Maternal ethanol consumption and hyperactivity in cross-fostered offspring

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Both cross-fostered and natural-mother raised male offspring whose dams had received 8.5 g/kg/day of ethanol by intubation during the entire gestational period exhibited significantly increased locomotor activity at 2 months of age, as compared with equivalent groups whose mothers received saline intubation during that period. Weight differences were excluded as a causal factor. This lends support to the hypothesis that the direct effect of alcohol on the fetus through placental transmission is the cause of increased activity or hyperactivity in offspring. The hypotheses of genetic transmission and maternal interaction effects received no support.

Hyperactivity in children has been correlated with parental alcoholism (Cantwell, 1972; Goodwin, Schulsinger, & Hermansen, 1975; Morrison & Stewart, 1971), but not with alcoholism in adoptive parents. Genetic transmission has been hypothesized, since a proportion of the biologic parents were also hyperactive as children (Morrison & Stewart, 1973). Another viable explanatory hypothesis for these data is that alcohol affects fetal development directly, either through placental diffusion (Dilts, 1970; Idänpään-Heikkilä, Fritchie, Ho, & McIsaac, 1971) or by affecting the sperm (Badr & Badr, 1975; Mann, 1968).

Studies which utilize an animal model are able to resolve the question of etiology, since animals are from a common genetic pool and are randomly assigned to treatment. In addition, either the male or female parent can be selectively exposed to the drug.

Two recent studies have found increased activity in the offspring of mice (Elis, Kršiak, Pöschlová, & Mäsek, 1976) and rats (Bond & diGiusto, 1976) whose mothers had received ethanol during all or part of the gestational period. However, both studies suffered from methodological deficiencies. Neither study employed maternal pair-feeding, weight-matching of offspring or cross-fostering techniques. Lowered body weights have been found to correlate in a dose-response fashion with in-

creased activity (Moskowitz, 1959). Since maternal alcohol administration results in weight loss during pregnancy and lighter weight offspring (Martin, Martin, Sigman, & Radow, 1977; Phillips & Štainbrook, 1976; Tze & Lee, 1975), the control offspring must be matched for weight when tested for valid conclusions to be drawn. This can be achieved most easily by pair feeding the pregnant control dams to the alcohol dams, which also matches the groups for undernutrition.

Unless the offspring have been cross-fostered at birth, pre- and postnatal factors are confounded and etiology is uncertain. Undernutrition may play an additional role if there is a carryover effect of the drug during the neonatal period, since alcohol affects oxytocin release by pituitary, which, in turn, affects milk release at doses exceeding 1.0 g/kg (Cobo, 1973; Fuchs, 1969). In addition, behavior patterns may differ in dams who have been receiving alcohol, and hence caretaking may be affected. Environmental influences through the dam cannot be discounted in the Bond study, since the young remained with their biological mothers and increased activity has been found to follow short-term alcohol ingestion by adult animals (Bond & diGiusto, 1975).

The following study was designed in an attempt to answer some of the etiological questions raised by the hyperactivity studies by separating pre- and postnatal factors through cross-fostering, pair feeding the control dams to control for the effects of undernutrition on offspring, and by administering the ethanol to female parents only. Hyperactivity was operationally defined as an increase in activity from control levels.

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METHOD

Subjects

Seventy-five Sprague-Dawley-derived barrier-sustained primiparous rats weighing between 250 and 275 g were delivered to our laboratory on the morning following evening impregnation as determined by vaginal lavage (Tyler Labs, Bellevue, Washington). This was designated as Day 1 of pregnancy. Twenty-seven were randomly assigned to the ethanol treatment condition, and 24 each to either the saline or no-treatment condition. Of the initial group, 7 from the ethanol, 5 from the saline, and 3 from the no-treatment group were excluded from all analysis due to non-delivery (10), death prior to delivery (3), inability to match delivery dates for cross-fostering (1), or a missed delivery (1). Thus 20 remained in the ethanol, 19 in the saline, and 21 in the no-treatment conditions.

