Published in final edited form as:

Alcohol. 2009 September ; 43(6): 453–463. doi:10.1016/j.alcohol.2009.08.001.

Binge ethanol exposure in late gestation induces ethanol aversion in the dam but enhances ethanol intake in the offspring and affects their postnatal learning about ethanol

M. Gabriela Chotro¹, Carlos Arias², and Norman E. Spear³

¹Universidad del País Vasco UPV-EHU, 20018 San Sebastián, Spain

²Instituto de Investigación Médica M. y M. Ferreyra, 5000 Córdoba, Argentina

³Binghamton University, Binghamton, NY 13902-6000, U.S.A.

Abstract

Previous studies show that exposure to 1 or 2 g/kg ethanol during the last days of gestation increases ethanol acceptance in infant rats. We tested whether prenatal exposure to 3 g/kg, a relatively high ethanol dose, generates an aversion to ethanol in both the dam and offspring, and whether this prenatal experience affects the expression of learning derived from ethanol exposure postnatally. The answer was uncertain, since postnatal administration of a 3 g/kg ethanol dose induces an aversion to ethanol after postnatal day 10 but increases ethanol acceptance when administered during the first postnatal week. In the present study pregnant rats received intragastric administrations of water or ethanol (3 g/kg) on gestation days 17–20. On postnatal days 7–8 or 10–11 the offspring were administered water or ethanol (3 g/kg). Intake of ethanol and water, locomotor activity in an open-field and ethanol odor preference were evaluated in the pups, while the mothers were evaluated in terms of ethanol intake. Results indicated an aversion to ethanol in dams that had been administered ethanol during gestation, despite a general increase in ethanol intake observed in their pups relative to controls. The prenatal ethanol exposure also potentiated the increase in ethanol intake observed after intoxication on postnatal days 7–8. Ethanol intoxication on postnatal days 10–11 reduced ethanol consumption; this ethanol aversion was still evident in infant rats exposed prenatally to ethanol despite their general increase in ethanol intake. No effects of prenatal ethanol exposure were observed in terms of motor activity or odor preference. It is concluded that prenatal exposure to ethanol, even in a dose that induces ethanol aversion in the gestating dam, increases ethanol intake in infant rats and that this experience modulates age-related differences in subsequent postnatal learning about ethanol.

Keywords

prenatal ethanol; postnatal intoxication; ethanol aversion; ethanol intake; infant rat

© 2009 Elsevier Inc. All rights reserved.

Corresponding author: M. Gabriela Chotro, Facultad de Psicología, Universidad del País Vasco, Avda de Tolosa, 70, 20018 San Sebastian, Spain, Phone: 34 943 015 730, Fax: 34 943 015 670, g.chotro@ehu.es.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

A growing number of studies using rodents demonstrate consistently that prenatal ethanol exposure induces increased intake of ethanol postnatally. This effect has been observed in studies in which ethanol was administered to the pregnant dam during most of gestation (Chotro et al., 2007; Youngentob et al., 2007b), and also when rat fetuses were exposed for only a few days before birth (gestation days 17-20) to ethanol doses (1 or 2 g/kg) that can be considered relatively low or moderate (Abate et al., 2008; Chotro and Arias, 2006).

The capacity of the rat fetus for perceiving chemosensory properties of the amniotic fluid and other substances present in their prenatal environment, from at least gestational day 17 until parturition, has been well documented (Pedersen et al., 1986; Smotherman and Robinson, 1985, 1988). This fetal ability has a direct relationship with postnatal responses towards those substances: contamination of the amniotic fluid with a flavor increases intake of that flavor later in life (Smotherman, 1982a). It has also been shown that the rat fetus can acquire conditioned responses to chemosensory stimuli in the amnion and that this prenatal associative memory can be retained and expressed more than two weeks later (Abate et al., 2002; Gruet et al., 2004; Molina and Chotro, 1991; Stickrod et al., 1982).

Taking into consideration these fetal capacities, one hypothesis is that the increased ethanol intake observed in studies with prenatal ethanol exposure, or at least in those in which ethanol (1 or 2 g/kg) was administered in a binge-like manner during the last days of gestation, is the result of a learned appetitive response for ethanol (e.g., Spear & Molina, 2005). This appetitive response presumably is acquired during the four days in which the fetuses are exposed repeatedly to ethanol's chemosensory aspects (Dominguez et al., 1996) contiguous with its pharmacological effects, which apparently provide appetitive reinforcement, mediated in part by the opioid system (Arias and Chotro, 2005a; Chotro and Arias, 2003). Although conditioned aversions in fetuses have been obtained by administering LiCl as the unconditioned stimulus (Gruet et al., 2004; Smotherman, 1982b; Stickrod et al., 1982), it is puzzling that administration of ethanol during gestation has most often yielded increased acceptance of ethanol (Chotro et al., 2007; Spear and Molina, 2005). Perhaps the ethanol doses administered in these experiments (1-2 g/kg) were not high enough to induce an aversion at this age. In other words, there is no evidence that the fetus can acquire an aversion to ethanol, even after intoxication with doses that have been found to be clearly aversive in infant and adult rats (Busse et al., 2005b; Fidler et al., 2004; Hunt et al., 1990; Molina et al., 1996; Pautassi et al., 2005; Roma et al., 2007).

Why is it relevant that the particular dose of ethanol experienced by dam and fetus during late gestation might determine whether the offspring subsequently have greater or less attraction for ethanol? First there is clinical evidence that humans exposed prenatally to moderate levels of ethanol have increased susceptibility to alcohol abuse in adolescence and childhood (Baer et al., 1998; Baer et al., 2003) and with animal models, the neurobiological basis of this effect has begun to be studied (e.g., (Arias and Chotro, 2005a). Second, there is this paradox: Given that an ethanol dose such as 1 or 2 g/kg is often sufficient to induce an aversion to ethanol in adult rats, perhaps even a pregnant dam, why should it induce a subsequently increased acceptance of ethanol in the offspring? Does pregnancy, for instance, protect against acquisition of conditioned ethanol aversion by the dam or is the fetus especially resistant to ethanol aversions in particular? A third reason is to help determine whether the effect of prenatal ethanol on postnatal acceptance of ethanol is most profitably viewed as due to an association acquired by the fetus between ethanol's chemosensory and pharmacological attributes or to the perceptual-affective consequences of mere exposure to ethanol (Spear & Molina, 2005). The primary issue, fundamental to the present study, is whether a dose of ethanol that induces

a conditioned aversion in a dam also induces an aversion in the offspring, or alternatively, results in enhanced drinking by the offspring?

In adult rats ethanol is readily shown to have aversive unconditioned properties. For example, ethanol can induce conditioned place aversions when administered acutely either intraperitoneal (i.p.) or intragastric (i.g.) in doses ranging from 1 to 2 g/kg (Busse et al., 2005a; Busse et al., 2005b; Fidler et al., 2004). Conditioned taste aversions (CTA) have also been described in adult rats using as the US an i.g. administration of 1.5 g/kg ethanol (Ciccocioppo et al., 1999), or with 2 g/kg injected i.p. (Ciccocioppo et al., 1999; Escarabajal et al., 2003; Roma et al., 2007). In infant rats ethanol has been also shown to be an effective aversive US for obtaining conditioned taste aversions (i.g., 1.2 g/kg, for example; (Hunt et al., 1990), or even texture aversions (2 or 2.5 g/kg; (Molina et al., 1996; Pautassi et al., 2005). In addition and more relevant for the goals of the present study, when ethanol is administered i.g. to the infant rat a subsequent change in response to ethanol occurs: the rat acquires an apparently conditioned response to ethanol. This conditioned response during ethanol intoxication apparently results from the association between ethanol's flavor (perceived mainly through respiration and saliva) and the pharmacological effects of the drug (Molina et al., 1989).

