NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION
RESEARCH ARTICLE
A. Ieraci, D. G. Herrera / Neuroscience xxx (2020) xxx–xxx

Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood

Alessandro Ieraci *† **and Daniel G. Herrera** ‡

Department of Psychiatry, Weill Medical College of Cornell University, New York, NY 10065, USA

Abstract—Drinking alcohol during pregnancy is particularly detrimental for the developing brain and may cause a broad spectrum of cognitive and behavioral impairments, collectively known as fetal alcohol spectrum disorders (FASDs). While behavioral abnormalities and brain damage have been widely investigated in animal models of FASD, the sex differences in the vulnerability to perinatal ethanol exposure have received less consideration. Here we investigated the long-term behavioral and molecular effects of acute ethanol-binge like exposure during the early postnatal period (equivalent to the third trimester of human pregnancy) in adult male and female mice. CD1 mice received a single ethanol exposure on P7 and were analyzed starting from P60. We found that ethanol-exposed mice showed increased activity in the open field test and in the plus-maze test, regardless of the sex. Interestingly, only ethanol-exposed adult male mice exhibited memory impairment in the water maze and fear-conditioning tests. Remarkably, hippocampal levels of NMDA-R2B were reduced only in ethanol-exposed male, while total BDNF levels were increased in both male and female ethanol-exposed mice. Our data suggest a different susceptibility of early postnatal ethanol exposure in male and female CD1 mice.

Key words: fetal alcohol syndrome, ethanol, brain-derived neurotrophic factor, hippocampus, NMDA, Mice, rodents, memory impairments, hyperactivity, sex difference.

INTRODUCTION

Consuming alcohol during gestation is particularly damaging for the developing brain and may cause fetal alcohol spectrum disorder (FASD) or fetal alcohol syndrome (FAS) depending on the gravity. FASD is an umbrella term describing the multiple effects that can occurs in individuals who are exposed to alcohol during the prenatal period. These effects may include physical, mental, behavioral, and/or learning impairments with potential lifespan consequences. FAS refers to the most severe form of the FASD spectrum, which is diagnosed by the contemporary presence of three features: facial malformations, growth restriction and brain abnormalities (Sokol et al., 2003). FASD is the leading preventable cause of mental retardation in western countries, affecting around 2–5% of the population (Glass et al., 2014). However, despite the effort to inform on the deleterious effects of drinking alcohol during pregnancy, it was estimated that globally around 10% of women consume alcohol regularly during pregnancy (May et al., 2009; Fontaine et al., 2016; Popova et al., 2017). The prevalence of binge drinking (four or more drinks on a single occasion) during pregnancy, which is particularly detrimental to the developing brain, has been estimated to range from 2 to 3% (Bonthius and West, 1990; Popova et al., 2017). Similar to human, animal models of FASD show several behavioral alterations following perinatal ethanol exposure, including hyperactivity, learning and memory deficits, anxiety (Chokroborty-Hoque et al., 2014; Fontaine et al., 2016; Marquardt and Brigman, 2016; Rojas-Mayorquín et al., 2016), and therefore are an useful tool to investigate the biological mechanisms underlying these behavioral impairments. Whereas the behavioral and neurocognitive effects of alcohol exposure during brain development have been considerably investigated (Mattson et al., 2011), the sex differences in vulnerability to perinatal alcohol exposure have received less attention.

*Corresponding author. Address: Laboratory of Neuropsychopharmacology and Functional Neurogenomics, Department of Pharmaceutical Sciences, Università di Milano, Via Balzaretti 9, 20133 Milano, Italy. Fax: +39-02-50318278.
E-mail address: alessandro.ieraci@unimi.it (A. Ieraci).
† Present address: Laboratory of Neuropsychopharmacology and Functional Neurogenomics – Department of Pharmaceutical Sciences, Università di Milano, Milano, Italy.
‡ Present address: Department of Psychiatry, Cambridge Health Alliance, Harvard Medical School Cambridge, MA, USA.

https://doi.org/10.1016/j.neuroscience.2019.11.045
0306-4522/

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

The developing hippocampus is particularly sensitive to the deleterious effects of ethanol during the third trimester and binge-drinking drastically modifies the hippocampal volume, structure and function in both mice and humans (Willoughby et al., 2008; Parnell et al., 2009). Several studies in animal models have shown that postnatal ethanol exposure, which corresponds to the third trimester of human pregnancy (Bayer et al., 1993), enhances apoptosis and neuronal cells loss (Ikonomidou et al., 2000; Ieraci and Herrera, 2006, 2018; Olney, 2014; Joshi et al., 2019); reduces adult hippocampal neurogenesis, decreases dendritic spines density and impairs synaptic plasticity (Ieraci and Herrera, 2007; Gil-Mohapel et al., 2010; De Giorgio and Granato, 2015; Fontaine et al., 2016). Moreover, perinatal ethanol exposure alters the levels of many molecules that play an important role in synaptic activity, mood, learning, and memory such as neurotrophins, glutamate receptors, and astroglial proteins (Guerri et al., 2001; Parks et al., 2008; Samudio-Ruiz et al., 2010; Goodfellow et al., 2016; Boschen and Klintsova, 2017). Notably, the majority of these results were observed in male rodents, and only a few studies have examined sex differences in either FASD humans or rodent models.

An emerging body of research suggests that alcohol exposure during pregnancy differentially affects male and female children. For example, it has been described as a higher incidence of FASD in young boys than in girls, although these sex differences were not manifest later in life (Thanh et al., 2014). FASD males were significantly more likely to be diagnosed with attention-deficit/hyperactivity disorder than FASD females (Herman et al., 2008). In contrast, the association between low levels of alcohol intake during pregnancy and mental disorders was more evident in girls than boys (Sayal et al., 2007). Although relatively few studies have explored sex differences in FASD animal models, some significant sex modifications have been reported. Prenatal ethanol administration reduced the survival of new hippocampal cells in male but not in female rats (Sliwowska et al., 2010; Uban et al., 2010). Hypothalamic–pituitary–adrenal (HPA) axis hyperactivity was described mainly in prenatal ethanol-exposed females but not in males, although the results were depended on the type and the time of the stressors (Weinberg et al., 2008; Fontaine et al., 2016). Ethanol exposure during brain development impaired memory duration but not memory encoding in male rats while having opposite effects in female rats (Kelly et al., 2009). Long-term potentiation was reduced only in ethanol-exposed male rats but not in females (Sickmann et al., 2014). However, other studies were not able to replicate such sex differences (Subbanna et al., 2018; Joshi et al., 2019). Moreover, the molecular mechanisms underlying these sex differences are not yet well understood.

Here we investigated whether early postnatal acute ethanol exposure, which mimics a binge-like alcohol consumption during the third trimester of pregnancy, differentially promotes behavioral and molecular changes in adult male and female mice.

EXPERIMENTAL PROCEDURES

Animals

Pregnant CD1 female mice were purchased from Charles River Laboratories. Postnatal seven-day-old (P7) CD1 mice were injected subcutaneously with 20% ethanol in saline solution delivering 5 g/kg body weight. This protocol of ethanol administration allow to reach a blood alcohol concentration above the toxic threshold of 200–400 mg/dL for several hours (Ieraci and Herrera, 2006, 2018). An equal volume of saline was injected as controls. Mice were weaned at P21 and then separated by sex and maintained in a temperature- and humidity-controlled room with a 12 h light/dark cycle. A total of 107 mice were used for all the studies (64 mice for the behavioral tests; 43 for the body weight measurements and 24 of these for the molecular analysis). All animal procedures were approved by the Institutional Animal Care and Use Committees of Weill Cornell Medical College and were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Behavioral analysis

Behavioral tests were conducted on 9–10 weeks-old mice and the total time necessary to run all the tests was around 6 weeks. Male and female mice were tested on separate days. Behavioral tests were conducted in a blind manner. To minimize possible interference across the different tests, mice were tested from the least stressful to the most stressful test with an interval of one week from one test to the other (Fig. 1A). Mice were tested in the following order: elevated plus-maze, open field, water maze and fear conditioning test (Fig. 1A).

