EFFECTS OF ISOLATED OR ENRICHED HOUSING AT ADOLESCENCE UPON ETHANOL INTAKE AND ANXIETY RESPONSES, IN RATS EXPOSED TO PRENATAL ETHANOL

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Abstract

Prenatal alcohol exposure (PAE) is highly prevalent worldwide, and can affect alcohol intake and anxiety responses in the offspring. It is still relatively unknown how PAE interacts with stress exposure at adolescence, and there is a scarcity of treatments to reduce the impact of PAE. The present study assessed alcohol (ethanol) intake and anxiety responses after PAE and tested the modulation of these responses by different housing conditions during early adolescence. Pregnant dams were exposed for 22 hours/day, from gestational day 0, throughout pregnancy, and until postnatal day 7 (PD 7), to a single bottle of 10% ethanol that was mixed in tap water and sucralose (EtOH Group), or to a single bottle of tap water and sucralose (CTRL Group). During PDs 21-42 the offspring was exposed to standard pair housing, isolated housing or enriched housing. Immediately after, they were pair-housed and tested for ethanol intake in 24-hour access, intermittent 2-bottle choice sessions conducted over 4 weeks, and for anxiety responses in the light-dark box test. The EtOH offspring exhibited heightened anxiety (i.e., greater avoidance of the white area of the light-dark box) at PD21, when compared to control (CTRL) rats. Isolated housing induced greater avoidance of the white area, when compared to standard or enriched housing, on PDs 42 and 70. There were no significant EtOH versus CTRL differences in relative (i.e., percent preference vs. water) ethanol intake, yet overall fluid intake and absolute (g/kg) ethanol intake were significantly lower in EtOH versus CTRL rats. Enriched housing during adolescence had a suppressing effect upon absolute ethanol intake during the first week of testing, an effect more noticeable in CTRL than in PAE rats. The present study indicates that PAE or isolated housing is associated with an anxiety-like phenotype. The protracted PAE protocol here employed caused a generalized reduction of intake behaviours. Environmental enrichment was associated with a subtle, yet significant, reduction in absolute ethanol intake.

Keywords: ethanol, prenatal alcohol exposure, anxiety, ethanol intake.
Prenatal alcohol exposure (PAE) is highly prevalent worldwide. In Argentina, López et al. observed that 15% of a sample of 614 women had consumed ≥5 drinks in a single drinking session, at least once during pregnancy. A recent study conducted in the US reported evidence of PAE within 1 month of delivery in 8.4% of their sample, whereas Lekettey et al. reported PAE in 48% of pregnant women in Ghana. Babies exposed to alcohol (hereinafter also referred to as ethanol) can exhibit fetal alcohol spectrum disorders, which involve reduced growth, birth defects and neurologic problems.

Even in the absence of a full-blown disorder, individuals that suffered PAE can exhibit hyperactivity, anxiety or greater likelihood of using alcohol themselves. In a classic study, Baer et al. reported that PAE significantly predicted alcohol consumption at age 14 or 21 years. These findings are consistent with pre-clinical studies indicating that PAE enhances voluntary ethanol intake in the offspring, an effect associated with an anxiety-like phenotype. An important caveat is that many of the pre-clinical studies that analyzed the effects of PAE on ethanol intake in the offspring assessed this behaviour only in a few testing sessions. For instance, Contreras et al. reported heightened intake of 10% ethanol in rats that underwent PAE throughout pregnancy and during the first postnatal week. Yet this ethanol intake was assessed in a single 24-hour test, and with ethanol as the only available fluid.

The therapeutic arsenal to treat the effects of PAE is much reduced. The possibility that rearing conditions during infancy and adolescence modulate the consequences of PAE has attracted attention, particularly in light of suggestions that PAE heightens stress-induced drinking. Previous studies have shown that environmental enrichment (EE) reduces and social isolation stress enhances, ethanol intake. EE involves prolonged home-cage exposure to conspecifics and objects such as toys, ladders, tunnels, and the availability of voluntary physical activity. There have been conflicting reports, however, with other studies indicating no effect of isolation housing on ethanol intake (for review), or suggesting that EE may enhance ethanol intake in adolescents.

