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Long lasting effects of rearing by an ethanol-consuming dam on voluntary ethanol consumption by rats

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Abstract

For exposure to alcohol early in life to potentiate alcohol abuse in adolescence or adulthood, consequences of early exposure to alcohol must be of considerable duration. In two experiments using Norway rats as subjects, we examined effects of exposure during weaning to a dam consuming ethanol on adolescents' later affinity for ethanol. In a preliminary experiment, we offered rat pups a choice between 8% ethanol and water for 7 days immediately after they were weaned at 26 days of age. Pups whose dam had ingested 8% ethanol for 6 days either immediately or 1 week before we weaned them drank more ethanol than pups whose dam drank only water during the same period. Independent groups of rats reared by a dam consuming 8% ethanol from postnatal days 18 to 26 and tested 1, 2, 4 or 6 weeks later all drank significantly more 8% ethanol at testing than did pups whose dam drank only water. Our data also provided confirmation of previous reports of an experience-independent greater affinity for ethanol in younger rats.

Keywords: Rats; Ethanol; Early experience; Social learning

Introduction

Adult offspring of alcoholics drink more alcohol and are at greater risk of becoming alcoholic than are children of non-alcoholics (Cloninger et al., 1988). Although such familial susceptibility to alcoholism suggests a heritable predisposition for alcohol consumption, in epidemiological studies of human populations genetic and experiential effects can be difficult to disentangle. Maternal alcohol use during pregnancy predicts both age at first intoxication and subsequent frequency of intoxication better than does genetic background (Baer, Barr, Bookstein, Sampson & Streissguth, 1998; Streissguth, Barr, Bookstein, Sampson & Carmichael Olson, 1999), and studies of adoptive and stepfamilies indicate that drinking by alloparents increases likelihood of abuse of alcohol by foster children with neither genetic predisposition to ingest alcohol nor exposure to ethanol during gestation (Newlin, Miles, van den Bree, Gupman & Pickens, 2000).

Studies designed to examine Fetal Alcohol Syndrome, like studies of adoptive and stepfamilies, provide evidence of a possible role of early exposure to alcohol in the development of an appetite for alcohol. Children of women who drank alcohol while pregnant, and who fail to develop full Fetal Alcohol Syndrome, are at enhanced risk of alcohol use when adolescent. However, because women who drink alcohol while pregnant are unlikely to abstain from alcohol after childbirth (e.g. Little & Streissguth, 1978), the greater alcohol intake of children born to drinking mothers might result from either prenatal or postnatal exposure to alcohol.

Animal studies of effects of early exposure to ethanol can be used to examine separately effects of pre- and postnatal exposure to ethanol on subsequent ethanol intake. Results of such studies have revealed that exposure to ethanol before birth can lead to long lasting changes in responsiveness to ethanol (Molina, Dominguez, Lopez, Pepino & Faas, 1999). For example, Abel, Bush and Dintcheff (1981) exposed pregnant rats to substantial doses of ethanol throughout

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gestation and examined physiological responses of affected offspring beginning when they were 6 months of age. Abel et al. (1981) found that rats whose dams had been exposed to ethanol while gestating showed a smaller drop in body temperature in response to an ethanol challenge than did control rats not exposed to ethanol in utero, indicating that tolerance to ethanol lasted at least 6 months.

Exposure to ethanol during gestation can also produce changes in subsequent responsiveness to chemosensory properties of ethanol. Eight days after birth, rat pups that had been exposed to ethanol in utero spent more time near an ethanol odor than control subjects lacking such exposure (Chotro, Cordoba & Molina, 1991; Chotro & Molina, 1990). Bond and Di Giusto (1976) report that female rats that experienced chronic ethanol exposure during gestation drank more ethanol at 65 days of age than did controls, though others have failed to find this effect (Honey & Galef, 2003; McGivern, Clancy, Mousa, Couri & Noble, 1984; Reyes, Garcia & Jones, 1985).

Like prenatal exposure to ethanol, postnatal experience with ethanol or ethanol-consuming conspecifics also affects later responsiveness to ethanol-related cues. For example, experience with either a single dose of ethanol or with littermates that have ingested ethanol results in increased heart rate and decreased motor activity in the presence of ethanol odor 4 days later (Chotro, Kraebel, McKinzie, Molina & Spear, 1996). Similarly, experience with an ethanol-consuming littermate on postnatal days 12, 14 and 16 results in enhanced acceptance of ethanol, but not of other relatively unpalatable substances, introduced directly into the oral cavity via cannula 6 days later (Hunt, Holloway & Scordalakes, 2001; Hunt, Lant & Carroll, 2000). Longer-term effects of postnatal exposure to ethanol in animals have rarely been examined, although if exposure to ethanol-consuming conspecifics in infancy is to affect ethanol consumption in adolescence or adulthood, effects of early exposure to ethanol have to last weeks rather than days.