The nature of this conditioning depends importantly on the dose of ethanol administered. For example, i.g. administration of 3 g/kg ethanol to 10-11 day old rats induces a conditioned aversion to the taste and odor of ethanol (Arias and Chotro, 2006c; Molina and Chotro, 1989a; Molina et al., 1989). However, with i.g. doses below 2 g/kg no changes in subsequent response to ethanol have been reported, unless the ethanol administration has been explicitly paired with an aversive or an appetitive US (Hunt et al., 1990; Molina and Chotro, 1989a, b). The age of the pups seems also to be a critical factor for observing this ethanol-induced learning. Recent data have shown that when a 3 g/kg ethanol dose is administered i.g. to 7-8 days old rats no aversions are observed; there is instead an increase in ethanol intake as well as enhanced palatability of ethanol's flavor, both mediated by the opioid system (Arias and Chotro, 2006b; Chotro and Arias, 2007). The apparent absence of aversive effects of ethanol during earlier development is consistent with the hypothesis of a sensitive period that begins within days of birth and ends around postnatal days (PD) 9-10, in which infant rats are predisposed to learn preferences for olfactory stimuli even when paired with mildly aversive unconditioned stimuli (Sullivan et al., 2000a; Sullivan et al., 2000b). This appetitive learning acquired by very young pups also has been shown to be mediated in part by the opioid system (Kehoe, 1988; Roth and Sullivan, 2001, 2003).

As mentioned before, all previous studies analyzing ethanol intake in infant rats after ethanol exposure during the last days of gestation have used 1 or 2 g/kg ethanol doses, which generated increased ethanol acceptance and enhanced palatability of ethanol's flavor in the infant rats. The question remains whether the unborn rat would learn an aversion to ethanol if the mother were administered a higher dose, one that clearly induces ethanol aversions in infant or adult rats. The present study has been conceived with two goals: first, to test whether as a result of the administration of a relatively higher ethanol dose (3g/kg) to the pregnant dam the fetus would acquire an aversion to ethanol; and second, to study whether this prenatal ethanol treatment affects subsequent postnatal learning mediated by ethanol. To test postnatal learning about ethanol infant rats that had, or had not, been given prenatal ethanol treatment were administered a 3 g/kg ethanol dose on PD 7-8 or on PD 10-11. On the basis of the studies reviewed above, if fetuses exposed to the 3 g/kg ethanol during gestational days 17-20 were to acquire an aversion to the drug, this would be expected to attenuate the increased acceptance of ethanol observed when ethanol intoxication takes place on PD 7-8, but to potentiate the acquired ethanol aversion expected for pups intoxicated on PD 10-11. On the other hand, if the prenatal ethanol experience generates a preference for ethanol, it would be expected to

potentiate the enhanced affinity for ethanol on PD 7-8 and attenuate the ethanol aversion acquired on PD 10-11.

Based on the results of previous studies, ethanol acceptance was evaluated with ethanol intake tests at both ages. In addition, tests of ethanol odor preference were conducted in order to obtain further information about the hedonic value of the prenatal and postnatal ethanol experiences with the drug. Considering the relatively high ethanol dose employed in this study, the possible teratological effects of the prenatal ethanol exposure were assessed by comparing the pups' body weight at birth and at each postnatal treatment age. For similar reasons the offspring's spontaneous motor activity was registered, in view of previous studies reporting hyperactivity in pups exposed to binge-ethanol on the last days of gestation (Arias et al., 2008; Chotro and Spear, 1997; Riley, 1990), an apparently teratological effect that may be associated with enhanced ethanol intake after prenatal ethanol (Bond and Digiusto, 1976). Finally, we tested the impact of the repeated intoxication with ethanol during gestation on the dam's subsequent ethanol intake, to test the relationship between the effects of the ethanol intoxication in the mother and the offspring. For instance, in a study in which another aversive agent, LiCl, was administered paired with a flavor during the last period of gestation in the rat, a conditioned taste aversion was observed in both the mother and the offspring (Gruest et al., 2004). If this were the case in terms of ethanol, conditioned aversion to ethanol during pregnancy should predict a consequential reduction in ethanol acceptance among the offspring.

In summary, previous studies have indicated enhanced acceptance of ethanol among humans or rats exposed prenatally to moderate amounts of ethanol. Although this might be attributed to the rewarding consequences of ethanol for the fetus, the doses experienced by the dam and fetus have in other studies evoked aversive consequences for adult and infant rats, as evidenced by a conditioned aversion to ethanol's sensory attributes or associates. We assess in the present study the relationship between the consequences of gestational ethanol for dam and fetus -- whether both were rewarding or both aversive, or if an aversive consequence for the dam could occur despite a presumably rewarding effect for the fetus that promotes enhanced acceptance of ethanol after birth. Knowing this relationship could lead, for example, to therapeutic intervention directed by monitoring the pregnant woman's affective response to ethanol intake.

Materials and Methods

Subjects

Subjects for this study were 256 infant Wistar rats (128 males and 128 females) derived from 32 dams. They were born and reared in a temperature-controlled vivarium at the University of the Basque Country (Spain). The colony room was maintained on a 12-h light / 12-h dark illumination cycle, with light onset at 8 a.m. Female rats were time-mated to provide subject fetuses for this study, and the presence of sperm in vaginal smear was considered as gestation day 0. Pregnant females were housed by pairs in standard maternity cages with continuous access to food (Panlab, Spain, maternity formula) and water, and remained undisturbed until the initiation of the ethanol treatment on gestation day 17. At the end of this procedure, on gestation day 20, dams were housed individually and remained undisturbed for parturition. In addition, a separate group of twelve pregnant rats on gestation day 20 was employed for determination of ethanol levels in blood and amniotic fluid. European regulations for the care and treatment of experimental animals were followed, and procedures were controlled and approved by the Diputación Foral de Gipuzkoa, Spain, in compliance with the European Communities Council Directive (86/609/EEC).

Experimental design

The factorial design was defined by the following independent variables: Prenatal treatment (Water and EtOH), Age of the postnatal treatment (PD7 and PD10), Postnatal treatment (Water and EtOH), Sex (Female and Male) and Test (Intake and Activity plus odor preference). This design resulted in 32 independent groups. The number of pups in each group was 8, and only one subject per litter entered into each group: From the 32 litters used in this study, 16 were derived from dams treated with ethanol and 16 from water treated dams. From each prenatal treatment 8 litters were treated postnatally at PD 7 and 8 and the other 8 litters at PD 10 and 11. Each litter provided 4 females and 4 males; two females and 2 males were treated postnatally with water and the remaining 2 females and 2 males with EtOH; all pups from a given litter were treated at the same postnatal age. One pup per litter from a given sex and postnatal treatment was tested in the intake test and the other in the activity and odor preference tests.