The present study assessed ethanol intake after PAE and tested the modulatory effect of different, and ostensibly opposite, housing conditions during early adolescence, upon ethanol intake and anxiety reactivity in late adolescence. Wistar rats, males and females, underwent PAE throughout pregnancy and were exposed, during postnatal days (PD) 21 to 42, to standard pair housing, isolated housing or enriched housing. Immediately after they were pair-housed and tested for ethanol intake in 24-hour access, intermittent 2-bottle choice, sessions conducted over 4 weeks, and for anxiety responses in a light-dark box test. We recently found, in Sprague-Dawley rats, that isolation housing during adolescence unmasked the facilitative effect of PAE on alcohol intake at adulthood. Based on this, we expected isolated housing to interact with PAE to promote alcohol drinking at adolescence, an effect likely to be associated with enhanced anxiety responses.

MATERIAL AND METHODS

Experimental Design and Subjects

A 2 (Prenatal alcohol treatment: ethanol [EtOH] vs. control treatment [CTRL]) × 2 (Sex: male vs. female) × 3 (Housing Condition after weaning and until PD 42: enrichment, isolation or standard housing) factorial design was employed. Each group had 9 animals.

Subjects, Housing, and Control of Litter Effects

One-hundred and 8 outbred Wistar rats, derived from 20 dams (11 EtOH, 9 CTRL) born and reared at the Instituto de Investigaciones Médicas M. y M. Ferreyra (INIMEC-CONICET-UNC), were used. Litter effects were avoided by assigning no more than one male and one female of each litter to a given group of the experimental design. The animals were kept at 22°C, in a 12 hour/12 hour light/dark cycle. The maintenance and experimental procedures were approved by the Animal Care Committee of INIMEC-CONICET-UNC and complied with the Guide for the Care and Use of Laboratory Animals as promulgated by the National Institutes of Health and European Union.

Maternal Ethanol Exposure

We employed an adapted version of the ethanol exposure protocol of Contreras et al. Time-mated pregnant dams were singled-housed in standard maternity...
cages and exposed for 22 hours a day, from gestational day 0 (GD0), throughout pregnancy, and until PD7, to a single bottle of 10% ethanol that was mixed in tap water and sucralose (64 mg/L, EtOH Group), or to a single bottle of tap water and sucralose (64 mg/L, CTRL Group). During the remaining 2 hours of each day, and to prevent the possibility of dehydration in the EtOH dams, all rats had a bottle filled with 20 mL of tap water. Sucralose was used to promote relatively high levels of ethanol self-administration. Unlike sucrose and other sweeteners, sucralose does not have nutritional value and is mainly excreted unchanged via urination. The rationale for extending the ethanol access to PD7 was that the first postnatal week of the rat is similar, in terms of neurodevelopment, to the third trimester of pregnancy in humans.

In the dams, we measured ethanol consumption (g/kg/day; only for EtOH dams), fluid consumption (mL/100g of body weight/day), consumption of the additional water provided 2 hours/day (mL/100g of body weight/day) and body weight (g).

**Housing Conditions After Postnatal Day 21**

On PD21, the offspring were randomly assigned to individual housing (i.e., isolated housing group), or were housed in groups of 4 (i.e., standard housing group) in standard maternity cages measuring 60 cm length × 40 cm width × 20 cm height. Animals assigned to the environmental enrichment condition were exposed to the standard EE protocol of our lab, described in. Briefly, they were transferred to taller cages (60 cm length × 40 cm width × 40 cm height) equipped with 7 objects, including a running wheel, ladders or ramps, cylinders, pipes, and a plastic house-like object. These cages had 2 levels connected by the ramp and food was provided in a feeder placed in a corner. To prevent habituation, these objects were changed twice a week. The rats were kept under EE, isolation or standard housing until PD41. Then, they were all pair-housed in same-sex couples in standard maternity cages.

