Published in final edited form as:

Neurosci Lett. 2020 January 23; 718: 134700. doi:10.1016/j.neulet.2019.134700.

Moderate prenatal alcohol exposure reduces parvalbumin expressing GABAergic interneurons in the dorsal hippocampus of adult male and female rat offspring.

John T. Madden^a, Shannon M. Thompson^a, Christy M. Magcalas^a, Jennifer L. Wagner^b, Derek A. Hamilton^{a,b}, Daniel D. Savage^{a,b}, Benjamin J. Clark^a, Nathan S. Pentkowski^{a,*}

^aDepartment of Psychology, University of New Mexico, Albuquerque, New Mexico

^bDepartment of Neurosciences, University of New Mexico, Albuquerque, New Mexico

Abstract

Prenatal alcohol exposure (PAE) negatively impacts hippocampal development and impairs hippocampal-sensitive learning and memory. However, hippocampal neural adaptations in response to moderate PAE are not completely understood. To explore the effects of moderate PAE on GABAergic interneuron expression, this study used a rat model of moderate PAE to examine the effects of PAE on parvalbumin (PARV)-positive cells in fields CA1, CA3 and the dentate gyrus (DG) of the dorsal hippocampus (dHC). Long-Evans dams were given daily access to 5% (vol/vol) ethanol or saccharine (SAC) control solutions throughout the course of gestation. Offspring were divided into four separate groups: PAE (n=7) or SAC (n=7) males, or PAE (n=8) or SAC (n=8) females. All rats were aged to adulthood and, following testing in the Morris water task, their brains were analyzed for the expression of the GABAergic neuronal marker PARV. We report a main effect of PAE on GABAergic expression, with significant reductions in PARV-positive cells in area CA3 for males and the DG for females. There was also a trend for a reduction in PARV expressing neurons in fields CA1 and CA3 in females. The results are discussed in relation to hippocampal GABAergic interneuron function, PAE and behavior.

Keywords

PAE; prenatal alcohol exposure; fetal alcohol spectrum disorder; interneuron; memory

*Corresponding author: Nathan S. Pentkowski, 1-University of New Mexico, MSC03-2220, Albuquerque, NM 87131; pentkowski@unm.edu.

Author contributions

John T. Madden: Formal analysis, Investigation, Writing

Shannon M. Thompson: Investigation

Christy M. Magcalas: Investigation

Jennifer L. Wagner: Investigation

Derek A. Hamilton: Methodology, Resources, Writing, Funding acquisition, Supervision

Daniel D. Savage: Methodology, Resources, Writing, Funding acquisition, Supervision

Benjamin J. Clark: Conceptualization, Methodology, Resources, Writing

Nathan S. Pentkowski: Conceptualization, Methodology, Resources, Data curation, Writing, Supervision, Project administration

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

Declarations of interest: none

Introduction

Prenatal alcohol exposure (PAE) can cause a constellation of neurobiological alterations and deficits in children, collectively labeled as fetal alcohol spectrum disorders (FASD). Among these alterations include long-term changes in learning, behavior and executive function [1,2,3]. The prevalence of FASD in young children is estimated to be 2-5% in the US [4,5]. The frequency of alcohol consumption during pregnancy varies widely between individuals highlighting the need to elucidate the dose-dependent effects of PAE on neurodevelopmental and behavioral outcomes. Of interest is the impact of moderate PAE where learning deficits can arise at peak maternal serum ethanol levels as low as 30mg/dL in rats [6]. A recent meta-analysis reveals a negative association between moderate PAE and child behavior [7], and adolescent studies suggest a negative association between moderate PAE and verbal learning and memory [2]. PAE also produces a variety of detrimental outcomes in rats, including reduced synaptic plasticity in the hippocampus [8,9,10], impaired performance in object-place paired-association tasks [11], impairments in social behavior and motor coordination [12], and alterations in functional connectivity [13]; for a detailed review see [14,15].

A recent study using mice found that gradually increasing ethanol exposure on postnatal days (P) 2-9 (3-4 g/dL on P2-3, 5-6 g/dL on P4-5 and 7-8 g/dL on P6-9) produces lasting downregulations of GABAergic interneurons in fields CA1, CA3 and the dentate gyrus (DG) of the dorsal hippocampus [dHC; 16]. In early development, disruption to GABAergic interneuron activity can have detrimental effects on hippocampal neuronal migration, cell differentiation and synaptogenesis [17], which may contribute to the memory impairments frequently reported after developmental alcohol exposure. While there are considerable data describing the impact of moderate PAE on excitatory hippocampal signaling [9,10], no studies have evaluated the impact of moderate PAE on inhibitory hippocampal interneurons. To address this gap in knowledge, the present study examined the effects of moderate PAE, where the mean maternal peak serum ethanol concentration is 60.8 mg/dL [18], on parvalbumin (PARV) expressing (+) GABAergic interneurons in fields CA1, CA3 and the DG throughout the dorsal hippocampus (dHC). The interneuron marker PARV was selected based on previous research indicating that PAE reduces PARV+ GABAergic interneurons in the cingulate cortex [19].