Elevated plus maze. The maze consisted of two open arms (30 × 5 cm), two closed arms (30 × 5 cm with 15 cm high black wall), which were elevated to 60 cm above from the floor. The test is based on the conflict between the aversion to open spaces and the natural exploratory behavior of rodents. Time and number of entries in the open arms correlate with the anxiety-like phenotype, while total entries into all the arms is related with hyperactivity. A single animal was positioned in the center facing an open arm and then allowed to explore the apparatus for 5 min. All the tests were videotaped and total entries into all arms, total entries into the open arms, and total time spent in the open arms were scored.

Open field. For analysis of spontaneous motor activity, single animals were placed in the center of a 50 × 50 cm square apparatus for 5 min. The floor was separated into nine equal squares. Each session was videotaped. Time taken to leave the center, time spent in the center, number of entries in the center, horizontal lines crossed, and rearing activities were measured.

Water maze. Mice were tested in a pool of 100 cm of diameter. Milk powder was added to the water and the temperature was maintained at 20–22 °C. A 10 cm diameter Plexiglass platform was hidden 1 cm below the

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

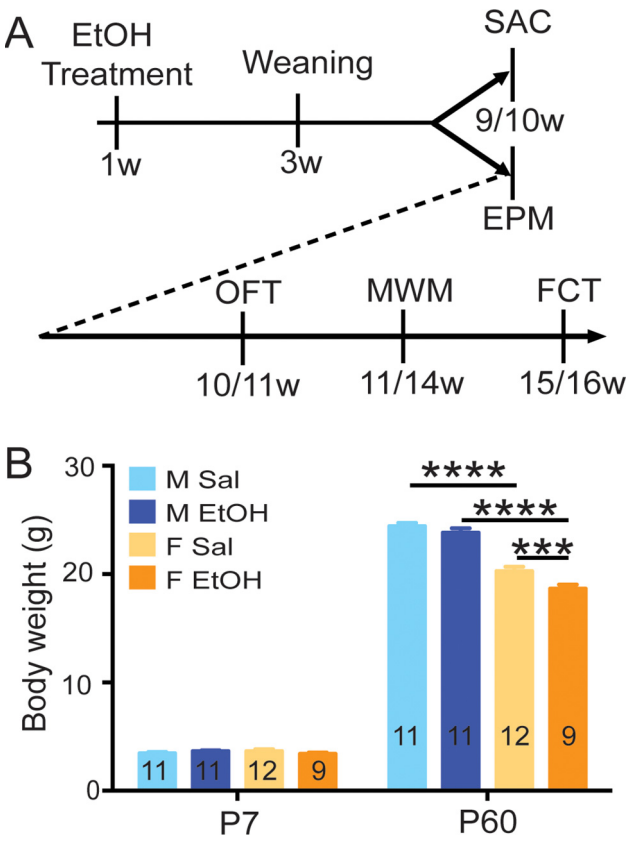

Fig. 1. Post-natal ethanol exposure reduces total body weight increase in female mice. (**A**) Experimental time-table. (**B**) Mice were weighted at P7, before ethanol exposure (5 mg/kg), and at P60, before the sacrifices. Data are presented as mean ± SEM; (n = 9–12 mice per group). Two-way ANOVA followed by Newman–Keuls multiple comparisons analysis. $^{***}P < 0.001$; $^{****}P < 0.0001$. EPM: Elevated Plus Maze; OFT: Open Field Test; MWM: Morris Water Maze; FCT: Fear Conditioning Test; W: weeks; SAC: sacrifice; M: Male; F: Female; Sal: Saline; EtOH; Ethanol.

water surface. Visual cues were positioned on the wall of the room. Each mouse was subjected to four trials per day with an inter-trial interval of about 60 min for seven consecutive days. In each trial, a single mouse was placed into the water, in a different quadrant, facing the wall of the pool. Mice were allowed to search for the platform for a maximum time of 60 s. If mice failed to find the platform, they were gently conducted there. Mice were allowed to stay on the platform for 15 s before being returned to their cage. On day 8 a probe trial, in which the platform was removed from the pool, was performed. Mice were placed in the opposite quadrant to the previous location of the platform and were allowed to swim for 60 s. The total time spent searching for the platform in every single quadrant was manually scored and expressed as a percentage of the total time (60 s). The visible platform task was performed 24 h after the completion of the probe trial. All the visual cues were removed, and the platform positioned randomly in one of the quadrants. Two different trials were performed for each mouse.

Fear conditioning. Mice were individually positioned into the conditioning chamber (Coulbourn Instrument, Allentown, PA). After 120 s of habituation, mice received three tone-shock pairs (tone: 70 db, 2.9 kHz 20 s; foot shock: 0.7 mA, 1 s) with an intertrial interval of 60 s. Sixty seconds after the last shock, animals were returned to their home cage. Twenty-four hours later, mice were positioned in the same chambers (contextual conditioning) and the total freezing time (cessation of all movement other than respiration) was measured for 5 min. Twenty-four hours after the contextual conditioning test, mice were placed in a different chamber. After 120 s of habituation, three tone (70 db, 2.9 kHz 20 s) were delivered at 1-min intervals (cued conditioning). The basal level of freezing in the mice was scored for 120 s in the new chamber before the presentation of the tone (pre-tone), to exclude the possibility that differences in freezing were due to altered activity.

Western blot

Isolated hippocampi were homogenized in ice-cold RIPA buffer (0.15 mM NaCl, 0.05 mM Tris HCl, pH 7.2, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) with Protease Inhibitor Cocktail (Sigma, St. Louis, MO, USA), briefly sonicated and centrifuged at 14,000*g* for 20 min. DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA) was used to measure protein concentration. Proteins were loaded in SDS-PAGE gel and blotted to a PVDF membrane (Immobilon P, Millipore, Bedford, MA, USA). After 1 h of saturation with 5% nonfat milk in TBS-T membranes were incubated overnight at 4 °C with the following primary antibodies: NMDA-R2A (1:1000; Millipore), NMDA-R2B (1:1000; Millipore), GFAP (1:1000; Sigma), alpha-tubulin antibody (1:40,000; Sigma). Membranes were washed several times with TBS-T to remove the excess of primary antibodies and then incubated with secondary antibodies. Peroxidase immunoreactivity bands were revealed by chemiluminescence method (Pierce, Rockford, IL, USA), acquired with a scanner and analyzed by the NIH Image software (Scion, Frederick, MD, USA).

BDNF ELISA

Hippocampal BDNF protein levels were measured using an anti-BDNF sandwich enzyme-linked immunosorbent assay (ELISA) method (BDNF Emax Immunoassay System, Promega, Madison, WI) with recombinant BDNF as a standard (ranging from 7.8 to 500 pg/mL), following the manufacturer's instructions. BDNF levels were adjusted based on the protein concentration (Tornese et al., 2019).

Data analysis

Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). Data are presented as the mean ± standard error of the mean (SEM). Normal distributions and equal variances were verified respectively by the Kolmogorov–Smirnov's test and Bartlett's test. A two-way analysis of variance

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

(ANOVA) followed a Newman–Keuls post hoc correction was used for multiple comparisons statistical analysis.

RESULTS

Post-natal ethanol exposure decreased total body weight in adult female mice

Mice were weighted on P7 and P60 (before the sacrifice). A repeated two-way ANOVA revealed a significant effect for the time ($F_{(1,39)} = 8717$; $P < 0.0001$), groups ($F_{(3,39)} = 43.13$; $P < 0.0001$) and an interaction of the two ($F_{(3,39)} = 48.32$; $P < 0.0001$). All the groups showed a significant increase in weight between P7 and P60 ($P < 0.0001$), with females being smaller than males at P60 ($P < 0.0001$). Interestingly, a post-hoc analysis revealed a significant reduction in total body weight at P60 only in ethanol-exposed females compared to control females ($P < 0.001$), but not in males ($p > 0.05$) (Fig. 1B).

Post-natal ethanol exposure induced hyperactivity and reduced anxiety-like phenotype in adult mice

Activity in the elevated plus-maze was significantly different in ethanol-exposed group and female group compared to control and male groups respectively. Ethanol-treated mice showed an increase in the total number of entries (treatment effect: $F_{(1,60)} = 9.620$; $P = 0.0029$); in the percentage of entries in the open arms (treatment effect: $F_{(1,60)} = 7.848$; $P < 0.007$) and in the percentage of time spent in the open arms (treatment effect: $F_{(1,60)} = 7.327$; $P = 0.009$). Female mice showed a higher number of percentage of entries in the open arms (gender effect: $F_{(1,60)} = 4.928$; $P = 0.0302$) and in the percentage of time spend in the open arm (gender effect: $F_{(1,60)} = 6.169$; $P = 0.016$) (Fig. 2).