We have shown previously that rearing by an ethanol-consuming dam from postnatal day 14 to 26 leads to enhanced ethanol consumption in pups tested immediately after weaning (Honey & Galef, 2003). In the experiments described below, we first determined whether rat pups are more sensitive to effects of living with an ethanol-consuming dam early or late in lactation (Experiment 1) and then examined the duration of effects of exposure to an ethanol-consuming dam during lactation (Experiment 2).

Experiment 1: exposure to ethanol early and late in weaning

Rat pups begin to eat solid food on about day 14 postpartum, and are capable of maintaining their body weight if weaned on day 18, although dams will continue to supply milk to pups until they are 30 days of age or older (Cramer, Thiels & Alberts, 1990; Galef, 1979; Thiels, Alberts & Kramer, 1990). We undertook Experiment 1 to determine whether there was a sensitive period during weaning when exposure to an ethanol-consuming dam would enhance rat pups' subsequent voluntary intake of ethanol.

Method

Subjects

Sixty adolescent rats, born to 27 female Long-Evans rats purchased from Charles River Breeding Farms (St Constant, Quebec) and maintained in the vivarium of the McMaster University Psychology Department served as subjects. Within 48 h of birth (Day 0), we culled each litter to eight pups (whenever possible, four pups of each sex), and randomly assigned the litter to one of three treatment conditions described in Section 2.1.3.

Apparatus

Birth to Day 14. Until pups were 14 days of age, we housed each dam with her litter in a transparent shoebox cage, measuring 36×31×17 cm. The wire lid of the cage held both food pellets (PMI Rodent Diet 5001, Brentwood, MO) and a water bottle, and each cage contained a 15 cm piece of 8 cm diameter, polyvinylchloride (PVC) conduit for environmental enrichment.

Day 14 to 26. From Day 14 to 26, we housed each dam and litter in a 'dual cage' created by connecting two shoebox cages, one measuring 36×31×17 cm and the other 46×26×20 cm, with a length of 7.5 cm diameter PVC conduit. To connect the cages, we drilled holes 8 cm in diameter in one end of each cage and joined the cages with threaded PVC plumbing joints. The PVC conduit allowed dams and litters to move freely between the two cages, the smaller of which served as a 'nest area' and the larger as a 'drinking area' that is depicted in Fig. 1.

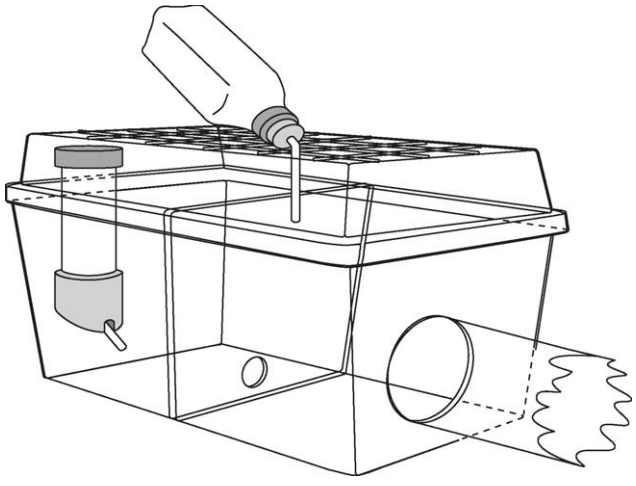

Fig. 1. Drinking area of the dual cage.

The cage serving as a nest area was closed with a wire lid holding food pellets, and contained a 15 cm length of PVC conduit for environmental enrichment. The cage serving as a drinking area was closed with a plastic lid intended to hold a filter (Micro Barrier Top MBT1019HT; Allentown Caging Equipment, Allentown, PA), and was divided into two chambers of equal size by a transparent Plexiglas partition. Dams could drink in the chamber nearest the nest area from a sipper tube that protruded through the lid of the cage serving as a drinking area. The end of this sipper tube was approximately 19 cm from the floor of the enclosure, and 6 cm from its roof, and could not be reached by pups whether they were standing on the cage floor or climbing across the cage lid.

We drilled a hole 3 cm in diameter through the Plexiglas partition that divided the drinking area to allow pups, but not their dams, access to the chamber at the back of the drinking area where water was available ad libitum. The apparatus permitted us to expose rat dams to ethanol without stressing them by removing them from their pups, injecting or intubating them while denying pups direct access to ethanol and providing them with ad libitum access to both food and water. Consequently, pups were never food or fluid deprived even if dams did not nurse them or pups rejected dams' ethanol-tainted milk. We determined, in a pilot study, that adult female rats maintained under conditions similar to those in which we maintained rats in the present experiments consumed 62.8 ± 3.9 ml of ethanol/day and showed blood-ethanol concentrations of 209 ± 27.2 mg% in the middle of the dark cycle (Honey, 2002). Ethanol concentration in mother's milk and mother's blood are similar (Mennella, 1999).

Day 26 to 33. To test adolescents' affinity for ethanol, beginning on Day 26, we housed adolescents in individual shoebox cages like those in which dams and litters had been housed from Days 0 to 14. During testing, each subject had access to two 50 ml test tubes, each closed with a rubber stopper and stainless steel sipper tube, one containing tap water and the other 8% (v/v) ethanol.

