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Effects of ethanol consumption by adult female rats on subsequent consumption by adolescents

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Abstract

We used a two-bottle choice test to measure voluntary ethanol consumption by adolescent rats that had lived with ethanol-consuming or water-consuming adult conspecifics. We found that housing weanlings with either a virgin or a lactating adult female rat that ingested ethanol increased the weanlings' subsequent voluntary intake of ethanol when they were fluid-deprived and provided with choices between 8% ethanol solution and water for 2 h/day. Rats housed with both an ethanol-consuming virgin female and their water-consuming dam drank more ethanol than did rats housed with a dam and virgin female, both consuming water. Rats housed with an ethanol-consuming dam and ethanol consuming adult virgin did not drink more ethanol than did rats housed with an ethanol-consuming dam and a water-consuming virgin female.

In sum: (1) young rats learned socially to consume ethanol. (2) Exposure to ethanol in mother's milk was not necessary for such social learning to occur, and (3) living with an ethanol-consuming unfamiliar, virgin female conspecific resulted in enhanced ethanol intake by adolescent rats, even if a water-consuming dam was also present.

Keywords: Ethanol; Rats; Early exposure; Social learning

Introduction

The food choices of rodents, like those of human beings, can be profoundly affected by social interactions. In general, focal animals tend to increase their intake of substances ingested by conspecifics that focal animals have interacted with. Such social effects on food choice can result from a variety of different types of interaction between a focal animal and conspecifics that have ingested substances unfamiliar to the focal animal (see Galef, 1996 for review). For example, and of particular relevance to the studies reported here: (1) experience of the flavor of a food in mother's milk increases preference for that food in both human infants and weanling rats (Galef & Sherry, 1973; Mennella & Beauchamp, 1997) in part because the act of suckling is itself reinforcing (Hunt, Kraebel, Rabine, Spear, & Spear, 1993; Martin & Alberts, 1979). (2) Exposure to the scent of a food, even an inherently unpalatable one (Galef, 1989), on the breath of an adult rat that has eaten the food (Galef & Stein, 1985) results in enhanced intake of it (Galef, Whiskin, & Bielavska, 1997), and (3) presence at a feeding site of either other rats or residual cues they deposit there enhances pups' intake of food available at that site (Galef & Clark, 1971; Galef & Heiber, 1976). Ethanol is a source of energy, and therefore, food of a sort. Consequently, any process that supports social influence on food choice should also affect development of affinity for ethanol.

The multiplicity of process that can increase preference for a food complicates analyses of the causes of enhanced ethanol intake of young rodents reared by dams that consume ethanol. For example, Randall & Lester (1975) reported that DBA mice (that normally avoid ethanol) reared by C57Bl dams (that voluntarily ingest ethanol) in an environment that provided pups access to ethanol drank more ethanol than did DBA mice reared in the same environment by foster dams of their own strain. Randall and Lester attributed the observed enhanced ethanol consumption by DBA mice reared by C57Bl foster dams to

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experience with ethanol in mothers' milk. However, pups reared by C57Bl foster dams in experiment of Randall & Lester (1975) were exposed to ethanol not only through their foster dams' milk, but also through social contact with both their dams and their ethanol-ingesting siblings, and by presence of conspecifics at the site where their dams and siblings were drinking ethanol (Thorpe, 1963).

Hunt et al., 1993 found that both 12- and 16-day-old rat pups that had previously ingested ethanol-tainted milk while suckling from an anaesthetized dam more readily accept ethanol administered directly into their mouths through an intraoral cannula than did pups lacking prior exposure to ethanol-tainted milk. However, pups in the study of Hunt et al. (1993) like those in experiment of Randall & Lester (1975), experienced more than one type of exposure to ethanol. In particular, each pup exposed to ethanol while suckling was also exposed to an adult that had been drinking ethanol and to siblings that had consumed ethanol in milk. Rat pups that interact with intoxicated siblings ingest more ethanol administered directly into their mouths than do pups that interact with sober littermates (Hunt, Lant, & Carroll, 2000), and exposure to an ethanol-consuming adult's breath may enhance pups' subsequent appetence for ethanol.