Ethanol-treated mice showed an increase in the number of lines crossed (horizontal activity). A two-way ANOVA showed a main effect on treatment ($F_{(1,60)} = 11.6$; $P = 0.0012$) but not in the gender or in the interaction of the two. We also found that females have a tendency to spend less time in the center (gender effect $F_{(1,60)} = 3.74$; $P = 0.058$). No significant differences were found in the number of rearing and in the number of entries in the center (Fig. 3). Altogether these results suggest that postnatal ethanol exposure increased hyperactivity and decreased anxiety-like behavior in both male and female mice.

Post-natal ethanol exposure induced memory impairment only in adult male mice

To determine whether early postnatal subcutaneous alcohol administration could impair spatial learning and memory, adult mice were tested in the Morris water maze using the hidden platform version of this task. Repeated measurements with ANOVA for the latency to find the hidden platform across the training days indicated a significant effect for the groups ($F_{(3,60)} = 6.824$; $P = 0.0005$) and days ($F_{(1,6)} = 83.44$; $P < 0.0001$), without a significant interaction effect of the two (Fig. 4A). All groups learned where the platform was located, but the ethanol-treated mice took a longer time to find the hidden platform than control mice. In

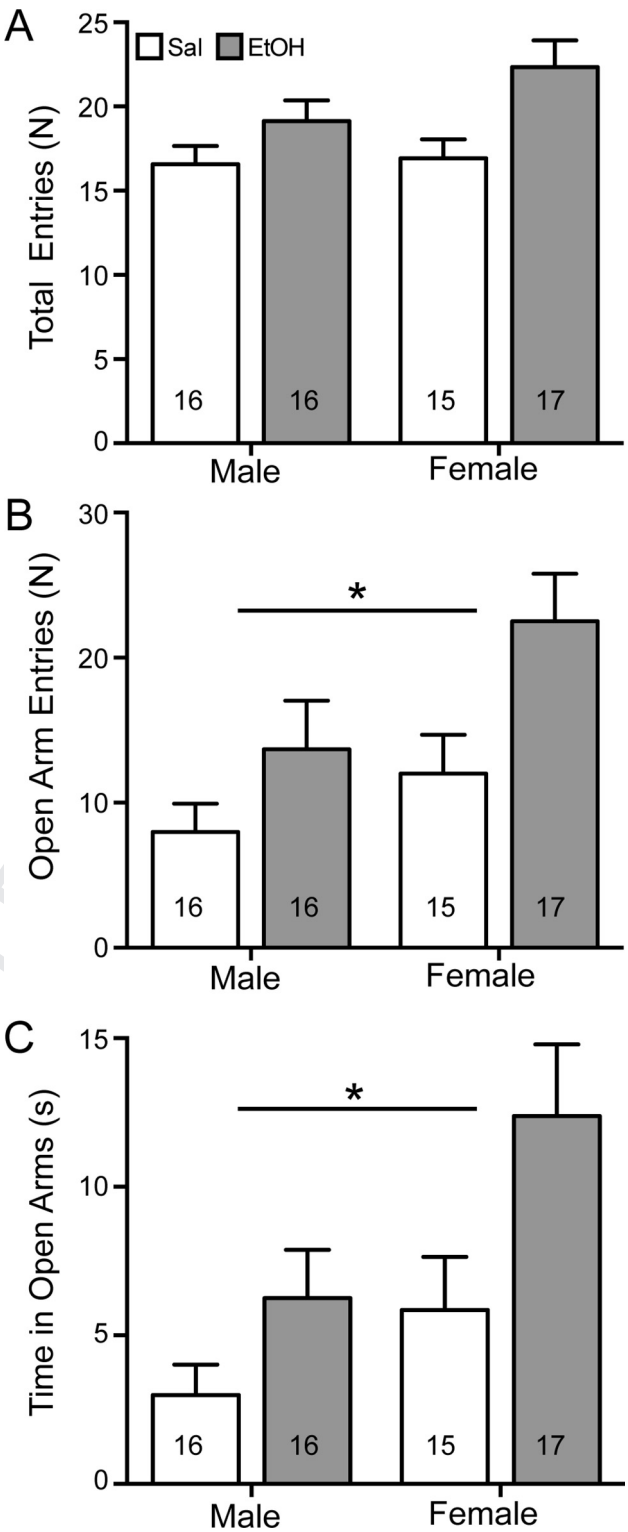

Fig. 2. Effect of postnatal ethanol exposure in the elevated plus maze test in adult mice. Total entries in the arm (**A**), in the open arms (**B**) and the percentage of time spent in the open arm (**C**). Data are presented as mean ± SEM; (n = 15–17 per group). Two-way ANOVA. *$P < 0.05$; **$P < 0.01$. Sal: Saline; EtOH; Ethanol.

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

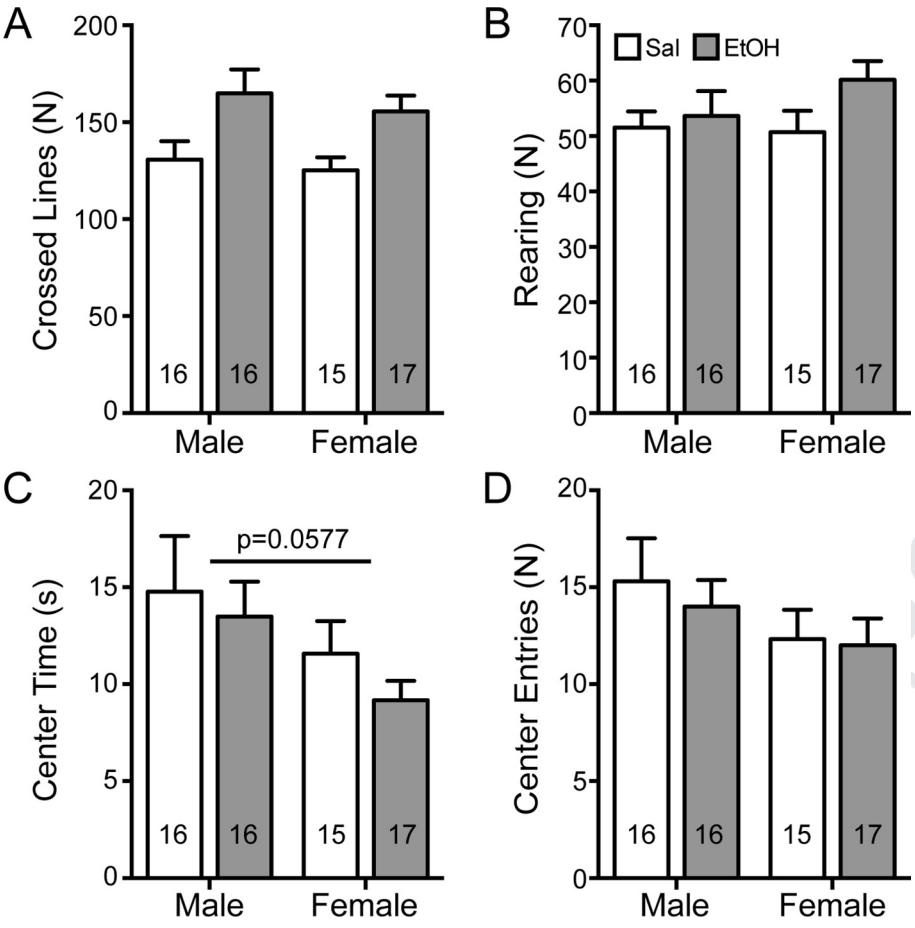

Fig. 3. Effect of postnatal ethanol exposure in the open field test in adult mice. Number of crossed lines (**A**), rearing (**B**), entries in the center (**C**) and time spent in the center (**D**). Data are presented as mean ± SEM; (n = 15–17 per group). Two-way ANOVA. *$P < 0.05$; **$P < 0.01$. Sal: Saline; EtOH; Ethanol.

particular female mice took a significantly longer time on days 2, 3 and 4 (Fig. 4A), while ethanol-exposed male mice took a longer time to reach the platform on day 4 (Fig. 4A). Animals were then tested in the probe trial, the proper criterion to attest the memory acquisition of the water maze test. A two way-ANOVA analysis revealed a main quadrant effect ($F_{(3,240)} = 144$; $p < 0.0001$) and an interaction between groups and quadrants ($F_{(9,240)} = 1.972$; $p = 0.043$) (Fig. 4B). The following Newman–Keuls multiple comparison analysis showed a significant difference only for ethanol-exposed male compared to control, but not for female, for the time spent in the target quadrant ($p < 0.05$). These differences were not due to a possible impairment in motor or visual functioning as there were no significant differences in the latency time to reach a visible platform among the different groups (Fig. 4C).