Material and methods

Animals

All rodent husbandry and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of New Mexico. Rats were housed at 22 °C on a reverse 12-hour dark/light cycle. Harlan 2920 irradiated rodent diet chow and tap water were available *ad libitum* throughout the study.

Prenatal Alcohol Exposure

Details of the moderate PAE paradigm employed in these studies was previously reported [18]. Nine-week-old female Long-Evans rats were single-housed and acclimated to their

housing conditions for 1 week prior to ethanol exposure. For the first 2 days, the rats were given free access to tap water containing 0.066% saccharin (SAC) and 0% ethanol between 10:00 am to 2:00 pm. Next, the ethanol content was increased to 2.5% (v/v) for 2 days, and then to 5% for 2 weeks. Ethanol consumption was monitored and recorded each day. Once the 2-week prepregnancy consumption period ended, each female was housed with a male breeder until pregnant, as evidenced by the presence of a vaginal plug. Subsequently, rat dams were assigned to 1 of 2 groups that were given free access to either 5% ethanol (PAE) or SAC for 4 hours per day throughout pregnancy. Daily consumption of ethanol was monitored and recorded. At birth, litters were culled to ten pups in each litter, weaned at twenty-four days of age and group-housed by litter with two males or three females per cage. Adult offspring from each litter, aged 180 to 190 days of age were used in these studies (PAE males: n = 7; SAC males: n = 7; PAE females: n = 8; SAC females: n = 8). No more than one offspring of each sex from each litter was used to avoid litter effects.

Morris Water Task

Behavioral testing procedures were conducted as previously described [12]. Briefly, the experiments consisted of 5 days of acquisition. Each acquisition day consisted of 3 testing blocks, with each block utilizing 4 uniform release points (NW, NE, SW & SE quadrants). Trials ended when rats reached the escape platform (15 cm x 15 cm) or 60 sec had elapsed. The position of the escape platform was counterbalanced across PAE and SAC groups. Following testing, videos were analyzed for latency and path to the escape platform.

Tissue Collection

Following testing in the Morris water task, adult rats were deeply anesthetized with Fatal Plus® (195 mg/kg, i.p.) and were perfused transcardially with ice cold 0.1M phosphate buffered saline (PBS) followed by 4% fresh 0.1M paraformaldehyde (PFA). The extracted brains were post-fixed in 4% fresh PFA for 24 hours, and then cryoprotected in 15% and then 30% sucrose solutions (24 - 48 hours each). Brains were mounted on disks with Optimal Cutting Temperature compound and a series of 40 µm coronal sections were collected throughout the entire rostral-caudal extent of the dHC (−2.30 mm to −3.30 mm from bregma); sections were separated by 120 µm. Sections were stored in anti-freezing solution at −20°C until processing.

Parvalbumin Immunohistochemistry

Staining procedures were conducted as previously described [20]. Briefly, tissue sections were thawed and washed in 0.1M PBS. Next, sections were incubated in 1% hydrogen peroxide for 30 minutes, followed by a nonspecific rinse consisting of 3% normal goat serum (NGS), 0.4% Triton X-100 in 0.1M PBS for 1 hour at room temperature. The tissue was then transferred to a primary antibody solution consisting of 2% NGS, 0.4% Triton X-100 and monoclonal anti-PARV primary antibody (1:2000; Sigma Aldrich) in 0.02M PBS for 48 hours. Subsequently, the tissue was washed and incubated in the secondary antibody (1:500; Sigma Aldrich) solution for 90 min at room temperature, followed by 60-min incubation in an Avidin Biotin Complex containing 0.4% triton X-100 in 0.1M PBS. Lastly, the tissue was incubated in a nickel-enhanced 3,3'-diaminobenzidine (DAB) solution

consisting of 0.02% DAB, 2.5 % nickel and 0.0005% hydrogen peroxide. Tissue sections were mounted on slides and cover-slipped using Eukitt quick-hardening mounting medium.

Imaging

Brightfield images of dHC sections were captured using a BX51 Olympus Microscope (Olympus America Inc.). To identify the regions of interest (fields CA1, CA3 and the DG) and assess for possible tissue damage, hippocampal sections were visualized at 10X magnification. Sections that were damaged or missing regions of interest were discarded. Next, images of fields CA1, CA3 and the DG were captured at 40X magnification; both the left and right hemispheres were included with a minimum of 4 sections per rat. Images were captured for each hippocampal field using a sampling area of 0.0894 mm². To maintain uniformity of images, the center point of each hippocampal field was captured for subsequent analysis (Figure 1A) and all images were collected with an exposure time of 400 ms and ISO sensitivity of 400.