We have recently reported that early social exposure to ethanol can enhance voluntary consumption of ethanol by rats tested when they reach 26 days of age (Honey & Galef, 2003). We exposed pups to ethanol-consuming dams in an environment where the pups did not have direct access to ethanol and found that exposure to an ethanol-consuming dam from Day 14 to 26 postpartum, but not from birth to Day 14, enhanced pups' subsequent ethanol consumption. The effect of exposure to an ethanol-consuming dam from Day 14 to 26 that we observed could reflect exposure to ethanol in mothers' milk, exposure to an adult that has ingested ethanol, exposure to siblings that have ingested ethanol, or some interaction among these factors.

Here, we describe three experiments concerned with ways in which adult rats may enhance voluntary ingestion of ethanol by juveniles. In the first experiment, we determined whether, as some previous researchers have assumed (Randall & Lester, 1975), consumption of mothers' milk tainted with ethanol is necessary to enhance affinity for ethanol in juvenile rodents that interacted with an ethanol-consuming dam. In Experiments 2 and 3, to determine whether lactating rat dams were more effective than other adult rats in influencing alcohol intake by juveniles, we assessed the ethanol consumption of adolescent rats that had lived with both their dam and a virgin adult female, only one of whom consumed ethanol.

Experiment 1

We undertook Experiment 1 to determine whether consumption of milk from a lactating rat ingesting ethanol is necessary for development of enhanced affinity for ethanol in adolescent rats (Honey & Galef, 2003). We removed rat dams from their litters on Day 18 postpartum, and replaced them with either a lactating or non-lactating female rat that we gave access to only a single fluid, either tap water or 8% ethanol. When pups were weaned at 26 days of age (adolescent), we tested them individually for ethanol consumption by giving them a series of choices between water and 8% ethanol for 2 h/day.

We began our experimental manipulations on Day 18 because rats stressed by early weaning subsequently drink more ethanol than rats weaned at a later age (Rockman, Hall, & Markert, 1987). At 18 days of age, rat pups invariably wean successfully, but will nurse from a lactating female, if given the opportunity to do so.

Method

Subjects

Forty-eight rat pups, born to 24, 3-month-old, 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. The vivarium was maintained at 21°C on a 12:12 h light:dark cycle, with light onset at 7:00 am local time. Twelve virgin female rats of the same age and from the same source as the mothers of litters, as well as 12 of the mothers of litters served as 'foster females' (see Procedure).

We culled litters to eight (when possible, four pups of each sex) within 48 h of birth (Day 0), and randomly assigned the litter to one of the four treatment conditions described in Procedure. We tested two adolescent subjects from each litter for voluntary ethanol consumption for 1 week starting when pups were 26 days old.

Apparatus

Birth to Day 14. Until pups were 14 days of age, we housed each dam with her litter in a transparent polypropylene shoe-box cage measuring $36 \times 31 \times 17$ cm^3. The wire lid of each cage held both food pellets (PMI Rodent Diet 5001, Brentwood, MO) and a water bottle. The floor of each cage was covered with wood-chip bedding, and for environmental enrichment, we placed a piece of polyvinylchloride (PVC) conduit, approximately 15 cm in length, in each cage.

Day 14–18. From Day 14 to 18, each dam and litter occupied a floor enclosure constructed of angle iron and hardware cloth, measuring $92 \times 92 \times 31$ cm^3. The galvanized, sheet-metal floor of the enclosure was carpeted with wood shavings to a depth of approximately 4 cm, and we provided each enclosure with a wooden nest box ($30 \times 30 \times 18$ cm^3), a food container and a water bottle that we attached to one of the enclosure's walls.

Day 18–26. On Day 18, just before we removed a mother and replaced her with a foster female, we separated each foster female's source of fluid from that of pups. We enclosed the sipper tube of the water bottle within a 30-cm^3