**Light-Dark Box (LDB) Tests**

On PD21 (immediately before the rats were assigned to standard housing, enriched housing or isolated housing), PD42 (immediately before the rats were pair-housed in same-sex couples in standard maternity cages) and at PD72 (48 hours after the last ethanol intake test), the rats were tested for anxiety response in a LDB test. The rats were placed for 5 minutes in a rectangular-shaped apparatus, composed of 2 sections connected by an opening at floor level. One of the sections was black with no illumination (i.e., 0 lux) whereas the other was white and brightly lit (300 lux). The test began by placing the animal in the white sector, opposite to the door opening. The test was captured in a digital camera for subsequent measurement of the following variables: time spent (s) in the white compartment, first latency (s) to enter into the dark compartment and number of transfers between compartments.

**Intermittent Ethanol Intake Assessment**

From PD42 to PD68 (i.e., 4 weeks, 12 intake tests), the offspring was tested for ethanol intake in an intermittent ethanol intake protocol (18). At 900 AM on Mondays, Wednesdays, and Fridays, the rats were individually housed in half of their home-cage, separated from their conspecific by an opaque Plexiglas divider. A special lid allowed equipping each half with 2 bottles, one filled with an ethanol (Porta Hnos, Cordoba, Cordoba, Argentina) solution, and the other filled with vehicle. This procedure allows the rats to smell, but not touch, each other, thus reducing the isolation stress of the test. Between sessions, the Plexiglas divider was removed and the animals had ad-libitum access to water and food.

During the first (PD42, PD44 and PD46) and second (PD49, PD51 and PD53) testing week ethanol (5%) was mixed with 1% sucrose (Parker Davis, Charlotte, NC, USA), or 0.5% sucrose, respectively. During PD56, PD58, and PD60 (third testing week) one of the bottles was filled with 5% ethanol that was mixed with tap water, and the other bottle contained only tap water. During PD63, PD65, and PD67 one of the bottles was filled with 10% ethanol that was mixed with tap water, and the other bottle contained only tap water. The change of the concentrations of ethanol and sucrose between intermittent ethanol intake sessions aimed at promoting the intake of ethanol concentrations ≥ 5%. Our studies suggest that uninitiated adolescent rats drink very little ethanol at concentrations ≥ 6% (19), unless the drug is mixed...
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with a sweetener. The protocol we used is similar to those used to train animals to drink ethanol, first by presenting a sweetened ethanol solution, followed by a gradual decrease in the sweetener and an increase in the EtOH concentration.20

The bottles were weighed before and after each intake session, to obtain a measure of raw intake, which was corrected for leakage. Specifically, a bottle of ethanol and a bottle of vehicle were introduced in an empty box and the post-pre liquid subtraction from each bottle served to correct the raw values of the tested rats. The corrected measures of ethanol and water intake were then used to calculate g/kg of ethanol ingested, percentage of ethanol preference, and overall liquid consumption per 100 grams of body weight ([total consumption of liquids (mL) × 100 g] / body weight).

DATA ANALYSIS

Student’s t-tests were employed to analyze differences between EtOH and CTRL dams, in the following variables: number of pups at birth, male/female ratio of pups at birth, fluid consumption (mL/100g/day; average across pregnancy days), consumption of the additional water provided 2 hours/day (mL/100g/day; average across days) and average maternal weight (g) during pregnancy or during breastfeeding. The ethanol intake of the EtOH dams was calculated (g/kg/day; average across pregnancy or nursing days) and reported as mean±SEM. Body weight (g) of the offspring at PD21 was analyzed in a subset of the rats (50% of the sample, randomly selected) using a factorial analysis of variance (ANOVA), with Prenatal alcohol exposure (PAE) (EtOH, CTRL) and Sex (male, female) as between-group factors. Data analysis was conducted using Statistica 8.0 (Dell software, Round Rock, Texas).