Cell Counts

The number of PARV+ cells were counted using open source image processing software, Fiji (<http://fiji.sc/Fiji>). Using the “grid” function (Plugins → analyze → grid) a 9 x 11 grid was placed over the images of each region, dividing each image into 99 separate sections. Next, manual counts of PARV+ cells were performed across each section of the grid using the cell counter plugin in Fiji (http://fiji.sc/Cell_Counter). To assist in the identification and counting of PARV+ cells, a separate copy of the same image was converted to a black and white (Image → Type → RGB stack), and a threshold was set that would convert the image to binary and eliminate all pixels above a specific pixel intensity (Image → Adjust → Threshold). The threshold was determined by calculating the average pixel intensity across all PARV+ cells. Using the Fiji rectangular selection tool, the somas of 100 PARV+ cells were selected (from randomly selected images), and the average pixel intensities of all these cells were recorded. The threshold was set at two standard deviations above the grand average pixel intensity. This threshold binary image was used as a reference by the experimenter while manually counting cells. Throughout the imaging and analysis, the experimenter was blinded to experimental conditions.

Data Analysis

All data are reported as mean ± standard error. Analyses were conducted using SPSS 25 with thresholds for significance set at $\alpha < 0.05$. Figures were created using GraphPad Prism 7.0. Mixed factor ANOVAs were conducted to examine changes in latency and path distance to the escape platform across acquisition, with sex and prenatal diet as between subject factors and acquisition trials as the within subjects factor. Separate two-way ANOVAs were conducted for each hippocampal region of interest with sex and prenatal diet group as between subject factors. Significant effects were followed by independent sample t-tests to examine differences in GABAergic interneuron counts between PAE and SAC in both males and females. Hedges g was computed to provide effect sizes for significant results. No post-hoc corrections were utilized. Two female rats, one each from the PAE and SAC conditions, were identified as outliers (more than 2 standard deviations from their group means) and were excluded from the analysis.

Results

The rat dams generating the PAE offspring used in this study consumed 1.89 ± 0.13 grams of ethanol per day throughout pregnancy (Table 1). Alcohol consumption did not affect maternal weight gain, offspring litter size or pup birthweight compared to controls. This level of ethanol consumption is similar to the levels recently reported in a separate set of rat dams where the mean peak maternal serum ethanol concentration was 60.8 ± 5.8 mg/dL [18].

Table 2 provides mean ($\pm$ SEM) values for latency and path length for each acquisition day. There were no significant PAE by sex by latency interactions across acquisition trials (Greenhouse-Geisser, $F(4,26) = 0.203$, $p = 0.936$).

Figure 1 provides a schematic representation of the range and location of dorsal hippocampal subfields analyzed (1A), as well as representative PARV stained images from the dHC and field CA1 at low (IB; 4X) and high (1C; 40X) magnification, respectively. Examples of PARV+ cells (arrows) from a PAE rat are evident by dark brown/black stained cells.

Figure 2 summarizes the PARV+ cell count data across the three hippocampal subfields. There were no prenatal diet by sex interactions for fields CA1 ($F(1,24) = 1.00$, $p = 0.33$, $\eta_p^2 = 0.04$), CA3 ($F(1,24) = 0.00$, $p = 0.99$, $\eta_p^2 < 0.001$) or the DG ($F(1,24) = 1.36$, $p = 0.26$, $\eta_p^2 = 0.05$); however, PAE produced significant main effects in fields CA1 ($F(1,26) = 4.29$, $p = 0.049$, $\eta_p^2 = 0.15$), CA3 ($F(1,26) = 9.45$, $p = 0.005$, $\eta_p^2 = 0.28$) and the DG ($F(1,26) = 6.64$, $p = 0.02$, $\eta_p^2 = 0.22$). Across each field, PAE rats exhibited significantly reduced PARV+ cell counts compared to SAC controls (2A). There was not a main effect of sex in fields CA3 or the DG ($p > 0.21$ in each case), but there was a trend in field CA1 ($F(1,26) = 4.17$, $p = 0.05$, $\eta_p^2 = 0.15$), with more PARV+ cells detected in females. To further explore the influence of PAE on GABAergic interneuron expression, we separately analyzed PARV+ cells in both males and females. In males, PAE significantly reduced PARV+ cells in field CA3 ($t(12) = 3.14$, $p = 0.01$, $g = 1.57$), but the reductions in fields CA1 and the DG were not significant ($p > 0.34$ in each case; 2B.). In females, PAE significantly reduced PARV+ cells in the DG ($t(12) = 2.82$, $p = 0.02$, $g = 1.41$), and produced a trend towards reduced cell counts in fields CA1 ($t(12) = 1.83$, $p = 0.09$, $g = 0.91$) and CA3 ($t(12) = 1.77$, $p = 0.1$, $g = 0.87$; 2C).

