Received: 25 November 2019 | Revised: 2 February 2020 | Accepted: 13 February 2020

DOI: 10.1002/jdn.10017

RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF DEVELOPMENTAL NEUROSCIENCE

WILEY

Ethanol exposure during the brain growth spurt affects social behavior and increases susceptibility to acute ethanol effects during adolescence in male mice

Kelly C. Demarque[1] | **Ana C. Dutra-Tavares**[2] | **André L. Nunes-Freitas**[2] | **Ulisses C. Araújo**[2] | **Alex C. Manhães**[2] | **Yael Abreu-Villaça**[2] | **Cláudio C. Filgueiras**[2] | **Anderson Ribeiro-Carvalho**[3]

[1]Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

[2]Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

[3]Departamento de Ciências, Faculdade de Formação de Professores da Universidade do Estado do Rio de Janeiro, São Gonçalo, Brazil

Correspondence
Anderson Ribeiro-Carvalho, Departamento de Ciências, Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro, Rua Dr. Francisco Portela, 1470, Patronato, São Gonçalo, RJ 24435-005, Brazil.
Email: ribeiro_carvalho@yahoo.com.br

Funding information
This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and fellowships by Sub-reitoria de Pós-graduação e Pesquisa da Universidade do Estado do Rio de Janeiro (SR2-UERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and FAPERJ

Abstract

The brain is particularly vulnerable to ethanol effects during its growth spurt. Outcomes of early ethanol exposure such as hyperactivity have been extensively investigated; however, persons with fetal alcohol spectrum disorder frequently have social impairments and are heavy drinkers. Despite that, scant information is available regarding the neurobiological basis of these latter behavioral issues. Here, Swiss mice exposed to ethanol (Etoh, 5 g/kg i.p., alternate days) or saline during the brain growth spurt [postnatal day (PN) 2 to 8] were used to assess social behavior after an ethanol challenging during adolescence. At PN39, animals were administered with a single ethanol dose (1 g/Kg) or water by gavage and were then evaluated in the three-chamber sociability test. We also evaluated corticosterone serum levels and the frontal cerebral cortex serotoninergic system. Etoh males showed reductions in sociability. Ethanol challenging reverted these alterations in social behavior, reduced corticosterone levels, and increased the 5-HT_2 receptor binding of male Etoh mice. No alterations were observed in 5-HT and 5-HIAA contents. These data support the idea that ethanol exposure during the brain growth spurt impacts social abilities during adolescence, alters ethanol reexposure effects, and suggests that stress response and serotoninergic system play roles in this phenomenon.

KEYWORDS

corticosterone, development, serotoninergic system, social behavior

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BCA kit, bicinchoninic acid kit; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; FASD, fetal alcohol spectrum disorder; FPLSD, Fisher's protected least significant difference tests; HPA, hypothalamic–pituitary–adrenal axis; HPLC, high-performance liquid chromatography; i.p., intraperitoneal injection; PN, postnatal days; rANOVA, repeated measures analyses of variance; uANOVA, univariate analyses of variance.

1 | INTRODUCTION

Ethanol is a potent teratogenic agent and, despite the known harmful effects on the offspring's health, ethanol consumption prevalence during pregnancy has not changed considerably over the past two decades (CDC, 2009; Denny, Acero, Naimi, & Kim, 2019). Indeed, 13%–20% of pregnant women consume alcoholic beverages at some point during gestation (Tatiana et al., 2013). Fetal exposure to alcohol remains a major preventable cause of infant morbidity. Early exposure to alcohol is associated with a multitude of long-term adverse effects collectively named under the umbrella term fetal alcohol spectrum disorder (FASD) (Marquardt & Brigman, 2016). Hyperactivity, deficits in cognition and in social behavior are among the most common adverse behavioral outcomes observed in children with FASD (Niccols, 2007).

Alterations in the social domain have a great impact on personal life. FASD individuals have problems with social cues and display difficulties in forming and maintaining reciprocal friendships (Rasmussen, Becker, Mclennan, Urichuk, & Andrew, 2011; Roebuck, Mattson, & Riley, 1999). FASD individuals also have increased ethanol-related problems later in life, including ethanol-induced social conflicts and aggressive behavior (Momino et al., 2012; Niccols, 2007). Evidence that developmental ethanol exposure is associated with deficient social behavior later in life (Momino et al., 2012) and that ethanol consumption during adolescence per se increases social disruptive behavior (Diestelkamp et al., 2015) raises the possibility that individuals with FASD show a worsened social deficit as a consequence of ethanol exposure during adolescence. This problem is compounded by the fact that ethanol exposure during development is also associated with the development of alcohol use and abuse during adolescence (Alati et al., 2008; Malone, Mcgue, & Iacono, 2011), a period when exploratory ethanol use typically begins (Spear, 2000).

Scant information exists concerning the neural mechanisms implicated in the behavioral consequences of early ethanol exposure on the social domain. The frontal cortex is decisively associated with the cognitive processes of social behavior (Hamilton et al., 2014). Serotoninergic abnormalities seem to be a contributing factor regarding social impairment. Imaging studies demonstrate reductions in serotonin transporters in the cortex of individuals with autism (Makkonen, Riikonen, Kokki, Airaksinen, & Kuikka, 2008; Nakamura et al., 2010). In addition, postmortem studies indicate an increase in the number of cortical serotonergic terminals in individuals diagnosed with autism (Azmitia, Singh, & Whitaker-Azmitia, 2011) as well as an association between reduced 5-HT_{2A} receptor binding and abnormal social communication in Asperger's syndrome patients (Murphy et al., 2006). Long-term serotonergic neuronal deficits are also caused by prenatal alcohol exposure in mice. Sari and Zhou (2004) demonstrated that ethanol exposure early in gestation reduces the number and delays the migration of serotonergic neurons in the raphe nuclei of these animals (Sari & Zhou, 2004). So, long-term alterations in serotonergic neurotransmission may play a role in early alcohol exposure-evoked social impairment. Interestingly, Hofmann, Ellis, Yu, and Weinberg (2007) demonstrated that prenatal ethanol exposure has differential long-term effects on the 5-HT_{2A}-mediated response in the hypothalamic–pituitary–adrenal axis (Hofmann et al., 2007). Since alterations in hypothalamic–pituitary–adrenal (HPA) axis functions are associated with the development of antisocial behavior in children, the social behavior impairment generated by early ethanol exposure may also be influenced by the direct modulation of HPA axis activity by ethanol. In fact, early ethanol exposure alters the development of the HPA axis (Hellemans et al., 2010, https://doi.org/10.1016/j.neubiorev.2009.06.004). Furthermore, social stress is a major trigger in the initiation and maintenance of ethanol consumption (Morrow, Porcu, Boyd, & Grant, 2006). Considering the aforementioned, the effect of ethanol consumption on HPA function in FASD individuals constitutes an important issue to be investigated.

