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Ethanol Consumption by Rat Dams During Gestation, Lactation and Weaning Increases Ethanol Consumption by Their Adolescent Young

ABSTRACT: *In two experiments, we examined effects of ethanol consumption in rat dams during gestation, lactation, and weaning on voluntary ethanol consumption by their adolescent young. We found that exposure to an ethanol-ingesting dam throughout gestation, lactation, and weaning enhanced voluntary ethanol consumption by 26- to 33-day-old adolescents. We systematically examined effects on adolescent ethanol intake or requiring dams to drink ethanol during various periods in their pups' development. We found that exposure to an ethanol-consuming dam during weaning enhanced adolescent ethanol consumption and exposure to a dam drinking ethanol during either gestation or while nursing enhanced adolescents' ethanol consumption only if pups also had access to ethanol during the weaning period. © 2003 Wiley Periodicals, Inc. Dev Psychobiol 42: 252–260, 2003.*

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Perhaps the most common way in which human children are exposed to alcohol is by interacting with parents who consume alcoholic beverages. Maternal consumption of alcohol during pregnancy and nursing leads to blood-ethanol levels in fetus or infant roughly equivalent to those found in the their mothers (Abel, 1984), and ethanol can be detected in both the amniotic fluid and milk of mothers who drink (Chotro & Molina, 1990; Mennella & Beauchamp, 1997). Furthermore, parents and other adults often drink alcoholic beverages when children are present,

and such modeling of alcohol consumption may induce alcohol ingestion by children who observe it.

Results of the Seattle Longitudinal Prospective Study (ongoing since 1974) suggest that prenatal exposure to alcohol is a risk factor for alcohol use by adolescents (Streissguth, Barr, Bookstein, Samson, & Carmichael Olson, 1999). However, effects of maternally mediated prenatal and postnatal exposure to alcohol are difficult to separate in epidemiological studies. For example, many women, including alcoholics, may cease consuming alcohol during pregnancy and resume consumption postnatally (Little & Streissguth, 1978). However, women are unlikely to drink during pregnancy and abstain after delivery, making it difficult to isolate effects of drinking while pregnant on subsequent alcohol use by children of drinking mothers.

Legal and ethical constraints on research with humans make it impossible to require human parents to either drink alcohol or abstain from alcohol consumption at various stages while rearing young. Consequently, animal models are needed to determine the effects, if any, of exposure to ethanol at various stages in development on subsequent ethanol intake.

It has been suggested that, in rats, exposure to ethanol during gestation leads to increased voluntary consumption of ethanol later in life (Bond & Di Gusto, 1976; Nash, Weaver, Cowen, Davis, & Tramill, 1984; Phillips & Stainbrook, 1976). However, effects of exposure to ethanol in utero have not been found by all investigators (Abel & York, 1979; Grace, Rockman, and Glavin, 1986; McGivern, Clancy, Mousa, Couri, & Noble, 1984). Even those researchers who have reported an effect of exposure to ethanol in utero on subsequent affinity for ethanol have not provided compelling evidence of such an outcome. For example, Bond and Di Gusto (1976) examined ethanol consumption of only three ethanol-exposed litters. Furthermore, although they found differences in ethanol consumption by ethanol-exposed and naïve subjects when offered solutions containing 6% ethanol or less, they found no differences when such subjects had access to 7 or 8% ethanol solutions. Reyes, Garcia, and Jones (1985) found only intermittent effects of prenatal exposure to ethanol when they tested their subjects at 45 days of age. Phillips and Stainbrook (1976) exposed their animals to ethanol not only during gestation but also throughout lactation and weaning. Consequently, effects of exposure to ethanol during gestation on later ethanol consumption could not be determined.

Information about ethanol, such as that concerning other flavors (Galef & Henderson, 1972; Galef & Sherry, 1973), is transmitted through mothers' milk (Mennella & Beauchamp, 1997). The act of suckling is reinforcing to nurslings, and positive hedonic associations with ethanol, resulting from experience of ethanol in a nursing context, can result in subsequent enhanced acceptance of low to moderate concentrations of ethanol (Hunt, Kraebel, Rabine, Spear, & Spear, 1993). Hunt et al. (1993) found that 12- and 16-day-old rat pups that experienced ethanol while suckling swallowed more 5.6% ethanol introduced into their mouths via intraoral cannula than did control pups that had no prior experience of ethanol. Such enhanced ingestion of ethanol introduced directly into the oral cavity reflects social learning about ethanol during lactation. However, there have been no tests of effects of exposure to ethanol in mothers' milk on more natural seeking and consumption of ethanol after weaning.