Beginning with Day 1 of pregnancy and continuing through Day 21, the ethanol dams were intubated twice daily at 9 a.m. and 4 p.m. with 4.25 g/kg of a 20% ethanol solution in 0.9% isotonic saline for a total of 8.5 g/kg/day. The saline dams were intubated with the same amount of the vehicle alone, and the no-treatment dams were not intubated. A No. 10 French catheter (Cutter-Resiflex) was lubricated with surgical jelly (Lubafax, Burroughs-Wellcome) and attached to a 10 cc disposable syringe. All animals were weighed daily prior to the morning intubation. They were housed individually in Lucite cages with Purina Chow and water available continuously. The saline and no-treatment dams were fed only the mean amount consumed on a given day by the alcohol animals. Each rack of cages had its own temperature and humidity control and air system and was separated from the room by a glass door.

Cross-Fostering

Half of the ethanol and half of the saline dams were randomly assigned to have their litters cross-fostered to no-treatment dams. The other half remained with their litters. On the same day that a no-treatment dam delivered, her litter was removed and sacrificed and a litter from either a saline or ethanol dam substituted. The ethanol or saline dam was then sacrificed. In a few instances, a saline or ethanol litter was not available until the day following no-treatment delivery, in which case the no-treatment litter was left with its mother for an additional day. In no instance was an ethanol or saline litter left with its mother past the delivery day, and in no instance did the no-treatment dams fail to accept the new litter.

Offspring

The gravid rats were checked twice daily for delivery beginning with Day 21, and newborns were counted and weighed as soon as they had been gathered into the nest. Since this strain of rats cannibalizes its dead and moribund young, there is no good indicant of stillbirths. On Day 4, the litters were sexed and culled to eight, saving as many males as possible for later behavioral studies. Thereafter, they were weighed weekly and weaned on Day 23. On Days 28-32, female offspring were sacrificed and males placed with littermates, two to four to a cage. On Days 32-41, each litter was randomly reduced to two males each with no more than two selected from each litter.

Developmental Measures

On Day 3, the newborns were checked for ear pinna uncurling, and tested for a righting response from a dorsal position within a 3-sec time period. On Day 5, gums were checked for incisor eruption. On Days 7, 11, 15, and 18, the distance traveled in a forward direction within a 5-sec time period was measured, and on Days 14, 16, and 18, eye opening was checked.

Activity

Between 60 and 69 days of age, 18 male rats in each of the four treatment conditions were weighed and placed in Wahmann activity wheels a 4 p.m. and removed the following morning at 8 a.m.

Statistics

A chi-square analysis was performed on gestational length, since the data were analyzed dichotomously. One-way analyses of variance were performed on the raw data for other dependent measures with arcsin transformations to stabilize the variances. A square-root transformation was applied to the activity counts and a *t* test performed between treatment conditions. An analysis of variance was first performed on weights, since Moskowitz (1959) had found that activity varies directly with body weight. Statistical methods are described in Winer (1972).

All of the analyses were performed on the number of litters rather than the number of individual offspring for the vital and developmental measures. There were three reasons for this procedure: (1) The performance of individuals within a litter could not be assumed to be independent due to the common genetic components and common intra-extrauterine environments; (2) intralitter effects could either enhance or obscure treatment effects; and (3) the extremely large number of individual offspring might result in statistically significant results, but without meaningful differences between the groups.

RESULTS

Vital Measures

Length of gestation. A chi square of 10.56 ($df = 4$, $p = .03$) was obtained on the number of days until delivery. Although the majority of females in all three groups delivered on Day 23, the distribution was skewed towards longer gestational periods for the ethanol mothers and towards shorter periods for the no-treatment mothers. Fifteen percent of the ethanol-intubated mothers delivered on Days 24-25, and none from the saline or no-treatment groups delivered later than Day 23. This pattern of longer gestational periods as a result of maternal ethanol exposure has been found previously in the laboratory (Martin et al., 1977) and was not unexpected, since alcohol has been used extensively clinically to retard premature labor (Castr, Gummerus, & Saarikoski, 1975).