The outcomes of developmental ethanol exposure are highly dependent on the window of time within which exposure occurs. Particularly, ethanol exposure during the period of the brain growth spurt has significant deleterious effects. This period is characterized by rapid increases in brain size, associated with intense neurogenesis, neural cell migration, and synaptogenesis (Bandeira, Lent, & Herculano-Houzel, 2009). In humans, the brain growth spurt occurs during the third trimester of gestation, while, in rodents, it happens during the first 10 postnatal days (PN). Animal models demonstrate that ethanol exposure during this period induces cell death and reduced neurogenesis (Gil-Mohapel, Boehme, Kainer, & Christie, 2010; Olney et al., 2002) and evokes adverse behavioral outcomes characteristic of FASD, such as locomotor hyperactivity and memory/learning deficits (Abreu-Villaça et al., 2018; Filgueiras, Krahe, & Medina, 2010; Nunes et al., 2011). Ethanol exposure during the brain growth spurt also has epidemiological relevance. Despite evidence that most women stop drinking when they verify that they are pregnant, the consumption of ethanol by pregnant women during the third trimester of gestation is frequent (Ethen et al., 2009). In

Highlights

Ethanol exposure during the brain growth spurt reduces adolescent male sociability. Ethanol exposure during the brain growth spurt alters ethanol effects later in life. Ethanol re-exposure during adolescence reverted the alterations in social behavior. Stress response and the serotoninergic system play roles in this phenomenon.

the present study, we assessed the effects of ethanol exposure during the brain growth spurt period on social behavior in male and female mice during adolescence. In addition, we investigated the effects of acute ethanol reexposure at this age. We also evaluated the effects on corticosterone serum levels and serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the frontal cerebral cortex. Finally, we assessed the 5HT2 receptor and presynaptic 5HT transporter bindings. Our hypothesis is that the brain growth spurt is a critical period concerning ethanol-induced deficits in social behavior and that ethanol reexposure during adolescence may mitigate these deficits. Considering that social anxiety disorder as a risk factor for alcohol use disorders (Buckner & Turner, 2009), if the present hypothesis is corroborated, alterations on a social domain could represent a relevant factor for the development of alcohol use and abuse during adolescence in FASD individuals.

2 | MATERIALS AND METHODS

All experiments were carried out under institutional approval of the Animal Care and Use Committee of the Universidade do Estado do Rio de Janeiro (CEUA/006/2018). All Swiss mice were bred and maintained in our animal facility. The animal colony was kept under controlled temperature (around 21°C) on a 12:12 hr light/dark cycle (lights on at 1:00 a.m.). Access to food and water was unrestricted. All behavioral tests were carried out in a sound-attenuated room adjoining the animal facility. The day of parturition was considered postnatal Day 1 (PN1). Ethanol (Etoh, 5 g/kg, i.p., 25%, v/v) or saline (Cont) was injected in the pups every other day from PN2 to PN8. We have previously demonstrated that this ethanol exposure model generates blood ethanol concentrations within the range that a human fetus would be exposed to after the maternal ingestion of a moderate to a heavy dose of ethanol (about 170 mg/dl (Filgueiras, Ribeiro-Carvalho, Nunes, Abreu-Villaça, & Manhães, 2009). Body mass was assessed every other day from PN2 to PN8 and we also evaluated the period of teeth eruption as well as ears and eye openings. We only used litters that, at birth, had 8 to 12 pups. Whenever applicable, data from males or females of the same litter were averaged within each group to minimize litter effects and avoid over-sampling. At weaning (PN23), animals were separated by sex in groups of two to five mice and allowed free access to food and water. At PN39, the behavioral tests were performed during the lights-on period (8:00–10:00 hr).

2.1 | Three-chamber sociability test

The apparatus is a rectangular clear plexiglass box divided into three chambers. The chambers are 12 cm (width) × 26 cm (length) × 50 cm (height) with 10-cm wide openings into the two end chambers. The chambers are isolated with two dividing plexiglass walls. In each side chamber, there is a cylinder made of grid bars (1-cm distance between adjacent grid bars). The social side was the side in which one mouse ("unfamiliar") was placed and the other was an empty cylinder. The "unfamiliar" mouse was of the same sex and age of the experimental animal and the social side was randomly assigned. The experimental mouse was habituated in the middle chamber for 5 min and then allowed to explore the entire apparatus for 10 min. The percent time in the social side ((social side/social side + empty side) * 100) and the latency for a first entry into the social side were measured. The mouse was considered to be in a given chamber when its head and four paws had entered the chamber. The chambers were thoroughly cleaned with 35% ethanol between tests. To reduce the likelihood that the ethanol odor would affect mice behavior, after the ethanol solution, the chambers were washed with water and dried out before the next testing session. One hour before the test, the experimental animals were administered with a low ethanol dose (1 g/Kg) or water by gavage, a procedure that resulted in four experimental groups: animals from the Cont (Cont–water, $n = 8$ for females and $n = 7$ for males) and Etoh groups that received water (Etoh–water, $n = 7$ for females and $n = 7$ for males) and animals from the Cont (Cont–etoh, $n = 9$ for females and $n = 6$ for males) and Etoh (Etoh–etoh, $n = 8$ for females and $n = 7$ for males) groups that received ethanol. The low ethanol dose was chosen because it did not induce nonspecific motor effects (such as locomotor stimulation) and seemed to affect social behavior in mice (Favoretto, Macedo, & Quadros, 2017; López-Cruz et al., 2016). Two hours after the gavage, blood and frontal cerebral cortices were collected and stored at −80°C for hormone and serotoninergic evaluation.

2.2 | Serum corticosterone levels

Blood samples were centrifuged (3,500× g, 10 min) to obtain serum, which was stored at −80°C. Total corticosterone levels were measured using a commercially available corticosterone EIA kit (Corticosterone Elisa Kit ab108821, Abcam) in accordance with the instructions provided by the manufacturer.

2.3 | Serotonergic evaluation

2.3.1 | High-performance liquid chromatography analysis

5HT and 5HIAA contents were analyzed in the frontal cerebral cortex by High-Performance Liquid Chromatography

(HPLC), using a fluorescence detector (Shimadzu Prominence LC-20AT, RF-20A detector). Our methodology was adapted from Yoshitake et al. (2004). In summary, each tissue was thawed and homogenized with 200 µl of HClO4 (0.1 M) plus sodium ascorbate (20 µM) (Precellys, BERTIN Technologies, Montigny-le-Bretonneux, France), diluted in 4 vols of Milli-Q water and centrifuged at 5,200× *g* for 30 min (4°C). The supernatant was filtrated in a 0.22 µm PVDF filter (Millipore) and 5HT and 5HIAA derivatization reaction was accomplished using 10 µl of standard solution or tissue sample with 50 µl of HCl 0.1 M plus glycine 0.1 M and more 50 µl of benzylamine derivatization reagent solution (3 M benzylamine/0.3 M CAPS buffer pH 10.0/60 µM potassium hexacyanoferrate III/methanol, proportion of 2:6:2:24, v/v). After 3 min in ambient temperature, a 20 µl of portion of the final reaction mixture was injected into the chromatograph. The detector wavelengths of excitation/emission were 345 nm/480 nm. The mobile phase was a gradient formed by acetonitrile and acetate buffer (20 mM, pH = 4.5) with EDTA (0.5 mM). We used a 0.1 ml/min flow rate and column temperature of 30°C. A reverse-phase column (150 mm × 2.6 mm i.d., packed with C 18 silica, 3 µm) was used for separation. 5HT and 5HIAA concentrations were calculated based on respective standard calibration curves and corrected by diluting factors. Results were converted and expressed as nmoL/g of the brain tissue. The precision of the method is less than 10% *SD* and the inferior limit of quantification is 0.1 pmoL per injection.