Procedure

Until pups were 14 days of age, each dam and litter remained undisturbed in a shoebox cage with ad libitum access to both food and water.

Early condition (20 pups from 10 litters). From Day 14 until 26, we housed each dam and litter assigned to the Early condition in a dual cage and, from noon on Day 14 to noon on Day 20, restricted dams' fluid intake to 8% ethanol dispensed from the sipper tube protruding through the lid of the dam's drinking chamber. From noon on Day 20 to noon on Day 26 dams had access only to tap water from the same sipper tube.

Late condition (20 pups from 10 litters). We treated litters assigned to the Late condition exactly as we treated those assigned to the Early condition, except that dams assigned to the Late condition had access only to water from Day 14 to 20, and only to 8% ethanol from Day 20 to 26.

Control condition (20 pups from 10 litters). We treated litters assigned to the Control condition exactly as we treated litters assigned to Early and Late conditions, except that dams assigned to the Control condition drank only tap water from Days 14 to 26.

Testing. On Day 26 postpartum, we randomly selected one male and one female adolescent from each litter, weighed them, and placed them in individual shoebox cages providing ad libitum access to food. We first deprived subjects of fluid for 22 h, then gave them access to both water and 8% ethanol for 2 h/day for 7 consecutive days. We determined the number of grams of both water and ethanol taken by subjects during each 2 h drinking session, and weighed subjects every second day.

Data analysis

In pilot studies, we had found that drinking tubes leaked approximately 0.1 g of fluid during each 2 h test session. We, therefore, subtracted.1 g from the weight of each drinking tube each day before undertaking further calculations. Further, if a subject drank no water during a drinking session, we discarded data for that subject for that day (10 of 756 data points) because lack of water intake resulted from blockage of a drinking spout.

We used g/kg intake as a dependent measure to provide an index of absolute ethanol intake by pups and gram alcohol ingested as a percentage of total fluid intake as an index of affinity for ethanol. We analysed the results using a 3 (Condition) $\times$ 2 (Sex) ANOVA. In addition, we used planned orthogonal contrasts between exposed conditions (Early and Late) and the Control condition, as well as between the Early and Late conditions. We did not use a repeated-measures ANOVA to examine differences in intake across the 7 days of testing, because in previous experiments (Honey & Galef, 2003; Honey, Varley, & Galef, in press) we have not found significant effects of day of testing on intake.

Results

The main results of Experiment 1 are presented in Fig. 2 that shows, in upper and lower panels respectively, for the 7 days of testing, the mean amount of ethanol ingested by subjects and the mean percentage of subjects' total fluid intake that was ethanol. Because we found no effect of sex of subject on ethanol consumption (see below), we combined data from male and female subjects in both panels of the figure.

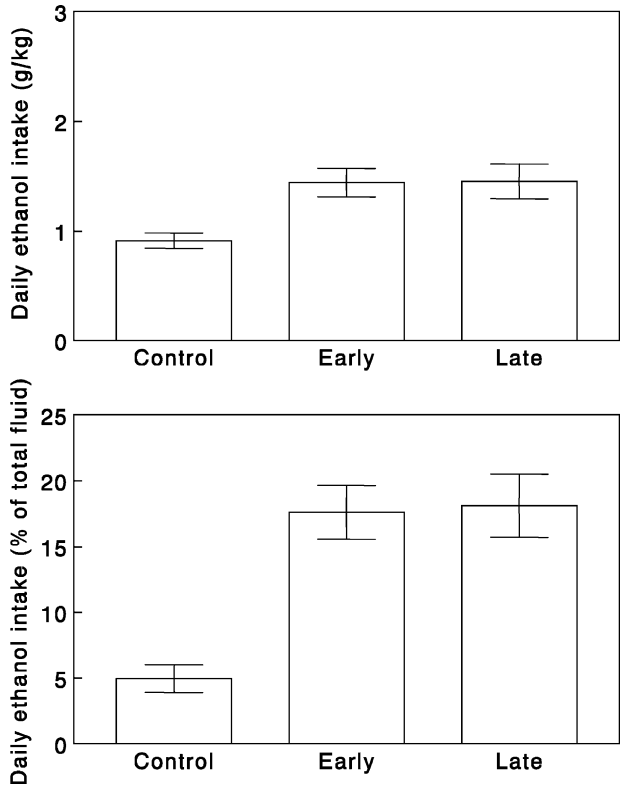

Fig. 2. Mean (±SEM) daily ethanol intake, measured in g/kg (upper panel) and as a percentage of total fluid intake (lower panel), by adolescent rats assigned to each of the three treatment conditions in Experiment 1.

Six days of exposure to an ethanol-consuming dam during the weaning period resulted in enhanced voluntary intake of ethanol by adolescent rats regardless of whether their exposure to ethanol occurred early or late in weaning (Fig. 2, upper panel). We found a significant main effect of exposure to an ethanol consuming dam ($F(1,54)=6.38$, $p<0.05$) but no effect of sex ($F(1,54)=1.50$, ns) and no significant interaction ($F(2,54)=0.50$, ns).