Maternal Treatment

Pregnant rats received, during gestational days 17, 18, 19 and 20, one daily intragastric (i.g.) administration of water (prenatal treatment Water) or 3 g/kg ethanol (prenatal treatment EtOH). This ethanol dose resulted from the administration of a volume equivalent to 0.015 ml of a 25.2 % v/v ethanol solution in water per gram of body weight. Dams from group Water received similar volume of only the vehicle. Intragastric administrations were performed using a 15-cm length of polyethylene tubing (PE-50 Clay Adams, Parsippany, New Jersey) attached to a 10 ml syringe with a 24-gauge needle. This tubing was gently introduced through the mouth and slowly pushed into the stomach. The entire procedure takes around 15 seconds per rat and was performed at 10:00 am. The day of birth was considered postnatal day 0. Within 24 h of birth all litters were culled to 8-10 pups (keeping at least 4 females and 4 males in each litter) and body weights of all pups were registered.

Postnatal treatment

On two consecutive days, postpartum days 7 and 8 (age group PD7) or on days 10-11 (age group PD10), 2 females and 2 males from each litter and prenatal treatment received an i.g. administration of water (postnatal treatment Water) and the remaining 2 females and 2 males from each litter received the administration of ethanol (postnatal treatment EtOH). Pups were separated from the mother and placed, grouped by treatment, in holding chambers (15 × 8 × 15 cm) maintained at 28-30° C with heating pads. Subjects from postnatal treatment EtOH received a 3 g/kg ethanol dose and the volume administered was 0.015 ml per gram of body weight of a 25.2 % v/v ethanol solution in water. Pups from postnatal treatment Water were administered an equivalent volume of the vehicle. Intragastric administrations were performed using a 10-cm length of polyethylene tubing (PE-10 Clay Adams, Parsippany, New Jersey) attached to a 1 ml syringe with a 27-gauge needle. This tubing was gently introduced through the mouth and slowly pushed into the stomach. The entire procedure takes around 15 seconds per pup. Three hours after the i.g. administration all pups were returned to the maternity cage.

Intake test

Half of the females and males of each treatment and age group were evaluated in terms of water and ethanol intake (two tests separated by 1-hour interval) on two consecutive days: postnatal days 9-10 (group PD7) or on days 12-13 (group PD10). Two hours before the first intake test (water), pups were separated from the mother and placed, grouped by litter, in heated holding chambers. Each pup was implanted with an intraoral cannula through the cheek using a procedure already described in previous studies (for example see: (Arias and Chotro, 2007; Hall and Rosenblatt, 1977; Spear et al., 1989). These cannulae were later used to infuse the solutions during the intake tests. A few minutes before the test, pups' bladders were voided by gentle brushing of the anogenital area and their body weights were registered. Pups were tested

in individual clear plastic chambers (8 cm × 8 cm × 25 cm). In the first intake test pups received intraoral infusions of water during 15 min. The fluid was delivered with a syringe infusion pump (KD Scientific, Holliston, Massachusetts) connected to the oral cannula of each pup by a polyethylene catheter (PE 50, Clay Adams, Parsippany, New Jersey). The volume administered to each subject's mouth was equivalent to 5.5 % of their body weight and was infused with continuous flow. During the test, pups could either consume or reject the infused fluid. At the end of the test post-infusion weights were registered and pups returned to the holding cages. One hour later pups were tested in terms of ethanol (6% v/v) consumption, with all procedures and parameters the same as those described for the water intake test. Intake of water and ethanol was calculated considering pre- and post-infusion body weights and expressed as percentage of body weight gained (%BWG). The test for ethanol intake always followed the test for water intake to avoid disrupting consequences of the pharmacological effects of the consumed ethanol (sedation, hypnosis) on performance of the water intake test.

Activity and Odor Preference Tests

The remaining pups from each litter and treatment, which were not given the intake tests, were evaluated first in terms of spontaneous locomotor activity in an open field followed by an odor preference test. These tests were performed the day following the last postnatal ethanol administration, i.e. on postnatal day 9 for group PD7, or on day 12 for group PD10. On the day of the test, pups were separated from the mother, weighed and placed in heated holding chambers. To assess locomotor activity pups were tested individually for 5 min, after being placed in the center of a square open-field (30 cm × 30 cm × 30 cm) made of black opaque Plexiglas. Motor activity (sec) was automatically registered and processed with a computerized video-tracking system (SAR, Donostia, Spain). After the open-field test pups were placed back in the holding chamber. Thirty minutes after the open-field test, these same pups were evaluated individually in a 3-min odor preference test. This test was conducted in a rectangular (30 cm × 15 cm × 15 cm) opaque black chamber. The floor of the chamber was covered with a wire-mesh platform elevated 4 cm above it. Two pieces of sponge (3 cm × 15 cm × 1 cm) were placed underneath the wire-mesh platform, one at each extreme of the apparatus. One of the sponges was soaked with 2 ml of ethanol (190 proof) and the other with 2 ml of an emulsion of lemon essence in water (0.01 % v/v). Pups were placed in the center of the chamber and preference for each odor was determined by the time pups spent on the corresponding side of the apparatus. This was registered and processed with a computerized video-tracking system (SAR, Donostia, Spain) set to consider the testing chamber divided in three sections: A central neutral section of 6 cm and two lateral 12-cm sections corresponding to each odor. Absolute and percentage time spent by a pup on the ethanol odor section were the dependent variables under consideration. After each pup was tested the chamber was wiped with a clean paper towel and the position of the odors was counterbalanced between conditions. All pups were sacrificed on postnatal day 13.

Maternal intake test

Maternal ethanol and water consumption was also measured during three consecutive days; two days after all pups were sacrificed. For this purpose, on days 15, 16 and 17 after parturition two bottles were introduced in the dam's cages, one bottle containing 100 ml of water and the other a similar volume of a 6% v/v ethanol solution. At 10 am dam's weights were registered. Then, bottles were filled with the corresponding fluid, weighed to the nearest 0.1 g (pre-test weight) and placed in the cages. Twenty-four hours later, bottles were carefully removed and weighed again (post-test weight). This complete procedure was repeated two more days, and the position of the bottles was counterbalanced. Water and ethanol consumption of each dam was calculated in ml by differences between pre-test and post-test bottle weights, considering the equivalence 1 g to 1 ml. Ethanol intake was analyzed as ml and in g/kg.

Ethanol levels in maternal blood and amniotic fluid

A separate group of 12 pregnant females on gestational days 20 were employed to analyze ethanol levels in maternal blood and amniotic fluid. Samples of both fluids were taken at 30, 90 and 270 min after an i.g. administration of 3 g/kg ethanol to the dams. At the corresponding post-administration time females were sacrificed in a CO₂ gas chamber. Blood was immediately taken from the jugular vein and stored under refrigeration at 4° C. A ventral laparotomy was then performed and the amniotic fluid was extracted from each amniotic sac, placed in sterilized tubes and kept refrigerated at 4° C. Four samples of amniotic fluid and of maternal blood from each dam were analyzed. Ethanol levels in all these samples were determined with head-space gas chromatography analysis (Hewlett-Packard, model 5890 Series II GC with Autosampler model 7673B) in accord with techniques described in previous papers (Molina et al., 1992; Pepino et al., 1998), and were expressed as mg of ethanol per dl of amniotic fluid or maternal blood (mg/dl = mg %).