2.3.2 | Binding analysis of $5HT_2$ receptors, and 5HT transporter

The binding method has been described in detail in previous papers (Lima et al., 2011, 2013). Briefly, tissues were thawed and homogenized (Ultra-Turrax T10 basic, IKA, São Paulo, SP) in 20 volumes of ice-cold 50 mM Tris–HCl (pH 7.4), the homogenates were sedimented at 40,000× *g* for 10 min, and the supernatant solution was discarded. The membrane pellet was resuspended (Ultra-Turrax) in the previous volume of buffer, resedimented, and the pellet was resuspended in 10 volumes (based on the mass of the tissue) of the same buffer using a smooth glass homogenizer fitted with a Teflon pestle. Aliquots were withdrawn for the measurements of binding and membrane protein evaluation (Bicinchoninic Acid kit). The serotonergic biomarkers were evaluated using 0.4 nM [3H] ketanserin for 5HT2 receptors and 85 pM [3H]paroxetine for presynaptic 5HT transporter. For 5HT2 receptors, incubations lasted for 15 min at 37ºC in 50 nM Tris (pH 7.4) and specific binding was displaced with 10 µM methysergide. For binding to the presynaptic 5HT transporter, incubations lasted for 120 min at 20ºC in a buffer consisting of 50 mM Tris (pH 7.4), 120 mM NaCl, and 5 mM KCl; 100 µM 5HT was used to displace specific binding. Labeled membranes were trapped by rapid vacuum filtration onto glass fiber filters that were presoaked in 0.15% polyethyleneimine. Data were obtained by calculating the specific binding per mg of membrane protein.

TABLE 1 Body mass (g)

	Cont	Etoh	% of Difference
PN2	2.1 ± 0.06	2.0 ± 0.04	4.8
PN4	3.0 ± 0.11	2.7 ± 0.09*	10.0
PN6	4.2 ± 0.16	3.7 ± 0.12*	11.9
PN8	5.6 ± 0.21	4.9 ± 0.13**	12.5
At weaning	16.2 ± 0.83	14.6 ± 0.58	9.9
PN39	33.1 ± 0.80	31.3 ± 0.80	5.5

Note: % of Reduction was calculated by the percent change between Cont and Etoh groups.

*$p < .05$; **$p < .01$ versus Cont.

2.4 | Materials

Bovine albumin, BCA kit, serotonin, methysergide, and polyethyleneimine were purchased from Sigma Chemical Co. (St. Louis, MO). Radioisotopically labeled compounds were purchased from PerkinElmer Life Sciences (Boston, MA). VETEC Química Fina Ltda (Rio de Janeiro, RJ) was the source for all other reagents.

2.5 | Statistics

Body mass data were evaluated using repeated measuares analyses of variance (rANOVA). Exposure to ethanol during the brain growth spurt was considered the between-subjects factor. The day was considered the within-subjects factor. A separated univariate ANOVA (uANOVA) was performed for body mass at the behavioral test day; in this case, Sex was also used as a between-subjects factor. For the percent time in the social side and the latency for the first entry into the social side in the three-chamber sociability test as well as for all serotonergic variables, Treatment (Cont–water, Etoh–water, Cont–etoh and Etoh–etoh) and Sex were considered between-subjects factors. Post hoc analyses were carried out by Fisher's Protected Least Significant Difference (FPLSD) tests. All data are shown as mean ± *SEM* (unless otherwise mentioned).

3 | RESULTS

3.1 | Somatic development

Early ethanol affected the pups' body mass (Treatment × Day: $F_{(1.3;32.5)} = 8.3$, $p < .001$). Although body mass increased in all groups (Day: $F_{(1.3;\ 32.5)} = 800.4$, $p < .001$), from PN4

onward, ethanol-exposed pups showed less mass gain when compared to controls (Table 1). This difference was mitigated after weaning (Table 1). As expected, males were heavier than females at PN39 ($F_{(1,49)} = 59.0$, $p < .001$), but there were no Sex × Treatment interactions. No significant differences in other developmental characteristics were observed between the groups (data not shown).

3.2 | Social behavior

Ethanol exposure during the development affected social behavior only in males (Figure 1). The Etoh group showed a reduction in the % Time in the social side ($F_{(3,26)} = 3.6$, $p < .05$; Cont–water > Etoh–water, $p < .01$) and increase latency for the first entry into the social side ($F_{(3,26)} = 3.6$, $p < .05$; Cont–water < Etoh–water, $p < .01$). Acute ethanol reexposure reverted these effects (Figure 1b,d). No differences were observed among groups in females (Figure 1a,c,e).

3.3 | Corticosterone levels

Regarding corticosterone levels, we also only observed significant differences among the groups in males ($F_{(3,18)} = 4.4$, $p < .05$). In this case, acute ethanol exposure during adolescence reduced corticosterone levels, but this effect only reached statistical significance in animals that were exposed to ethanol during the development (Figure 2). In control mice, there was only a trend toward reduced corticosterone levels in mice acutely exposed to ethanol ($p < .09$). These results indicate that ethanol exposure during the brain growth spurt potentiates ethanol-induced reduction in corticosterone levels in adolescent males.

3.4 | Serotoninergic measures

Ethanol exposure during the development or ethanol gavage during adolescence did not affect serotonin, 5-HIAA content or 5-HIAA/5-HT ratio in the frontal cerebral cortex (Table 2). Regarding 5-HT2 receptor binding, we observed significant differences among the groups in males (Figure 3, $F_{(3,22)} = 4.2$, $p < .05$). Ethanol gavage during adolescence promoted the upregulation of 5-HT_2 receptor in animals that were exposed to ethanol during the development (Figure 3b; Etoh–etoh > Cont–water, $p < .01$; Etoh–etoh > Etoh–water, $p < .01$; and Etoh–etoh > Cont-etoh, $p < .05$). No differences were observed among the groups for 5-HTT binding (Figure 3c,d).