Planned orthogonal comparisons revealed significantly greater ethanol consumption by adolescents assigned to both Early and Late than by adolescents assigned to the Control condition ($t_{\Psi 1}=1.73$, $p<0.05$) and no difference between subjects assigned to Early and Late conditions in ethanol consumption (($t_{\Psi 2}=0.02$, ns). When we examined ethanol intake relative to total fluid intake (Fig. 2, lower panel), results were the same as when we examined absolute ethanol intake: a significant main effect of exposure to an ethanol-consuming dam ($F(2,54)=21.65$, $p<0.001$, no main effect of sex ($F(1,54)=3.57$, ns) and no interaction between main effects ($F(2,54)=2.53$, ns). Again, rats that had lived with an ethanol consuming dam drank more ethanol than did rats that had lived with a water-consuming dam ($t_{\Psi 1}=1.89$, $p<0.05$), and there was no significant difference in ethanol consumption between adolescents assigned to Early and Late conditions ($t_{\Psi 2}=0.71$, ns).

Although rearing by an ethanol-consuming dam had profound effects on subsequent ethanol intake by pups, such rearing had no effect on pup weight at 26 days of age (Table 1). A 3 (Conditions)×2 (Sex) ANOVA showed an expected main effect of sex on body weight ($F(1,54)=31.98$, $p<0.001$), no effect of experimental condition on body weight ($F(2,54)=0.12$, ns), and no interaction between main effects ($F(2,54)=0.69$, ns). The similarity of body weights of pups in all conditions suggests that rearing by an alcohol-consuming dam did not result in a nutritional deficiency.

Table 1
Mean (±SEM) weights (g) of subjects in Experiment 1 on the first day of testing

Condition	Males	Females
Early	79.2±1.2	74.0±2.1
Late	81.0±2.4	72.9±1.7
Control	80.9±1.6	72.1±1.6

Discussion

After 6 days of social exposure to ethanol during weaning, adolescent rats voluntarily consumed more ethanol than did adolescents that had no exposure to ethanol via their dam. Exposure to an alcohol-consuming dam early and late in weaning resulted in equivalent subsequent ethanol consumption.

It is relevant to note that we tested subjects assigned to the Late condition immediately after they were weaned from contact with their ethanol-consuming dam, whereas subjects assigned to the Early condition had no contact with ethanol for 6 days before we started to test them. Thus, a 6-day delay between exposure to an ethanol consuming dam and subsequent opportunity to ingest alcohol did not have an effect on ethanol consumption by exposed adolescents. This result suggests that effects of exposure to an alcohol-consuming dam might be long lasting.

Experiment 2: duration of effects of early exposure to ethanol

We undertook Experiment 2 to determine whether exposure to ethanol-consuming dams has long-lasting effects on voluntary ethanol consumption by pups. We exposed pups to an ethanol-consuming dam for 8 days prior to weaning, and 1, 2, 4, or 6 weeks later compared the ethanol consumption of independent groups of subjects with that of subjects of equal age whose dams had no exposure to ethanol.

Methods

Subjects

One hundred and sixty adolescent rats, born to 20 female Long-Evans rats served as subjects. They were acquired and maintained as were the participants in Experiment 1.

Procedure

Until pups were 18 days of age, we left each dam and her litter undisturbed in a shoebox cage with ad libitum access to both food and water.

Ethanol condition (80 pups from 10 litters). From Day 18 to 26, we housed each dam and litter in one of the dual-cages partially illustrated in Fig. 1. The fluid intake of dams was restricted to 8% ethanol, and that of pups to water.

Control condition (80 pups from 10 litters). We treated litters assigned to the Control condition as we treated litters assigned to the Ethanol-exposure condition, except that dams assigned to the Control condition had access only to water from Day 18 to 26.

Delays. On Day 26 postpartum, we removed all pups from their dam and housed them with their same-sex littermates until testing. We tested each subject at only one of the four delay intervals, and tested a pair of subjects of the same sex from Control and Ethanol conditions 1, 2, 4 or 6 weeks after weaning on Day 26, counterbalancing across litters the sex of subjects tested at each delay interval.

By housing and testing subjects in this way, we could avoid stressing pups by housing some of them alone for prolonged periods. To avoid exposing untested subjects to littermates that had been exposed to ethanol during testing, we housed tested subjects separately from their untested siblings.

Testing. As in Experiment 1, 22 h before we started to test a pair subjects, we transferred each subject to an individual shoebox cage and deprived it of all fluids, but not of food, for 22 h. At the end of the deprivation period, we gave each subject access to both water and 8% ethanol for 2 h/day for 7 consecutive days.

Data analysis

As in Experiment 1, we weighed subjects every second day and determined the weight of each test tube before and after each 2 h drinking session. We also corrected for leakage and discarded data for days on which a subject's water tube was blocked (28 of 2240 data points). We analysed the results using a 2 (Condition)×2 (Sex)×4 (Delay) ANOVA. The purpose of this experiment was to determine whether independent groups of adolescents that experienced different delays between exposure and testing would demonstrate different levels of ethanol consumption, so we planned orthogonal contrasts among the individual delay conditions (1 Week vs 2 Weeks; 2 Weeks vs 4 weeks; 4 weeks vs 6 Weeks).