Discussion

The present study is the first to report a detrimental effect of moderate PAE [18,21] on PARV+ GABAergic interneurons in the dHC of male and female adult rats. Specifically, there was a main effect of PAE, with ethanol-exposed rats exhibiting significantly lower PARV+ cell counts throughout fields CA1, CA3 and the DG. Further analysis revealed a sex-specific effect, with reduced numbers of PARV+ cells in field CA3 in males and the DG in females; there was also a trend toward reduced PARV+ cells in fields CA1 and CA3 in females. These data extend the literature describing the negative impact of postnatal developmental ethanol exposure on dHC interneuron expression and suggest that moderate

PAE (maternal peak serum ethanol concentration: 60 ± 5.8 mg/dL) produces a more selective sex- and subfield-specific effect on dHC interneuron expression. For example, heavy ethanol exposure during the early postnatal period (P2-9; mean serum ethanol concentration: 221.6 ± 18.3 mg/dl) reduces dorsal hippocampal GABAergic interneurons in adult mice, particularly males [16]. Additionally, postnatal binge-like exposure (P7; mean serum ethanol concentration: 0.5 g/dl) reduces PARV+ cell counts in the dHC regardless of sex [22]. These sex- and subfield-specific differences in hippocampal interneuron expression may be due to the model species, specific interneuron type, and/or the timing, frequency and dose of ethanol.

Research also suggests that non-hippocampal GABAergic interneurons are detrimentally impacted by early ethanol exposure. For example, PARV+ interneurons are reduced in the cingulate cortex following PAE [19] and display shorter dendrites in the striatum following alcohol exposure on P2-6 [23]. In contrast, using a 3-day binge-like in utero ethanol exposure paradigm on embryonic days 13.5-16.5, Skorput and colleagues found persisting increases in PARV+ migrating interneurons in the mPFC [24]. These alterations in PARV+ neurons may involve ethanol-induced apoptosis, cell proliferation, differentiation or migration [for review see 17 and 25]. For example, the number of apoptotic cells in both the primary somatosensory cortex and hippocampus are increased in mice following early ethanol exposure [26]. Collectively, these results add to a growing body of research indicating that PAE causes a variety of detrimental neurobiological outcomes in developing brains, including deficits in growth [27] and neuronal loss across multiple brain regions [28].

The present findings suggest that moderate PAE-induced reductions in PARV+ cells could have long-term implications for normal brain development and behavior [for a comprehensive review see 29]. Indeed, ionotropic GABA_A receptors have a critical role in the regulation and development of neural networks. *In-vivo* studies demonstrate that the early postnatal depolarizing to hyperpolarizing shift in GABA_A receptors (also known as the GABA-switch) contributes to neural development, with alterations to this shift causing long-term changes in morphology of cortical neurons [30]. Viral-mediated removal of PARV+ cells in dorsal hippocampal CA1 disrupts spatial working memory in mice [31]. Chemogenetic activation of PARV+ cells in the DG in mice facilitates fear extinction [32] and produces an anxiolytic-like effect [32]. Lastly postmortem studies have found reductions in PARV+ cells in patients with schizophrenia and bipolar disorder [33, 34]. Collectively, these studies suggest that moderate PAE-induced loss of PARV+ GABAergic interneurons in field CA1 and the DG may be one factor contributing to hippocampal synaptic plasticity deficits observed in PAE offspring [8, 9, 10] and may enhance vulnerability to several neuropsychological disorders.

Although not the primary focus of the present study, prior to histological assessment, all rats were tested in a standard hidden platform Morris water task procedure. The behavioral results are consistent with previous reports suggesting that acquisition in the Morris water task is spared following moderate PAE [12, 13, 35]. In addition, the present study did not find sex differences in behavioral performance. While previous research has reported sex differences in performance on hippocampal-based tasks, the findings are mixed. Male offspring from gestating rat dams continually exposed to liquid diets of 35% ethanol-derived

calories show greater spatial processing deficits than females in Morris water task probe trials [36]. In contrast, female offspring from gestating rat dams given an acute high dose of ethanol (5.8 mg/kg, ip, gestational day 8) show greater spatial learning deficits in Morris water task probe trials [37]. However, other studies report no sex differences in performance on hippocampal-dependent tasks following PAE. For instance, one study found no sex differences on acquisition trials of the Morris water task following moderate PAE [13] and another study detected no sex differences on temporal discrimination tasks following moderate PAE [38].

The behavioral studies summarized above vary in the frequency, time-points and degrees of PAE exposure, highlighting the need for elucidating PAE's sex-specific effects on hippocampal development. To partially address this knowledge gap, the present study examined sex differences in PARV expressing GABAergic interneurons in the dHC in rats exposed to moderate PAE. PAE females showed higher intraneuronal counts than PAE males in field CA1. Additionally, compared to SAC controls PAE males exhibited lower interneuron counts in field CA3, while PAE females exhibited lower interneuron counts in the DG, and trends toward lower cell counts in fields CA1 and CA3. Based on these results, future research should characterize how PAE differentially influences males and females on behavioral tasks selectively dependent on DG or CA3 processing [39]. For instance, damage to the DG can produce impairments on tasks requiring animals to discriminate between spatial locations with a high degree of similarity [40]. A recent study reported that PARV+ cells provide the main source of inhibition over granule cells in the DG and may play a central role in this cognitive process [41]. Within areas CA1 and CA3, fast spiking interneurons, which are typically PARV+ cells, innervate large populations of pyramidal cells and coordinate hippocampal network oscillations thought to have a critical role in long-term memory [42, 43, 44]. Notably, moderate PAE can impair performance by adult male rats in spatial discrimination and object-place paired associate tasks, both of which are dependent on intact DG and CA3, respectively [11, 45, 46].