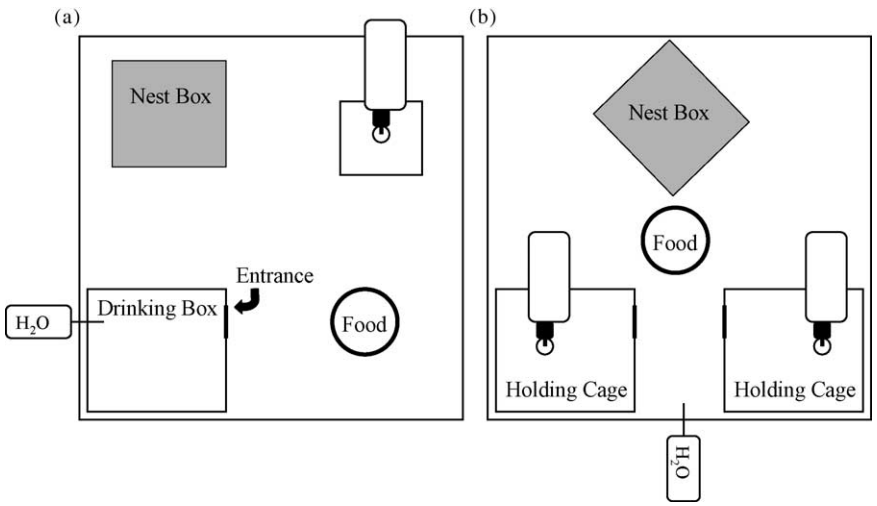

Fig. 1. Schematic drawing of the layout of floor enclosures used in: (a) in Experiment 1 and (b) Experiments 2 and 3. Pups in Experiment 1 could access water in the drinking box (where adults could not enter) but could not reach the sipper tube that dispensed fluid for their foster females (presented in the unlabeled bottle). Pups in Experiments 2 and 3 could access water from the bottle mounted outside the holding cages, and could freely visit each adult female, but could not reach the sipper tube in the lid of each holding cage.

transparent Plexiglas drinking box (Fig. 1a). The drinking box had a circular entrance 2.5-cm in diameter that permitted pups, but not adults, to enter.

We mounted a second bottle containing fluid on the wire-mesh lid of each enclosure with its sipper tube protruding through both the lid and a 20 cm^2 Plexiglas plate attached to the underside of the lid. The Plexiglas plate prevented pups from climbing along the underside of the wire-mesh lid to drink the fluid intended for their foster female. While standing on the floor of the enclosure, pups could not reach the sipper tube which was approximately 25 cm above the floor of the enclosure. The sipper tube was readily accessible to adults.

A small glass container filled with absorbent wood shavings and closed with wire mesh placed directly beneath the sipper tube inserted through the cage lid collected any fluid dripping from the sipper tube and prevented pups from sampling any leaked fluid.

Day 26–33. To measure adolescents' affinity for ethanol, beginning on Day 26, we housed them individually in shoebox cages like those in which dams and litters had been housed from Day 0 to 14. During a choice test, each subject had available two 50-ml test tubes, one containing tap water and the other 8% ethanol, and each closed with a rubber stopper and stainless steel sipper tube.

Procedure

Lactating-Female/Ethanol (LE) Condition (n = 6 litters). From Day 14 to 26, we housed each litter assigned to the LE Condition in a floor enclosure like that illustrated in Fig. 1a. From Day 14 to 18, dams remained with their litters. On Day 18, we removed each dam and replaced her with a lactating female that we had just removed from another litter. We then restricted foster females' fluid intake to 8% ethanol placed in the bottle mounted on the lid of the floor enclosure. Pups had access only to water in the drinking box that their foster female could not enter.

Lactating-Female/Water (LW) Condition (n = 6 litters). We treated litters assigned to the LW Condition just as we treated litters assigned to the LE Condition except that lactating foster females drank water rather than 8% ethanol from the sipper tube protruding through the lid of the enclosure.

Virgin-Female/Ethanol (VE) Condition (n = 6 litters). We treated litters assigned to the VE Condition just as we treated those assigned to the LE Condition, except that foster females introduced into floor enclosures with pups were non-lactating, virgin, rather than lactating, adult females.

Virgin-Female/Water (VW) Condition (n = 6 litters). We treated litters assigned to the VW Condition just as we treated those assigned to the LW Condition, except that foster females were virgin, rather than lactating, adult females.

Observation. Starting on Day 18, we observed each litter and foster female daily on closed-circuit television to determine whether foster females were behaving maternally toward their foster litters.

Testing. On Day 26 postpartum, we selected one male and one female adolescent at random from each litter, and transferred them to individual shoebox cages. Each subject was fluid-deprived for 22 h/day and had access to both water and 8% ethanol for 2 h/day for seven consecutive days of testing. We determined the weight of each test tube before and after each 2-h drinking session, and weighed subjects every second day.