Results

The main results of Experiment 2 are presented in Fig. 3 that shows, in upper and lower panels respectively,

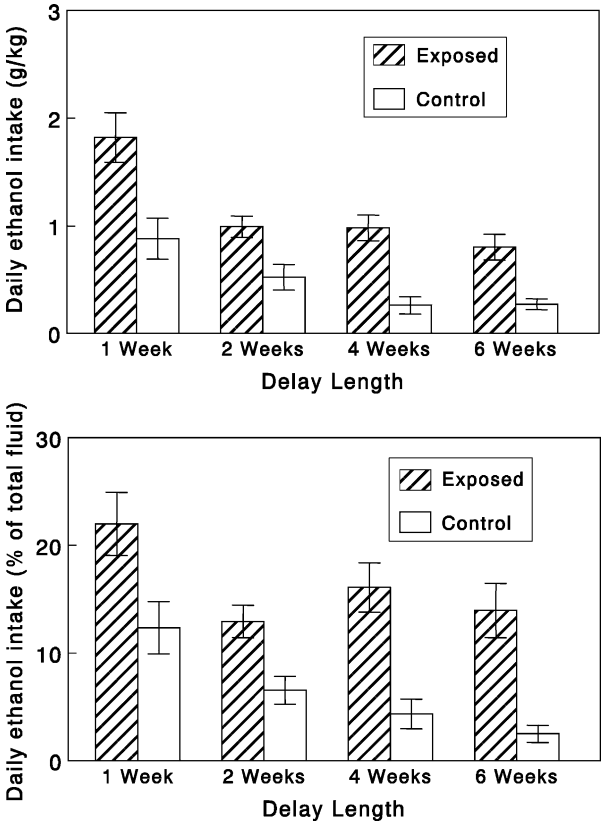

Fig. 3. Mean (±SEM) daily ethanol intake, measured in g/kg (upper panel) and as a percentage of total fluid intake (lower panel), by adolescent rats assigned to each of the two treatment conditions and each of the four delay conditions in Experiment 2.

the meant amount and mean percent ethanol consumption of subjects assigned to Control and Ethanol-exposure conditions and tested at each of the four delay intervals. As is evident in the upper panel of Fig. 3, 8 days of exposure to an ethanol-consuming dam before weaning resulted in enhanced voluntary intake of ethanol by adolescent rats that persisted for at least 6 weeks. Statistical analyses revealed significant main effects of ethanol exposure ($F(1,64)=52.41$, $p<0.001$) and delay ($F(3,64)=15.94$, $p<0.001$), but no main effect of sex ($F(1,64)=2.47$, ns), and no interactions among main effects. Analysis of ethanol intake as a percentage of total fluid consumption (Fig. 3, lower panel) revealed the same pattern: significant main effects of condition ($F(1,64)=49.28$, $p<0.001$) and delay ($F(3,64)=7.96$, $p<0.05$), but no main effect of sex ($F(1,64)=3.03$, ns) and no interactions among main effects.

As can also be seen in Fig. 3, rats assigned to both ethanol-exposure and Control Conditions drank more ethanol (relative both to body weight and total fluid intake) when 33 days old (1 week delay) than when tested at later ages (g/kg ethanol intake: $t_{\psi 1a}=6.69$, $p<0.001$; percent ethanol ingested: $t_{\psi 1b}=4.85$, $p<0.001$). There were however, no significant differences on either measure of alcohol consumption during the next 5 weeks (all $t_{\psi j}<1.10$, all ns).

Table 2
Mean (±SEM) weights (g) of subjects in Experiment 2 on the first day of testing

Delay	Males		Females	
	Ethanol	Control	Ethanol	Control
One week	127.3±2.2	120.0±6.5	104.8±1.4	113.5±4.7
Two weeks	183.3±6.1	179.2±4.4	121.1±2.2	128.4±5.1
Four weeks	237.9±11.1	240.5±10.0	160.1±9.1	154.6±3.8
Six weeks	247.6±20.3	241.7±22.9	192.3±8.0	196.6±6.9

The greater affinity for ethanol in younger than older rats is consistent with results of several recent studies demonstrating age-related differences in sensitivity to a variety of drugs (Brasser & Spear, 2002; McKinzie, McBride, Murphy, Lumeng & Li, 1999; O'Callaghan, Croft, Watson, Brooks & Little, 2002; Silveri & Spear, 1998; Varlinskaya, & Spear, 2002; Vastola, Douglas, Varlinskaya & Spear, 2002). It has been hypothesised that this heightened responsiveness in younger animals may reflect a catecholaminergic hyposensitivity (Shalaby & Spear, 1980).

As in Experiment 1, we found no significant effects of exposure to an ethanol-consuming dam on body weight ($F(1,64)=0.12$, ns; Table 2), although, as expected, males were heavier than females ($F(1,64)=165.49$, $p<0.001$) and the weight of all rats increased with age ($F(3,64)=126.11$, $p<0.001$).