In conclusion, the present results indicate that adult male and female rats exposed to moderate PAE exhibit reduced PARV+ cell counts in the dHC compared to rats prenatally exposed to SAC. This downregulation of GABAergic interneurons could have long-term implications for the development of neural networks, and subsequently, performance on learning and memory tasks, as well as enhanced vulnerability for a variety of neuropsychiatric conditions. Although the present study did not detect impairments in Morris water task acquisition after moderate PAE, future work should relate PAE-induced loss of GABAergic function with performance on additional spatial memory impairments in the Morris water task, such as retention or reversal deficits, that are observed with this alcohol exposure protocol [21], or more complex discrimination tasks that are hippocampal dependent (e.g., [11, 38]). Indeed, research indicates that spatial working memory in the radial arm water maze is negatively affected by CA1 PARV+ cell loss [31]. In addition to reduced PARV expressing interneurons reported in the present study, future work should examine the effects of moderate PAE on different subsets of hippocampal GABAergic interneurons (e.g., cholecystokinin- or somatostatin-positive, chandelier and/or O-LM cells). Lastly, future research should characterize how other brain regions, such as the ventral hippocampus, are impacted by moderate PAE, how the timing of PAE correlates with

reductions in GABAergic interneurons, and how these alterations influence neurobiological development and behavior.

Acknowledgments

This work was supported by grants from NIAAA (R21 AA024983 and P50 AA022534). The authors wish to thank Suzy Davies, Kyle Christensen and Morgan Pruitt for their technical assistance with animal husbandry and the prenatal ethanol exposure paradigm.

References

- [1]. Hamilton DA, Kodituwakku P, Sutherland RJ, and Savage DD (2003). Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task. *Behavioural brain research*, 143(1), 85–94. 10.1016/S0166-4328(03)00028-7 [PubMed: 12842299]
- [2]. Willford JA, Leech SL, & Day NL (2006). Moderate prenatal alcohol exposure and cognitive status of children at age 10. *Alcoholism: Clinical and Experimental Research*, 30(6), 1051–1059. 10.1111/j.1530-0277.2006.00119.x
- [3]. Kodituwakku PW (2007). Defining the behavioral phenotype in children with fetal alcohol spectrum disorders: a review. *Neuroscience & Biobehavioral Reviews*, 31(2), 192–201. 10.1016/j.neubiorev.2006.06.020 [PubMed: 16930704]
- [4]. May PA, Baete A, Russo J, Elliott AJ, Blankenship J, Kalberg WO, Buckley B, Brooks M, Hasken J, Abdul-Rahman O, Adam MP, Robinson LK, Manning M, & Hoyme HE (2014). Prevalence and characteristics of fetal alcohol spectrum disorders. *Pediatrics*, 134(5), 855 <https://dx.doi.org/10.1542%2Fpeds.2013-3319> [PubMed: 25349310]
- [5]. May PA, Chambers CD, Kalberg WO, Zellner J, Feldman FL, Buckley D, Kopald D, Hasken JM, Xu R, Honerkamp-Smith G, Taras H, Manning MA, Robinson LK, Adam MP, Abdul-Rahman O, Vaux K, Jewett T, Elliott AJ, Kable JA, Akshoomoff N, Falk D, Arroyo JA, Hereld D, Riley EP, Chamess ME, Coles CD, Warren KR, Jones KL, & Hoyme HE (2018). Prevalence of Fetal Alcohol Spectrum Disorders in 4 US Communities. *JAMA*. 2018 319(5):474–482. doi: 10.1001/jama.2017.21896.
- [6]. Savage DD, Becher M, de la Torre AJ, & Sutherland RJ (2002). Dose-dependent effects of prenatal ethanol exposure on synaptic plasticity and learning in mature offspring. *Alcoholism: Clinical and Experimental Research*, 26(11), 1752–1758. 10.1111/j.1530-0277.2002.tb02480.x
- [7]. Flak AL, Su S, Bertrand J, Denny CH, Kesmodel US, & Cogswell ME (2014). The association of mild, moderate, and binge prenatal alcohol exposure and child neuropsychological outcomes: a meta-analysis. *Alcoholism: Clinical and Experimental Research*, 38(1), 214–226. DOI: 10.1111/acer.12214
- [8]. Sutherland RJ, McDonald RJ & Savage DD (1997). Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring. *Hippocampus* 7(2):232–8. 10.1002/(SICI)1098-1063(1997)7:2<232::AID-HIPO9>3.0.CO;2-O [PubMed: 9136052]
- [9]. Varaschin RK, Akers KG, Rosenberg MJ, Hamilton DA & Savage DD (2010). Effects of the cognition-enhancing agent ABT-239 on fetal ethanol-induced deficits in dentate gyrus synaptic plasticity. *Pharmacol Exp Ther*. 334(1): 191–8. doi: 10.1124/jpet.109.165027.
- [10]. Brady ML, Diaz MR, Iuso A, Everett JC, Valenzuela CF, & Caldwell KK (2013). Moderate prenatal alcohol exposure reduces plasticity and alters NMDA receptor subunit composition in the dentate gyrus. *Journal of Neuroscience*, 33(3), 1062–1067. 10.1523/JNEUROSCI.1217-12.2013. [PubMed: 23325244]
- [11]. Sanchez LM, Goss J, Wagner J, Davies S, Savage DD, Hamilton DA, & Clark J (2019). Moderate prenatal alcohol exposure impairs performance by adult male rats in an object-place paired-associate task. *Behavioural brain research*, 360, 228–234. 10.1016/j.bbr.2018.12.014 [PubMed: 30529401]