4 | DISCUSSION

Social disruptive behaviors are among the most common adverse outcomes observed in individuals with FASD. In the present study, we demonstrated that ethanol exposure during

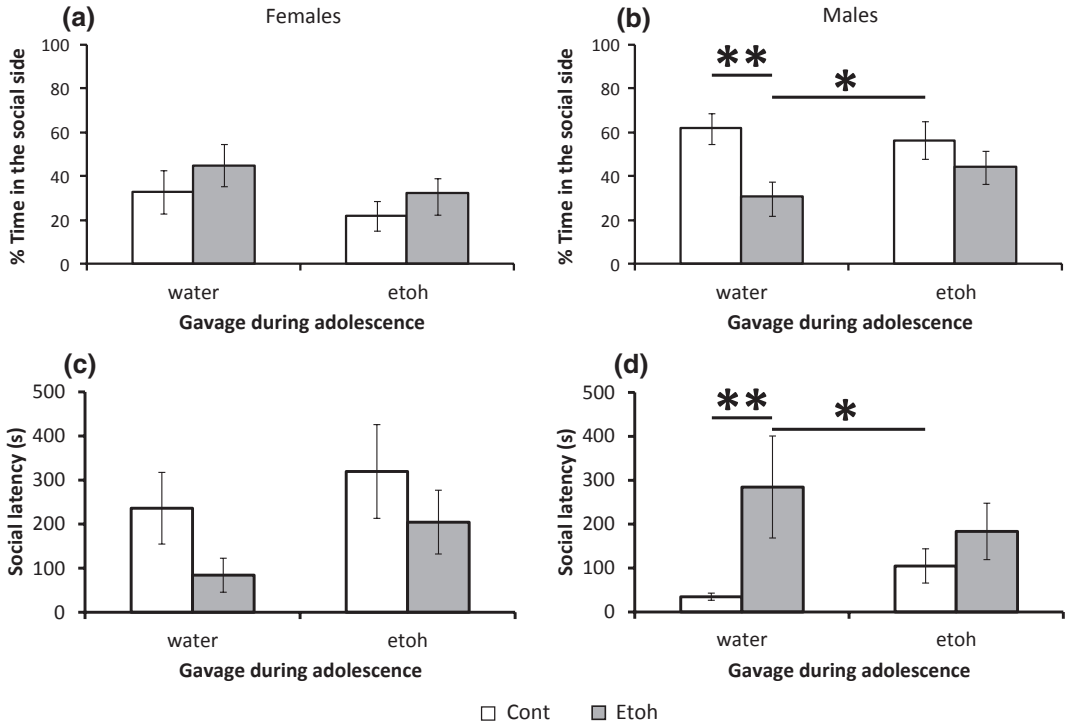

FIGURE 1 Effects of ethanol exposure during the brain growth spurt period on social behavior in female (a and c) and male (b and d) mice during adolescence after a challenge with either water or ethanol. (a) and (b) percent of time on the social side; (c) and (d) latency for a first entry into the social side. Values are means ± *SEM*. *$p < .05$; **$p < .01$

the brain growth spurt reduces adolescent male sociability. Interestingly, early ethanol-exposed male mice responded distinctively to acute low ethanol reexposure. In males, ethanol reexposure during adolescence reverted the alterations in social behavior, reduced corticosterone levels, and increased 5-HT_2 receptor binding in the frontal cerebral cortex. These mice also showed a reduction in body mass gain during the period of early ethanol exposure. However, this reduction was reverted at weaning.

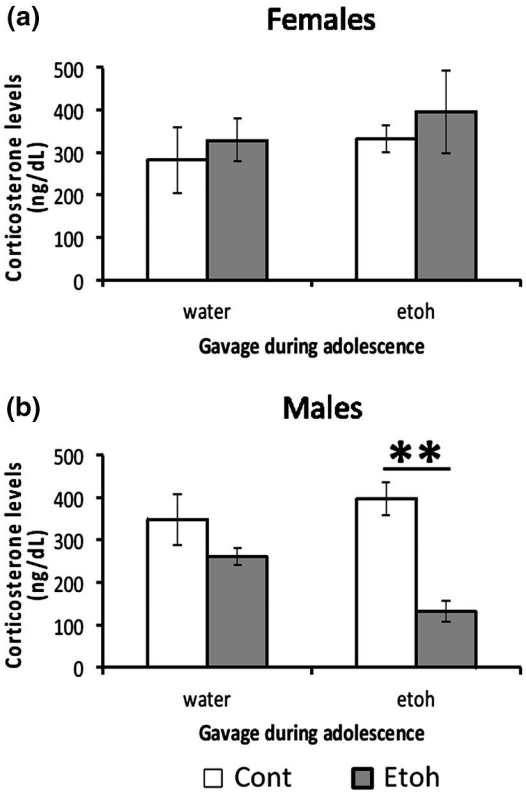

FIGURE 2 Effects of ethanol exposure during the brain growth spurt period on corticosterone levels in female (a) and male (b) mice during adolescence after a challenge with either water or ethanol. Values are means ± *SEM*. **$p < .01$, Etoh–etoh versus Cont–etoh. The post hoc analysis also indicated that the Etoh–etoh group is statistically different from Cont–water ($p < .01$) and Etoh–water ($p < .05$)

The aforementioned effects were not accompanied by developmental alterations such as altered time-course of teeth eruption, ears and eye openings. Other studies further showed that, in the FASD model used here, the great majority of the animals survive until adolescence and the mortality rates do not differ between the experimental groups (Filgueiras et al., 2009; Nunes et al., 2011). An additional methodological issue resides in the possibility that the i.p. injections used in our model, irrespective of the presence of saline or ethanol in the syringe, could have generated, on its own, an inflammatory response and/or an increased stress level that would constitute confounding factors in the analysis of our results. The inclusion of a naïve group in the experimental design could have helped in addressing this issue, however it should be pointed out that mice injected with i.p. saline are usually the only control animals in studies that specifically analyze the response to inflammatory stimuli (e.g., Barth et al., 2016; Bolognese et al., 2018; Li, Ke, Peng, Wu, & Song, 2018), which indicates that this control group (i.p. saline) does not show untoward responses that could compromise the proper assessment of ethanol effects in our experimental model (Abreu-Villaça et al., 2018; Krahe, Filgueiras, & Medina, 2016; Nunes et al., 2011). Given the multiple procedures needed to create our control group (separation from the dam and littermates, manipulation by the experimenter, actual injection, etc.), the use of a naïve group would not have allowed us to clearly define which factor (or factors) was (were), indeed, relevant in causing eventual differences among groups, making the use of naïve animals less justifiable.

Several effects of developmental exposure to ethanol are sexually dimorphic. Despite the fact that it has been described that females are more vulnerable than males to the inflammatory effects of binge ethanol exposure (Pascual et al., 2017), our findings show that the effects of early ethanol exposure on both social interaction and corticosterone levels were restricted to male mice. These data are in

TABLE 2 Serotonin and 5-hydroxyindole acetic acid and 5-HIAA/5-HT ratio in the frontal cerebral cortex

	Cont		Etoh	
	Females	**Males**	**Females**	**Males**
H_2O gavage				
5-HT (nmol/g)	1.6 ± 0.2	1.7 ± 0.2	1.93 ± 0.1	1.6 ± 0.3
5-HIAA (nmol/g)	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.3	2.1 ± 0.3
5-HIAA/5-HT	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.6 ± 0.2
Etoh gavage				
5-HT (nmol/g)	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.1	2.0 ± 0.2
5-HIAA (nmol/g)	2.2 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	1.9 ± 0.1
5-HIAA/5-HT	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.1	1.0 ± 0.1

Abbreviations: 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin.

accordance with studies demonstrating that alterations on social behavior and structural plasticity are more apparent in males than females (Diaz, Mooney, & Varlinskaya, 2016; Hamilton et al., 2010; Mooney & Varlinskaya, 2011). It is possible to speculate that hormonal fluctuations during the estrous cycle contribute to the absence of effects of ethanol in females. However, no clear differences in variability between males and females were observed. Interestingly, sex-dependent effects of early ethanol exposure on stress response can already be seen before puberty. Girls show greater changes in heart rate and negative affect and boys in cortisol levels during the still-face procedure (Haley, Handmaker, & Lowe, 2006). In fact, the sexually dimorphic effects of developmental exposure to ethanol seem to be dependent on the functional domains of the brain that were evaluated. For example, acute ethanol exposure during neurulation increases the activity in the elevated plus-maze in male mice, while, in females, it increases exploratory behavior in the open field and transiently impairs rotarod performance (Fish et al., 2016).