Baseline level of anxiety response in EtOH and CTRL pups was assessed by analyzing latency to exit the white compartment for the first time, time spent in the white compartment, and number of transfers between compartments, in the test conducted at PD21. Specifically, the scores registered for each of these variables at PD 21, before commencement of the differential housing conditions, were independently analyzed using a factorial ANOVA, with PAE and Sex as between-group factors. The performance in the LDB test at PD42 and PD70 was assessed by four-way mixed ANOVAs, with PAE, Sex and Housing conditions at PDs 21-42 (standard, isolated, enriched) as between-group factors and day of assessment (PD 42, PD70) as the repeated measure. The offsprings’s ethanol intake [absolute (g/kg) and percent (%) preference], overall fluid consumption (mL/100 g) and body weight (g) prior to each intake session were assessed via separate repeated-measures ANOVAs. PAE, Sex and Housing conditions at PDs 21-42 served as between-group factors, and day of ethanol intake assessment (Days 1–12) was the repeated measure. The intake data of 2 subjects was lost due to technical issues. These data were not replaced.

Tukey’s test was used, as a post-hoc test, to analyze the locus of the significant main effects and the significant interactions yielded by the ANOVA, and the partial eta squared ($\eta^2_p$) was used to inform effect size. The $\alpha$ level was set at $p \leq 0.05$. Descriptive data are presented as mean ± SEM.

RESULTS

The average ethanol intake (g/kg) in the dams was 5.67 ± 2.05 and 7.04 ± 1.74 during gestation and lactation, respectively. The number of pups at birth was similar between EtOH and CTRL dams (11.00 ± 1.16 vs. 12.66 ± 0.83, $p > 0.05$), as was the ratio of female/male offspring (data not shown, $p > 0.05$). Overall liquid intake (mL/100 g of body weight) during pregnancy was significantly reduced in EtOH versus CTRL dams (7.29 ± 0.77 vs. 17.90 ± 1.50, respectively; $t_{18} = –6.64$, $p < 0.0001$), whereas water consumption (mL/100g/2 hours a day; averaged across pregnancy days) was significantly greater in EtOH versus CTRL dams (4.12 ± 0.26 vs. 1.46 ± 0.23, respectively; $t_{18} = 7.39$, $p < 0.0001$). Body weight was similar in EtOH and CTRL dams during pregnancy or breastfeeding (data not shown, $p > 0.05$). The offspring’s weight at PD21 was significantly greater in female than in male rats ($F_{1,92} = 8.1$, $p < 0.005$, $\eta^2_p = 0.08$), yet similar in EtOH versus CTRL rats (179.5 ± 5.43 and 184.4 ± 7.03, respectively).
The ANOVA conducted on time spent in the white section of the LDB apparatus before the offspring was exposed to isolated, enriched or control housing (PD21; i.e., baseline measurement), indicated a significant main effect of Prenatal treatment ($F_{1,92} = 8.1, p < 0.005, \eta^2_p = 0.08$), with EtOH rats exhibiting greater avoidance of the white section than CTRL peers. The ANOVAs for latency to escape from the white compartment and number of transfers between compartments at PD21 did not yield significant main effects or significant interactions. Mean and SEM for each of these variables are presented in Table 1.

Independent ANOVAs analyzed the performance in the LDB at PD42 and PD72. The analysis of time spent in the white section revealed significant main effects of Sex, Housing Condition and Day of testing ($F_{1,96} = 22.28, p < 0.0005, \eta^2_p = 0.19, F_{2,96} = 8.99, p < 0.003, \eta^2_p = 0.15, F_{1,96} = 97.34, p < 0.0001, \eta^2_p = 0.50$; respectively). The post-hoc tests revealed that male rats, or rats that had been chronically isolated, exhibited a reduced time spent in the white section than female rats or rats housed in standard or enriched conditions. The 2 latter groups did not differ between each other, and there was no significant main effect of PAE nor significant interactions involving PAE. The post-hoc tests also revealed that time spent in the white compartment also increased from PD 42 to PD 70, across groups. Latency to escape from the white area at PD42 and PD72 was significantly lower in enriched rats than in isolated rats (significant main effect of Housing Condition, $F_{2,96} = 3.80, p < 0.05, \eta^2_p = 0.07$). The analysis of number of transfers revealed heightened frequency of this behaviour in female than in male rats and in the third test at PD70 compared to the second test ($F_{1,96} = 16.41, p < 0.0005, \eta^2_p = 0.14$ and $F_{1,96} = 55.42, p < 0.0001, \eta^2_p = 0.34$, respectively). The analysis also showed greater number of transfers in rats that had been exposed to chronic isolation than in enriched or standard-housed counterparts (significant main effects of Housing Condition, $F_{2,96} = 9.21, p < 0.0002, \eta^2_p = 0.16$). Figure 1 depicts these results.