In a pilot study, we had found that drinking tubes leaked approximately 0.1 g of fluid during each 2-h test session. We, therefore, subtracted 0.1 g from the weight of each

drinking tube each day before undertaking further calculations.

We used g/kg/day intake as a dependent measure to compensate for differences in subjects' body weights. To provide an index of fluid choice, we also report amount of ethanol consumed as a percentage of total fluid intake.

If a subject drank no water during a drinking session, data for that subject for that day (seven of 336 data points) were discarded because lack of water intake was generally caused by an air bubble blocking a sipper tube. We averaged scores for males and females in each litter (after conducting preliminary *t*-tests to check for an effect of sex) so that only one score from each litter entered into any statistical comparison.

Results

Lactating foster females started to nurse pups within 24 h of placement with litters and continued to nurse foster pups until they were removed on Day 26. Each virgin foster female was observed both to groom pups and to huddle with them.

As can be seen in Table 1, which shows adolescent rats' mean fluid intake per day during 7 days of testing, fluid intake by subjects in Experiment 1 ranged from 11.0 to 11.6 g ($F(3, 20) = 0.29$ ns). Virgin females that had 8% ethanol as their only source of fluid consumed approximately 20 g/kg/day and lactating females approximately 24 g/kg/day.

To determine whether subjects exhibited changes in ethanol intake across the 7 days of testing, we used a repeated-measures ANOVA. We found no significant effect of day of testing on the amount of ethanol consumed as a percentage of total fluid intake ($F(6, 120) = 2.10$ ns). There was, however, a statistically significant effect of day of testing on mean daily g/kg total intake of ethanol ($F(6, 120) = 4.14$, $p < 0.01$) reflecting subjects drinking more g/kg ethanol on the second and third days of testing than on other days. We consider this statistically significant result an artifact, as we have not seen such an outcome in more than 20 similar tests. There was no interaction between day of testing and group assignment ($F(6, 120) = 0.71$ ns).

Table 1
Daily total fluid intake and ethanol intake as a percentage of total fluid intake by adolescent rats in Experiments 1, 2 and 3

Group	Total fluid (g)	Ethanol/total fluid (%)
Experiment 1		
LE	11.6 ± 0.9	18.2 ± 2.8
VE	11.0 ± 1.1	22.0 ± 3.2
LW	11.6 ± 0.8	11.4 ± 2.5
VW	11.3 ± 1.0	9.0 ± 2.3
Experiment 2		
WW	11.8 ± 0.9	7.5 ± 0.7
EW	11.5 ± 0.8	14.2 ± 1.6
Experiment 3		
WE	11.4 ± 0.8	21.0 ± 4.9
EE	11.3 ± 1.0	20.0 ± 1.5

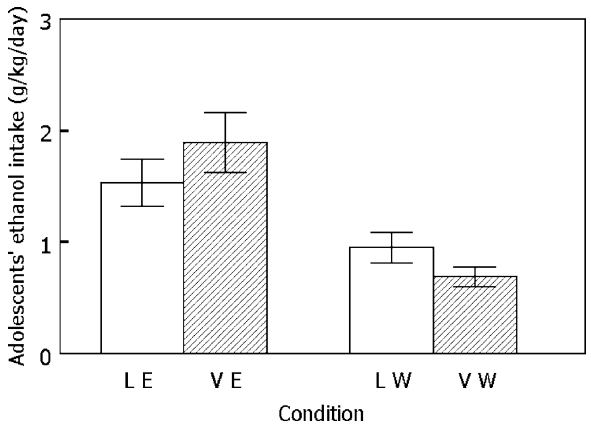

Fig. 2. Mean (±SEM) daily g/kg ethanol intake by adolescent rats in Experiment 1 that had lived with virgin or lactating foster females. Rats that had lived with an ethanol-consuming foster female consumed more ethanol than did rats that had lived with a water consuming foster female ($F(1, 20) = 18.26$, $p < 0.001$) but there were no differences in ethanol consumption as a result of whether the foster female was lactating or virgin ($F(1, 20) = 0.54$ ns).