Social experience with ethanol-consuming conspecifics have, however, been shown to cause changes in appetitive behaviors directed toward ethanol. Randall and Lester (1975) found that mice of the DBA strain (that normally drink very little ethanol), if reared for 7 weeks by ethanol-preferring mice of the C57bl strain that had access to ethanol and ingested considerable amounts of it, subsequently drank more ethanol than mice of their strain typically consume. Exposure of adolescent rats to intoxicated siblings enhanced ethanol intake of rats given a choice between ethanol and an equipalatable coffee solution (Hunt, Holloway, & Skordalakes, 2001).

Even brief exposure to ethanol-consuming conspecifics was found to have an impact on subsequent ethanol intake by rat pups. After a 30-min interaction with an intoxicated littermate, 8-, 12-, and 16-day-old rats swallowed more ethanol introduced into their mouths than did controls (Hunt, Lant, & Carroll, 2000), and repeated exposure to intoxicated siblings on days 12, 14, and 16 postpartum led to enhanced acceptance of ethanol after weaning (Day 22), suggesting that social exposure has a lasting effect on ethanol acceptance (Hunt et al., 2000).

Clearly, results of a variety of previous studies indicate that early exposure to ethanol alters rodents' subsequent responses to ethanol solutions. However, these findings are difficult to integrate. Different laboratories use different strains or species, different doses, routes and schedules of administration of ethanol, and test subjects at different ages and using different techniques. Consequently, it is difficult to combine findings to produce a comprehensive picture of effects of ethanol exposure during early development on subsequent affinity for ethanol.

We conducted the present series of experiments to systematically investigate the impact of a rat dam's consumption of moderate doses of ethanol during gestation, lactation, and weaning on voluntary ethanol consumption by her adolescent offspring.

For brevity and clarity, we define several terms used below. Rats up to 26 days of age that remain with their dam are referred to here as "pups," even though they were well past the age when they could survive on their own. Young recently separated from their dam are referred to as "adolescents." The term "maternal exposure" is used to refer to any exposure to ethanol mediated by the dam. Thus, prenatal exposure to an ethanol-ingesting dam, exposure to such a dam while suckling, or exposure to ethanol and ethanol-related cues during interactions with a dam are all referred to as maternal exposure. The term "asocial exposure" is used to refer to pups' exposure to ethanol and ethanol-related cues experienced when their dam is absent. For example, pups smelling or sampling ethanol from a source that a dam cannot reach are referred to as experiencing asocial exposure, though there may be social aspects to such events because of the presence of littermates.

EXPERIMENT 1

We undertook Experiment 1 to examine the impact on adolescents' ethanol consumption of maternal exposure to ethanol during gestation, lactation, and weaning. Dams of

pups maternally exposed to ethanol drank 4% (v/v) ethanol in tap water. Four percent ethanol solution is generally accepted by naïve rats (Samson, Pfeffer, & Tolliver, 1988) and its ad libitum consumption by rat dams during gestation does not cause physical abnormalities in their offspring (Abel, 1984).

During both the weaning period and testing we provided pups and dams with access to an 8% ethanol solution that is not readily accepted by naïve rats (Samson et al., 1988). By exposing pups to a relatively unpalatable ethanol solution during both weaning and testing, we were able to reduce spontaneous intake of ethanol by naïve pups. We compared ingestion of 8% ethanol by adolescent rats exposed to ethanol throughout early development with ethanol intake of adolescent rats exposed to ethanol either during restricted portions of their early development or not at all.

Subjects and Methods

Subjects. One hundred twenty-four rat pups, born to 62 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec) and maintained in the vivarium of the McMaster University Psychology Department served as subjects. Within 48 hr of birth (Day 0), we culled each litter to eight pups (where possible, four pups of each sex) and randomly assigned each litter to one of the five treatment conditions described below.

Apparatus. Until pups were 14 days old, we housed each dam with her litter in a shoebox cage measuring $36 \times 31 \times 17$ cm. The top of each cage was covered with a wire lid that held food (PMI Rodent Diet 5001, Brentwood, MO) and a bottle. The bottle held either tap water or a solution of 4% ethanol in tap water (all ethanol solutions were prepared v/v). The floor of the cage was covered with wood-chip bedding. For environmental enrichment, each cage was provided with a polyvinylchloride (PVC) conduit 10 cm in diameter and approximately 15 cm long.