The impact of ethanol in social behavior varies as a function of the timing of exposure (For a review see, [Marquardt & Brigman, 2016]). Lugo, Marino, Cronise, and Kelly (2003) demonstrated that exposure to ethanol from the first gestational day to PN10 (a period considered equivalent to the entire gestational period in humans) increase social interactions during the adolescence of rats (Lugo et al., 2003). However, when ethanol exposure was restricted to the rats' gestation (first and second trimester equivalent to human gestation), it reduced social interactions in male rats (Hellemans, verma, et al., 2010, https://doi.org/10.1111/j.1530-0277.2009.01132.x). In the present study, ethanol exposure during the third trimester equivalent of human gestation decreased the sociability of adolescent male mice. This outcome was associated with an increase in latency to the first entry on the chamber that has the "unfamiliar" animal in the three-chamber paradigm test, a finding suggestive of social anxiety. Accordingly, some studies have shown that third trimester-equivalent ethanol exposure increases anxiety-like behaviors (Baculis, Diaz, & Fernando Valenzuela, 2015; Mantha, Kleiber, & Singh, 2013). Mice exposed to ethanol at PN4 and PN7 spend significantly less time than control ones in the center zone of the open-field arena (Mantha et al., 2013). Interestingly, in the same study, it was demonstrated that ethanol exposure during the second trimester-equivalent decreases anxiety levels (Mantha et al., 2013). The increase in anxiety observed in rats exposed to ethanol during the equivalent to the last trimester of human pregnancy has been associated with a persistent increase in excitatory inputs to pyramidal neurons in the basolateral amygdala (Baculis et al., 2015). The possible relationship between social impairment and anxiety in FASD is an interesting issue to be investigated in the future.

In the present study, the acute low-dose ethanol reexposure reverted early ethanol effects on social domain. We hypothesized that ethanol exposure during the brain growth spurt increases social anxiety, which could be counteracted by the anxiolytic effects of the acute ethanol exposure during adolescence. Since corticosterone levels in control mice were not affected by ethanol gavage, our results suggest that alterations in HPA axis function do not play a role in the reduction of social interaction in early ethanol-exposed males, but that the decreased corticosterone levels in response to the ethanol challenge during adolescence could explain, at least in part, the reversion of the reduced social interaction caused by early ethanol exposure. Epidemiological studies indicate a frequent co-occurrence of social anxiety and alcohol use disorders (Chow et al., 2018; Smith & Randall, 2012). In addition, alcohol drinking is believed to reduce tension in social contexts (Gilles, Turk, & Fresco, 2006). In mice, chronic social stress increases ethanol consumption during adolescence, suggesting that social stress at this age is an important risk factor for later alcohol use (Caruso et al., 2018). Here, both increases in social anxiety and the anxiolytic effects of the acute ethanol exposure could represent a reason for the development of alcohol use disorder during adolescence in FASD individuals.

Serotoninergic abnormalities in the frontal cortices seem to be a contributing factor to social impairment in FASD individuals. Our data did not show alterations in the basal levels of serotonin and 5-HIAA content, as well as 5-HTT binding in the frontal cortex. However, acute ethanol induced 5-HT_2 upregulation in the cerebral cortex of male mice exposed to ethanol during the brain growth spurt. Even though we did not observe alterations in the basal levels of serotonin, microdialysis studies have indicated that ethanol induces enhancements in serotoninergic neurotransmission (meta-analysis from Brand, Fliegel, Spanagel, & Noori, 2013). Persistent stimulation of 5-HT_2 receptors results in time-dependent desensitization and increases in binding only 2 hr after serotonin or agonist administration (Akiyoshi, Hough, & Chuang, 1993). Here, we speculate that exposure to ethanol during the brain growth spurt increases this serotonin effect on 5-HT_2 receptor binding. Alterations in neuronal intracellular signaling could play a role in these aforementioned effects. In fact, our previous findings using the FASD model used here indicated reductions in cAMP and cGMP levels in the cerebral cortex that were linked to hyperactivity and memory/learning deficits (Abreu-Villaça et al., 2018; Nunes et al., 2011). In this sense, exposure to ethanol during the brain growth spurt promotes long term disruption of neuronal intracellular signaling that could affect receptor metabolism and affinity. It should be noted that these alterations could also affect other neurotransmitter systems and participate in FASD pathophysiology. In this regard, the cholinergic system undergoes an intense period of maturation during the postnatal period and disturbances of its function in the prefrontal cortex is associated with social impairment caused by the prenatal administration of