- [12]. Hamilton DA, Barto D, Rodriguez CI, Magcalas CM, Fink BC, Rice JP, Bird CW, Davies S, Savage DD (2014). Effects of moderate prenatal ethanol exposure and age on social behavior, spatial response perseveration errors and motor behavior. *Behavioural brain research*, 269, 44–54. 10.1016/j.bbr.2014.04.029 [PubMed: 24769174]
- [13]. Rodriguez CI, Davies S, Calhoun V, Savage DD, & Hamilton DA (2016). Moderate prenatal alcohol exposure alters functional connectivity in the adult rat brain. *Alcoholism: Clinical and Experimental Research*, 40(10), 2134–2146. 10.1111/acer.13175
- [14]. Berman RF, and Hannigan JH (2000). Effects of prenatal alcohol exposure on the hippocampus: Spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus*, 10, 94–110. [https://doi.org/10.1002/\(SICI\)1098-1063\(2000\)10:1%3C94::AID-HIPO11%3E3.0.CO;2-T](https://doi.org/10.1002/(SICI)1098-1063(2000)10:1%3C94::AID-HIPO11%3E3.0.CO;2-T) [PubMed: 10706221]
- [15]. Valenzuela CF, Morton RA, Diaz MR, & Topper L (2012). Does moderate drinking harm the fetal brain? Insights from animal models. *Trends in neurosciences*, 55(5), 284– 292. 10.1016/j.tins.2012.01.006
- [16]. Bird CW, Taylor DH, Pinkowski NJ, Chavez GJ, & Valenzuela CF (2018). Longterm Reductions in the Population of GABAergic Interneurons in the Mouse Hippocampus following Developmental Ethanol Exposure. *Neuroscience*, 383, 60–73. 10.1016/j.neuroscience.2018.05.003 [PubMed: 29753864]
- [17]. Isayama RN, Leite PEC, Lima JPM, Uziel D, & Yamasaki EN (2009). Impact of ethanol on the developing GABAergic system. *The Anatomical Record*, 292, 1922–1939. 10.1002/ar.20966 [PubMed: 19943346]
- [18]. Davies S, Ballesteros-Merino C, Pruitt ME, Christensen KH & Savage DD (2018). Impact of moderate prenatal ethanol exposure on markers of histaminergic neurotransmission in different brain regions of adult rat offspring. *Alcohol*, 76, 47–57. 10.1016/j.alcohol.2018.07.007 [PubMed: 30557779]
- [19]. Moore DB, Quintero MA, Ruygrok AC, Walker DW, & Heaton MB (1998). Prenatal ethanol exposure reduces parvalbumin-immunoreactive GABAergic neuronal number in the adult rat cingulate cortex. *Neuroscience letters*, 249 (1), 25–28. 10.1016/S0304-3940(98)00378-4 [PubMed: 9672380]
- [20]. Wilber AA, Clark BJ, Demecha AJ, Mesina L, Vos JM, & McNaughton BL (2015). Cortical connectivity maps reveal anatomically distinct areas in the parietal cortex of the rat. *Frontiers in Neural Circuits*, 5(146). doi: 10.3389/fncir.2014.00146
- [21]. Savage DD, Rosenberg MJ, Wolff CR, Akers KG, El-Emawy A, Staples MC, Varaschin RK, Wright CA, Seidel JL, Caldwell KK, & Hamilton DA (2010). Effects of a Novel Cognition-Enhancing Agent on Fetal Ethanol-Induced Learning Deficits. *Alcohol. Clin. Exp. Res* 24, 1793–1802. 10.1111/j.1530-0277.2010.01266.x
- [22]. Sadrian B, Lopez-Guzman M, Wilson DA, & Saito M (2014). Distinct neurobehavioral dysfunction based on the timing of developmental binge-like alcohol exposure. *Neuroscience*, 280, 204–219. 10.1016/j.neuroscience.2014.09.008 [PubMed: 25241068]
- [23]. De Giorgio A, Comparini SE, Intra FS, & Granato A (2012). Long-term alterations of striatal parvalbumin interneurons in a rat model of early exposure to alcohol. *Journal of neurodevelopmental disorders*, 4 (1), 2 10.1186/1866-1955-4-18 [PubMed: 22958782]
- [24]. Skorput AG, Gupta VP, Yeh PW, & Yeh HH (2015). Persistent interneuronopathy in the prefrontal cortex of young adult offspring exposed to ethanol in utero. *Journal of Neuroscience*, 55(31), 10977–10988. DOI: 10.1523/JNEUROSCI.1462-15.2015
- [25]. Moreno MC, Ignacio C, Burke P, & Middleton FA (2016). Alcohol intake and apoptosis: a review and examination of molecular mechanisms in the central nervous system In *Molecular aspects of alcohol and nutrition* (pp. 45–61). Academic Press 10.1016/B978-0-12-800773-0.00004-5
- [26]. Moreno MC, Mooney SM, & Middleton FA (2017). Heterogeneity of p53 dependent genomic responses following ethanol exposure in a developmental mouse model of fetal alcohol spectrum disorder. *PloS one*, 12(7), e0180873 10.1371/journal.pone.0180873 [PubMed: 28723918]
- [27]. Maier SE, Miller JA, & West JR (1999). Prenatal binge-like alcohol exposure in the rat results in region-specific deficits in brain growth. *Neurotoxicology and teratology*, 21(3), 285–291. 10.1016/S0892-0362(98)00056-7 [PubMed: 10386832]