From Day 14 to Day 26, each dam and litter occupied a large floor enclosure constructed of galvanized metal frame and wire-mesh, measuring $184 \times 92 \times 31$ cm (Figure 1). The floor of this enclosure was carpeted with wood shavings to a depth of approximately 4 cm, and we provided each enclosure with two $30 \times 30 \times 18$ cm wooden nest boxes, two food containers, and two 30-cm^3 transparent Plexiglas drinking boxes.

One of the two drinking boxes in each enclosure had a 5-cm^2 entrance that allowed both dam and pups to enter, while the other drinking box had a round entrance, 2.5 cm in diameter, that permitted access only to pups. Each box contained a bowl filled with either tap water or 8% ethanol in tap water. We prepared fresh ethanol solutions daily and
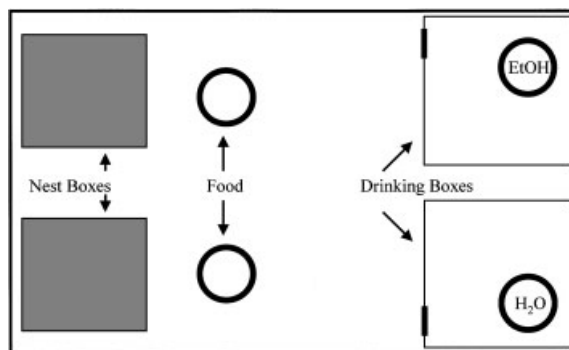
FIGURE 1 Schematic drawing of floor enclosures used in experiments.

replaced both the ethanol and water in drinking boxes every morning.

On Day 26, we moved adolescent rats from the large enclosure to individual shoebox cages, such as those described above, for ethanol-choice testing. During a choice test, all adolescents chose between two 50-ml test tubes, one containing tap water and the other containing 8% ethanol in tap water. Each test tube was closed with a rubber stopper and a stainless steel sipper tube.

Procedure (See Table 1)

Gestation, Lactation, and Weaning (GLW) Condition ($n = 14$ Litters). On approximately Day 7 of gestation, when weight gain allowed us to determine that a female was pregnant, we gave her 4% ethanol in tap water to drink. This ethanol solution provided her sole source of fluid during the last 2 weeks of gestation and the first 2 weeks postpartum. From Day 14 until Day 26, we housed each GLW litter in one of the floor enclosures described above (Fig. 1).

In a pilot study, we had observed that pups housed in floor enclosures began to drink from fluid containers on or about Day 18. Consequently, by placing each litter in a floor enclosure from Day 14 to 26, we allowed approximately 4 days for litters to become acclimatized to the enclosure and approximately 1 week for pups to experience access to both 8% ethanol solution and water.

While dams and young resided in floor enclosures, dams' fluid intake was restricted to 8% ethanol by placing a container of 8% ethanol in the drinking box with the larger opening. Pups had access to both 8% ethanol from the same source as their dam and to water in the drinking box with the smaller opening.

Gestation and Lactation (GL) Condition ($n = 8$ Litters). Until Day 14, we treated litters assigned to the GL condition just as we treated litters assigned to the GLW condition. From Day 14 to Day 26, dams assigned to

Table 1. Procedure for Experiment 1

| Condition | Dam Access | | Pup Access |
	Prior to Day 14	Day 14–Day 26	Day 14–Day 26
GLW	Ethanol	Ethanol	Ethanol and water
GL	Ethanol	Water	Ethanol and water
W	Water	Ethanol	Ethanol and water
A	Water	Water	Ethanol and water
C	Water	Water	Water

Note: GLW, gestation, lactation, and weaning; GL, gestation and lactation; W, weaning; A, access; C, control.

the GL condition had access to water rather than 8% ethanol in the drinking box with the larger opening, while their pups had access both to water in the drinking box with the larger opening and to 8% ethanol in the drinking box with the smaller opening.

Weaning (W) Condition ($n = 12$ Litters). Litters assigned to the W condition were not exposed to ethanol before day 14. On Day 14, we moved each litter assigned to the W condition to a floor enclosure and treated them just as we had treated litters assigned to the GLW condition, i.e., we restricted dams to drinking 8% ethanol from the drinking box with the larger opening, while pups had access to both 8% ethanol in the drinking box with the larger opening and water in the drinking box with the smaller opening.