The main results of Experiment 1 are presented in Fig. 2 that shows the mean daily ethanol intake (g/kg/day) of adolescents during testing. As can be seen in the figure, regardless of whether the foster female rearing a litter was lactating or virgin, exposure to an ethanol-consuming foster female from Day 18 to 26 resulted in enhanced voluntary intake of ethanol by adolescent rats. A two-way ANOVA revealed a main effect of the fluid consumed by foster females (either ethanol or water; $F(1, 20) = 18.26$, $p < 0.001$), but no main effect of whether a foster female was lactating or virgin ($F(1, 20) = 0.54$ ns), and no interaction between main effects ($F(1, 20) = 2.23$ 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 as when ethanol consumption was measured in g/kg/day (Table 1). There was a significant effect of the fluid consumed by foster females on percent ethanol intake of adolescents ($F(1, 20) = 15.82$, $p < 0.001$), but no main effect of whether a foster female was lactating or virgin ($F(1, 20) = 0.09$ ns) and no interaction between main effects ($F(1, 20) = 1.61$ ns).

Ethanol intake by adolescents that had lived with an ethanol-consuming foster female from Day 18 to 26 was >1.5 g/kg/day (VE Condition = 1.89 ± 0.27 g/kg/day, LE Condition = 1.53 ± 0.21 g/kg/day), comparable to the ethanol consumption of adolescent subjects in previous studies that interacted with ethanol-consuming dams from Day 14 to 26 postpartum (Honey & Galef, 2003). Ethanol intake by adolescents that had lived with a water-consuming virgin tended to be less than

1 g/kg/day (VW Condition = 0.69 ± 0.09 g/kg/day, LW Condition = 0.95 ± 0.14 g/kg/day), a level of intake consistent with that of adolescents that had lived with water-consuming dams in previous studies (Honey & Galef, 2003).

To avoid stressing foster females in the present experiments, we did not take blood samples, so we do not know their circulating levels of blood–ethanol. However, in a pilot study, we found that adult females with ad libitum access to 8% ethanol consumed an average of 62.8 ± 3.9 ml of ethanol/day and showed blood–ethanol concentrations of 209 ± 27.2 mg% when we sampled blood in the middle of the dark cycle. Ethanol concentration in mother's milk and mother's blood are similar (Mennella, 1999).

Discussion

The results of Experiment 1 replicate our previous finding that adolescent rats that have lived with an ethanol-consuming lactating female during weaning drink more ethanol than do adolescents that have lived with a water-consuming lactating female (Honey & Galef, 2003).

Adolescent rats that lived with an ethanol-consuming, virgin adult female from Day 18 to 26 were exposed to neither ethanol in mother's milk nor ethanol-intoxicated siblings. However, their levels of ethanol intake after weaning were comparable to those of adolescents that had experienced both exposure to ethanol in milk and interaction with siblings that had ingested ethanol-tainted milk. Thus, the present results also show that enhanced voluntary consumption of ethanol by fluid-deprived adolescent rats need not depend on either previous experience of ethanol in mothers' milk or previous interaction with intoxicated siblings. Rather, social exposure to an ethanol-consuming adult female rat, like exposure to ethanol in mothers' milk (Hunt et al., 1993) and exposure to intoxicated siblings (Hunt, Holloway, & Skordalakes, 2001; Hunt et al., 2000) results in enhanced ethanol intake by adolescent rats.

The failure to find a difference in the effects of lactating and virgin foster females drinking ethanol on subsequent ethanol intake of adolescent pups that they reared may reflect the level of exposure to ethanol received by pups that interacted with a lactating foster female. Relevant is Hunt et al. (2001) finding that young rats that interacted with intoxicated siblings intubated with 1.5 g/kg of ethanol in a single bolus demonstrated enhanced ethanol intake, whereas subjects whose siblings had been intubated with either 1.0 or 3.0 g/kg did not.

Experiment 2

In Experiment 1, levels of ethanol consumption by adolescent rats that had lived with an ethanol-consuming foster female were comparable to those of adolescent rats in other experiments in our laboratory that interacted with their own ethanol-consuming dams (Honey & Galef, 2003). This similarity in ethanol intake by pups reared by ethanol-consuming dams whether related and familiar or unrelated and unfamiliar suggests that exposure to any ethanol-consuming adult female is equally effective in enhancing affinity for ethanol in young rats. However, it is possible that pups exposed to more than one source of social information about what to ingest are more strongly influenced by their dam than by other adults.