**TABLE 1** Variables registered in the light-dark box (LDB) test conducted at postnatal day 21 (PD 21), in male and female Wistar rats as a function of maternal ethanol exposure throughout gestation until PD7. The dams in the ethanol (EtOH) condition were given 22 hours’ access per day to a bottle that contained 10% ethanol (v/v) that was mixed in tap water and sucralose. Control (CTRL) dams were given only tap water and sucralose. n.s = non-significant. The data are expressed as mean ± SEM.
FIG. 1 Variables registered in the light-dark box (LDB) test conducted at postnatal day 42 (PD42) and PD72, as a function of maternal ethanol (EtOH) or vehicle (CTRL) exposure throughout gestation until PD7. The dams in the EtOH condition were given 22 h access per day to a bottle that contained 10% ethanol (v/v) that was mixed in tap water and sucralose (64 mg/L). CTRL dams were given only tap water and sucralose. From PD21 to PD42 the offspring was exposed to standard control housing, social isolation housing or enriched housing. Panels A, B, and C depict latency (s) to enter into the dark compartment, number of transfers between compartments and time spent (s) in the white compartment, respectively. The asterisk sign indicates a significant main effect of Housing Condition, in each of the variables. Latency to escape from the white area at PD42 and PD72 was significantly lower in enriched rats than in isolated rats. Number of transfers was significantly greater in rats that had been exposed to chronic isolation than in enriched or standard-housed counterparts; and rats that had been chronically isolated exhibited a reduced time spent in the white section than rats housed in standard or enriched conditions. Please refer to the text for a full account of the significant main effects and significant interactions yielded by the ANOVA. The data are expressed as mean ± SEM. For visualization purposes, the data were collapsed across males and females.
The ANOVA for overall liquid intake in the offspring, during the two-bottle intake tests, revealed significant main effects of Sex, Prenatal treatment and Session, \((F_{1,93} = 20.87, p < 0.01, \eta^2_p = 0.18, F_{1,93} = 7.88, p < 0.05, \eta^2_p = 0.08; F_{11,1023} = 80.96, p < 0.01, \eta^2_p = 0.46; \text{respectively})\). As shown in Figure 2 and confirmed by the post-hoc tests, the overall volume of liquid consumed significantly decreased across tests and was significantly lower in females and in rats exposed to ethanol during gestation.

Given the difference in overall liquid intake between EtOH and CTRL offspring, it is not surprising that EtOH rats consumed significantly less ethanol in a gram per kilogram basis than controls exposed to vehicle. Specifically, the ANOVA for absolute ethanol ingested \((g/kg)\) revealed significant main effects of Prenatal treatment and Intake sessions \((F_{1,93} = 4.63, p < 0.05, \eta^2_p = 0.05, F_{11,1023} = 5.31, p < 0.001, \eta^2_p = 0.05; \text{respectively})\). The interactions between Sex and Intake session, and between Housing Condition and Intake session \((F_{11,1023} = 2.56, p < 0.001, \eta^2_p = 0.02, F_{22,1023} = 1.59, p < 0.05, \eta^2_p = 0.03; \text{respectively})\) were also significant. The post-hoc tests revealed that males drank significantly more ethanol than females from the end of the second testing week onwards. The post-hoc tests also revealed that, during sessions 1 and 2, enriched animals consumed significantly less ethanol compared to peers that had been isolated. Enriched animals also drank significantly less absolute ethanol than controls, on session 2. Ethanol preference was significantly greater in males vs. females in sessions 6 to 12 (significant Sex x Session interaction, \(F_{11,1023} = 4.04, p < 0.01, \eta^2_p = 0.04\), yet not affected by Prenatal treatment or Housing conditions. These results are depicted in Figure 3 and 4. Both figures represent absolute and percent ethanol intake as a function of Prenatal treatment and Housing conditions. Figure 3 depicts the data for every intake session conducted (1–12), whereas Figure 4 depicts the average drinking scores, across the 12 sessions.