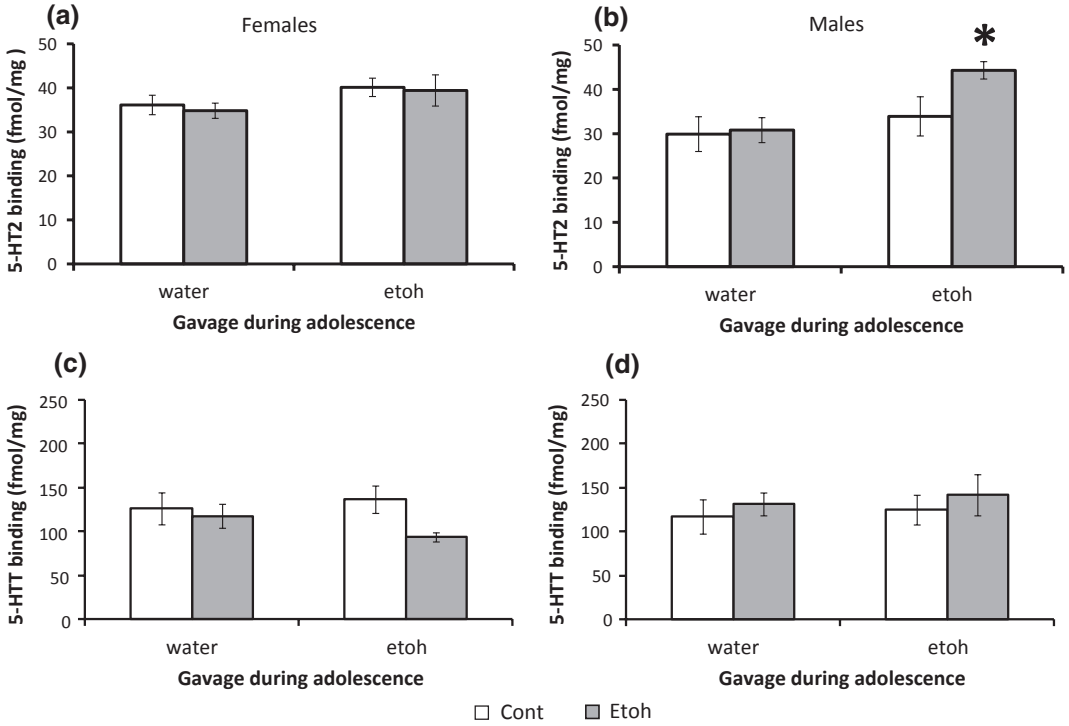

FIGURE 3 Effects of ethanol exposure during the brain growth spurt period on serotoninergic markers in female (a and c) and male (b and d) mice during adolescence after a challenge with either water or ethanol. (a) and (b) $5HT_2$ receptor binding; (c) and (d) 5HTT binding. Values are means ± *SEM*. $5HT_2$ serotonin receptor subtype 2; 5HTT, serotonin presynaptic transporter; *$p < .05$ versus all other groups

valproic acid (Kim et al., 2014), which raises the possibility that it might be susceptible to early ethanol exposure.

In conclusion, our data indicate that ethanol exposure during the brain growth spurt impairs social behavior in adolescent male mice. We also demonstrate that early exposure to ethanol increases susceptibility to the acute effects of ethanol during adolescence. Reexposure reduced corticosterone levels, evoked 5-HT_2 receptors upregulation in the frontal cerebral cortex, and reverted the social impairment generated by early ethanol exposure. These data support the idea that developmental ethanol exposure during the brain growth spurt alters ethanol effects later in life and suggests that stress response mechanisms and the serotoninergic system play roles in this phenomenon.

ACKNOWLEDGEMENTS

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and fellowships by Sub-reitoria de Pós-graduação e Pesquisa da Universidade do Estado do Rio de Janeiro (SR2-UERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and FAPERJ. The authors are thankful to Ulisses Risso for animal care.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

AR-C wrote the manuscript. All authors read and approved the final manuscript. All authors contributed equally to this manuscript and had full access to the data in the study and take responsibility for its integrity.

ETHICAL STATEMENT

All experiments were carried out under institutional approval of the Animal Care and Use Committee of the Universidade do Estado do Rio de Janeiro.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Anderson Ribeiro-Carvalho https://orcid.org/0000-0003-4324-1413

REFERENCES

Abreu-Villaça, Y., Carvalho-Graça, A. C., Skinner, G., Lotufo, B. M., Duarte-Pinheiro, V. H. S., Ribeiro-Carvalho, A., … Filgueiras, C. C. (2018). Hyperactivity and memory/learning deficits evoked by developmental exposure to nicotine and/or ethanol are mitigated by cAMP and cGMP signaling cascades activation. *Neurotoxicology*, *66*, 150–159. https://doi.org/10.1016/j.neuro.2018.04.003

Akiyoshi, J., Hough, C., & Chuang, D. M. (1993). Paradoxical increase of 5-hydroxytryptamine2 receptors and 5-hydroxytryptamine2 receptor mRNA in cerebellar granule cells after persistent 5-hydroxytryptamine2 receptor stimulation. *Molecular Pharmacology*, *43*, 349–355.

Alati, R., Clavarino, A., Najman, J. M., O'Callaghan, M., Bor, W., Mamun, A. A., & Williams, G. M. (2008). The developmental origin of adolescent alcohol use: Findings from the Mater University Study of Pregnancy and its outcomes. *Drug and Alcohol Dependence*, *98*, 136–143. https://doi.org/10.1016/j.drugalcdep.2008.05.011

Azmitia, E. C., Singh, J. S., & Whitaker-Azmitia, P. M. (2011). Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology*, *60*, 1347–1354. https://doi.org/10.1016/j.neuropharm.2011.02.002

Baculis, B. C., Diaz, M. R., & Fernando Valenzuela, C. (2015). Third trimester-equivalent ethanol exposure increases anxiety-like behavior and glutamatergic transmission in the basolateral amygdala. *Pharmacology, Biochemistry and Behavior*, *137*, 78–85. https://doi.org/10.1016/j.pbb.2015.08.009

Bandeira, F., Lent, R., & Herculano-Houzel, S. (2009). Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. *Proceedings of the National Academy of Sciences*, *106*, 14108–14113. https://doi.org/10.1073/pnas.0804650106

Barth, C. R., Luft, C., Funchal, G. A., de Oliveira, J. R., Porto, B. N., & Donadio, M. V. F. (2016). LPS-induced neonatal stress in mice affects the response profile to an inflammatory stimulus in an age and sex-dependent manner. *Developmental Psychobiology*, *58*, 600–613. https://doi.org/10.1002/dev.21404

Bolognese, A. C., Yang, W. L., Hansen, L. W., Sharma, A., Nicastro, J. M., Coppa, G. F., & Wang, P. (2018). Activation of invariant natural killer T cells redirects the inflammatory response in neonatal sepsis. *Frontiers in Immunology*, *9*, 833. https://doi.org/10.3389/fimmu.2018.00833

Brand, I., Fliegel, S., Spanagel, R., & Noori, H. R. (2013). Global ethanol-induced enhancements of monoaminergic neurotransmission: A meta-analysis study. *Alcoholism, Clinical and Experimental Research*, *37*, 2048–2057. https://doi.org/10.1111/acer.12207

Buckner, J. D., & Turner, R. J. (2009). Social anxiety disorder as a risk factor for alcohol use disorders: A prospective examination of parental and peer influences. *Drug and Alcohol Dependence*, *100*, 128–137. https://doi.org/10.1016/j.drugalcdep.2008.09.018

Caruso, M. J., Seemiller, L. R., Fetherston, T. B., Miller, C. N., Reiss, D. E., Cavigelli, S. A., & Kamens, H. M. (2018). Adolescent social stress increases anxiety-like behavior and ethanol consumption in adult male and female C57BL/6J mice. *Scientific Reports*, *8*, 10040. https://doi.org/10.1038/s41598-018-28381-2

CDC. (2009). Alcohol use among pregnant and nonpregnant women of childbearing age: United States, 1991–2005. *Morbidity and Mortality Weekly Report*, *58*, 529–532.