General discussion

Although we have provided evidence of a long-lasting effect of ethanol ingestion by rat dams on their pups' subsequent appetite for ethanol, questions remain as to how a dam drinking ethanol causes her young to subsequently seek out and ingest a relatively unpalatable drug, when they have access to a more palatable fluid. There are, obviously, several quite different ways in which maternal ingestion of ethanol might increase appetite for ethanol in juveniles, and we consider some of these below.

Social influences on flavor preference

Research on the development of flavor preferences has shown that young animals will consume an unpalatable or toxic food after interacting with a conspecific that has eaten that food (Galef, 1989). Galef and his colleagues have shown repeatedly that exposure to both flavour cues contained in mother's milk (e.g. Galef & Sherry, 1973) and olfactory cues carried on the breath of a conspecific demonstrator (e.g. Galef, 1996) result in enhanced intake of foods with the flavor experienced during social interaction.

Effects of such interactions are both robust and long lasting (Galef, 1989), and simple exposure to the taste or smell of a food is not nearly so effective in increasing intake of the associated food as is exposure to it in a social context (e.g. Galef, Mason, Preti & Bean, 1988). Bannoura, Kraebel, Spear and Spear (1998) reported that simple exposure of rat pups to the odor of 100% ethanol from postnatal day 1 to 22 enhances both odor preference for ethanol and subsequent intake of 6% ethanol (v/v). However, we have found in a previous experiment, with parameters very similar to those in the present experiments, that simple exposure to 8% ethanol for 12 days during weaning does not enhance ethanol consumption after weaning, whereas exposure to a dam consuming 8% ethanol does (Honey & Galef, 2003). The discrepancy between our results and those of Bannoura et al. (1998) probably reflects differences in the concentration of the ethanol to which pups were exposed. Not only is 100% ethanol a more potent chemosensory stimulus than is 8% ethanol, but its vapors are also intoxicating.

Seeking calories

It might be argued that pups in our experiments that were being reared by an ethanol-consuming dam drank ethanol because their mothers were not providing them with sufficient milk, and the pups needed calories from ethanol to sustain growth. However, pups in ethanol-exposed and control animals in our experiments did not differ in weight at the start of testing. Additionally, in a previous experiment where pups were weaned at 18 days of age, such reduction in maternally derived nourishment did not result in enhanced ethanol intake by adolescents (Honey et al., 2004).

Tolerance for ethanol

Exposure to ethanol in mother's milk can result in increased tolerance for ethanol (Abel et al., 1981; Perez, Gonzalez & Smith, 1983; Reyes, Duran & Swizer, 1993). Consequently, rats reared by ethanol-consuming dams in our experiments might have consumed greater quantities of ethanol than control rats without experiencing unpleasant drug effects.

We do not know whether reactivity to ethanol or metabolism of ethanol change as a result of exposure to ethanol in the milk of an ethanol-consuming dam. However, we have reported previously that rats that have interacted from day 18 to 26 postpartum with either a lactating female drinking ethanol or a virgin female drinking ethanol show equal intake of ethanol when tested at 26 days of age (Honey, et al., 2004). Clearly, direct exposure to ethanol in mother's milk is not necessary to produce the effect of interacting with an ethanol-consuming dam reported here. Further, rat pups whose mothers drank 8% ethanol throughout both gestation and the first 2 weeks of life do not show enhanced ethanol intake when tested at weaning

(Honey & Galef, 2003), although they surely had opportunity to develop a tolerance for ethanol.

Effects of stress

Physiologically or psychologically stressed animals show an increase in ethanol consumption (Mello, 1973; Mills & Bean, 1978; Lynch, Kushner, Rawleigh, Fiszdon & Carroll, 1999), and stress is often considered an important factor in development of alcoholism in humans (Mello, 1973). To minimize stressing of subjects participating in the present experiments, we minimized handling of pups, did not collect blood samples from pups or dams, and administered ethanol to dams via normal ingestion rather than intubation or injection. Pups in the present studies were both deprived of fluid and housed in isolation during testing and, thus subjected to some stress. However, subjects reared by ethanol-consuming dams and control subjects both experienced similar deprivation and isolation, so it is not obvious how deprivation or isolation could be responsible for differences between Control and Experimental Groups in ethanol intake.

It remains possible that interaction with a chronically intoxicated dam is stressful to pups and induces an affinity for ethanol. Differentiating effects on ethanol intake of stress resulting from interacting with an intoxicated dam and effects on intake of ethanol of exposure to ethanol-related cues experienced while interacting with an intoxicated dam will prove challenging.

Conclusion

Exposure to an ethanol-consuming dam for 6 or 7 days during weaning led to enhanced ethanol intake by adolescent rats. Such exposure enhanced ethanol intake both immediately after it occurred, and later when subjects were adult, suggesting that exposure to an ethanol-consuming dam has profound, long-term effects on ethanol affinity in rats.