The baseline differences in overall liquid intake between EtOH and CTRL offspring can confound the effect of Sex or Housing conditions on absolute ethanol intake \((g/kg)\). Therefore, we conducted separate Sex x Housing Conditions ANOVAs on ethanol intake \((g/kg)\) scores, one for each prenatal condition. To clarify the data analysis, these mixed follow-up ANOVAs were conducted on the average \(g/kg\) drank in each week (i.e., Week 1 to 4 served as repeated measure). The ANOVA for the CTRL condition revealed a significant main effect of Week and significant interactions between Week and Sex, and between Week and Housing Condition \((F_{3,141} = 2.71, p < 0.05, \eta^2_p = 0.05, F_{3,141} = 3.85, p < 0.01, \eta^2_p = 0.07, F_{6,141} = 2.24, p < 0.05, \eta^2_p = 0.08; \text{respectively})\). Tukey’s comparisons revealed that males drank significantly more than females on week 3; and that enriched rats drank significantly less ethanol than isolated or control rats across week 1. The ANOVA for the EtOH rats only yielded a significant main effect of Week \((F_{3,138} = 4.99, p < 0.01, \eta^2_p = 0.09)\). CTRL rats exhibited peak ethanol intake during the Week 2.

**DISCUSSION**

In the present study PAE enhanced anxiety responses at weaning. EtOH rats exhibited greater avoidance of the white area of the LDB, when compared to CTRL rats. This is consistent with studies from our lab that employed shorter protocols of prenatal ethanol exposure and was not confounded by alterations in the overall level of activity, nor was associated with significant EtOH versus CTRL differences in relative (percent preference vs. water) ethanol intake. Previously, we found that brief PAE \((2.0 \text{ g/kg, daily intubations from gestational days 17 to 20})\) induced greater avoidance of the white area of a LDB and enhanced shelter-seeking in a concentric square field, with concomitant changes in gene expression of transmitters systems that regulate stress responses.