In Experiment 2, we determined whether an ethanol-consuming adult virgin female could induce ethanol appetence in young rats when their water-consuming dam was also present.

Method

Subjects

Forty pups, born to 20, 3-month-old female Long-Evans rats served as subjects. An additional 20 adult virgin females, the same age as the dams that we paired them with (see Procedure) served as virgin foster females.

Apparatus

Birth to Day 18. As in Experiment 1, until pups were 14 days of age, we housed each dam and her litter in a transparent polypropylene shoebox cage. From Day 14 to 18, each dam and her litter, together with a virgin female (introduced on Day 14), resided in a floor enclosure containing a nest box and providing ad libitum access to food and water.

Days 18–26. On Day 18, we placed two Plexiglas holding cages in each large floor enclosure (Fig. 1b) and introduced the pups' dam into one holding cage and a virgin adult female into the other. The 2.5-cm entrance to each holding cage permitted pups to enter, but prevented adults from leaving. Each holding cage contained a food container, a 15-cm length of PVC conduit and had a bottle mounted on its lid. The sipper tube closing the bottle protruded into the holding cage through its lid and was suspended approximately 25 cm from the floor of the enclosure.

Testing. As in Experiment 1, on Day 26, we removed adolescents from floor enclosures and placed two adolescents from each litter in individual cages for testing.

Procedure

Throughout the experiment, pups assigned to both WW and EW conditions described below had ad libitum access to food and water and could interact with both adult females in their respective holding cages. However, pups could not reach the sipper tubes protruding through the lids of the holding cages containing their dam and the unrelated virgin female.

Virgin-Water/Dam-Water (WW) Condition (n = 10 litters). From Day 18 to 26, we restricted the fluid intake

of both dams and virgin females to water dispensed through the sipper tube mounted on the lid of each holding cage.

Virgin-Ethanol/Dam-Water (EW) Condition (n = 10 litters). We treated litters assigned to the EW Condition just as we treated litters assigned to the WW Condition, except that virgin females assigned to the EW drank only 8% ethanol from Day 18 to 26.

Testing. We tested adolescents as in Experiment 1.

Results

As can be seen in Table 1, subjects assigned to the WW Condition drank 11.5 g ± 0.4 of fluid each day, and subjects assigned to the EW Condition drank 11.8 g ± 0.2 per day (Student's *t*-test, $t(15) = 0.68$ ns).

The main results of Experiment 2 are presented in Fig. 3 that shows the mean daily ethanol intake of adolescent rats assigned to EW and WW Conditions. Adolescent subjects that had interacted during weaning with an ethanol-consuming virgin female drank more ethanol than did adolescents that had interacted with a water-consuming virgin female whether ethanol intake was measured in g/kg/day (Repeated-measures ANOVA; $F(1, 18) = 5.98$, $p < 0.05$) or relative to total fluid intake ($F(1, 18) = 11.37$, $p < 0.01$) (Table 1). Thus, even when pups' water-consuming dam was present, an adult virgin female that consumed ethanol had a significant effect on the ethanol affinity of pups that interacted with her.