In the experiments described here, rat pups reared by an ethanol-consuming dam were exposed to ethanol in their dams' milk and to ethanol odor in their home cages, on their dam's breath and during interactions with littermates that had consumed ethanol-tainted milk. Each of these sources of exposure to ethanol have been shown to enhance subsequent ethanol intake under some circumstances (Bannoura et al., 1998; Honey, 2002; Honey & Galef, 2003; Honey et al., 2004; Hunt, Kraebel, Rabine, Spear & Spear, 1993; Hunt et al., 2000).

The complex exposure to ethanol-related cues experienced by rat pups reared by an ethanol-consuming dam is, of course, not unlike the experience of young humans who may not only experience ethanol in mother's milk and on mother's breath but also observe ethanol consumption by and interact with an ethanol-consuming parent. The results of the present studies show that exposure of rat pups to an ethanol-consuming mother can have long-lasting effects on the pups' subsequent voluntary intake of ethanol. A similar effect may remain to be demonstrated in humans.

Acknowledgements

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References

Abel, E. L., Bush, R., & Dintcheff, B. A. (1981). Exposure of rats to alcohol in utero alters drug sensitivity in adulthood. *Science*, *212*, 1531–1533.

Baer, J. S., Barr, H. M., Bookstein, F. L., Sampson, P. D., & Streissguth, A. P. (1998). Prenatal alcohol exposure and family history of alcoholism in the etiology of adolescent alcohol problems. *Journal of Studies on Alcohol*, *59*, 533–543.

Bannoura, M. D., Kraebel, K. S., Spear, L. P., & Spear, N. E. (1998). Effects of preweanling ethanol odor exposure on ethanol preference. *Alcohol*, *15*, 213–217.

Bond, N. W., & Di Gusto, E. L. (1976). Effects of prenatal alcohol consumption on open-field behaviour and alcohol preference in rats. *Psychopharmacologia*, *46*, 163–165.

Brasser, S. M., & Spear, N. E. (2002). Physiological and behavioral effects of acute ethanol hangover in juvenile, adolescent and adult rats. *Behavioral Neuroscience*, *116*, 302–320.

Chotro, M. G., Cordoba, N. E., & Molina, J. C. (1991). Acute prenatal experience with alcohol in the amniotic fluid: Interactions with aversive and appetitive alcohol orosensory learning in the rat pup. *Developmental Psychobiology*, *24*, 431–451.

Chotro, M. G., Kraebel, K. S., McKinzie, D. L., Molina, J. C., & Spear, N. E. (1996). Prenatal and postnatal ethanol exposure influences preweanling rats' behavioral and autonomic responding to ethanol odor. *Alcohol*, *13*, 377–385.

Chotro, M. G., & Molina, J. C. (1990). Acute ethanol contamination of the amniotic fluid during gestational day 21: Postnatal changes in alcohol responsiveness in rats. *Developmental Psychobiology*, *23*, 535–547.

Cloninger, C. R., Sigvardsson, S., Gilligan, S. B., von Knorring, A-L., Reich, T., & Bohman, M. (1988). Genetic heterogeneity and the classification of alcoholism. *Advances in Alcohol and Substance Abuse*, *7*, 3–16.

Cramer, C. P., Thiels, E., & Alberts, J. R. (1990). Weaning in rats: I Maternal Behavior. *Developmental Psychobiology*, *23*, 479–493.

Galef, B. G., Jr. (1979). Investigation of the functions of coprophagy in juvenile rats. *Journal of Comparative and Physiological Psychology*, *93*, 295–305.

Galef, B. G., Jr. (1989). Enduring social enhancement of rats' preferences for the palatable and the piquant. *Appetite*, *13*, 81–92.

Galef, B. G., Jr.. (1996). Social influences on food preferences and feeding behaviors of vertebrates. In E. D. Capaldi (Ed.), *Why we eat what we eat: the psychology of eating* (pp. 207–230). Washington, DC: American Psychological Association.

Galef, B. G., Jr., Mason, J. R., Preti, G., & Bean, N. J. (1988). Carbon disulfide: a semiochemical mediating socially-induced diet choice in rats. *Physiology and Behavior*, *42*, 119–124.

Galef, B. G., Jr., & Sherry, D. F. (1973). Mother's milk: a medium for the transmission of cues reflecting the flavor of mother's diet. *Journal of Comparative and Physiological Psychology*, *83*, 374–378.

Honey, P. L., (2002). *Social induction of ethanol consumption in adolescent rats, Rattus norvegicus*. Unpublished doctoral dissertation. McMaster University, Hamilton, Ontario.

Honey, P. L., & Galef, B. G., Jr. (2003). Ethanol consumption by rat dams during gestation, lactation and weaning increases ethanol consumption by their adolescent young. *Developmental Psychobiology*, *42*, 252–260.

Honey, P. L., Varley, K. R., & Galef, B. G., Jr.. (2004). Effects of ethanol consumption by adult female rats on subsequent consumption by adolescents. *Appetite*, *42*, 299–306.

Hunt, P. S., Holloway, J. L., & Scordalakes, E. M. (2001). Social interaction with an intoxicated sibling can result in increased intake of ethanol by periadolescent rats. *Developmental Psychobiology*, *38*, 101–109.