The rats exposed to EtOH drank significantly less ethanol, in a gram per kilogram basis, than control peers. The discussion of the levels of ingestion of ethanol in the offspring, however, needs to be interpreted in the context of 2 important caveats. First, there was no significant main effect of prenatal treatment on ethanol preference. Perhaps more important, the PAE offspring exhibited significantly less overall liquid intake than CTRL peers. We can only speculate, but this may reflect teratological consequences of PAE, affecting intake behaviours. Despite EtOH dams exhibiting similar body weight to CTRL dams, EtOH dams exhibited a significant, two-fold, decrease of the amount of liquid...
FIG. 2 Overall fluid intake (ml/100 g of body weight) during intake sessions 1 to 12 in Wistar rats as a function of maternal ethanol exposure throughout gestation until PD7 (panel A) or as a function of sex (panel B). The dams in the ethanol (EtOH) condition were given 22 h access per day to 10% ethanol (v/v) that was mixed in tap water and sucralose. Control (CTRL) dams were given only tap water and sucralose. From postnatal day 21 (PD21, weaning) to PD42 the offspring was exposed to standard, isolated or enriched housing. Two-bottle intake sessions (5% ethanol vs. vehicle) were conducted on Monday, Wednesday, and Friday (22-h session length) for 4 weeks, between PD42 and PD68. The ANOVA yielded significant main effects of maternal ethanol exposure and sex. Overall fluid intake was significantly lower in EtOH vs. CTRL rats, and in male vs. female rats. The asterisk sign indicates these effects. Please refer to the text for a full account of the significant main effects and significant interactions yielded by the ANOVAs. The data are expressed as mean ± SEM. For visualization purposes, the data were collapsed across males and females.
FIG. 3 Ethanol intake (g/kg, A–B) and percent ethanol preference (C–D) during intake sessions 1 to 12 in Wistar rats as a function of maternal ethanol exposure throughout gestation until PD7. The dams in the ethanol (EtOH) condition were given 22 h access per day to 10% ethanol (v/v) that was mixed in tap water and sucralose. Control (CTRL) dams were given only tap water and sucralose. From postnatal day 21 (PD 21, weaning) to PD 42 the offspring was exposed to standard, isolated or enriched housing. Two-bottle intake sessions (5% ethanol vs. vehicle) were conducted on Monday, Wednesday, and Friday (22-hour session length) for 4 weeks, between PD42 and PD68. Please refer to the text for a full account of the significant main effects and significant interactions yielded by the ANOVA. The data are expressed as mean ± SEM. For visualization purposes, the data were collapsed across males and females.
ingested during gestation and pregnancy. Moreover, when given water each day (session length: 2 hours, up to 20 mL), EtOH dams consumed significantly more than CTRL dams. These results suggest that EtOH dams experienced dehydration, which could have affected the basal drinking of water, or the renal regulatory responses, of the offspring. In favour of this hypothesis, Godino et al.\textsuperscript{21} reported that PAE was associated with reduced drinking of sodium and water, after furosemide-induced sodium depletion.

It is worth noting that in Fernández et al.\textsuperscript{14} we employed a similar PAE protocol, yet did not observe reduced overall liquid intake in the EtOH offspring. There are, however, some differences between the studies. Notably, in Fernández et al.\textsuperscript{14} the ethanol intake tests began 2 weeks later than in the present study and Sprague-Dawley, instead of Wistar, rats were employed.

Social isolation housing resulted in greater anxiety in the present report, as indicated by avoidance of the white section and motor hyperactivity in the LDB test. This result is consistent with studies indicating that single housing throughout adolescence enhanced anxiety behaviours in an open field\textsuperscript{12} or in the elevated plus maze.\textsuperscript{11} Yet unlike the latter report and others,\textsuperscript{23,24} we did not observe greater ethanol ingestion after social isolation, nor observed synergistic effects between social isolation and PAE. This discrepancy could obey to the fact that we tested our rats for ethanol intake while they were still adolescents, instead of imposing a delay until testing in adulthood, a common methodological element of many studies.
assessing the lingering effects of housing conditions on anxiety response or ethanol intake.

The effect of EE was, compared to that of social isolation, more difficult to predict. There is conflicting evidence concerning the consequences of this treatment on ethanol’s effects and intake.\textsuperscript{13–15} In this study, EE exerted a suppressing effect upon absolute ethanol intake during the first week of testing. Although short lasting, the effect is important given previous suggestions of this treatment having deleterious effects when applied during adolescence.\textsuperscript{13,25} Moreover, the follow-up ANOVAs conducted to analyze the effect of housing conditions in each prenatal group suggested that the protective effect of EE upon alcohol intake was observed in CTRL, but not in PAE, rats.

There were few sex-related differences, although males displayed greater levels of anxiety in the LDB tests, than females. This was associated with greater percent predilection for ethanol and with greater overall levels of fluid intake.

Overall, the present study cements the hypothesis that PAE or isolated housing are associated with an anxiety-like phenotype. It also indicates that the protracted PAE protocol here employed causes a generalized reduction of intake behaviours in Wistar rats. EE was associated with a subtle, yet significant, reduction in absolute ethanol intake, which was more noticeable in CTRL than in PAE rats. Further work is needed to test if EE can be used to reduce alcohol drinking after PAE.

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