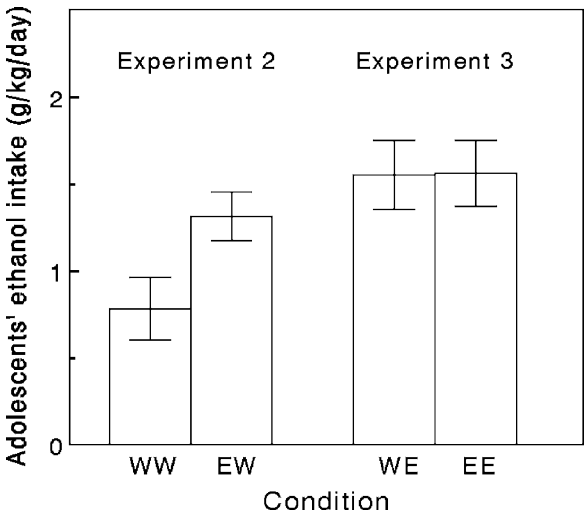

Fig. 3. Mean (±SEM) daily ethanol intake by adolescent rats in Experiments 2 and 3 that had lived with their water-consuming dam and a virgin female consuming either ethanol or water (Experiment 2) or their ethanol-consuming dam and a virgin female consuming either ethanol or water (Experiment 3). Exposure to an ethanol-consuming virgin female led to enhanced ethanol intake by adolescent rats in Experiment 2 ($F(1, 18) = 5.98$, $p < 0.05$). However, in Experiment 3, exposure to an ethanol-consuming foster female did not enhance adolescent ethanol intake beyond effects of exposure to an ethanol consuming dam ($F(1, 120) = 0.02$ ns).

There was no effect of day of testing on either g/kg/day ethanol intake ($F(6, 48) = 0.94$ ns) or ethanol intake relative to total fluid intake ($F(6, 48) = 0.64$ ns), nor was there any interaction between main effects (g/kg intake: $F(6, 48) = 0.45$ ns; percent ethanol intake: $F(6, 48) = 0.50$ ns).

Discussion

Adolescent rats that interacted with an ethanol-consuming virgin female drank more ethanol than did adolescents that interacted with a water-consuming virgin female. However, the amount of ethanol consumed by adolescents exposed to an ethanol-consuming female (1.30 ± 0.10 g/kg/day) was somewhat less than the 1.5 g/kg/day or more of ethanol that subjects exposed to an ethanol consuming adult during the weaning period usually consume during testing (Experiment 1, Honey & Galef, 2003).

There are several reasons why ethanol intake by adolescents in the present experiment might have been lower than ethanol intake by pups in our previous experiments. First, modeling of water consumption by the dam may compete with modeling of ethanol consumption by another female to produce a reduction in subsequent ethanol consumption by adolescents. Chou and Richerson (1992) and Galef, Attenborough, and Whiskin (1990) have both reported that interaction with models that have ingested two different foods reduces the magnitude of each model's effect on the diet choices of observers offered a choice between the two foods that their models ate.

Second, as Boyd and Richerson (1985) have suggested, in social learning situations where more than one model is present, observers may be biased to copy either a model engaged in behaviors that are inherently rewarding to their observers (direct bias) or a model that has particular characteristics (indirect bias). Given the greater attractiveness to rat pups of lactating than of virgin female rats (Leon, 1974), the former may be more potent models for juveniles than the latter.

We made time-lapse videotape recordings of four litters for 4 h on each of Days 19 and 25, and determined the number of pups in each of the two holding cages, one containing the pups' dam and other the unfamiliar female, once every 10 min. On Day 19, an average of 7.4 ± 0.4 pups were with their dam and only 0.2 ± 0.04 were with the virgin female. Similarly on Day 25, on average, 5.4 ± 0.3 pups were with their dam and only 1.2 ± 0.1 pups were with the virgin female. We never saw more pups with the virgin female than with the dam (Mann–Whitney *U* tests, $U = 0$, $p < 0.02$). Although the sample sizes are small, the results are suggestive.

On the other hand, given the greater palatability of water than of 8% ethanol to rats (Samson, Pfeffer, & Tolliver, 1988), direct bias would lead pups to be more strongly influenced by an adult model drinking water than by one drinking 8% ethanol.

Experiment 3

We undertook Experiment 3 to determine whether presence of either an ethanol- or water-consuming virgin female, in addition to a dam, would alter the influence of an ethanol-consuming dam on adolescents' affinity for ethanol. If pups are susceptible only to direct bias (Boyd & Richerson, 1985), then even when dams model ethanol consumption, modeling of water consumption by a virgin female should result in reduced ethanol consumption by adolescents. If, to the contrary, pups are influenced only by indirect bias (Boyd & Richerson, 1985), then: (1) modeling of water consumption by a virgin female rat should not reduce the impact of modeled ethanol consumption by pups' own dam, and (2) levels of ethanol consumption by adolescents should be similar to those seen previously in experiments other than Experiment 2 in the present series.

Method

Subjects

Forty rat pups, born to 20 female Long-Evans rats served as subjects. We used an additional 20 adult virgin female rats as foster females. We randomly assigned litters to one of the two conditions described in Procedure.