Statistical analyses

Pup's body weights at birth (PD 0), and before each postnatal treatment (PD 7 and PD 10), were analyzed with separate 2-way Prenatal Treatment x Sex ANOVAs. Data in the first analysis (PD 0) were the averages of each litter by sex, so that each litter contributed only one data point to the analysis. Water and ethanol intake data for each age group were analyzed with 4-way mixed ANOVAs: Prenatal treatment x Postnatal treatment x Sex x Day (1st and 2nd). Total time of locomotor activity and percent time on ethanol odor were also analyzed for each test and age group with 3-way ANOVAs: Prenatal treatment x Postnatal treatment x Sex. Maternal intake data (water in ml and intake of ethanol in ml and as g/kg) were analyzed with 3-way mixed ANOVAs: Prenatal treatment x Age (at which the pups were treated, PD7 or PD10) x Day of testing (1, 2 and 3). Alcohol levels (mg/dl) in maternal blood and amniotic fluid were analyzed with a 2-way mixed ANOVA: Fluid (maternal blood and amniotic fluid) x Post-administration time (30, 90 and 270). Between-within interactions were further explored with follow-up ANOVAs for each level of the within variable and factorial interactions were explored with Newman-Keuls post-hoc tests. The alpha level was set a priori at $p < 0.05$ for all analyses; p values between 0.05 and 0.10 were considered trends.

Results

Body weight

Analyses of the body weight data at PD 0 indicated significant effects of Prenatal Treatment, with neonates exposed prenatally to ethanol weighing significantly less than pups from water treated mothers [$F(1,28) = 23.60$, $p < 0.001$]. Body weights on the first postnatal treatment day for subjects treated on PD 7 indicated that at this age the effect of Prenatal treatment was not significant although borderline [$F(1,28) = 3.84$, $p = 0.060$]. No significant effects were detected when analyzing body weight data from pups on PD 10 on their first postnatal treatment day. These results show that the prenatal ethanol exposure induced a transient reduction in the pup's body weight that lasted almost a week. Body weight data of all pups at birth and on postnatal days 7 and 10, are presented as a function of Prenatal treatment in Table 1.

Intake test

Water and ethanol intake data from group PD7 are depicted in Figure 1A. As can be observed in this figure, no differences in water intake were observed as a function of any of the treatments. This was corroborated by the ANOVA indicating no significant effects of any of the factors included in the analysis. Nevertheless, in the ethanol intake test it can be observed that pups exposed prenatally and postnatally to ethanol consumed more than control pups, pups exposed only postnatally to ethanol and even pups with only prenatal exposure, although this

last difference appeared on the second testing day. The results of the statistical analysis partially confirmed these impressions, indicating significant main effects of Prenatal treatment [$F(1,56) = 80.04$, $p < 0.001$], and Postnatal treatment [$F(1,56) = 30.40$, $p < 0.005$], as well as a significant interaction between these two variables [$F(1,56) = 9.74$, $p < 0.005$]. The interaction of Prenatal treatment x Postnatal treatment x Day was not statistically significant [$F(1,56) = 3.59$, $p = 0.063$]. No significant effect of Sex was observed in this analysis. Post-hoc analyses of the significant 2-way interaction revealed that group EtOH-EtOH differed from all other groups, and that groups EtOH-Water and Water-EtOH did not differ, although both differed significantly from group Water-Water.

Water and ethanol intake data from group PD10 are depicted in Figure 1B. As was the case with the younger pups, no significant differences were detected in water intake data at this age, whereas in terms of ethanol intake there were significant effects of Prenatal treatment [$F(1,56) = 45.02$, $p < 0.001$], Postnatal treatment [$F(1,56) = 5.94$, $p < 0.05$], and Day [$F(1,56) = 7.40$, $p < 0.01$]. The interactions Prenatal treatment x Day [$F(1,56) = 7.48$, $p < 0.01$] and Prenatal treatment x Postnatal treatment x Day [$F(1,56) = 7.02$, $p < 0.05$] were also obtained. No significant effects of Sex were observed in the analysis. The 3-way interaction was further examined with follow-up 2-way ANOVAs including Prenatal treatment and Postnatal treatment as the between factors, for each day. The ANOVA of ethanol intake data on testing day 1 indicated significant effects of Prenatal treatment [$F(1,56) = 59.01$, $p < 0.001$], Postnatal treatment [$F(1,56) = 22.86$, $p < 0.001$], and the significant interaction Prenatal treatment x Postnatal treatment [$F(1,56) = 4.06$, $p < 0.05$]. Post-hoc analyses of this interaction showed that on this first testing day group EtOH-Water consumed significantly more than group EtOH-EtOH, although the latter consumed significantly more than group Water-EtOH. The difference between groups Water-Water and Water-EtOH did not quite attain statistical ($p = 0.054$). Analysis of ethanol intake on day 2 indicated only a significant effect of Prenatal treatment [$F(1,56) = 14.80$, $p < 0.001$], with pups prenatally exposed to ethanol consuming more ethanol than pups from mothers administered water during gestation, independent of the postnatal ethanol administration.

Activity test

Activity data are depicted in Figure 2. As can be observed in this figure, no differences were found when measuring this index. The ANOVA with these data indicated no significant effects of any of the variables analyzed at either age.

Odor preference test

Odor preference data are depicted in Figure 3. The analysis of data from PD7 group indicated no significant effect of any of the factors analyzed, whereas the ANOVA with the PD10 group showed a significant effect of Postnatal treatment [$F(1,72) = 8.40$, $p < 0.005$]: pups administered ethanol on days 10-11 spent less time over ethanol odor than their corresponding water controls. These results show that pups at the older age expressed an aversion to the odor of ethanol, if exposed to ethanol prenatally; no effect of ethanol previous exposure was detected in the younger pups with this index.

Maternal intake test

For simplification, only ethanol intake data expressed as g/kg are depicted in Figure 4. The ANOVA with the water intake data indicated no significant effects or interactions between variables, while the analysis of ethanol intake in ml indicated a significant effect of Day [$F(2,60) = 59.45$, $p < 0.001$], and the interaction Day x Prenatal treatment [$F(2,60) = 3.58$, $p < 0.05$]. Further analyses of this interaction revealed that mothers administered ethanol during pregnancy consumed less ethanol than water treated females, but only on the first testing day. Similar results were obtained when ethanol intake was based on the body weight of the dams

(g/kg): Day [$F(2,60) = 45.52, p < 0.001$] and Day x Prenatal treatment [$F(2,60) = 4.13, p < 0.05$]. Results indicate that adult females acquired an aversion to ethanol as a result of the repeated i.g. administration of the 3 g/kg ethanol dose during late gestation of their offspring.