Given our results from the water maze test, we questioned whether we could detect a similar impairment in another hippocampal-dependent memory task, such as the fear-conditioning test. In this test, mice learn to associate a context (experimental chamber) or a cue (tone) with a foot shock. Context fear conditioning is hippocampus-dependent, while cued fear conditioning is hippocampus-independent. Twenty-four hours after training, ethanol-treated mice froze less than control mice in response to spatial context (treatment effect $F_{(1,60)} = 14.74$; $P = 0.0003$). Moreover, there was also a significant interaction effect for treatment and gender ($F_{(1,60)} = 4.155$; $P = 0.0459$) (Fig. 5A). Interestingly, the further post-hoc analysis revealed a significant difference only in ethanol-exposed male mice compared to control ($p < 0.001$), but not in female ($p > 0.05$). In contrast, there were no differences in hippocampal-independent memory. All mice spent similar freezing time in a novel context both before and during the presentation of the cued (tone), 48 hours after the training (Tone: $F_{(3,120)} = 77.29$; $P < 0.0001$; Groups: $F_{(1,120)} = 0.27$; $P = 0.6$; Interaction $F_{(1,60)} = 0.64$; $P = 0.59$) (Fig. 5B).

Adult hippocampal BDNF levels are augmented in postnatally ethanol-exposed mice

To investigate the possible molecular mechanism(s) underlying the memory impairment specifically revealed in male exposed to ethanol postnatally, we assessed the levels of various proteins implicated in neuronal plasticity, learning and memory.

We measured hippocampal BDNF protein levels by using a BDNF ELISA kit. A two-way ANOVA analysis revealed a significant effect of treatment ($F_{(1,20)} = 11.04$; $p = 0.0034$) and only a trend of gender ($F_{(1,20)} = 4.29$; $p = 0.0515$), but not an interaction of the two ($F_{(1,20)} = 0.33$; $p = 0.856$). The levels of BDNF were overall higher in ethanol-exposed mice compared to control mice, both in male ($p < 0.05$) and in female ($p < 0.05$) (Fig. 6A).

Adult hippocampal NMDA-R2B levels are decreased only in postnatally ethanol-exposed male mice

Next, we assessed the hippocampal protein levels of NMDA-R2A and NMDA-R2B by western blot analysis. We found a significant reduction of NMDA-R2B levels specifically in the HPC of ethanol-exposed male mice compared to male control ($p < 0.05$), but not in female ($p > 0.05$) (treatment: $F_{(1,20)} = 2.719$; $p = 0.115$; gender $F_{(1,20)} = 0.126$; $p = 0.726$; interaction $F_{(1,20)} = 4.153$; $p = 0.0463$) (Fig. 6C). There were no significant differences in the NMDA-R2A protein levels (treatment: $F_{(1,20)} = 0.052$; $p = 0.82$; gender

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

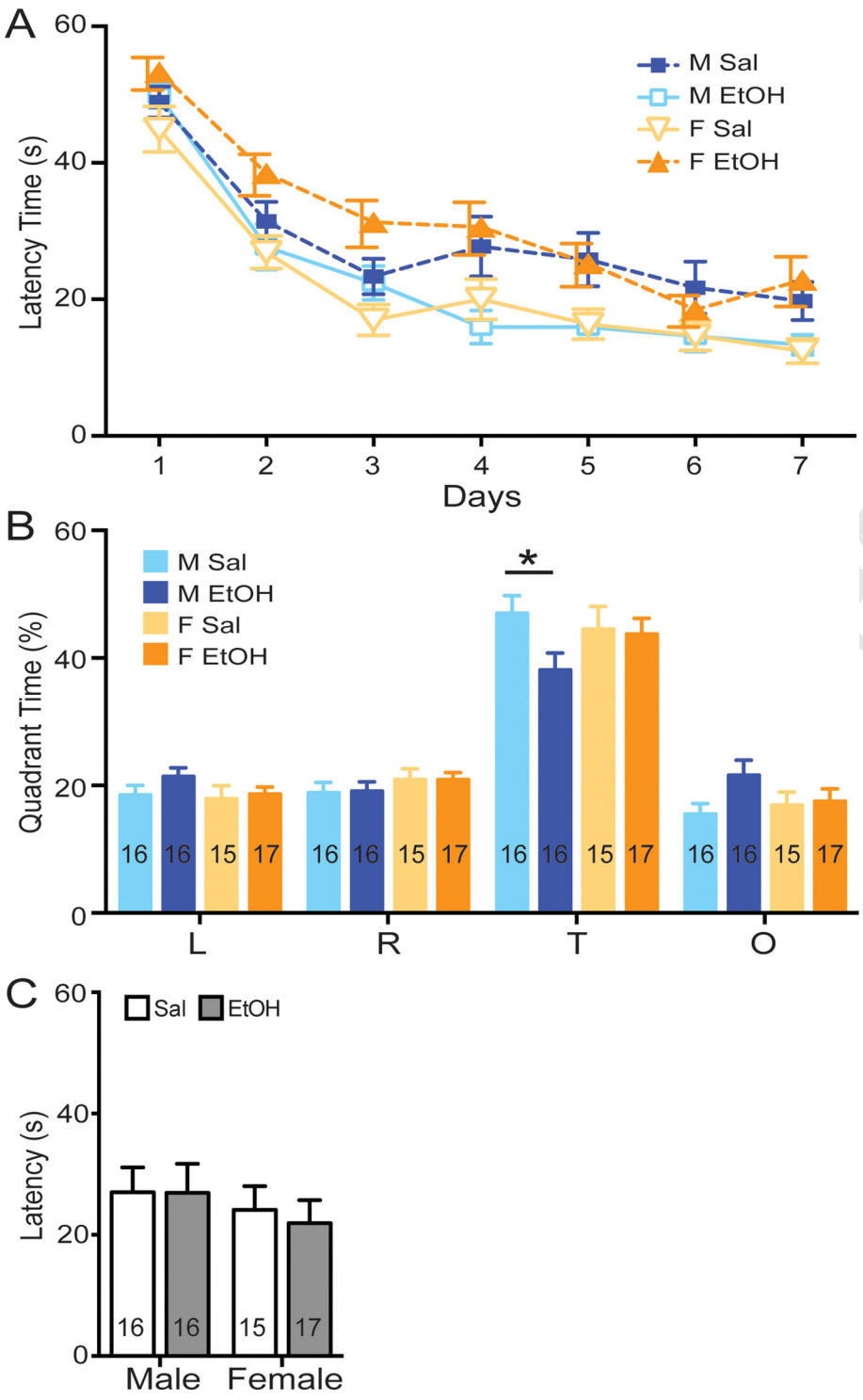

Fig. 4. Effect of postnatal ethanol exposure in the water maze test in adult mice. Water maze learning (**A**), probe test (**B**) and visible platform test (**C**) in control and ethanol postnatal exposed mice. Data are presented as mean ± SEM; (n = 15–17 per group). Two-way ANOVA. $^{**}P < 0.01$. M: Male; F: Female; Sal: Saline; EtOH; Ethanol: T: target; O: opposite; L: left; R: Right.

$F_{(1,20)} = 0.023$; $p = 0.88$; interaction $F_{(1,20)} = 2.54$; $p = 0.124$) (Fig. 6B).

Adult hippocampal GFAP levels are unchanged in postnatally ethanol-exposed mice

Finally, we measured hippocampal GFAP protein levels by western blot. Two-way ANOVA did not reveal any differences in the hippocampal levels of GFAP among all the groups (treatment: $F_{(1,20)} = 0.0009$; $p = 0.976$; gender $F_{(1,20)} = 0.045$; $p = 0.833$; interaction $F_{(1,20)} = 0.27$; $p = 0.609$) (Fig. 6D).