Apparatus

We used the same apparatus that we used in Experiment 2.

Procedure

Pups in both the EW and EE Conditions described below had ad libitum access to both food and water throughout the experiment and could interact with both adult females restrained in holding cages, but could not reach the sipper tubes protruding through the lids of holding cages. Dams drank only ethanol from Day 18 to 26, and virgin females assigned to EW and EE Conditions drank, respectively, either water or 8% ethanol.

Virgin-Water/Dam-Ethanol (WE) Condition ($n = 10$ litters). From Day 18 to 26, we restricted each dam's fluid intake to 8% ethanol dispensed through the sipper tube mounted on the lid of her holding cage, while virgin females were restricted to tap water.

Virgin-Ethanol/Dam-Ethanol (EE) Condition ($n = 10$ litters). We treated litters assigned to the EE Condition as we treated litters assigned to the WE Condition, except that both dams and virgin females assigned to the EE Condition drank 8% ethanol from Day 18 to 26.

Testing. We tested adolescents as in Experiments 1 and 2.

Results and discussion

As in Experiments 1 and 2, in Experiment 3 pups' total fluid intake (Table 1) did not differ between groups (Student's *t*-test, $t(12) = 0.18$ *ns*).

The main results of Experiment 3 are presented in Fig. 3 that shows the mean daily ethanol intake by pups that had lived with their ethanol-consuming dam and either a water- or ethanol-consuming unfamiliar virgin female from Day 18 to 26. There were no differences between groups in either g/kg/day intake of ethanol (Repeated-measures ANOVA; $F(1, 120) = 0.002$ ns), or ethanol intake relative to total fluid intake ($F(1, 120) = 0.04$ ns); Table 1). Further, there was no effect of day of testing on either measure (g/kg/day intake: $F(6, 48) = 0.59$ ns; percent ethanol intake: $F(6, 48) = 0.51$ ns). Adolescents in both EE and WE Conditions drank an average of more than 1.5 g/kg/day ethanol, the level of ethanol intake found both in Experiment 1 and all of our earlier experiments (Honey & Galef, 2003).

As in Experiment 2, pups were more likely to be found with their dam than with the virgin female (Day 19: 7.2 ± 0.1 pups with dam; 0.2 ± 0.02 pups with virgin female: Day 25: 5.1 ± 0.3 pups with dam, 1.2 ± 0.2 pups with virgin female), and the number of pups in their dams' holding cage was always greater than the number of pups in the virgin female's holding cage ($U = 0$, $p < 0.02$).

The results are consistent with the view that indirect rather than direct bias (Boyd & Richerson, 1985) determines the relative effectiveness of pups' own dams and virgin females in influencing pups ethanol affinity.

General discussion

The results of the present series of experiments suggest that simple social exposure to an ethanol-consuming adult female during weaning leads to enhanced ethanol intake by adolescent rats. In Experiment 1, we found that rearing by an ethanol consuming dam that provided exposure to ethanol both in mothers' milk and via siblings did not result in greater ethanol consumption than rearing by an ethanol-consuming, non-lactating virgin female that provided neither exposure to ethanol-tainted milk nor ethanol-consuming siblings. In Experiments 2, we again found that interaction with any adult female can enhance ethanol consumption by the young with whom she interacts.

The differences in ethanol intake between ethanol-exposed and non-exposed adolescents in the present experiments were not large, possibly because we tested subjects when fluid deprived. However, the increased ethanol consumption we found in adolescents previously exposed to an ethanol-consuming adult may have occurred early in a trajectory either toward or away from risk of developing an ethanol use 'disorder.' We are currently examining the duration of effects of early exposure to an ethanol-consuming adult to determine whether: (1) increased ethanol intake is seen in adulthood and (2) ethanol intake is greater in adults exposed to ethanol consuming adults when young and granted access to ethanol while they mature.

It has been previously demonstrated that both exposure to ethanol in mother's milk (Hunt et al., 1993) and exposure to intoxicated siblings (Hunt et al., 2000, 2001) can enhance ethanol intake by adolescent rats. The results of the present experiments indicate that simple exposure to an ethanol-consuming adult can have similar effects. Interaction with an adult consuming ethanol increases the subsequent level of ethanol consumption by juveniles with whom the adult interacted.

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