Ethanol levels in maternal blood and amniotic fluid

As expected, ethanol levels in amniotic fluid and in maternal blood varied as a function of the post administration period (see Figure 5), and a significant positive correlation was obtained between ethanol concentrations in these two fluids [Pearson's correlation coefficient: $r(12) = 0.83, p < 0.001$]. The ANOVA comparing ethanol content in both fluids indicated a significant effect of Post-administration time [$F(2,9) = 30.28, p < 0.001$], and a significant interaction of Post-administration time x Fluid, [$F(2,9) = 41.03, p < 0.001$]. Further analyses of this interaction revealed that 30 min after the i.g. administration ethanol concentration in maternal blood was higher than in amniotic fluid, at 90 min there were no differences, and at 270 min ethanol concentration was higher in amniotic fluid than in maternal blood. Comparisons at different post-administration times indicated that ethanol levels in both fluids increased significantly from 30 min to 90 min, then decreased significantly between 90 min and 270 min.

Discussion

The administration of a 3 g/kg ethanol dose to pregnant rats during the last days of gestation resulted in a clear increase in ethanol intake by the offspring despite, paradoxically, an aversion to ethanol in the dams. Additionally, the prenatal ethanol exposure potentiated the increased ethanol acceptance observed in pups intoxicated with ethanol on postnatal days 7-8. In contrast, when postnatal ethanol intoxication occurred at a later age, on PD 10-11, an aversion was observed; and this aversion was still evident within the general augmented ethanol intake induced by the prenatal ethanol experience.

Increased ethanol intake after prenatal binge ethanol exposure has been found in several studies, yet in none of them were ethanol doses above 2 g/kg (Chotro et al., 2007). The present results demonstrate that after a higher ethanol dose prenatally, which induces peak blood ethanol levels around 190 mg%, similarly increased ethanol intake is observed. Experimental tests of this effect have supported the hypothesis of an appetitive conditioned response to the flavor of ethanol, acquired when the fetus associates ethanol's flavor in the amniotic fluid along with its apparently appetitive pharmacological effects. In favor of this associative explanation are the results showing that pups exposed prenatally to ethanol also show enhanced consumption of a sweet-bitter solution of sucrose and quinine, which has been found to share taste chemosensory characteristics with ethanol without its pharmacological consequences (Arias and Chotro, 2005b; Molina et al., 1995). In addition, it has been found that administration of the opioid antagonist naloxone together with ethanol to the pregnant dam eliminates the increased ethanol intake effect as well as the enhanced palatability of ethanol's flavor displayed by subjects with prenatal ethanol exposure (Arias and Chotro, 2005a; Chotro and Arias, 2003). Nevertheless, another non-exclusive hypothesis, which assumes sensitization to the positive reinforcing effects of ethanol, should be considered for explaining the outcome of this study (Nizhnikov et al., 2006). According to this, subjects exposed prenatally to ethanol subsequently consume more ethanol than non-exposed controls because they are more susceptible to the positive reinforcing effects of relatively low ethanol levels in blood during the intake test (Abate et al., 2008; Nizhnikov et al., 2006). Although in the present study blood ethanol levels of the infant rats were not measured during or after the ethanol intake test, the average of .65 - 1.22 g/kg ethanol ingested on the first testing day are comparable to doses that have been found to act as an appetitive reinforcer in Pavlovian and operant conditioning in newborn and older infant rats (Bordner et al., 2008; Nizhnikov et al., 2006; Petrov et al., 2003).

With respect to the differential effect of postnatal ethanol intoxication as a function of age, the present data also concur with previous studies in showing that the effects of ethanol intoxication on pups younger than a week or so are comparable to those on near-term fetuses, since at both stages exposure to relatively high doses of ethanol induce enhanced ethanol acceptance. Also in agreement with earlier studies was an apparent change around postnatal day 10 in motivational effects of ethanol that yield ethanol-induced aversion (Arias and Chotro, 2006b; Chotro and Arias, 2007). This likely coincides with the end of a period that has been described as critical for learning of attraction and/or preference for stimuli paired with reinforcers that are aversive only a few days later in life (Roth and Sullivan, 2001, 2003; Sullivan et al., 2000a).

The interaction between pre and postnatal ethanol exposure observed in the present study also confirms that fetal experience with ethanol can influence subsequent learning about ethanol's consequences. Previous studies have found that prenatal ethanol exposure (1 or 2 g/kg) either enhances reinforcing effects and detection of relatively low ethanol doses (Nizhnikov et al., 2006; Pueta et al., 2005), or attenuates the aversive effects of a relatively high ethanol dose (Arias and Chotro, 2006c). The present study adds that prenatal exposure to an ethanol dose considered unequivocally aversive for adults (3g/kg) seems to potentiate the appetitive value of the drug near the end of the first postnatal week. The effects in the older pups could be also interpreted as an attenuation of the taste aversion observed after postnatal intoxication, since pups exposed pre and postnatally to ethanol showed higher intake scores than pups exposed only postnatally to the drug. This latter interpretation cannot be fully supported here given absence of an unequivocal reduction in ethanol among pups exposed pre and postnatally to ethanol. A more clearly attenuated aversion reported in a previous study was obtained with slightly older pups and more ethanol intoxication trials (Arias and Chotro, 2006c).

Notably, the effect of increased ethanol acceptance generated by the prenatal experience with the drug occurred even with a dose known to induce conditioned taste aversion to ethanol in either infant (Arias and Chotro, 2006b; Chotro et al., 1991) or adult rats (Deutsch and Eisner, 1977; Miceli et al., 1980; Sinclair, 1984). This paradox is further corroborated by the present effect of the gestational ethanol on the dam. Aversion to ethanol after peripheral administration of the drug without explicit exposure to its flavor, as occurs with intragastric (i.g.) administration, has been rarely reported in adult rats (Miceli et al., 1980). It is supposed that, as occurs with infant rats given i.g. ethanol, adults given i.g. ethanol subsequently perceive ethanol's orosensory aspects -- derived from excretion of the drug via saliva, urine, expired breath, as well as through hematogenic stimulation of the olfactory epithelium (Maruniak et al., 1983; Molina et al., 1999) -- in association with ethanol's pharmacological effects, which would be aversive with high doses. It appears that although adult metabolism of ethanol, and especially that of pregnant rats (Shankar et al., 2007), is more efficient than that of infants (Kelly et al., 1987; Zorzano and Herrera, 1989) this non-metabolized fraction of ethanol is still sufficient in adults to be perceived and associated with ethanol's aversive post-absorptive effects. That the aversion acquired by pregnant females was independent of their pups' response to ethanol eliminates the possibility that mothers learned the aversion from observing their intoxicated pups, i.e. "poisoned partner" effect (Revusky et al., 1982). It is notable that the aversion observed in the dams was relatively weak and extinguished after one testing trial.

Changes in ethanol metabolism as a function of age or prenatal ethanol exposure cannot be dismissed as an explanation for the present results. Although data from previous studies have found no differences in peak blood alcohol levels or elimination rates with the present ages and ethanol doses (Arias and Chotro, 2006a; Kelly et al., 1987; Zorzano and Herrera, 1989), one study did suggest an effect of prenatal ethanol on ethanol metabolism in neonates (Nizhnikov et al., 2006). Further studies of the effect of prenatal ethanol on ethanol metabolism during early postnatal development are necessary, especially considering the changes that seem

to take place in acceptance of ethanol and learning about this drug during early development (Sanders and Spear, 2007).

