Published in final edited form as:

Psychoneuroendocrinology. 2015 July ; 57: 37–50. doi:10.1016/j.psyneuen.2015.03.001.

Alcohol and pregnancy: effects on maternal care, HPA axis function, and hippocampal neurogenesis in adult females

Joanna L. Workman^{#1,†}, Charlis Raineke^{#2}, Joanne Weinberg^{#1,2,3,*}, and Liisa A. M. Galea^{#1,3,*}

¹Department of Psychology University of British Columbia 2136 West Mall Vancouver, BC, Canada V6T 1Z4

²Department of Cellular & Physiological Sciences University of British Columbia 2350 Health Sciences Mall Vancouver, BC, Canada V6T 1Z3

³Djavad Mowafaghian Centre for Brain Health 2215 Wesbrook Mall Vancouver, BC Canada V6T 1Z3

These authors contributed equally to this work.

Abstract

Chronic alcohol consumption negatively affects health, and has additional consequences if consumption occurs during pregnancy as prenatal alcohol exposure adversely affects offspring development. While much is known on the effects of prenatal alcohol exposure in offspring less is known about effects of alcohol in dams. Here, we examine whether chronic alcohol consumption during gestation alters maternal behavior, hippocampal neurogenesis and HPA axis activity in late postpartum female rats compared with nulliparous females. Rats were assigned to alcohol, pair-fed or *ad libitum* control treatment groups for 21 days (for pregnant rats, this occurred gestation days 1 – 21). Maternal behavior was assessed throughout the postpartum period. Twenty-one days after alcohol exposure, we assessed doublecortin (DCX) (an endogenous protein expressed in immature neurons) expression in the dorsal and ventral hippocampus and HPA axis activity. Alcohol consumption during pregnancy reduced nursing and increased self-directed and negative behaviors, but spared licking and grooming behavior. Alcohol consumption increased corticosterone and adrenal mass only in nulliparous females. Surprisingly, alcohol consumption did not alter DCX-expressing cell density. However, postpartum females had fewer DCX-expressing cells (and of these cells more immature proliferating cells but fewer postmitotic cells)

© 2015 Published by Elsevier Ltd.

*Corresponding Authors: lgalea@psych.ubc.ca Tel: 604-822-3941 joanne.weinberg@ubc.ca Tel: 604-822-6214.

†Present address: University at Albany, State University of New York Department of Psychology 1400 Washington Ave. Albany, NY 12222 USA

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

Contributors

All authors participated in experimental design. JLW and CR collected and analyzed data and wrote the manuscript. LAMG and JW edited the manuscript.

Conflict of Interest

All authors report no conflict of interest.

than nulliparous females. Collectively, these data suggest that alcohol consumption during pregnancy disrupts maternal care without affecting HPA function or neurogenesis in dams. Conversely, alcohol altered HPA function in nulliparous females only, suggesting that reproductive experience buffers the long-term effects of alcohol on the HPA axis.

Keywords

alcohol; maternal behavior; corticosterone; doublecortin; reproductive experience; postpartum

1. Introduction

Clinical and pre-clinical studies have demonstrated that chronic alcohol consumption has negative short- and long-term consequences for physical and mental health. These problems are substantially exacerbated when alcohol consumption occurs during pregnancy, as it can alter the developmental trajectory of the fetus and lead to enduring cognitive, physiological, morphological, neurobiological, and neurobehavioral deficits (Hellemans et al., 2010; Riley et al., 2011; Schneider et al., 2011; Valenzuela et al., 2012; Weinberg et al., 2008). The negative effects of alcohol consumption during pregnancy on offspring development can extend beyond the *in utero* effects, as maternal care may also be altered (O'Connor and Paley, 2006; Pearson et al., 2012). Indeed, the quality of maternal care has long-lasting consequences for the physical and mental health of infants and children (Gershon et al., 2013; Hofer, 1994; Hofer et al., 2008; Kim and Cicchetti, 2006; Murray et al., 1996), findings supported by studies in rodents demonstrating the significant developmental consequences of maternal care (Barha et al., 2007; Champagne et al., 2003; Hellstrom et al., 2012; Lindeyer et al., 2013; Raine et al., 2012; Weaver et al., 2004). Studies using animal models to investigate the consequences of alcohol consumption during pregnancy on maternal care have reported inconsistent results. Some studies showed that alcohol during pregnancy did not alter maternal behavior (Anandam et al., 1980; Ewart and Cutler, 1979), others showed that pup retrieval was delayed or reduced (Abel, 1978; Ness and Franchina, 1990), and one on the combined exposure to alcohol and nicotine found increased time away from pups (McMurray et al., 2008).