DISCUSSION

The goal of this work was to investigate potential sex differences in behavioral alterations and hippocampal molecular changes in mice following a single binge-like ethanol exposure during the third trimester-equivalent (P7). We found that only ethanol-exposed adult male mice showed significant hippocampal memory impairments measured in the water maze and fear-conditioning tests. Interestingly, these deficits were paralleled by a reduction of hippocampal NMDA-R2B levels in ethanol-exposed males but not in females. On the contrary, hyperactivity and hippocampal BDNF levels were increased in adult mice exposed postnatally to ethanol, regardless of the sex.

A coherent finding both in individuals with FASD and preclinical animal models of FAS is a deficit in spatial learning and memory (Valenzuela et al., 2012; Patten et al., 2014; Marquardt and Brigman, 2016). However, few studies have investigated sex difference in memory performance in adult animals exposed to ethanol perinatally (Goodlett and Peterson, 1995; Johnson and Goodlett, 2002; Wagner et al., 2014; Goodfellow et al., 2016; Subbanna et al., 2018; Xu et al., 2018; Joshi et al., 2019). Here we report that a single exposure to ethanol in the early postnatal period induced a long-lasting hippocampal-dependent spatial memory deficit in adult male mice but not in female mice. Consistent with this study it has been previously reported

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

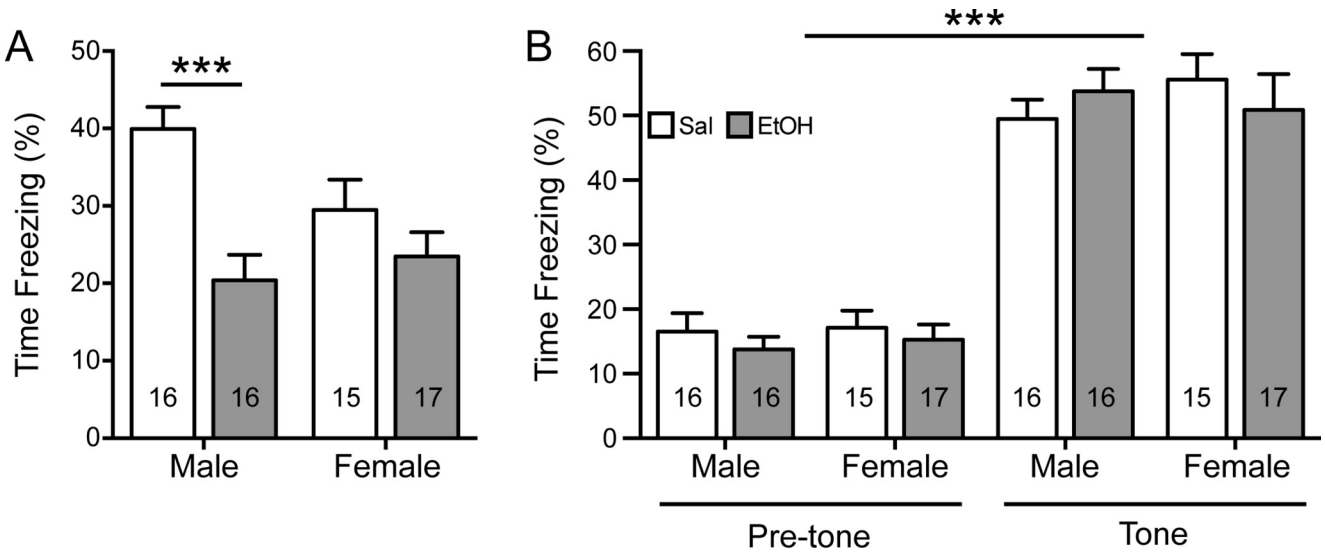

Fig. 5. Effect of postnatal ethanol exposure in the fear-conditioning test in adult mice. Context indicates the freezing levels in the shocking chamber after training (**A**). Pre-tone indicates the freezing levels in a new chamber after training and before the tone testing (**B**). Tone indicates the freezing levels in the new chamber during the presentation of tone (**B**). Context was done 24 h after training and tone was done 48 h after training. Data are presented as mean ± SEM; (n = 15–17 per group). Two-way ANOVA followed by Newman–Keuls multiple comparisons analysis. $^{***}P < 0.001$; $^{****}P < 0.0001$. Sal: Saline; EtOH; Ethanol.

that even 3-days of ethanol exposure (P7-9) induced spatial learning deficits in both juvenile and adult male but not in female rats (Goodlett and Peterson, 1995; Johnson and Goodlett, 2002) while longer postnatal Ethanol exposure (over 5–6 days) promoted memory impairments both in male and female rats (Goodfellow et al., 2016; Xu et al., 2018). In contrast to our results, Wagner et al. found that one or three days of postnatal alcohol exposure resulted in significant spatial learning impairments in the water maze in both male and female adult mice (Wagner et al., 2014). These contrasting results may be due to some experimental differences in ethanol administration, the strain of mice and behavioral analysis between our studies and the previous one. For example, in the Wagner et al. study, mice were given a shorter period on the platform after finding the submerged platform (10 s compared to 15 s in this study) and a shorter inter-trial interval (3 min compared to 60 min in this study). Moreover, the pool used in Wagner et al was larger (125 cm vs 100 cm), yielding a more searchable surface area, which may increase the sensitivity of the task (Wagner et al., 2014). Altogether, these results suggest that after episodes of binge-like ethanol exposure during the third trimester, males are more susceptible to long-lasting memory impairments in less challenging tasks than females. However, future studies will be necessary to clarify whether multiple ethanol injections or the oral administration of ethanol to the mother during pregnancy and the postnatal period, a more physiological model of FASD, are able to produce similar effects in both sexes, or if males are similarly more sensitive than females in conditions of greater ethanol exposure.

NMDA are ionotropic glutamatergic receptors important during brain development, synaptic plasticity and learning and memory processes. It is well known that ethanol is an NMDA antagonist and previous studies have reported that ethanol exposure during pregnancy altered the NMDA subunits expression in the adult brain. In particular NR2B, a dynamic NMDA subunit which is expressed early during brain development and plays an important role in adult brain function is very sensitive to alcohol exposure. Indeed, it has been previously reported that prenatal ethanol exposure reduced the NR2B expression in both juvenile and adult hippocampus (Zhang et al., 2005; Toso et al., 2006; Incerti et al., 2010). Our result, showing that postnatal ethanol administration down-regulated the NR2B expression in the adult male hippocampus, confirms and extends these previous findings, suggesting that even a single binge-like ethanol episode in the postnatal mice brain development, equivalent to the third trimester in human, is sufficient to significantly reduce the NR2B expression, at least, in the adult male hippocampus. Moreover, this decrease of NR2B may partially explain the memory impairments observed only in ethanol-exposed male mice. Indeed, it has been shown that transgenic mice overexpressing NR2B by different strategies show improved learning and memory and enhanced long term potentiation, suggesting an important role for NR2B-containing NMDARs in the adult brain (Tang et al., 1999; von Engelhardt et al., 2008).

BDNF is a neurotrophin which plays a key role in brain development, neuroplasticity, synaptic function, behavior, learning and memory (Ieraci et al., 2016; Mitre et al., 2016; Boschen and Klintsova, 2017; Mallei et al., 2018; von Bohlen und Halbach and von Bohlen und Halbach, 2018). Although several studies have extensively investigated the consequences of ethanol exposure on BDNF levels in the adult brain and the possible contribution of BDNF to FASD pathophysiology has been hypothesized,