Hunt, P. S., Lant, G. M., & Carroll, C. A. (2000). Enhanced intake of ethanol in preweanling rats following interactions with intoxicated siblings. *Developmental Psychobiology*, *37*, 90–99.

Hunt, P. S., Kraebel, K. S., Rabine, H., Spear, L. P., & Spear, N. E. (1993). Enhanced ethanol intake in preweanling rats following exposure to ethanol in a nursing context. *Developmental Psychobiology*, *26*, 133–153.

Little, L. E., & Streissguth, A. P. (1978). Drinking during pregnancy in alcoholic women. *Alcoholism: Clinical and Experimental Research*, *2*, 179–183.

Lynch, W. J., Kushner, M. G., Rawleigh, J. M., Fiszdon, J., & Carroll, M. E. (1999). The effects of restraint stress on voluntary ethanol consumption in rats. *Experimental and Clinical Psychopharmacology*, *7*, 318–323.

McGivern, R. F., Clancy, A. N., Mousa, S., Couri, D., & Noble, E. P. (1984). Prenatal alcohol exposure alters enkephalin levels, without affecting ethanol preference. *Life Sciences*, *34*, 585–589.

McKinzie, D. L., McBride, W. J., Murphy, J. M., Lumeng, L., & Li, T.-K. (1999). Rat lines selectively bred for alcohol preference: A potential animal model of adolescent alcohol drinking. In J. H. Hannigan, L. P. Spear, N. E. Spear, & C. R. Goodlett (Eds.), *Alcohol and alcoholism: Effects on brain and development*. New Jersey: Erlbaum.

Mello, N. K. (1973). A review of methods to induce alcohol addiction in animals. *Pharmacology, Biochemistry and Behaviour*, *1*, 89–101.

Mennella, J. A. (1999). The transfer of alcohol to human milk: Sensory implications and effects on mother-infant interaction. In J. H. Hannigan, L. P. Spear, N. E. Spear, & C. R. Goodlett (Eds.), *Alcohol and alcoholism: Effects on brain and development* (pp. 177–198). Hillsdale, NJ: Erlbaum.

Mills, K. C., & Bean, J. W. (1978). The caloric and intoxicating properties of fluid intake as components of stress-induced ethanol consumption in rats. *Psychopharmacology*, *57*, 27–31.

Molina, J. C., Dominguez, H. D., Lopez, M. F., Pepino, M. Y., & Faas, A. E. (1999). The role of fetal and infantile experience with alcohol in later recognition and acceptance patterns of the drug. In J. H. Hannigan, L. P. Spear, N. E. Spear, & C. R. Goodlett (Eds.), *Alcohol and alcoholism: Effects on brain and development*. New Jersey: Erlbaum.

Newlin, D. B., Miles, D. R., van den Bree, M. B. M., Gupman, A. E., & Pickens, R. W. (2000). Environmental transmission of DSM-IV substance use disorders in adoptive and step families. *Alcoholism: Clinical and Experimental Research*, *24*, 1785–1794.

O'Callaghan, M. J., Croft, A. P., Watson, W. P., Brooks, S. P., & Little, H. J. (2002). Low alcohol preference among the high alcohol preference C57/BL10 mice; factors affecting such preference. *Pharmacology, Biochemistry and Behavior*, *72*, 475–481.

Perez, V. J., Gonzalez, G. E., & Smith, C. J. (1983). Exposure to ethanol during pregnancy in mice: Potential importance of dose for the development of tolerance in offspring. *Physiology and Behavior*, *30*, 485–488.

Reyes, E., Duran, E., & Switzer, S. H. (1993). Effects of in utero administration of alcohol on alcohol sensitivity in adult rats. *Pharmacology, Biochemistry and Behavior*, *44*, 307–312.

Reyes, E., Garcia, K. D., & Jones, B. C. (1985). Effects of the maternal consumption of alcohol on alcohol selection in rats. *Alcohol*, *2*, 323–326.

Shalaby, I. A., & Spear, L. P. (1980). Psychopharmacological effects of low and high doses of apomorphine during ontogeny. *European Journal of Pharmacology*, *67*, 451–459.

Silveri, M. M., & Spear, L. P. (1998). Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. *Alcoholism: Clinical and Experimental Research*, *22*, 670–676.

Streissguth, A. P., Barr, H. M., Bookstein, F. L., Sampson, P. D., & Carmichael Olson, H. (1999). The long-term neurocognitive consequences of prenatal alcohol exposure: A 14-year study. *Psychological Science*, *10*, 186–190.

Thiels, E., Alberts, J. R., & Cramer, C. P. (1990). Weaning in rats: II Pup behavior patterns. *Developmental Psychobiology*, *23*, 495–510.

Varlinskaya, E. I., & Spear, L. P. (2002). Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcoholism: Clinical and Experimental Research*, *26*, 1502–1511.

Vastola, B. J., Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2002). Nicotine-induced conditioned place preference in adolescent and adult rats. *Physiology and Behavior*, *77*, 104–114.