- [28]. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovská V, Horster F, Tenkova T, Dikranian K, & Olney JW (2000). Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science*, 287(5455), 1056–1060. 10.1126/science.287.5455.1056 [PubMed: 10669420]
- [29]. Pelkey KA, Chittajallu R, Craig MT, Tricoire L, Wester JC, & McBain CJ (2017). Hippocampal GABAergic inhibitory interneurons. *Physiological reviews*, 97(4), 1619–1747. 10.1152/physrev.00007.2017 [PubMed: 28954853]
- [30]. Cancedda L, Fiumelli H, Chen K, & Poo MM (2007). Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. *Journal of Neuroscience*, 27(19), 5224–5235. 10.1523/JNEUROSCI.5169-06.2007 [PubMed: 17494709]
- [31]. Murray AJ, Sauer JF, Riedel G, McClure C, Ansel L, Cheyne L, Bartos M, Wisden W, & Wulff P (2011). Parvalbumin-positive CA1 interneurons are required for spatial working but not for reference memory. *Nature neuroscience*, 14(3), 297 <https://dx.doi.org/10.1038%2Fnn.2751> [PubMed: 21278730]
- [32]. Zou D, Chen L, Deng D, Jiang D, Dong F, McSweeney C, Zhou Y, Liu L, Chen G, Wu Y, & Mao Y (2016). DREADD in parvalbumin interneurons of the dentate gyrus modulates anxiety, social interaction and memory extinction. *Current molecular medicine*, 16(1), 91–102. <https://dx.doi.org/10.2174%2F1566524016666151222150024> [PubMed: 26733123]
- [33]. Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, & Knable MB (2005). Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biological psychiatry*, 57(3), 252–260. 10.1016/j.biopsych.2004.10.019 [PubMed: 15691526]
- [34]. Konradi C, Zimmerman EI, Yang CK, Lohmann KM, Gresch P, Pantazopoulos H, Berretta S, & Heckers S (2011). Hippocampal interneurons in bipolar disorder. *Archives of general psychiatry*, 68(4), 340–350. doi:10.1001/archgenpsychiatry.2010.175 [PubMed: 21135314]
- [35]. 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. 10.1016/j.alcohol.2015.12.002 [PubMed: 26992695]
- [36]. Blanchard BA, Riley EP, & Hannigan JH (1987). Deficits on a spatial navigation task following prenatal exposure to ethanol. *Neurotoxicology and teratology*, 9(3), 253–258. 10.1016/0892-0362(87)90010-9 [PubMed: 3627089]
- [37]. Minetti A, Arolfo MP, Virgolini MB, Brioni JD, & Fulginiti S (1996). Spatial learning in rats exposed to acute ethanol intoxication on gestational day 8. *Pharmacology Biochemistry and Behavior*, 53(2), 361–367. 10.1016/0091-3057(95)02035-7
- [38]. Patten AR, Sawchuk S, Wortman RC, Brocardo PS, Gil-Mohapel J, & Christie BR (2016). Prenatal ethanol exposure impairs temporal ordering behaviours in young adult rats. *Behavioural brain research*, 299, 81–89. 10.1016/j.bbr.2015.11.032 [PubMed: 26632335]
- [39]. Harvey RE, Berkowitz LE, Hamilton DA, & Clark BJ (2019). The Effects of Developmental Alcohol Exposure on the Neurobiology of Spatial Processing. *Neuroscience & Biobehavioral Reviews*, 10.1016/j.neubiorev.2019.09.018
- [40]. Yassa MA, & Stark CE (2011). Pattern separation in the hippocampus. *Trends in neurosciences*, 34(10), 515–525. 10.1016/j.tins.2011.06.006 [PubMed: 21788086]
- [41]. Espinoza C, Guzman SJ, Zhang X, & Jonas P (2018). Parvalbumin+ interneurons obey unique connectivity rules and establish a powerful lateral-inhibition microcircuit in dentate gyrus. *Nature communications*, 9(1), 4605 10.1038/s41467-018-06899-3.
- [42]. Buzsáki G, & Wang XJ (2012). Mechanisms of gamma oscillations. *Annual review of neuroscience*, 35, 203–225. 10.1146/annurev-neuro-062111-150444
- [43]. Ognjanovski N, Schaeffer S, Wu J, Mofakham S, Maruyama D, Zochowski M, & Aton SJ (2017). Parvalbumin-expressing interneurons coordinate hippocampal network dynamics required for memory consolidation. *Nature communications*, 8, 15039 10.1038/ncomms15039
- [44]. Stark E, Roux L, Eichler R, Senzai Y, Royer S, & Buzsáki G (2014). Pyramidal cell-interneuron interactions underlie hippocampal ripple oscillations. *Neuron*, 83(2), 467–480. <https://dx.doi.org/10.1016%2Fj.neuron.2014.06.023> [PubMed: 25033186]

