a subsequent trend analysis indicated a significant linear function, $F(1,16)=29.40$, $p<0.01$, which accounted for 87% of the variance attributable to groups.

The standard errors presented in Fig. 1 also appear to be a

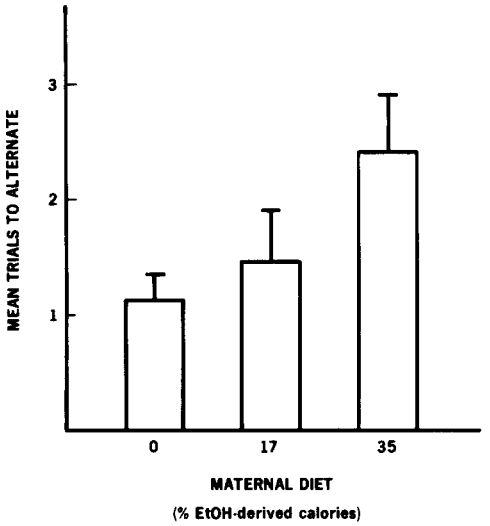

FIG. 1. The mean number of trials (± SE) required to alternate to the goal opposite that chosen on the first trial for offspring in the various diet groups. Data used to compute these means were litter averages.

function of prenatal alcohol exposure, reflecting a tendency for the within-group variability to increase as the amount of EDC consumed by the mother increased. Levene's test [12] was used to ascertain whether this trend was significant, and a significant linear trend component was noted, $F(1,16)=5.61$, $p<0.05$, indicating that the within-group variability increased proportionately with the extent of prenatal alcohol exposure. It appeared from the data (again using litter averages) that the groups might also differ on first trial latencies. The 35% animals averaged 45.29 sec prior to reaching the goal box compared to 76.48 sec and 96.82 sec for the 17% and 0% groups, respectively. However, the linear component of this analysis failed to reach significance, $F(1,16)=4.04$, $p=0.06$.

Separate analyses between lab chow animals and 0% control subjects were conducted, since the lab chow control group is not on the same variable dimension that identifies the liquid diet groups. Therefore, its inclusion in any overall analysis would render subsequent trend analysis inappropriate. All comparisons between the offspring of the 0% group and the lab chow control group failed to approach significance indicating that there was no effect of liquid diet administration.

EXPERIMENT 2

METHOD

Animals

Female offspring of the mothers described in Experiment 1 served in Experiment 2. Animals were randomly selected from the various litters such that each litter was represented. All animals were housed with their mothers who had free access to food and water and were maintained under a 12 hr light-dark cycle. Ten animals from the 35% group and eight from each of the other groups were tested at 20–21 days of age.

Apparatus

The testing apparatus was a white Plexiglas T-maze. The alley was 28.5 cm long, 6 cm wide, and 10.25 cm high, and contained a 7.5 cm start box. The alley led to the two arms of the maze, each 14.37 cm long and 6.75 cm wide, which were crossed by infrared photobeams 5 cm from the end of each arm. The floor of the apparatus consisted of 0.3 cm dia. stainless steel bars spaced 1.5 cm from center to center. Shock, 0.25 mA, was delivered by means of a Lafayette shock generator and scrambler (Model No. A 615C). The lid of the maze was clear Plexiglas, allowing the animal to be observed by the experimenter. Testing was conducted in a dimly lit room equipped with white masking noise.

Procedure

Each animal was removed from the home cage and placed in a holding cage for 3 min. Following this, the pup was placed into the start box facing towards the door and 3 sec later the door was raised. This activated an automatic timer and the shock source. On the first trial animals were required to enter both arms before shock was terminated. The initial arm entered on Trial 1 was made the incorrect choice during the acquisition phase of the study. Following seven consecutive correct responses the acquisition criterion was achieved and the reversal phase was started. During this phase the formerly incorrect response now terminated shock, while responses to the previously correct goal were ineffective. The criterion during reversal learning was also seven consecutive choices. Errors throughout the study were defined as moving past the choice point with all four feet toward the incorrect goal. Each trial was terminated automatically when the appropriate photobeam was interrupted and all trials were separated by a 30 sec intertrial interval, during which the pup was confined to the holding cage. On a rare occasion an animal failed to reach the correct goal within 180 sec; if this occurred the pup was gently prodded to the correct arm.

RESULTS

Analyses for this study were computed on raw scores and not litter means because each litter was typically represented by one or two subjects. In only one 17% litter were three littermates tested.

No differences were found between the 35, 17 and 0% groups on either first trial latencies, $F<1$, or trials to criterion during acquisition, $F<1$. During this phase all groups performed equally, requiring approximately eight trials to reach criterion. However, during reversal learning the results were somewhat different. The mean number of mistakes are presented in Fig. 2. As can be seen, the 35% group made more mistakes than either the 17 or 0% groups. An analysis of variance on these data proved significant, $F(2,22)=8.22$, $p<0.01$, and subsequent decomposition indicated a linear trend, $F(1,22)=12.62$, $p<0.01$, accounting for about 77% of the total group variance. Furthermore, the variances of these three groups were tested by Levene's technique and found to be significantly different, $F(2,22)=6.14$, $p<0.01$. Decomposition indicated a significant linear function, $F(1,22)=11.94$, $p<0.01$, indicating that as the amount of maternal calories ingested as alcohol increased, the variance within each group also increased.