Chow, P. I., Portnow, S., Zhang, D., Salemink, E., Wiers, R. W., & Teachman, B. A. (2018). Comorbid interpretation and expectancy bias in social anxiety and alcohol use. *Anxiety, Stress, & Coping*, *31*, 669–685. https://doi.org/10.1080/10615806.2018.1521958

Denny, C. H., Acero, C. S., Naimi, T. S., & Kim, S. Y. (2019). Consumption of alcohol beverages and binge drinking among pregnant women aged 18–44 years—United States, 2015–2017. *Morbidity and Mortality Weekly Report*, *68*, 365–368. https://doi.org/10.15585/mmwr.mm6816a1

Diaz, M. R., Mooney, S. M., & Varlinskaya, E. I. (2016). Acute prenatal exposure to ethanol on gestational day 12 elicits opposing deficits in social behaviors and anxiety-like behaviors in Sprague Dawley rats. *Behavioral Brain Research*, *310*, 11–19. https://doi.org/10.1016/j.bbr.2016.05.003

Diestelkamp, S., Kriston, L., Arnaud, N., Wartberg, L., Sack, P. M., Härter, M., & Thomasius, R. (2015). Drinking patterns of alcohol intoxicated adolescents in the emergency department: A latent class analysis. *Addictive Behaviors*, *50*, 51–59. https://doi.org/10.1016/j.addbeh.2015.06.009

Ethen, M. K., Ramadhani, T. A., Scheuerle, A. E., Canfield, M. A., Wyszynski, D. F., Druschel, C. M., & Romitti, P. A. (2009). Alcohol consumption by women before and during pregnancy. *Maternal and Child Health Journal*, *13*, 274–285. https://doi.org/10.1007/s10995-008-0328-2

Favoretto, C. A., Macedo, G. C., & Quadros, I. M. H. (2017). Effects of ethanol on social avoidance induced by chronic social defeat stress in mice. *Stress*, *20*, 68–74. https://doi.org/10.1080/10253890.2017.1280667

Filgueiras, C. C., Krahe, T. E., & Medina, A. E. (2010). Phosphodiesterase type 1 inhibition improves learning in rats exposed to alcohol during the third trimester equivalent of human gestation. *Neuroscience Letters*, *473*, 202–207. https://doi.org/10.1016/j.neulet.2010.02.046

Filgueiras, C. C., Ribeiro-Carvalho, A., Nunes, F., Abreu-Villaça, Y., & Manhães, A. C. (2009). Early ethanol exposure in mice increases laterality of rotational side preference in the free-swimming test. *Pharmacology, Biochemistry and Behavior*, *93*, 148–154. https://doi.org/10.1016/j.pbb.2009.04.023

Fish, E. W., Holloway, H. T., Rumple, A., Baker, L. K., Wieczorek, L. A., Moy, S., … Parnell, S. E. (2016). Acute alcohol exposure during neurulation: Behavioral and brain structural consequences in adolescent C57BL/6J mice. *Behavioral Brain Research*, *311*, 70–80. https://doi.org/10.1016/j.bbr.2016.05.004

Gilles, D. M., Turk, C. L., & Fresco, D. M. (2006). Social anxiety, alcohol expectancies, and self-efficacy as predictors of heavy drinking in college students. *Addictive Behaviors*, *31*, 388–398. https://doi.org/10.1016/j.addbeh.2005.05.020

Gil-Mohapel, J., Boehme, F., Kainer, L., & Christie, B. R. (2010). Hippocampal cell loss and neurogenesis after fetal alcohol exposure: Insights from different rodent models. *Brain Research Reviews*, *64*, 283–303. https://doi.org/10.1016/j.brainresrev.2010.04.011

Haley, D. W., Handmaker, N. S., & Lowe, J. (2006). Infant stress reactivity and prenatal alcohol exposure. *Alcoholism, Clinical and Experimental Research*, *30*, 2055–2064. https://doi.org/10.1111/j.1530-0277.2006.00251.x

Hamilton, D. A., Akers, K. G., Rice, J. P., Johnson, T. E., Candelaria-Cook, F. T., Maes, L. I., … Savage, D. D. (2010). Prenatal exposure to moderate levels of ethanol alters social behavior in adult rats: Relationship to structural plasticity and immediate early gene expression in frontal cortex. *Behavioral Brain Research*, *207*, 290–304. https://doi.org/10.1016/j.bbr.2009.10.012

Hamilton, D. A., Barto, D., Rodriguez, C. I., Magcalas, C. M., Fink, B. C., Rice, J. P., … Savage, D. D. (2014). Effects of moderate prenatal ethanol exposure and age on social behavior, spatial response perseveration errors and motor behavior. *Behavioral Brain Research*, *269*, 44–54. https://doi.org/10.1016/j.bbr.2014.04.029

Hellemans, K. G. C., Sliwowska, J. H., Verma, P., & Weinberg, J. (2010). Prenatal alcohol exposure: Fetal programming and later

life vulnerability to stress, depression and anxiety disorders. *Neuroscience and Biobehavioral Reviews*, *34*, 791–807. https://doi.org/10.1016/j.neubiorev.2009.06.004

Hellemans, K. G. C., Verma, P., Yoon, E., Yu, W. K., Young, A. H., & Weinberg, J. (2010). Prenatal alcohol exposure and chronic mild stress differentially alter depressive- and anxiety-like behaviors in male and female offspring. *Alcoholism, Clinical and Experimental Research*, *34*, 633–645. https://doi.org/10.1111/j.1530-0277.2009.01132.x

Hofmann, C. E., Ellis, L., Yu, W. K., & Weinberg, J. (2007). Hypothalamic? Pituitary? Adrenal responses to 5-HT 1A and 5-HT 2A/C agonists are differentially altered in female and male rats prenatally exposed to ethanol. *Alcoholism, Clinical and Experimental Research*, *31*, 345–355. https://doi.org/10.1111/j.1530-0277.2006.00316.x

Kim, J.-W., Seung, H., Kwon, K. J., Ko, M. J., Lee, E. J., Oh, H. A., … Bahn, G. H. (2014). Subchronic treatment of Donepezil rescues impaired social, hyperactive, and stereotypic behavior in valproic acid-induced animal model of autism. *PLoS ONE*, *9*, e104927. https://doi.org/10.1371/journal.pone.0104927

Krahe, T. E., Filgueiras, C. C., & Medina, A. E. (2016). Effects of developmental alcohol and valproic acid exposure on play behavior of ferrets. *International Journal of Developmental Neuroscience*, *52*, 75–81. https://doi.org/10.1016/j.ijdevneu.2016.03.007

Li, Y., Ke, J., Peng, C., Wu, F., & Song, Y. (2018). microRNA-300/NAMPT regulates inflammatory responses through activation of AMPK/mTOR signaling pathway in neonatal sepsis. *Biomedicine & Pharmacotherapy*, *108*, 271–279. https://doi.org/10.1016/j.biopha.2018.08.064

Lima, C. S., Nunes-Freitas, A. L., Ribeiro-Carvalho, A., Filgueiras, C. C., Manhães, A. C., Meyer, A., & Abreu-Villaça, Y. (2011). Exposure to methamidophos at adulthood adversely affects serotonergic biomarkers in the mouse brain. *Neurotoxicology*, *32*, 718–724. https://doi.org/10.1016/j.neuro.2011.08.002