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

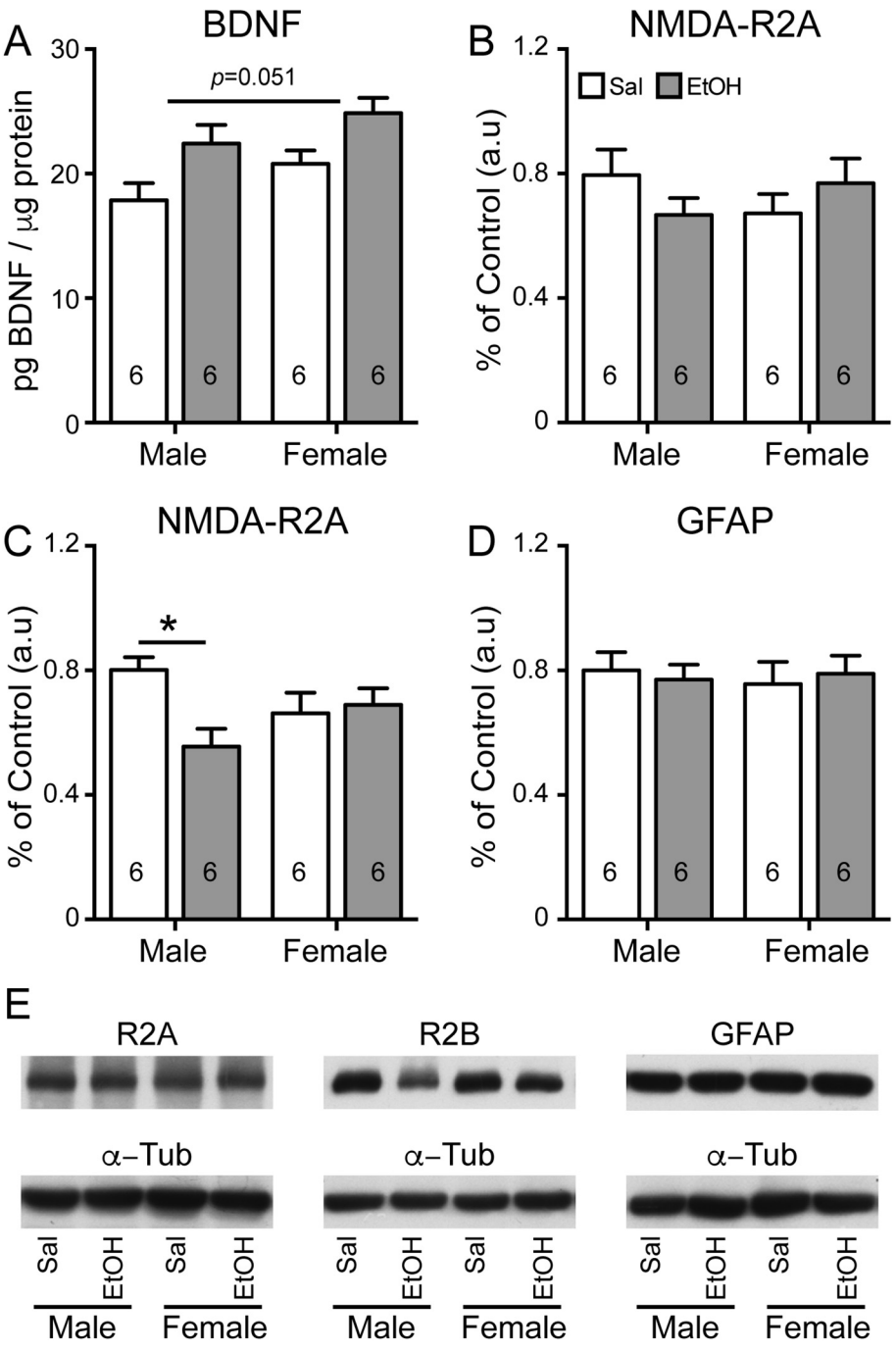

Fig. 6. Effect of postnatal ethanol exposure on hippocampal levels of BDNF, NR2A, NR2B and GFAP. Total hippocampal BDNF protein levels measured by ELISA (**A**). Representative western blot pictures of NR2A (**B**); NR2B (**C**) and GFAP (**D**) proteins from adult hippocampus and relative densitometric quantification (**B**–**D**). (**E**) Representative western blot images for R2A, R2B and GFAP proteins. Data are presented as mean ± SEM; (n = 6 per group). Two-way ANOVA followed by Newman–Keuls multiple comparisons analysis. $^{***}P < 0.001$; $^{****}P < 0.0001$. Sal: Saline; EtOH; Ethanol.

the long-term effects of perinatal ethanol exposure on BDNF levels in the adult brain have been relatively less explored (Davis, 2008; Boschen and Klintsova, 2017). To the best of our knowledge, the present study is the first to investigate the effects of post-natal binge-like ethanol exposure on hippocampal BDNF protein levels in adult CD1 mice. Previously, most of the studies addressing the variation of BDNF expression following EtOH exposure were conducted in rats (Light et al., 2001; Balaszczuk et al., 2013; Boschen et al., 2017) with BDNF levels being analyzed shortly after EtOH exposure, between 2 and 24 h (Light et al., 2001; Balaszczuk et al., 2013). Interestingly, it has been reported that six consecutive days of EtOH treatment (postnatal days 4–9) did not cause long-lasting alterations in hippocampal BDNF mRNA levels in the Long-Evans rat (Boschen et al., 2017). This suggests that the long-lasting BDNF alteration induced by postnatal EtOH administration may be different in mice and rats, or alternatively that postnatal EtOH exposure affects the BDNF protein levels, but not the mRNA levels. In contrast with previous results showing no effect of ethanol exposure during pregnancy on BDNF levels in adult hippocampus of C57BL/6J mice (Caldwell et al., 2008; Boehme et al., 2011), we found that a single ethanol exposure in the early postnatal period promotes an increase of BDNF protein levels in the hippocampus of both male and female mice.

These results may suggest that BDNF is more sensitive to ethanol exposure during postnatal period consistent with the evidence that BDNF expression is low in prenatal brain developing and start to increase in postnatal brain (Maisonpierre et al., 1990). In addition, we found higher levels of hippocampal BDNF in female compared to male CD1 mice. In some way, this might suggest that higher levels of BDNF are protective in females. However, it has been reported that chronic stress in young-adult mice promotes memory impairment only in male but not in female heterozygous BDNF +/− mice (Klug et al., 2012). Moreover, in the BDNF Val66Met, in which the activity-dependent release of BDNF is reduced, prenatal alcohol exposure promotes greater behavioral changes in male compared to female (Bird et al., 2019). Altogether these results suggest that female mice are protect from different

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

adverse stimuli even when BDNF activity/levels are reduced indicating that other protecting mechanisms are probably involved (e.g. hormones).

Here we found that ethanol-exposed animals were hyperactive in two different tests, OFT and EPM, regardless of the sex, suggesting that post-natal ethanol exposure promotes a generalized increase of locomotor activity. In addition, in the EPM test, we found both an augment of time spent and the percentage of entries in the open arms in ethanol-exposed mice compared to control mice. Although increased exploration in the open arms is usually associated with decreased anxiety-like behavior in rodents, this interpretation is complicated by the fact that mice in the present study also demonstrated locomotor hyperactivity, which may be attributable to an increase in novelty-seeking behavior and/or impulsivity, defined respectively as enhanced exploration of new environments and a tendency to act suddenly without foresight for the possible consequences. These observations are in line with previous data showing that hyperactivity, novelty-seeking behavior and impulsivity are some of the features observed in both animal models and FASD patients (Herman et al., 2008; Juárez et al., 2013; Kim et al., 2013; Atalar et al., 2016; Furtado and Roriz, 2016; Rojas-Mayorquín et al., 2016; Lange et al., 2018; Wang et al., 2019). Interestingly, overexpression of BDNF in the forebrain reduced the anxiety-like phenotype in mice (Weidner et al., 2014) and higher levels of BDNF have been found in the hippocampus of the hyperactive serotonin-2C receptor knock-out mice (Hill et al., 2011). High-novelty-seeking behavior in rodents has been associated with higher BDNF level in the hippocampus and cerebellum, compared with low-novelty-seeking animals (Duclot and Kabbaj, 2013; Laricchiuta et al., 2018) and infusion of BDNF in the cerebellum increased exploration and novelty-seeking behavior in mice (Laricchiuta et al., 2018). Moreover, a positive correlation between BDNF serum level and impulsivity has been found in post-traumatic stress disorders and major depressive disorders (Park et al., 2014; Martinotti et al., 2015). Consistent with this observation, we found that BDNF protein levels were increased in the hippocampus of ethanol-exposed mice, suggesting that BDNF might regulate some of these behavioral impairments, although future studies will be required to investigate the BDNF level in other brain regions and its role in the behavioral alteration induced by postnatal ethanol exposure.

In conclusion, the present study has shown that single binge-like alcohol exposure during the brain growth spurt, in the early postnatal period, induces behavior and molecular changes differentially in male and female mice. This highlights the risk of sporadic consumption of alcohol during pregnancy or early in life, given that the brain growth spurt period extends several years after birth in humans. Moreover, future studies examining perinatal drug and alcohol exposure should carefully analyze both males and females to reveal important and significant sex differences that might be useful for the diagnosis and/or therapeutic interventions targeting affected children.