Access (A) Condition ($n = 13$ Litters). Litters assigned to the A condition were not exposed to ethanol before day 14. On Day 14, we moved each litter to a floor enclosure where we restricted dams to drinking water from the drinking box with the larger opening while pups had access both to water from the same source as their dam and 8% ethanol in the drinking box with the smaller opening.

Control (C) Condition ($n = 15$ Litters). Litters assigned to the C condition were not exposed to ethanol before testing. On Day 14, we moved each litter to a large enclosure, where we restricted dams to drinking water from the drinking box with the larger opening. Pups could access water from the same source as their dam and also had access to another bowl of water in the drinking box with the smaller opening.

Testing. On Day 26 postpartum, we selected one male and one female adolescent at random from each litter, and housed each of these subjects individually. Each rat had access to both water and 8% ethanol for 2 h/day for each of 7 consecutive days of testing. We determined the weight of each fluid container before and after each 2-hr drinking session and to reduce effects, if any, of handling, we determined the weight of each subject every second

day. For the remaining 22 hr of each day, subjects had ad libitum access to pellets of rat chow but no access to fluids.

In a pilot study, we had found that our drinking tubes leaked approximately 0.1 g of fluid in each 2-hr session. We therefore subtracted 0.1 g from the weight of each drinking tube each day before undertaking further calculations. We used g/kg intake as a dependent measure to both compensate for body weight differences among adolescents and estimate levels of intoxication. We also determined pups' ethanol intakes as a proportion of total fluid intake.

If a subject drank no water during a drinking session, data for that subject for that day was discarded (27 of 434 data points) because lack of water intake was generally cause by an air bubble blocking a drinking spout. We also removed a subject from testing if it lost more than 10% of its body weight as a result of restricted fluid intake (2 of 124 pups). We averaged scores for males and females in each litter (after checking for an effect of sex) so that only one score from each litter entered into statistical comparisons.

Results

Across groups, mean total fluid intake/day ranged from 10.3 to 11.4 g [$F(4,57) = .98$, *ns*] and there was no effect of day of testing on the amount of ethanol consumed whether measured as g/kg total fluid [repeated-measures ANOVA, $F(6, 342) = .02$, *ns*] or percentage of total fluid intake [$F(6, 342) = .58$, *ns*].

The main results of Experiment 1 are presented in Figure 2. As can be seen in Figure 2, exposure to an ethanol-consuming dam during development resulted in enhanced voluntary intake of ethanol by adolescent rats [one-way ANOVA: $F(4, 57) = 7.07$, $p < .001$]. Planned orthogonal comparisons revealed that ethanol consumption by adolescents raised by an ethanol-consuming dam (conditions GLW, GL, and W) was significantly greater than ethanol consumption by adolescents from groups A and C whose mothers did not drink ethanol ($t_{\Psi 1} = 3.26$, $p < .001$). There were no differences in ethanol consumption among groups exposed to ethanol drinking dams
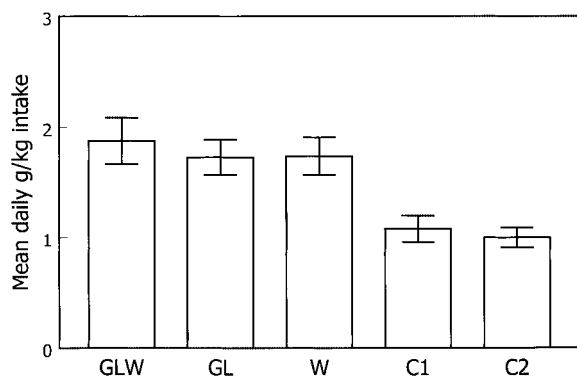
FIGURE 2 Daily ethanol intake (mean ± *SEM*) by adolescent rats in each of five treatment conditions in Experiment 1.

(GLW vs. GL and W, $t_{\Psi 2} = .21$, *ns*; GLW and GL vs. W, $t_{\Psi 3} = .47$, *ns*). Furthermore, there was no effect of mere access to ethanol during the weaning period on adolescent ethanol intake, since adolescents assigned to the Access condition did not drink more ethanol than did adolescents assigned to the Control condition ($t_{\Psi 4} = .20$, *ns*).