Ethanol odor preference was not affected by prenatal ethanol, although postnatal ethanol intoxication decreased selection of this odor. Pups intoxicated with ethanol on PD 10-11 subsequently avoided ethanol odor, which coincides with previous reports (Molina et al., 1989). Previously it was also observed, as in the present study, that the effects of prenatal ethanol detected in an ethanol intake test were not directly reflected in an odor preference test (Chotro et al., 1991). This could suggest that the two-way odor test used to assess the hedonic value of ethanol odor is not optimal for detecting changes in response to ethanol odor derived from prenatal exposure to this drug. A more effective test might be assessment of autonomic and motor responses to the odor of ethanol (Abate et al., 2004; Chotro et al., 1996; Chotro and Molina, 1992; Dominguez et al., 1996; Dominguez et al., 1998), reflexive sniffing response to ethanol odor (Middleton et al., 2009; Youngentob et al., 2007a), and responses to ethanol flavor assessed in intake or taste reactivity tests (Arias and Chotro, 2005b; Chotro and Arias, 2003; Dominguez et al., 1998).

Finally, although signs of teratology derived from this prenatal ethanol treatment were minimal -- transient reduction in birth weight and no sign of hyperactivity -- we cannot discard the possibility of more subtle effects of ethanol exposure on fetal neurophysiology that might have induced changes in response to ethanol. Hyperactivity has been frequently reported in studies in which relatively high ethanol doses were administered during most of gestation. This effect also has been observed with moderate ethanol doses in rat fetuses (Chotro and Spear, 1997), in neonates (Dominguez et al., 1998) and preweanlings (Arias et al., 2008). However, in all those cases hyperactivity was induced by a specific stimulus whereas in the present study pups were tested for spontaneous motor activity without any explicit manipulation.

In summary, the present results together with those of previous studies indicate that when the rat is exposed to ethanol during late gestation or infancy, even to a dose that induces ethanol aversion in adults, the subsequent consequence is greater ethanol intake than controls without prenatal ethanol exposure. In addition, this early experience modulates later acquisition of associative learning about ethanol in an age-dependent manner, potentiating appetitive learning about ethanol near the end of the first postnatal week and apparently reducing ethanol's aversive consequences during the second postnatal week.

Acknowledgements

The authors want to thank Teri Tanenhaus and J.C. de la Cruz, for their technical assistance. This work was supported by grants from NIAAA (R01 AA011960 and R01 AA13098) to N.E.S.; by grants from MEC (PSI2008-00412) and the Basque Government (IT-276-07) to M.G.C.; and by postdoctoral fellowships from MEC and CONICET to C.A.

References

- Abate P, Pepino MY, Spear NE, Molina JC. Fetal learning with ethanol: correlations between maternal hypothermia during pregnancy and neonatal responsiveness to chemosensory cues of the drug. *Alcohol. Clin. Exp. Res* 2004;28:805–815. [PubMed: 15166658]
- Abate P, Pueta M, Spear NE, Molina JC. Fetal learning about ethanol and later ethanol responsiveness: Evidence against “Safe” amounts of prenatal exposure. *Exp. Biol. Medicine* 2008;233:139–154.
- Abate P, Varlinskaya EI, Cheslock SJ, Spear NE, Molina JC. Neonatal activation of alcohol-related prenatal memories: impact on the first suckling response. *Alcohol. Clin. Exp. Res* 2002;26:1512–1522. [PubMed: 12394284]
- Arias C, Chotro MG. Increased palatability of ethanol after prenatal ethanol exposure is mediated by the opioid system. *Pharmacol. Biochem. Behav* 2005a;82:434–442. [PubMed: 16257045]

- Arias C, Chotro MG. Increased preference for ethanol in the infant rat after prenatal ethanol exposure, expressed on intake and taste reactivity tests. *Alcohol. Clin. Exp. Res* 2005b;29:337–346. [PubMed: 15770108]
- Arias C, Chotro MG. Early exposure to ethanol induces a conditioned preference for the drug's flavour. *Chemical Senses* 2006a;31:E90–E90.
- Arias C, Chotro MG. Ethanol-induced preferences or aversions as a function of age in preweanling rats. *Behav. Neurosci* 2006b;120:710–718. [PubMed: 16768622]
- Arias C, Chotro MG. Interactions between prenatal ethanol exposure and postnatal learning about ethanol in rat pups. *Alcohol* 2006c;40:51–59. [PubMed: 17157720]
- Arias C, Chotro MG. Amniotic fluid can act as an appetitive unconditioned stimulus in preweanling rats. *Dev. Psychobiol* 2007;49:139–149. [PubMed: 17299786]
- Arias C, Molina JC, Mlewski EC, Pautassi RM, Spear N. Acute sensitivity and acute tolerance to ethanol in preweanling rats with or without prenatal experience with the drug. *Pharmacol. Biochem. Behav* 2008;89:608–622. [PubMed: 18374972]
- Baer JS, Barr HM, Bookstein FL, Sampson PD, Streissguth AP. Prenatal alcohol exposure and family history of alcoholism in the etiology of adolescent alcohol problems. *J. Stud. Alcohol* 1998;59:533–543. [PubMed: 9718105]
- Baer JS, Sampson PD, Barr HM, Connor PD, Streissguth AP. A 21-year longitudinal analysis of the effects of prenatal alcohol exposure on young adult drinking. *Arch. Gen. Psychiatry* 2003;60:377–385. [PubMed: 12695315]
- Bond NW, Digiusto EL. Effects of prenatal alcohol consumption on open-field behaviour and alcohol preference in rats. *Psychopharmacologia* 1976;46:163–165. [PubMed: 951453]
- Bordner KA, Molina JC, Spear NE. Analysis of ethanol reinforcement in 1-day-old rats: assessment through a brief and novel operant procedure. *Alcohol. Clin. Exp. Res* 2008;32:580–592. [PubMed: 18366451]
- Busse GD, Lawrence ET, Riley AL. The effects of alcohol preexposure on cocaine, alcohol and cocaine/alcohol place conditioning. *Pharmacol. Biochem. Behav* 2005a;81:459–465. [PubMed: 15907338]
- Busse GD, Verendeev A, Jones J, Riley AL. The effects of cocaine, alcohol and cocaine/alcohol combinations in conditioned taste aversion learning. *Pharmacol. Biochem. Behav* 2005b;82:207–214. [PubMed: 16154625]
- Chotro MG, Arias C. Prenatal exposure to ethanol increases ethanol consumption: a conditioned response? *Alcohol* 2003;30:19–28. [PubMed: 12878271]
- Chotro MG, Arias C. Exposure to low and moderate doses of alcohol on late gestation modifies infantile response to and preference for alcohol in rats. *Ann. Ist Super. Sanita* 2006;42:22–30. [PubMed: 16801722]
- Chotro MG, Arias C. Ontogenetic difference in ethanol reinforcing properties: the role of the opioid system. *Behav. Pharmacol* 2007;18:661–666. [PubMed: 17912050]
- Chotro MG, Arias C, Laviola G. Increased ethanol intake after prenatal ethanol exposure: studies with animals. *Neurosci. Biobehav. Rev* 2007;31:181–191. [PubMed: 17010438]
- Chotro MG, Cordoba NE, Molina JC. Acute prenatal experience with alcohol in the amniotic fluid: interactions with aversive and appetitive alcohol orosensory learning in the rat pup. *Dev. Psychobiol* 1991;24:431–451. [PubMed: 1783223]
- Chotro MG, Kraebel KS, McKinzie DL, Molina JC, Spear N. Prenatal and postnatal ethanol exposure influences preweanling rats' behavioral and autonomic responding to ethanol odor. *Alcohol* 1996;13:377–385. [PubMed: 8836327]
- Chotro MG, Molina JC. Bradycardiac responses elicited by alcohol odor in rat neonates: influence of in utero experience with ethanol. *Psychopharmacology (Berl)* 1992;106:491–496. [PubMed: 1579622]
- Chotro MG, Spear NE. Repeated exposure to moderate doses of alcohol in the rat fetus: evidence of sensitization to toxic and chemosensory aspects of alcohol. *Alcohol. Clin. Exp. Res* 1997;21:360–367. [PubMed: 9113276]
- Ciccocioppo R, Angeletti S, Chhada M, Perfumi M, Frolidi R, Massi M. Conditioned taste aversion induced by ethanol in alcohol-preferring rats: Influence of the method of ethanol administration. *Pharmacol. Biochem. Behav* 1999;64:563–566. [PubMed: 10548272]