Also presented in Fig. 2 is the average number of mistakes per pre-criterion trial, since it was possible for an

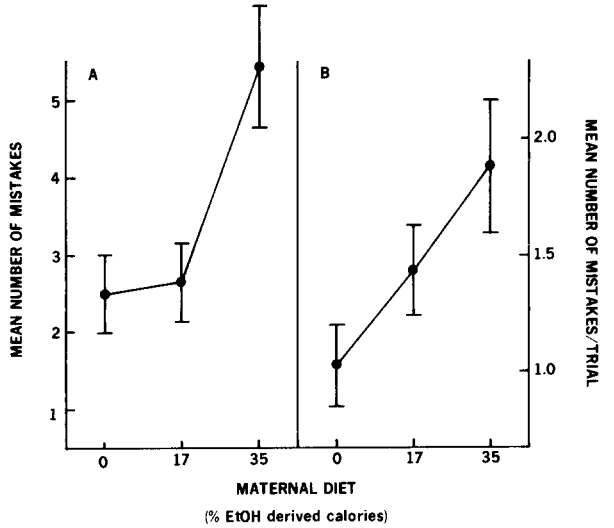

FIG. 2. Mean number of mistakes (± SE) made during reversal learning (A) and the average number of mistakes (± SE) per trial (B) as a function of maternal diet.

animal to retrace into the alley after making an incorrect turn and subsequently make the same mistake. An analysis of these data proved significant, $F(1,22)=3.79$, $p<0.05$, as did the linear trend component, $F(1,22)=7.56$, $p<0.025$. During five consecutive trials, one 35% subject either entered and remained in the incorrect arm or sat in the alley for the entire 180 sec, and was therefore excluded from data analysis for the reversal phase. All comparisons between the 0% and lab chow control groups failed to approach significance.

DISCUSSION

These two experiments illustrate that animals exposed to alcohol prenatally tend to perseverate on prepotent responses. In the first experiment, alcohol-exposed animals required a greater number of trials than control pups before choosing the opposite goal from the one initially entered. In Experiment 2, where an escape response was required, prenatally exposed rats had difficulty reversing to a new correct goal area. A previous report [21] proposed that pups prenatally exposed to alcohol show a deficit in their ability to withhold a response. This hypothesis was based upon the findings that alcohol-exposed offspring evidenced a passive avoidance deficit and showed less suppression of a conditioned taste aversion. The perseveration noted on the part of the alcohol-exposed pups in the present experiments might also result from a lack of response inhibition, since older prepotent responses must be inhibited prior to the acquisition of a new response.

The acquisition of spontaneous alternation in the rat is dependent upon a central cholinergic system which develops at about 20 days of age [7,20]. Furthermore, since it has been hypothesized that this cholinergic system is inhibitory [8] it leads to speculation concerning the deficiency noted in alcohol-exposed animals. Behaviorally, there are several similarities between animals exposed to alcohol prenatally and animals treated with anticholinergic drugs. These similarities include increases in general activity [3, 4, 6, 7, 23] and deficiencies in spontaneous alternation [9], maze learning [23,26], passive avoidance [19,21], and FR operant behavior [5,18]. These similarities and the present data suggest that alcohol exposure *in utero* may in some way interfere with the functioning of this central inhibitory system.

Animals exposed to alcohol prenatally often show age dependent changes in activity, being overactive at an early age but normalizing as they get older [4,23], and recently it was found that female mice exposed to alcohol *in utero* were delayed in sexual maturation [2]. It appears that prenatal alcohol exposure may cause a developmental lag and hence retard maturation. If this hypothesis is tenable, the present study on spontaneous alternation and the previous passive avoidance study optimized any behavioral differences between alcohol-exposed and control animals by testing at a time when the cholinergic inhibitory system first becomes functional.

It does not appear that all offspring in the alcohol treated groups were affected by the treatment, but rather the incidence as well as the severity of dysfunction was dependent upon the extent of *in utero* alcohol insult. This finding was supported by the significant linear function between groups for both mean differences in performance and for within-group variance differences. That is, as the level of maternal calories ingested in the form of ethanol increased, the level of dysfunction and the within-group variance increased. These data are important in light of a recent report, substantiating the claim that as the extent of maternal alcohol consumption increases from moderate to heavy levels, the risk of having a child with abnormal features also increases [27].

Teratological assessments conducted with diets similar in alcohol concentration to those used in these behavioral studies have indicated an increased risk of physical anomalies the greater the alcohol exposure. However, the frequency of behavioral dysfunction is much higher than that of physical anomalies. Therefore, it would appear that prenatal alcohol exposure can result in behavioral changes that are not associated with any of the gross, physical malformations typically seen in FAS. However, it should be cautioned that cross-fostering was not utilized in the present study. Thus, although attempts were made to minimize the more obvious postnatal maternal effects (e.g., diets being removed prior to parturition) subtle alteration in maternal behavior could have occurred and influenced the results. Given the absence of such maternal effects these data support the contention [22] of a broad concept of FAS, encompassing a range of symptoms from behavioral manifestations through severe morphological defects.

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