When we analyzed the data in terms of amount of ethanol consumed by pups as a percentage of total fluid intake, the outcome was the same. There was a significant effect of group assignment on percent ethanol intake [$F(4, 57) = 4.03$, $p < .006$], percent ethanol consumed by adolescents raised by an ethanol-consuming dam (Conditions GLW, GL, and W) was significantly greater than was percent ethanol consumed by adolescents whose mothers did not drink ethanol (conditions A and C; $t_{\Psi 1} = 3.92$, $p < .001$), and the percentage ethanol intake of adolescents assigned to groups A and C was equal ($t_{\Psi 2} = .80$, *ns*) as was that of adolescents assigned to groups GLW, GL, and W (GLW vs. GL and W, $t_{\Psi 3} = .51$, *ns*; GLW and GL vs. W, $t_{\Psi 4} = .69$, *ns*).

For subjects raised with an ethanol-consuming dam, the mean g/kg ethanol intake in 2 hr was greater than 1.5 g/kg (group GLW = 1.88 ± 0.21 g/kg, group GL = 1.74 ± 0.16, and group W = 1.74 ± 0.17). When ethanol is injected intraperitoneally, 1.5 g/kg produces intoxication (see Larson & Siegel, 1998, or Wenger, Tiffany, Bombardier, Nicholls, & Woods, 1981 for examples of ataxic effects of a 1.5 g/kg dose of ethanol).

When subjects in this experiment were given 2 hr to consume fluids, most completed drinking within 30 min of fluid presentation, so subjects that consumed a dose greater than 1.5 g/kg within a session might have experienced some intoxication. In fact, informal behavioral observations were consistent with the view that many subjects in groups GLW, GL, and W were intoxicated following test sessions. In particular, on days when individuals consumed more than 3.0 g/kg of 8% ethanol, they either showed loss of locomotor coordination or appeared sedated. We attempted to formally measure

ataxia in intoxicated 27- to 33-day-old rats using the procedures of Larson and Siegel (1998). Unfortunately, adolescent rats weighed so little that often, even when unconscious, they did not slide down the increasingly elevated inclined plane used to measure ataxia any sooner than did control subjects not suffering from alcohol intoxication.

Subjects raised by water drinking dams consumed smaller doses of ethanol (group A = 1.08 % 0.12 g/kg, group C = 1.00 % 0.09 g/kg) than did those reared by alcohol-consuming dams, and no animals assigned to groups A or C exhibited signs of intoxication.

Discussion

In addition to confirming that exposure to ethanol during early development can result in enhanced affinity for ethanol in adolescent rats, the results of the present experiment suggest that asocial exposure to ethanol is not in itself sufficient to enhance adolescent rats' ethanol consumption. Pups assigned to the Access condition, unlike pups assigned to the Control condition, had opportunity to drink ethanol throughout the weaning period (Day 14–26). However, we found no difference in ethanol consumption of adolescents assigned to the two conditions. Consumption of substantial quantities of ethanol by subjects assigned to conditions other than the Access and Control conditions must therefore have been due to something other than opportunity to consume ethanol during the weaning period.

Pups assigned to the Access condition were exposed not only to the smell and taste of ethanol but also to siblings that had ingested ethanol. Such subjects might therefore be expected to ingest more ethanol during testing than pups assigned to the Control condition that were not exposed to ethanol-consuming siblings (Hunt et al., 2001). However, Hunt et al. (2001) intubated siblings of focal subjects that drank excess ethanol with 1.5 g/kg of ethanol in a single bolus. Focal subjects whose siblings had been intubated with either 1.0 or 3.0 g/kg did not show increased ethanol intake.

In a pilot study, we found that pups aged 18 to 26 days, treated exactly as we treated subjects assigned to the Access condition in Experiment 1, never consumed more than 4g/kg of ethanol over 24 hr. Consequently, they were unlikely to provide siblings with the intensity of stimulation that provoked enhanced ethanol intake in the Hunt et al. (2001) study.

EXPERIMENT 2

Experiment 2 was designed (1) to isolate effects of exposure to ethanol during gestation from effects of exposure to ethanol during lactation, and (2) to remove effects of asocial exposure to ethanol during the weaning

period so that we could determine effects of maternal exposure to ethanol during gestation and lactation per se.

In Experiment 1, asocial exposure to ethanol during weaning had no effect on post-weaning ethanol intake of adolescent rats. However, asocial exposure to ethanol during weaning may have been experienced differently by weanlings assigned to the Access condition (that had no prior maternal exposure to ethanol) than by weanlings assigned to the GL condition (that had prior maternal exposure to ethanol).