- Deutsch JA, Eisner A. Ethanol self-administration in the rat induced by forced drinking of ethanol. *Behav. Biol* 1977;20:81–90. [PubMed: 869852]
- Dominguez HD, Lopez MF, Chotro MG, Molina JC. Perinatal responsiveness to alcohol's chemosensory cues as a function of prenatal alcohol administration during gestational days 17–20 in the rat. *Neurobiol. Learn. Memory* 1996;65:103–112.
- Dominguez HD, Lopez MF, Molina JC. Neonatal responsiveness to alcohol odor and infant alcohol intake as a function of alcohol experience during late gestation. *Alcohol* 1998;16:109–117. [PubMed: 9665312]
- Escarabajal MD, De Witte P, Quertemont E. Role of acetaldehyde in ethanol-induced conditioned taste aversion in rats. *Psychopharmacology (Berl.)* 2003;167:130–136. [PubMed: 12655465]
- Fidler TL, Bakner L, Cunningham CL. Conditioned place aversion induced by intragastric administration of ethanol in rats. *Pharmacol. Biochem. Behav* 2004;77:731–743. [PubMed: 15099918]
- Gruest N, Richer P, Hars B. Emergence of long-term memory for conditioned aversion in the rat fetus. *Devel. Psychobiology* 2004;44:189–198.
- Hall WG, Rosenblatt G. Suckling behavior and intake control in the developing rat pup. *J. Comp. Physiol. Psychol* 1977;91:1232–1247.
- Hunt PS, Molina JC, Spear LP, Spear NE. Ethanol-mediated taste aversions and state-dependency in preweanling (16-day-old) rats. *Behav. Neural Biol* 1990;54:300–322. [PubMed: 2078162]
- Kehoe, P. Opioids, behavior, and learning in mammalian development. In: Blass, EM., editor. *Handbook of Behavioral Neurobiology. Developmental Psychobiology and Behavioral Ecology*. Plenum Press; New York: 1988. p. 309–346.
- Kelly SJ, Bonthius DJ, West JR. Developmental changes in alcohol pharmacokinetics in rats. *Alcohol. Clin. Exp. Res* 1987;11:281–286. [PubMed: 3307494]
- Maruniak JA, Silver WL, Moulton DG. Olfactory receptors respond to blood-borne odorants. *Brain Res* 1983;265:312–316. [PubMed: 6850336]
- Miceli D, Marfaingjallat P, Lemagnen J. Ethanol Aversion Induced by Parenterally Administered Ethanol Acting Both as Cs and Ucs. *Physiological Psychol* 1980;8:433–436.
- Middleton FA, Carrierfenster K, Mooney SM, Youngentob SL. Gestational ethanol exposure alters the behavioral response to ethanol odor and the expression of neurotransmission genes in the olfactory bulb of adolescent rats. *Brain Res* 2009;1252:105–116. [PubMed: 19063871]
- Molina F, Bosquesendra JM, Pla A, Megias L. Determination of Ethanol in Biological Samples by Headspace Gas-Chromatography. *J. Pharmaceut. Biomed. Analysis* 1992;10:1069–1071.
- Molina JC, Bannoura MD, Chotro MG, McKinzie DL, Arnold HM, Spear NE. Alcohol-mediated tactile conditioned aversions in infant rats: Devaluation of conditioning through alcohol-sucrose associations. *Neurobiol. Learn. Memory* 1996;66:121–132.
- Molina JC, Chotro MG. Acute alcohol intoxication paired with appetitive reinforcement: effects upon ethanol intake in infant rats. *Behav. Neural Biol* 1989a;51:326–345. [PubMed: 2730497]
- Molina JC, Chotro MG. Acute alcohol intoxication paired with aversive reinforcement: ethanol odor as a conditioned reinforcer in rat pups. *Behav. Neural Biol* 1989b;52:1–19. [PubMed: 2502977]
- Molina JC, Chotro MG. Association between chemosensory stimuli and cesarean delivery in rat fetuses: neonatal presentation of similar stimuli increases motor activity. *Behav. Neural Biol* 1991;55:42–60. [PubMed: 1996947]
- Molina, JC.; Chotro, MG.; Dominguez, HD. Fetal alcohol learning derived from ethanol contamination of the prenatal environment. In: Lecanuet, JP.; Fifer, WP.; Krasnegor, N.; Smotherman, WP., editors. *Fetal development: A psychobiological perspective*. Lawrence Erlbaum Associates; Hillsdale, NJ: 1995. p. 419–438.
- Molina JC, Chotro MG, Spear NE. Early (preweanling) recognition of alcohol's orosensory cues resulting from acute ethanol intoxication. *Behav. Neural Biol* 1989;51:307–325. [PubMed: 2730496]
- Molina, JC.; Dominguez, HD.; Lopez, MF.; Pepino, MY.; Faas, AE. The role of fetal and infantile experience with alcohol in later recognition and acceptance patterns of the drug. In: Hannigan, JH., editor. *Alcohol and Alcoholism: Effects on Brain and Development*. Lawrence Erlbaum Associates; Mahwah, NJ: 1999. p. 199–228.
- Nizhnikov ME, Molina JC, Varlinskaya EI, Spear NE. Prenatal ethanol exposure increases ethanol reinforcement in neonatal rats. *Alcohol. Clin. Exp. Res* 2006;30:34–45. [PubMed: 16433730]