ACKNOWLEDGMENTS

A.I. was supported by the De Witt-Reader's Digest Fellowship and D.G.H. was supported by grants from the National Alliance for Research on Schizophrenia and Depression and the Reader's Digest Foundation. We thank Sebastian Herrera for revising the manuscript.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

AI and DGH designed the study, analyzed the data, and contributed to writing the paper. AI performed the experiments.

REFERENCES

Atalar EG, Uzbay T, Karakaş S (2016) Modeling symptoms of attention-deficit hyperactivity disorder in a rat model of fetal alcohol syndrome. Alcohol Alcohol 51:684–690.

Balaszczuk V, Bender C, Pereno G, Beltramino CA (2013) Binge alcohol-induced alterations in BDNF and GDNF expression in central extended amygdala and pyriform cortex on infant rats. Int J Dev Neurosci 31:287–296.

Bayer SA, Altman J, Russo RJ, Zhang X (1993) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14:83–144.

Bird CW, Baculis BC, Mayfield JJ, Chavez GJ, Ontiveros T, Paine DJ, Marks AJ, Gonzales AL, Ron D, Valenzuela CF (2019) The brain-derived neurotrophic factor VAL68MET polymorphism modulates how developmental ethanol exposure impacts the hippocampus. Genes Brain Behav 18 e12484.

Boehme F, Gil-Mohapel J, Cox A, Patten A, Giles E, Brocardo PS, Christie BR (2011) Voluntary exercise induces adult hippocampal neurogenesis and BDNF expression in a rodent model of fetal alcohol spectrum disorders. Eur J Neurosci 33:1799–1811.

Bonthius DJ, West JR (1990) Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. Alcohol Clin Exp Res 14:107–118.

Boschen KE, Klintsova AY (2017) Neurotrophins in the Brain. In: Vitamins and hormones, pp 197–242.

Boschen KE, McKeown SE, Roth TL, Klintsova AY (2017) Impact of exercise and a complex environment on hippocampal dendritic morphology, Bdnf gene expression, and DNA methylation in male rat pups neonatally exposed to alcohol. Dev Neurobiol 77:708–725.

Caldwell K, Sheema S, Paz R, Samudioruiz S, Laughlin M, Spence N, Roehlk M, Alcon S, Allan A (2008) Fetal alcohol spectrum disorder-associated depression: evidence for reductions in the levels of brain-derived neurotrophic factor in a mouse model. Pharmacol Biochem Behav 90:614–624.

Chokroborty-Hoque A, Alberry B, Singh SM (2014) Exploring the complexity of intellectual disability in fetal alcohol spectrum disorders. Front Pediatr 2:90.

Davis MI (2008) Ethanol-BDNF interactions: still more questions than answers. Pharmacol Ther 118:36–57.

De Giorgio A, Granato A (2015) Reduced density of dendritic spines in pyramidal neurons of rats exposed to alcohol during early postnatal life. Int J Dev Neurosci 41:74–79.

Duclot F, Kabbaj M (2013) Individual differences in novelty seeking predict subsequent vulnerability to social defeat through a differential epigenetic regulation of brain-derived neurotrophic factor expression. J Neurosci 33:11048–11060.

Fontaine CJ, Patten AR, Sickmann HM, Helfer JL, Christie BR (2016) Effects of pre-natal alcohol exposure on hippocampal synaptic plasticity: sex, age and methodological considerations.

Furtado EF, de S Roriz ST (2016) Inattention and impulsivity associated with prenatal alcohol exposure in a prospective cohort study with 11-years-old Brazilian children. Eur Child Adolesc Psychiatry 25:1327–1335.
Gil-Mohapel J, Boehme F, Kainer L, Christie BR (2010) Hippocampal cell loss and neurogenesis after fetal alcohol exposure: insights from different rodent models. Brain Res Rev 64:283–303.
Glass L, Ware AL, Mattson SN (2014) Neurobehavioral, neurologic, and neuroimaging characteristics of fetal alcohol spectrum disorders. Handb Clin Neurol 125:435–462.
Goodfellow MJ, Abdulla KA, Lindquist DH (2016) Neonatal ethanol exposure impairs trace fear conditioning and alters NMDA receptor subunit expression in adult male and female rats. Alcohol Clin Exp Res 40:309–318.
Goodlett CR, Peterson SD (1995) Sex differences in vulnerability to developmental spatial learning deficits induced by limited binge alcohol exposure in neonatal rats. Neurobiol Learn Mem 64:265–275.
Guerri C, Pascual M, Renau-Piqueras J (2001) Glia and fetal alcohol syndrome. Neurotoxicology 22:593–599.
Herman LE, Acosta MC, Chang P-NN (2008) Gender and attention deficits in children diagnosed with a Fetal Alcohol Spectrum Disorder. Can J Clin Pharmacol 15:e411–e419.
Hill RA, Murray SS, Halley PG, Binder MD, Martin SJ, van den Buuse M (2011) Brain-derived neurotrophic factor expression is increased in the hippocampus of 5-HT2C receptor knockout mice. Hippocampus 21:434–445.
Ieraci A, Herrera DG (2006) Nicotinamide protects against ethanol-induced apoptotic neurodegeneration in the developing mouse brain Spong CY, ed. PLoS Med 3 e101.
Ieraci A, Herrera DG (2018) Nicotinamide inhibits ethanol-induced caspase-3 and PARP-1 over-activation and subsequent neurodegeneration in the developing mouse cerebellum. The Cerebellum 17:326–335.
Ieraci A, Herrera DGDG (2007) Single alcohol exposure in early life damages hippocampal stem/progenitor cells and reduces adult neurogenesis. Neurobiol Dis 26:597–605.
Ieraci A, Madaio AI, Mallei A, Lee FS, Popoli M (2016) Brain-derived neurotrophic factor Val66Met human polymorphism impairs the beneficial exercise-induced neurobiological changes in mice. Neuropsychopharmacology 41:3070–3079.
Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Horster F, Tenkova T, Dikranian K, Olney JW (2000) Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science (80-) 287:1056–1060.
Incerti M, Vink J, Roberson R, Wood L, Abebe D, Spong CY (2010) Reversal of alcohol-induced learning deficits in the young adult in a model of fetal alcohol syndrome. Obstet Gynecol 115:350–356.
Johnson TB, Goodlett CR (2002) Selective and enduring deficits in spatial learning after limited neonatal binge alcohol exposure in male rats. Alcohol Clin Exp Res 26:83–93.
Joshi V, Subbanna S, Shivakumar M, Basavarajappa BS (2019) CB1R regulates CDK5 signaling and epigenetically controls Rac1 expression contributing to neurobehavioral abnormalities in mice postnatally exposed to ethanol. Neuropsychopharmacology 44:514–525.
Juárez J, Muñoz-Villegas P, Guerrero-Álvarez Á, Flores-Ocampo P (2013) Assessing impulsivity in prepubertal male rats: a novel device and method to assess motor and cognitive impulsivity. Exp Clin Psychopharmacol 21:315–322.
Kelly SJ, Leggett DC, Cronise K (2009) Sexually dimorphic effects of alcohol exposure during development on the processing of social cues. Alcohol Alcohol 44:555–560.
Kim P, Park JH, Choi CS, Choi I, Joo SH, Kim MK, Kim SY, Kim KC, Park SH, Kwon KJ, Lee J, Han S-H, Ryu JH, Cheong JH, Han JY, Ko KN, Shin CY (2013) Effects of ethanol exposure during early pregnancy in hyperactive, inattentive and impulsive behaviors and MeCP2 expression in rodent offspring. Neurochem Res 38:620–631.
Klug M, Hill RA, Choy KHC, Kyrios M, Hannan AJ, van den Buuse M (2012) Long-term behavioral and NMDA receptor effects of young-adult corticosterone treatment in BDNF heterozygous mice. Neurobiol Dis 46:722–731.
Lange S, Rehm J, Anagnostou E, Popova S (2018) Prevalence of externalizing disorders and Autism Spectrum Disorders among children with Fetal Alcohol Spectrum Disorder: systematic review and meta-analysis. Biochem Cell Biol 96:241–251.
Laricchiuta D, Andolina D, Angelucci F, Gelfo F, Berretta E, Puglisi-Allegra S, Petrosini L (2018) Cerebellar BDNF promotes exploration and seeking for novelty. Int J Neuropsychopharmacol 21:485–498.
Light KE, Ge Y, Belcher SM (2001) Early postnatal ethanol exposure selectively decreases BDNF and truncated TrkB-T2 receptor mRNA expression in the rat cerebellum. Brain Res Mol Brain Res 93:46–55.
Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD (1990) NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 5:501–509.
Mallei A, Ieraci A, Popoli M (2018) Chronic social defeat stress differentially regulates the expression of *BDNF* transcripts and epigenetic modifying enzymes in susceptible and resilient mice. World J Biol Psychiatry:1–12.
Marquardt K, Brigman JL (2016) The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: Insights from rodent models. Alcohol 51:1–15.
Martinotti G, Sepede G, Brunetti M, Ricci V, Gambi F, Chillemi E, Vellante F, Signorelli M, Pettorruso M, De Risio L, Aguglia E, Angelucci F, Caltagirone C, Di Giannantonio M (2015) BDNF concentration and impulsiveness level in post-traumatic stress disorder. Psychiatry Res 229:814–818.
Mattson SN, Crocker N, Nguyen TT (2011) Fetal alcohol spectrum disorders: neuropsychological and behavioral features. Neuropsychol Rev 21:81–101.
May PA, Gossage JP, Kalberg WO, Robinson LK, Buckley D, Manning M, Hoyme HE (2009) Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. Dev Disabil Res Rev 15:176–192.
Mitre M, Mariga A, Chao MV (2016) Neurotrophin signalling: novel insights into mechanisms and pathophysiology. Clin Sci 131:13–23.
Olney JW (2014) Focus on apoptosis to decipher how alcohol and many other drugs disrupt brain development. Front Pediatr 2:81.
Park Y-M, Lee B-H, Um TH, Kim S (2014) Serum BDNF levels in relation to illness severity, suicide attempts, and central serotonin activity in patients with major depressive disorder: a pilot study. Schmidt U, ed. PLoS One 9 e91061.
Parks EA, McMechan AP, Hannigan JH, Berman RF (2008) Environmental enrichment alters neurotrophin levels after fetal alcohol exposure in rats. Alcohol Clin Exp Res 32:1741–1751.
Parnell SE, O'Leary-Moore SK, Godin EA, Dehart DB, Johnson BW, Allan Johnson G, Styner MA, Sulik KK (2009) Magnetic resonance microscopy defines ethanol-induced brain abnormalities in prenatal mice: effects of acute insult on gestational day 8. Alcohol Clin Exp Res 33:1001–1011.
Patten AR, Fontaine CJ, Christie BR (2014) A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. Front Pediatr 2:93.
Popova S, Lange S, Probst C, Gmel G, Rehm J (2017) Global prevalence of alcohol use and binge drinking during pregnancy and fetal alcohol spectrum disorder. Biochem Cell Biol.
Rojas-Mayorquín AE, Padilla-Velarde E, Ortuño-Sahagún D (2016) Prenatal alcohol exposure in rodents as a promising model for the study of ADHD molecular basis. Front Neurosci 10:565.
Samudio-Ruiz SL, Allan AM, Sheema S, Caldwell KK (2010) Hippocampal N-methyl-d-aspartate receptor subunit expression profiles in a mouse model of prenatal alcohol exposure. Alcohol Clin Exp Res 34:342–353.
Sayal K, Heron J, Golding J, Emond A (2007) Prenatal alcohol exposure and gender differences in childhood mental health