Exposure to ethanol during pregnancy and lactation may have increased affinity for ethanol so that weanlings assigned to group GL were more likely to sample ethanol present in their cages from Days 14 to 26 than were weanlings assigned to the Access condition. If so, the enhanced ethanol intake observed in adolescent subjects assigned to the GL condition in Experiment 1 may have depended on their exposure to ethanol while weaning.

In Experiment 2, we examined ethanol intake of adolescence rats that had maternal exposure to ethanol during either gestation or lactation or during both gestation and lactation but had no asocial access to ethanol during the weaning period.

Method

Subjects. We randomly assigned 37 pregnant female Long-Evans rats to one of the five conditions described below. Within 48 hr of birth of a litter (Day 0), we recorded the number of live births and culled each litter to eight pups (where possible, four pups of each sex). We documented the mass of each litter on Day 7, and on Day 26 we randomly selected one pup of each sex from each litter for testing.

Apparatus

The apparatus was that used in Experiment 1.

Procedure (See Table 2)

Gestation and Lactation (GL2) Condition (n = 8 Litters). We gave pregnant rats assigned to the GL2 condition 4% ethanol in tap water as their sole source of fluid during the last 2 weeks of gestation and first 2 weeks postpartum. From Day 14 to Day 26 dams and their litters assigned to the GL2 condition, unlike dams and litters assigned to the GL condition in Experiment 1, had access only to tap water (Table 2).

Gestation (G) Condition (n = 8 Litters). We gave pregnant rats assigned to the G condition 4% ethanol as the sole source of fluid during the last 2 weeks of gestation and tap water from Day 0 until Day 26.

Lactation (L) condition (n = 8 litters): We gave pregnant rats assigned to this condition tap water throughout gestation. From Day 0 to Day 14, they had 4% ethanol as the sole source of fluid, and tap water from Day 14 to Day 26.

Control (C) Condition (n = 8 Litters). Dams and their litters assigned to the Control condition drank tap water throughout gestation, lactation, and until Day 26.

Blood-Ethanol Concentration (BEC) Condition (n = 5 Litters). Pregnant rats assigned to the BEC condition drank 4% ethanol during the last 2 weeks of gestation. Six hours after light offset, when circadian fluctuations in blood-ethanol concentrations are at a peak in rats given ad libitum access to ethanol (C. L. Randall, personal communication, April, 1999), on or about prenatal Day 16, we collected approximately 0.25 ml of blood from each rat dam via a 5-mm tail amputation. Blood was collected into heparinized tubes and analyzed for blood-ethanol concentration using a multipurpose biochemistry analyzer (YSI Model 2700 Biochemistry Analyzer; Yellow Springs Industries, Yellow Springs, OH). All blood-ethanol concentrations are presented as mg/100 ml of blood (mg %). No litters from this condition were used for ethanol choice testing.

Testing. Testing was conducted exactly as in Experiment 1.

Table 2.   Procedure for Experiment 2

	Dam Access		Dam and Pup Access
Condition	Prior to Day 0	Day 0–Day 14	Day 14–Day 26
GL	Ethanol	Ethanol	Water
G	Ethanol	Water	Water
L	Water	Ethanol	Water
C	Water	Water	Water
BEC	Ethanol	Water	Water

Note: GL, gestation and lactation; G, gestation; L, lactation; C, control; BEC, blood ethanol concentration.

Results

As in Experiment 1, we found no differences among groups in mean daily fluid intake, which ranged from 11.7 to 12.4 g [$F(3, 28) = .46$, *ns*]. We also found no differences across days of testing in either ethanol intake relative to total fluid intake (repeated-measures ANOVA, [$F(6, 168) = .45$, *ns*] or g/kg intake [$F(6, 168) = .11$, *ns*].

The main results of Experiment 2 are presented in Figure 3. A one-way ANOVA revealed no effect of group assignment on mean daily g/kg intake of ethanol [$F(3, 28) = .83$, *ns*]. When we analyzed ethanol consumption as a percentage of total daily fluid intake the results were the same [$F(3, 28) = .56$, *ns*].

Levels of ethanol consumption by adolescent rats were not affected by exposure to ethanol during either gestation or lactation, or throughout both stages of development if they had not had access to ethanol while weaning. No subjects in Experiment 2 exhibited signs of intoxication common in subjects assigned to GLW, GL, and W in Experiment 1.