Maternal weight gain. The ethanol dams gained 146 g, the saline dams 143 g, and the no-treatment dams 152 g over the 21-day gestational period: $F(2,57) = .87$, $p = .42$. This a considerably higher weight gain than is usual with ethanol treatment, but the ethanol group received less alcohol than is the case in studies which administered alcohol in drinking solutions. Only 24% of the daily diet was in alcohol (20.1 k/cal/day) as contrasted with 31%-41% possible with other oral methods (Martin et al., 1977). Since alcohol is caloric, the amount administered has an effect upon the amount of food consumed and, hence, weight gain. The other

two groups received the same amount of food consumed by the ethanol rats.

Litter size and weight. There were no significant differences in live litter size or weight at birth among the three groups. There were also no differences in litter weights on Postnatal Days 7, 14, 21, or 28.

Developmental Measures

Neither the *righting response* (Day 3), *incisor eruption* (Day 5), nor *distance traveled forward* (days 7, 11, and 15) differentiated between treatment conditions.

Ear-flap uncurling. Between 76% and 82% of the two saline groups had ear flaps uncurred by Day 3, but only 47% of the alcohol/mother offspring had uncurred pinnae. An $F(3,35) = 2.15$, $p = .11$, was obtained on the transformed data, and a contrast of the two alcohol vs. the two saline groups resulted in a rejection of the null hypothesis with a $p = .028$.

Eye opening. Eyes in the rat normally open between Postnatal Days 14-17 (Farris, 1949). "Open" was defined as a rounded aperture, not merely a slit. An $F(3,35) = 3.09$, $p = .03$, was obtained for the number of eyes open on Day 14. Only 4% of the alcohol/mother offspring had eyes opened by that date as opposed to 36% of the saline/cross-fostered offspring and 14%-15% of the other two groups. The interesting result here is that cross-fostering results in three times the number of eyes open for both alcohol and control groups as does the mother-raised condition. The null hypothesis was not rejected for the Day 16 eye-opening data, since 98% of all rats had open eyes by that date.

Locomotor Activity

An $F(3,68) = .26$, $p = .85$, was obtained on body weight on Day 60. The mean weights for each treatment condition were 383.1 g (alcohol/mothers), 382.8 g (alcohol/cross-fostered), 377.3 g (saline/mothers), and 376.9 g (saline/cross-fostered). The mean number of turns in the activity wheel over 1 night was 201 (alcohol/mothers), 226 (alcohol/cross-fostered), 149 (saline/mothers), and 123 (saline/cross-fostered). See Figure 1 for a graphic depiction. The scores of the two saline groups and the scores of the two alcohol groups were combined, and a square root transformation performed on the raw data to stabilize the variances. A $t = 2.15$, $p = .03$, was obtained. The maternal treatment effectively masked any cross-fostering effects which may have been in evidence.

Figure 1. Mean number of turns in the activity wheel as a function of maternal treatment. Standard errors of the mean are indicated.

DISCUSSION

Although the eye-opening data, in which the alcohol-mother offspring are more retarded than the alcohol cross-fostered or saline offspring, lends credence to a hypothesis of developmental delay due to lingering ethanol effects to the mother, the ear-flap data were more ambiguous, since both alcohol groups were more retarded than controls, which argued a transplacental effect. The activity data was unambiguous and demonstrated a transplacental effect of alcohol in utero upon increased activity in offspring. The hypothesis that the mother's behavior played a role in increased offspring activity was not a viable one, since the cross-fostered group was equally affected. Any genetic effect had been excluded initially by selecting rats from the same outbred strain and by using only one, or at most two, from a litter.

Hyperactivity, as defined in this study, was found to be due to an indirect effect of maternal alcohol intubation upon the fetus. Cross-fostering at birth did not result in an attenuation of the effects. These results cast doubt upon both the genetic

hypothesis and postnatal environmental factors and lend credence to the hypothesis that alcohol affects the fetus directly.

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