Expression of maternal behavior is the result of the activity of several interconnected brain areas including, the olfactory bulb, medial preoptic area, and amygdala (Olazabal et al., 2013). Although the hippocampus is not a major component of the maternal behavior circuitry, hippocampal lesions can disrupt maternal care – particularly pup retrieval (Kimble et al., 1967; Terlecki and Sainsbury, 1978). Moreover, the hippocampus is extremely vulnerable to long-term alcohol consumption (Beresford et al., 2006) and hippocampal degeneration may contribute to cognitive deficits and depression associated with alcoholism (Crews and Nixon, 2009; Nixon, 2006). Chronic and binge alcohol consumption suppress hippocampal neurogenesis by reducing cell proliferation and cell survival in male and female rodents immediately following exposure (Anderson et al., 2012; Crews et al., 2004; He et al., 2005; Herrera et al., 2003; Nixon and Crews, 2002). However, effects of alcohol may vary if assessed after a period of abstinence. For example, chronic consumption of alcohol (28 days for males, 6 weeks for females), reduced hippocampal neurogenesis after 2

weeks of abstinence in male and female mice (Pang et al., 2013; Stevenson et al., 2009), whereas in male and female rats, voluntary consumption of alcohol for 7 weeks followed by 4 weeks of abstinence increased neurogenesis by increasing cell proliferation (He et al., 2009). Together, these data suggest suppressed neurogenesis may be one component of alcohol-induced neurodegeneration, and increased cell proliferation during abstinence may represent a compensatory response to replace hippocampal neurons once alcohol consumption ceases.

The effects of prenatal alcohol exposure (PAE) on neurogenesis has also been investigated, with data suggesting significant adverse effects of PAE on neurogenesis in both male (Sliwowska et al., 2010) and female (Uban et al., 2010) offspring. However, dynamic effects of alcohol consumption on hippocampal neurogenesis in pregnant females have not been evaluated. Importantly, pregnancy and motherhood alter hippocampal structure and function. For instance, one reproductive experience (primiparity) reduced cell proliferation in the dentate gyrus in the early postpartum period (Darnaudery et al., 2007; Leuner et al., 2007; Pawluski et al., 2009b) and dendritic complexity in the CA3 and CA1 regions shortly after weaning in primiparous females (Pawluski et al., 2009a). In contrast, multiple reproductive experiences increased hippocampal neurogenesis in middle-aged females (Barha et al., 2011; Roes et al., 2014), and it is unknown whether there is a shift from reduced neurogenesis in the late postpartum.

Finally, chronic alcohol consumption can stimulate hypothalamic-pituitary-adrenal (HPA) axis activity in both non-pregnant (Becker, 2012) and pregnant females (Weinberg and Bezio, 1987). Additionally, administration of high concentrations of corticosterone reduced hippocampal neurogenesis in nulliparous (Brummelte and Galea, 2010) and postpartum females (Workman and Galea, unpublished observations). However, it is not known whether the increased HPA activity observed with alcohol intake during pregnancy (Weinberg and Bezio, 1987) persists into the postpartum period, and whether it affects hippocampal neurogenesis after a period of abstinence from alcohol.

Using an animal model of chronic alcohol consumption, we examine how alcohol consumption during pregnancy affects maternal behavior and HPA axis activity. We also assess hippocampal neurogenesis and developmental stage of immature neurons at the end of the postpartum period by staining for doublecortin (DCX), an endogenous protein expressed in immature neurons. As well, we compare the effects of chronic gestational alcohol consumption in postpartum females following their first reproductive experience and nulliparous (reproductively naïve) females to determine whether reproductive experience can buffer the effects of alcohol on neurogenesis and the HPA axis. We hypothesized that: 1) alcohol consumption during gestation will disrupt maternal care even when dams are not exposed to alcohol during the postpartum period; 2) chronic alcohol consumption will alter neurogenesis even after a period without alcohol exposure; 3) effects of alcohol on neurogenesis will differ in postpartum compared with nulliparous females; and 4) chronic alcohol consumption will alter corticosterone concentrations and adrenal mass in nulliparous and postpartum females and that the effects may differ depending on reproductive experience.

2. Methods

2.1 Animals

Male (N = 20) and female (N = 52) Sprague-Dawley rats were obtained from Charles River Laboratories (St. Constant, Quebec, Canada). Rats were housed with a same-sex cage mate and maintained at a constant temperature (21 ± 1 °C) and on a 12:12 light-dark cycle (lights on at 0700 h) with *ad libitum* access to water and standard laboratory chow (Harlan, Canada). Females were assigned to either be mated (postpartum females in their first reproductive experience, n = 9 – 13 per group) or to remain reproductively-naïve (nulliparous, n = 6 – 7 per group). All experiments were performed in accordance with National Institutes of Health (NIH) Guidelines For The Care And Use Of Laboratory Animals and Canadian Council on Animal Care guidelines and were approved by the University of British Columbia Animal Care Committee.

2.2 Breeding

After a 10-day acclimation period, males and females were paired for breeding. Vaginal smears were taken each morning, and the presence of sperm indicated gestational day 1 (G1), at which time pregnant rats were single-housed.