Failure in the present experiment to find any effect of early exposure to ethanol on adolescent ethanol consumption cannot be attributed to a lack of ethanol exposure during gestation. Dams assigned to the BEC condition consumed sufficient ethanol to result in a mean blood-ethanol concentration of 165.6 mg % ($\pm$ 21.7), and it is reasonable to assume that dams assigned to GL2 and G conditions had similar blood-ethanol titers. Blood-ethanol concentrations in this range are known to produce neurobehavioral changes, but not physiological anomalies, in exposed offspring (see Driscoll, Streissguth & Riley, 1990 for review).

Dams' fluid consumption during lactation was similar to that during gestation, so we assume that pups were exposed to moderate levels of ethanol during that developmental stage, as well. Exposure to ethanol did not affect litter size, or pup size, since both litter size and weight were similar across groups (Table 3).
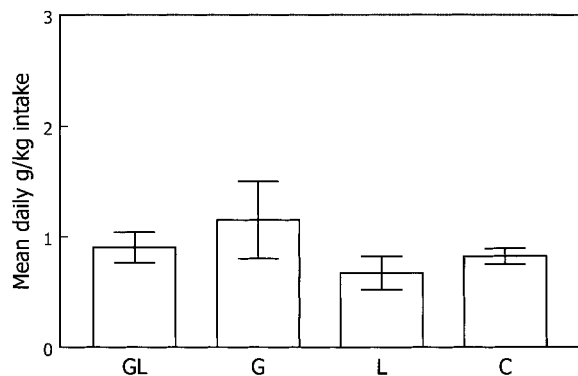
Table 3. Mean Litter Sizes and Litter Weights in Experiment 2

Condition	Litter Size (Pups)	Litter Weight, g (Day 7)
GL2	14.5	141.1
G	14.0	137.3
L	15.9	133.5
C	14.4	138.5
BEC	15.0	143.2

Note: Values are expressed as means.

Discussion

The results of Experiment 2, unlike those of Experiment 1, indicate that exposure to a moderate dose of ethanol during either gestation or lactation, or throughout both gestation and lactation, does not lead to enhanced ethanol affinity after weaning. The principle methodological difference between Experiments 1 and 2 that might account for the difference in outcome was removal of asocial exposure to ethanol during the weaning period. In Experiment 1, but not in Experiment 2, of the present series, pups had opportunity to sustain any maternally induced affinity for ethanol by ingesting ethanol directly. Maternal exposure to ethanol may have initiated a cascade of events that made asocial exposure to ethanol during the weaning period sufficient to cause enhanced affinity for ethanol in adolescents.

Second, both in Experiment 2 and in many experiments described in the literature (e.g., Abel & York, 1979; Reyes et al., 1985; McGivern et al., 1984), when examining effects of preweaning exposure to ethanol, considerable delay has been introduced between exposure to ethanol and testing for ethanol consumption. Possibly, such delay results in loss of any affinity for ethanol resulting from preweaning exposure to it.

A third plausible explanation for the discrepancy in results between Experiments 1 and 2 is related to differences in concentrations of ethanol used during exposure and testing. We exposed pups maternally to 4% ethanol during gestation and lactation in both Experiments 1 and 2. However, in Experiment 1, pups could also sample 8% ethanol (either with or without their dams) during weaning. During the test phase of both Experiments 1 and 2, we offered adolescents a choice between water and 8% ethanol.

Four and eight percent ethanol differ in both palatability and pharmacological effect. If pups' maternal exposure to ethanol during gestation and lactation had come from dams drinking 8% ethanol, perhaps exposure during gestation or lactation would have been sufficient to enhance ethanol consumption after weaning.

To test this hypothesis, we compared the ethanol choices of adolescent rats that had lived with dams that

FIGURE 3 Daily ethanol intake (mean $\pm$ *SEM*) by adolescent rats in each of four treatment conditions in Experiment 2.

consumed 8% ethanol throughout gestation and lactation ($n = 10$ litters) to the ethanol choice of adolescents that had lived with dams that had consumed 4% ethanol during the same period ($n = 10$ litters). We also documented the blood-ethanol concentration of six additional pregnant rats that drank 4% ethanol, and six that drank 8% ethanol.