- [45]. Gilbert PE, & Kesner RP (2003). Localization of function within the dorsal hippocampus: the role of the CA3 subregion in paired-associate learning. *Behavioral neuroscience*, 117(6), 1385 <https://psycnet.apa.org/doi/10.1037/0735-7044.117.6.1385> [PubMed: 14674856]
- [46]. Brady ML, Allan AM, & Caldwell KK (2012). A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. *Alcoholism: Clinical and Experimental Research*, 36(3), 457–466. 10.1111/j.1530-0277.2011.01644.x

Highlights

- Moderate prenatal alcohol exposure reduced GABAergic interneuron expression in area CA3 in adult male offspring.
- Moderate prenatal alcohol exposure reduced GABAergic interneuron expression in the dentate gyrus in adult female offspring.
- In female offspring there was a trend towards reduced GABAergic interneuron expression in fields CA1 and CA3.

Figure 1:
Schematic representation depicting the range of dorsal hippocampal subfields analyzed with black squares indicating the regions of interest imaged (1A), as well as representative PARV stained images of the dHC at low magnification (1B; 4X) and field CA1 at high magnification (1C; 40X). The white box indicates the region imaged in 1C. Arrows illustrate examples of PARV-positive cells. Abbreviations: parvalbumin (PARV).

Figure 2:

Effects of PAE on PARV expressing GABAergic interneuron counts in the dHC. There was a main effect of ethanol exposure (2A), with PAE rats ($n = 14$) exhibiting lower PARV-positive interneuron counts compared to SAC controls ($n = 14$) across all dHC regions. PAE males ($n = 7$) exhibited lower interneuron counts compared to SAC controls ($n = 7$) in all dHC regions, with a significant difference in the CA3 (2B). PAE females ($n = 7$) had lower interneuron counts than SAC controls ($n = 7$) in all three regions of interest, with a significant difference in the DG, and trends in fields CA1 and CA3 (2C). Data are expressed as the mean number of PARV+ cells per section $\pm$ SEM. The asterisk (*) and pound (#) represent significant differences and trends, respectively, between PAE and SAC controls. Abbreviations: parvalbumin expressing (PARV+); dorsal hippocampus (dHC); prenatal alcohol exposure (PAE); saccharin (SAC); standard error of the mean (SEM).

Table 1.

Effects of daily 4-h consumption of 5% ethanol on female rat dams and their offspring.

	Saccharin	5% Ethanol
Daily 4-hour 5% ethanol consumption	NA	1.89 ± 0.13^a (6)
Maternal serum ethanol concentration	NA	60 ± 5.8^b (62)
Maternal weight gain during pregnancy	89 ± 6^c (8)	87 ± 9 (6)
Litter size	10.9 ± 0.7^d (8)	10.0 ± 1.0 (6)
Pup birth weight	7.59 ± 0.45^e (8)	7.38 ± 0.17 (6)

^a-Mean $\pm$ SEM grams ethanol consumed/kg body weight/day^b-Mean $\pm$ SEM mg ethanol/dL serum, 45 minutes into the drinking period^c-Mean $\pm$ SEM grams increase in body weight from GD 1 through GD 18^d-Mean $\pm$ SEM number of live births/litter^e-Mean $\pm$ SEM grams pup birth weight

NA-not applicable

(n)-Group sample size

Table 2:

Effects of prenatal alcohol exposure (PAE) on acquisition latencies in the Morris water task as described in Hamilton et al. (2014). Data represent the average latencies (seconds) during the 12 training trials across 5 days (3 for each of the four starting locations).

Acquisition latencies	Males		Females	
	PAE	SAC	PAE	SAC
Day 1	14.39 ± 1.69	15.42 ± 1.02	13.23 ± 1.27	15.83 ± 2.42
Day 2	7.30 ± 0.83	5.32 ± 0.79	7.37 ± 2.09	5.58 ± 0.37
Day 3	5.20 ± 0.80	3.90 ± 0.41	4.90 ± 0.77	4.96 ± 0.57
Day 4	3.87 ± 0.32	3.36 ± 0.26	4.43 ± 0.51	4.72 ± 0.63
Day 5	3.83 ± 0.26	4.22 ± 0.44	6.07 ± 0.87	5.80 ± 1.35