Please cite this article in press as: Ieraci A, Herrera DG. Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. Neuroscience (2020), https://doi.org/10.1016/j.neuroscience.2019.11.045

problems: a longitudinal population-based study. Pediatrics 119: e426–e434.

Sickmann HMM, Patten ARR, Morch K, Sawchuk S, Zhang C, Parton R, Szlavik L, Christie BRR (2014) Prenatal ethanol exposure has sex-specific effects on hippocampal long-term potentiation. Hippocampus 24:54–64.

Sliwowska JH, Barker JM, Barha CK, Lan N, Weinberg J, Galea LA (2010) Stress-induced suppression of hippocampal neurogenesis in adult male rats is altered by prenatal ethanol exposure. Stress 13:301–313.

Sokol RJ, Delaney-Black V, Nordstrom B (2003) Fetal alcohol spectrum disorder. JAMA 290:2996–2999.

Subbanna S, Nagre NN, Shivakumar M, Joshi V, Psychoyos D, Kutlar A, Umapathy NS, Basavarajappa BS (2018) CB1R-mediated activation of caspase-3 causes epigenetic and neurobehavioral abnormalities in postnatal ethanol-exposed mice. Front Mol Neurosci 11:45.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic enhancement of learning and memory in mice. Nature 401:63–69.

Thanh NX, Jonsson E, Salmon A, Sebastianski M (2014) Incidence and prevalence of fetal alcohol spectrum disorder by sex and age group in Alberta, Canada. J Popul Ther Clin Pharmacol 21: e395–e404.

Tornese P, Sala N, Bonini D, Bonifacino T, La Via L, Milanese M, Treccani G, Seguini M, Ieraci A, Mingardi J, Nyengaard JR, Calza S, Bonanno G, Wegener G, Barbon A, Popoli M, Musazzi L (2019) Chronic mild stress induces anhedonic behavior and changes in glutamate release, BDNF trafficking and dendrite morphology only in stress vulnerable rats. The rapid restorative action of ketamine. Neurobiol Stress 10 100160.

Toso L, Poggi SH, Roberson R, Woodard J, Park J, Abebe D, Spong CY (2006) Prevention of alcohol-induced learning deficits in fetal alcohol syndrome mediated through NMDA and GABA receptors. Am J Obstet Gynecol 194:681–686.

Uban KA, Sliwowska JH, Lieblich S, Ellis LA, Yu WK, Weinberg J, Galea LAM (2010) Prenatal alcohol exposure reduces the proportion of newly produced neurons and glia in the dentate gyrus of the hippocampus in female rats. Horm Behav 58:835–843.

Valenzuela CF, Morton RA, Diaz MR, Topper L (2012) Does moderate drinking harm the fetal brain? Insights from animal models. Trends Neurosci 35:284–292.

von Bohlen und Halbach O, von Bohlen und Halbach V (2018) BDNF effects on dendritic spine morphology and hippocampal function. Cell Tissue Res 373:729–741.

von Engelhardt J, Doganci B, Jensen V, Hvalby Ø, Göngrich C, Taylor A, Barkus C, Sanderson DJ, Rawlins JNP, Seeburg PH, Bannerman DM, Monyer H (2008) Contribution of hippocampal and extra-hippocampal NR2B-containing NMDA receptors to performance on spatial learning tasks. Neuron 60:846–860.

Wagner JL, Zhou FC, Goodlett CR (2014) Effects of one- and three-day binge alcohol exposure in neonatal C57BL/6 mice on spatial learning and memory in adolescence and adulthood. Alcohol 48:99–111.

Wang R, Shen Y-L, Hausknecht KA, Chang L, Haj-Dahmane S, Vezina P, Shen R-Y (2019) Prenatal ethanol exposure increases risk of psychostimulant addiction. Behav Brain Res 356:51–61.

Weidner KL, Buenaventura DF, Chadman KK (2014) Mice over-expressing BDNF in forebrain neurons develop an altered behavioral phenotype with age. Behav Brain Res 268:222–228.

Weinberg J, Sliwowska JH, Lan N, Hellemans KGC (2008) Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. J Neuroendocr 20:470–488.

Willoughby KA, Sheard ED, Nash K, Rovet J (2008) Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. J Int Neuropsychol Soc 14:1022–1033.

Xu W, Hawkey AB, Li H, Dai L, Brim HH, Frank JA, Luo J, Barron S, Chen G (2018) Neonatal ethanol exposure causes behavioral deficits in young mice. Alcohol Clin Exp Res 42:743–750.

Zhang TA, Hendricson AW, Wilkemeyer MF, Lippmann MJ, Charness ME, Morrisett RA (2005) Synergistic effects of the peptide fragment D-NAPVSIPQ on ethanol inhibition of synaptic plasticity and NMDA receptors in rat hippocampus. Neuroscience 134:583–593.

(Received 23 July 2019, Accepted 26 November 2019)
(Available online xxxx)