We found no effect of ethanol concentration fed to dams on intake of 8% ethanol by their adolescent young. Mean ethanol intake by adolescents exposed to 4% ethanol was 0.94 g/kg $\pm$ 0.19, while mean ethanol intake by adolescents exposed to 8% ethanol was 1.02 g/kg $\pm$ 0.12 (Student's *t* test, $t = .39$, *ns*).

Failure to find an effect of ethanol concentration was not due to a lack of difference in blood-ethanol concentration between dams in the two conditions. Mean blood-ethanol concentration of pregnant females that drank 4% ethanol was 111.7 mg % $\pm$ 20, while mean blood-ethanol concentration achieved by pregnant rats that drank 8% ethanol was 239.3 mg % $\pm$ 37 ($t = 3.03$, $p < .05$). Thus, exposure to either 4% ethanol or 8% ethanol throughout gestation and lactation does not lead to enhanced voluntary consumption of 8% ethanol by adolescent rats.

GENERAL DISCUSSION

In Experiment 1, we found that adolescent rats that had lived with ethanol-consuming dams drank more ethanol than did adolescent rats that had lived with water consuming dams. This increased ethanol consumption by adolescents that followed prolonged exposure to an ethanol-consuming dam is consistent with results obtained by other researchers in both rats (Phillips & Stainbrook, 1976) and humans (Streissguth et al., 1999).

We also found in Experiment 1 that asocial exposure of pups to ethanol during the weaning period was not sufficient to enhance voluntary ethanol consumption after weaning. Thus, asocial exposure to the odor of ethanol and asocial opportunity to consume ethanol during weaning did not increase the probability that an adolescent rat would drink copious amounts of ethanol after weaning, whereas maternal exposure to ethanol did increase adolescents' ethanol consumption. This result is consistent with that that obtained by Randall and Lester (1975) who found that prolonged social exposure to ethanol preferring C57bl mice increased the strain-typical ethanol preferences of young DBA mice.

The results of Experiment 2 indicated that maternal exposure to ethanol during gestation and lactation in not only not necessary to enhance ethanol consumption by adolescent rats but that it is also not sufficient to do so. Neither maternal exposure to 4% ethanol nor maternal exposure to 8% ethanol throughout gestation and the first

2 weeks of lactation led to enhanced ethanol intake in exposed rats without either maternal or asocial exposure to ethanol during weaning, even though dams consuming ethanol during gestation achieved substantial blood-ethanol concentrations.

On the other hand, maternal exposure during gestation and lactation, combined with asocial access to ethanol during weaning, did result in substantial voluntary ethanol consumption by adolescent rats. By analogy, results of the Seattle Longitudinal Study (Streissguth et al., 1999), in which children of women who drank during pregnancy were found to be at enhanced risk in adolescence for alcohol abuse, may have reflected exposure to alcohol during pregnancy and continued exposure to alcohol in the home environment throughout childhood and early adolescence. Possibly, children with prenatal exposure to alcohol may be at reduced risk for subsequent alcohol abuse, if raised without further exposure to alcohol.

Although the results of our experiments may appear to conflict with those obtained by some other researchers who have used rodent models to examine effects of exposure to ethanol during gestation and lactation on later response to ethanol, the conflict is more apparent than real. Those finding effects of prenatal or nursing exposure to ethanol on response to ethanol have generally not measured active seeking and ingestion of ethanol. For example, Chotro and Molina (1990) found that a brief ethanol exposure during late gestation resulted in increased preference for ethanol odor, and increased ethanol acceptance by 8- and 9-day-old rat pups when ethanol was directly introduced into the mouth via cannula. Similarly, Hunt and colleagues (1993) demonstrated that experience with ethanol in a nursing context resulted in enhanced acceptance by 12- and 16-day-old rats of ethanol introduced directly into the oral cavity.

Taken together, such reports suggest that prenatal and early postnatal experience with ethanol does alter rat pups' responsiveness to ethanol. However, we did not find enhanced active seeking and ingestion of ethanol with similar early ethanol exposure. Possibly, pups in our experiments would have demonstrated enhanced acceptance of ethanol or demonstrated enhanced preference for ethanol odor, but we did not examine these dependent variables. Also, we tested our subjects for the first time when 26 days of age. We do not know how our subjects would have responded to ethanol at earlier ages. Conversely, it is not known whether pups in the experiments of Chotro and Molina (1990) or Hunt et al. (1993) would have sought ethanol voluntarily, or if the effects they have described last until after weaning. Such questions regarding the impact of maternal exposure to ethanol on subsequent affinity for ethanol remain to be answered.

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