- Pautassi RM, Ponce LF, Molina JC. Effects of early exposure to ethanol on subsequent learning mediated by the unconditional attributes of the drug. *Revista Latinoamericana De Psicología* 2005;37:149–166.
- Pedersen, PE.; Greer, CA.; Shepherd, GM. Early development of olfactory function. In: Blass, EM., editor. *Handbook of Behavioral Neurobiology. Developmental Psychobiology and Behavioral Neurobiology*. Plenum Press; New York: 1986. p. 163-204.
- Pepino MY, Kraebel KS, Lopez MF, Spear NE, Molina JC. Behavioral detection of low concentrations of ethanol in milk in the preweanling rat. *Alcohol* 1998;15:337–353. [PubMed: 9590520]
- Petrov ES, Varlinskaya EI, Spear NE. Reinforcement from pharmacological effects of ethanol in newborn rats. *Alcohol. Clin. Exp. Res* 2003;27:1583–1591. [PubMed: 14574228]
- Pueta M, Abate P, Spear NE, Molina JC. Interactions between ethanol experiences during late gestation and nursing: effects upon infantile and maternal responsiveness to ethanol. *Int. J. Comp. Psychol* 2005;18:207–224.
- Revusky S, Coombes S, Pohl RW. Us Preexposure - Effects on Flavor Aversions Produced by Pairing a Poisoned Partner with Ingestion. *Animal Learn. Behav* 1982;10:83–90.
- Riley EP. The long-term behavioral effects of prenatal alcohol exposure in rats. *Alcohol. Clin. Exp. Res* 1990;14:670–673. [PubMed: 2264595]
- Roma PG, Chen SA, Barr CS, Riley AL. Dissociation between the aversive and pharmacokinetic effects of ethanol in female Fischer and Lewis rats. *Behav. Brain Res* 2007;182:51–56. [PubMed: 17568694]
- Roth TL, Sullivan RM. Endogenous opioids and their role in odor preference acquisition and consolidation following odor-shock conditioning in infant rats. *Dev. Psychobiol* 2001;39:188–198. [PubMed: 11745312]
- Roth TL, Sullivan RM. Consolidation and expression of a shock-induced odor preference in rat pups is facilitated by opioids. *Physiol. Behav* 2003;78:135–142. [PubMed: 12536020]
- Sanders S, Spear NE. Ethanol acceptance is high during early infancy and becomes still higher after previous ethanol ingestion. *Alcohol. Clin. Exp. Res* 2007;31:1148–1158. [PubMed: 17451398]
- Shankar K, Ronis MJJ, Badger TM. Effects of pregnancy and nutritional status on alcohol metabolism. *Alcohol Res. Health* 2007;30:55–59. [PubMed: 17718402]
- Sinclair JD. Ethanol-induced conditioned taste aversion to ethanol. *Alcohol* 1984;1:223–227. [PubMed: 6536288]
- Smotherman WP. In utero Chemosensory Experience Alters Taste Preferences and Corticosterone Responsiveness. *Behav. Neural Biol* 1982a;36:61–68. [PubMed: 7168731]
- Smotherman WP. Odor Aversion Learning by the Rat Fetus. *Physiol. Behav* 1982b;29:769–771. [PubMed: 6296892]
- Smotherman WP, Robinson SR. The rat fetus in its environment: behavioral adjustments to novel, familiar, aversive, and conditioned stimuli presented in utero. *Behav. Neurosci* 1985;99:521–530. [PubMed: 3040034]
- Smotherman WP, Robinson SR. Behavior of rat fetuses following chemical or tactile stimulation. *Behav. Neurosci* 1988;102:24–34. [PubMed: 2833290]
- Spear LP, Specht SM, Kirstein CL, Kuhn CM. Anterior and posterior, but not cheek, intraoral cannulation procedures elevate serum corticosterone levels in neonatal rat pups. *Devel. Psychobiol* 1989;22:401–411. [PubMed: 2721821]
- Spear NE, Molina JC. Fetal or infantile exposure to ethanol promotes ethanol ingestion in adolescence and adulthood: a theoretical review. *Alcohol. Clin. Exp. Res* 2005;29:909–929. [PubMed: 15976517]
- Stickrod G, Kimble DP, Smotherman WP. In utero taste/odor aversion conditioning in the rat. *Physiol. Behav* 1982;28:5–7. [PubMed: 6281819]
- Sullivan RM, Landers M, Yeaman B, Wilson DA. Good memories of bad events in infancy. *Nature* 2000a;407:38–39. [PubMed: 10993064]
- Sullivan RM, Stackenwalt G, Nasr F, Lemon C, Wilson DA. Association of an odor with activation of olfactory bulb noradrenergic beta-receptors or locus coeruleus stimulation is sufficient to produce learned approach responses to that odor in neonatal rats. *Behav. Neurosci* 2000b;114:957–962. [PubMed: 11085610]

- Youngentob SL, Kent PF, Sheehe PR, Molina JC, Spear NE, Youngentob LM. Experience-induced fetal plasticity: The effect of gestational ethanol exposure on the behavioral and neurophysiologic olfactory response to ethanol odor in early postnatal and adult rats. *Behav. Neurosci* 2007a;121:1293–1305. [PubMed: 18085882]
- Youngentob SL, Molina JC, Spear NE, Youngentob LM. The effect of gestational ethanol exposure on voluntary ethanol intake in early postnatal and adult rats. *Behav. Neurosci* 2007b;121:1306–1315. [PubMed: 18085883]
- Zorzano A, Herrera E. Decreased in vivo rate of ethanol metabolism in the suckling rat. *Alcohol. Clin. Exp. Res* 1989;13:527–532. [PubMed: 2679210]

Figure 1. Mean ethanol intake (% body weight gain) of pups from group PD7 (A) or group PD10 (B) during two testing days as a function of the prenatal treatment (Water or EtOH) and the postnatal treatment (Water or EtOH). Vertical bars represent standard error of the mean.

Figure 2. Mean time (sec) pups from group PD7 (A) or group PD10 (B) spent locomoting in an open field, as a function of prenatal (Water or EtOH) and postnatal (Water or EtOH) treatments. Vertical bars represent standard error of the mean.

Figure 3. Mean time (%) spent on ethanol odor of pups from group PD7 (A) or group PD10 (B), in a two way odor preference test (ethanol vs. lemon), as a function of prenatal (Water or EtOH) and postnatal (Water or EtOH) treatments. Vertical bars represent standard error of the mean.

Figure 4. Mean ethanol intake (g/kg) of the mothers during three testing days as a function of the treatment received on gestation (Water or EtOH). Vertical bars represent standard error of the mean.

Figure 5. Mean ethanol levels (mg %) in maternal blood and amniotic fluid at 30, 90 and 270 minutes after the administration of a 3g/kg ethanol dose to pregnant dams on gestational day 20. Vertical bars represent standard error of the mean.

Table 1

Prenatal treatment	Sex	PD 0	PD 7	PD 10
Water	female	5.33 ± 0.04	14.37 ± 0.11	19.07 ± 0.27
	male	5.30 ± 0.03	14.44 ± 0.15	19.71 ± 0.32
EtOH	female	5.13 ± 0.03 [*]	14.03 ± 0.17	19.28 ± 0.29
	male	5.14 ± 0.05 [*]	14.18 ± 0.18	19.94 ± 0.25

Pup's mean weights (g) ± S.E.M., as a function of prenatal treatment (Water or EtOH), sex and postnatal day (PD 0, PD 7 or PD 10).

^{*} Significant difference from water control with $p < 0.001$.