Lima, S. C., Dutra-Tavares, A. C., Nunes, F., Nunes-Freitas, A. L., Ribeiro-Carvalho, A., Filgueiras, C. C., … Abreu-Villaça, Y. (2013). Methamidophos exposure during the early postnatal period of mice: Immediate and late-emergent effects on the cholinergic and serotonergic systems and behavior. *Toxicological Sciences*, *134*, 125–139. https://doi.org/10.1093/toxsci/kft095

López-Cruz, L., San-Miguel, N., Bayarri, P., Baqi, Y., Müller, C. E., Salamone, J. D., & Correa, M. (2016). Ethanol and caffeine effects on social interaction and recognition in mice: Involvement of adenosine A2A and A1 receptors. *Frontiers in Behavioural Neurosciences*, *10*, 1–15. https://doi.org/10.3389/fnbeh.2016.00206

Lugo, J., Marino, M., Cronise, K., & Kelly, S. (2003). Effects of alcohol exposure during development on social behavior in rats. *Physiology & Behavior*, *78*, 185–194. https://doi.org/10.1016/S0031-9384(02)00971-X

Makkonen, I., Riikonen, R., Kokki, H., Airaksinen, M. M., & Kuikka, J. T. (2008). Serotonin and dopamine transporter binding in children with autism determined by SPECT. *Developmental Medicine and Child Neurology*, *50*, 593–597. https://doi.org/10.1111/j.1469-8749.2008.03027.x

Malone, S. M., Mcgue, M., & Iacono, W. G. (2010). Mothers' maximum drinks ever consumed in 24 hours predicts mental health problems in adolescent offspring. *J Child Psychol Psychiatry*, *51*, 1067–1075. https://doi.org/10.1111/j.1469-7610.2010.02219.x.

Mantha, K., Kleiber, M., & Singh, S. (2013). Neurodevelopmental timing of ethanol exposure may contribute to observed heterogeneity of behavioral deficits in a mouse model of fetal alcohol spectrum disorder (FASD). *Journal of Behavioral and Brain Science*, *3*, 85–99.

Marquardt, K., & Brigman, J. L. (2016). The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: Insights from rodent models. *Alcohol*, *118*, 6072–6078. https://10.1016/j.alcohol.2015.12.002.

Momino, W., Félix, T. M., Abeche, A. M., Zandoná, D. I., Scheibler, G. G., Chambers, C., … Schüler-Faccini, L. (2012). Maternal drinking behavior and fetal alcohol spectrum disorders in adolescents with criminal behavior in southern Brazil. *Genetics and Molecular Biology*, *35*, 960–965. https://doi.org/10.1590/S1415-47572012000600011

Mooney, S. M., & Varlinskaya, E. I. (2011). Acute prenatal exposure to ethanol and social behavior: Effects of age, sex, and timing of exposure. *Behavioral Brain Research*, *216*, 358–364. https://doi.org/10.1016/j.bbr.2010.08.014

Morrow, A. L., Porcu, P., Boyd, K. N., & Grant, K. A. (2006). Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior. *Dialogues in Clinical Neuroscience*, *8*, 463–477.

Murphy, D. G. M., Daly, E., Schmitz, N., Toal, F., Murphy, K., Curran, S., … Travis, M. (2006). Cortical serotonin 5-HT 2A receptor binding and social communication in adults with Asperger's syndrome: An in vivo SPECT study. *American Journal of Psychiatry*, *163*, 934–936. https://doi.org/10.1176/ajp.2006.163.5.934

Nakamura, K., Sekine, Y., Ouchi, Y., Tsujii, M., Yoshikawa, E., Futatsubashi, M., … Mori, N. (2010). Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. *Archives of General Psychiatry*, *67*, 59. https://doi.org/10.1001/archgenpsychiatry.2009.137

Niccols, A. (2007). Fetal alcohol syndrome and the developing socio-emotional brain. *Brain and Cognition*, *65*, 135–142. https://doi.org/10.1016/j.bandc.2007.02.009

Nunes, F., Ferreira-Rosa, K., Pereira, M. D. S., Kubrusly, R. C., Manhães, A. C., Abreu-Villaça, Y., & Filgueiras, C. C. (2011). Acute administration of vinpocetine, a phosphodiesterase type 1 inhibitor, ameliorates hyperactivity in a mice model of fetal alcohol spectrum disorder. *Drug and Alcohol Dependence*, *119*, 81–87. https://doi.org/10.1016/j.drugalcdep.2011.05.024

Olney, J. W., Tenkova, T., Dikranian, K., Qin, Y.-Q., Labruyere, J., & Ikonomidou, C. (2002). Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. *Developmental Brain Research*, *133*, 115–126. https://doi.org/10.1016/S0165-3806(02)00279-1

Pascual, M., Montesinos, J., Marcos, M., Torres, J.-L., Costa-Alba, P., García-García, F., … Guerri, C. (2017). Gender differences in the inflammatory cytokine and chemokine profiles induced by binge ethanol drinking in adolescence. *Addiction Biology*, *22*, 1829–1841. https://doi.org/10.1111/adb.12461

Rasmussen, C., Becker, M., Mclennan, J., Urichuk, L., & Andrew, G. (2011). An evaluation of social skills in children with and without prenatal alcohol exposure. *Child: Care, Health and Development*, *37*, 711–718. https://doi.org/10.1111/j.1365-2214.2010.01152.x

Roebuck, T. M., Mattson, S. N., & Riley, E. P. (1999). Behavioral and psychosocial profiles of alcohol-exposed children. *Alcoholism: Clinical and Experimental Research*, *23*, 1070–1076.

Sari, Y., & Zhou, F. C. (2004). Prenatal alcohol exposure causes long-term serotonin neuron deficit in mice. *Alcoholism, Clinical and Experimental Research*, *28*, 941–948. https://doi.org/10.1097/01.ALC.0000128228.08472.39

Smith, J. P., & Randall, C. L. (2012). Anxiety and alcohol use disorders: Comorbidity and treatment considerations. *Alcohol Research*, *34*, 414–431.

Spear, L. P. 2000. The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*, *24*(4), 417–463.

Tatiana, B., Barbara, B., Chaffin, M., Bard, D., Isurina, G., Tsvetkova, L., & Volkova, E. (2013). Women's alcohol consumption and risk for alcohol-exposed pregnancies in russia. *Addiction*, *107*, 109–117. https://doi.org/10.1111/j.1360-0443.2011.03569.x.Women

Yoshitake, T., Kehr, J., Yoshitake, S., Fujino, K., Nohta, H., & Yamaguchi, M. (2004). Determination of serotonin, noradrenaline, dopamine and their metabolites in rat brain extracts and microdialysis samples by column liquid chromatography with fluorescence detection following derivatization with benzylamine and 1,2-diphenylethylenediamine. *Journal of Chromatography B*, *807*, 177–183. https://doi.org/10.1016/j.jchromb.2004.03.069

How to cite this article: Demarque KC, Dutra-Tavares AC, Nunes-Freitas AL, et al. Ethanol exposure during the brain growth spurt affects social behavior and increases susceptibility to acute ethanol effects during adolescence in male mice. *Int J Dev Neurosci*. 2020;80:197–207. https://doi.org/10.1002/jdn.10017