2.3 Alcohol Consumption

Pregnant rats at G1 and nulliparous females were randomly assigned to one of three treatment groups: alcohol, pair-fed or *ad libitum*-fed control. Animals in the alcohol group were offered *ad libitum* access to liquid ethanol diet with 36% ethanol-derived calories (Dyets Inc, Bethlehem, PA, USA). The liquid ethanol diet was introduced gradually over the first 3 days with bottles containing: Day 1 - 66% control diet, 34% ethanol diet; day 2 - 34% control diet, 66% ethanol diet; day 3 - 100% ethanol diet. This diet is formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake (Lan et al., 2006). Pair-fed animals were offered a liquid control diet with maltose-dextrin isocalorically substituted for ethanol, in an amount matched to that consumed by an ethanol-fed partner (g/Kg body weight/day of gestation or day of experiment). Control animals were offered *ad libitum* access to a pelleted form of the liquid control diet. All animals had *ad libitum* access to water, and were provided with fresh diet daily within 1 h prior to lights off to prevent a shift of corticosterone circadian rhythms, which occurs in animals that are on a restricted feeding schedule, such as pair-fed animals (Gallo and Weinberg, 1981). Experimental diets were continued for 21 days. Beginning on day 22 all animals were offered *ad libitum* access to standard laboratory chow and water, which they received throughout the remainder of the experiment. There was no alcohol consumption during the last 3 weeks of the experiment in either postpartum or nulliparous females. Animals were left undisturbed except for cage changing and weighing which occurred on G1, 7, 14, and 21 and at comparable times for nulliparous females. On the day of birth (postnatal day 1; PND 1), litters were weighed and culled to 10 – 12 pups with an attempt to preserve an equal number of males and females per litter.

2.4 Blood alcohol concentration (BAC)

To determinate the maximal or near maximal BAC achieved by alcohol consuming rats, tail blood samples were taken on day 15, approximately 2-3 h after lights off, which typically follows a major eating bout ($n = 3$ pregnant rats, $n = 7$ nulliparous rats). Blood samples were allowed to coagulate for 2 h at room temperature and then spun down at 3500 g for 20 min at 4 °C. Serum was collected and stored at -20 °C until the time of assay. BACs were measured using Pointe Scientific Inc. Alcohol Reagent Set (Lincoln Park, MI, USA); the minimum detectable concentration of alcohol is 2 mg/dl.

2.5 Maternal Behavior Observations

Observations of maternal behaviors occurred 3 times per day from postpartum days 2 through 8, and again on days 12, 16, and 20. Each observation consisted of a 75-min period during which each dam was observed once every 3 min for the following behaviors: licking and grooming (anogenital licking and body grooming were not distinguished in this experiment), nursing [arched-back, blanket, passive (nursing while supine or on side)], eating, drinking, self-grooming, sleeping, and off nest. Negative maternal behaviors were scored if dams were stepping on pups, dragging pups (i.e., moving around the cage if pups remained attached to the nipples), and handling pups roughly, as previously described (Rainecki et al., 2012).

2.6 Tissue collection

On PND 22, offspring were weaned and used in other studies. Dams were perfused immediately after weaning. In parallel, nulliparous females were perfused 22 days after ethanol diets stopped. All rats were given an overdose of sodium pentobarbital and weighed. Blood was collected via cardiac puncture and rats were perfused with 60 ml cold physiological saline followed by 120 ml cold 4% paraformaldehyde (PFA). Adrenal glands were collected and weighed. Relative adrenal mass was determined by dividing absolute adrenal mass by body mass. Blood samples were kept at 4 °C for 24 h, then centrifuged for 15 min at 3500 g. Serum was collected and frozen at -20 °C until radioimmunoassay. Brains were extracted and placed in 4% PFA at 4 °C to postfix overnight and then transferred to 30% sucrose in phosphate buffer at 4 °C until they sank to the bottom. Brains were rapidly frozen and sectioned using a freezing microtome (Leica, Richmond Hill, ON, Canada) at 40 µm in a series of 10. Sections were immediately stored in antifreeze (ethylene glycol/glycerol, Sigma) at -20 °C until immunohistochemistry for doublecortin (DCX), which is expressed in immature neurons approximately 1 – 21 days old (Brown et al., 2003). Bromodeoxyuridine (BrdU), a DNA synthesis marker which labels dividing progenitor cells and their progeny during a 2 h time period, was not used in this study as it could have unwanted effects on the offspring. When administered to pregnant dams, BrdU crosses the placenta and has numerous teratogenic effects (reviewed in (Taupin, 2007)). Similarly, BrdU could also transfer to pups via the milk and lead to neurodevelopmental deficits. Thus, we chose to examine expression of the endogenous protein DCX so as not to interfere with offspring development, as offspring from this breeding were used for other experiments.

2.7 Radioimmunoassay

Total corticosterone concentrations were measured in one assay using a double antibody ^{125}I radioimmunoassay kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. All reagents were halved and samples run in duplicate. The cross-reactivity with other steroids is less than 0.4%. The intra-assay coefficient of variation was less than 5%.

2.8 Immunohistochemistry

Sections were first washed in PBS 5×10 min and then treated with 0.3% hydrogen peroxide in dH_2O for 30 min before transferring into the primary antibody solution: 1:1000, goat anti-doublecortin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) with 0.04% Triton-X in PBS and 3% normal rabbit serum. Twenty-four hours later, sections were washed 5×10 min and transferred to the secondary antibody solution: 1:500, rabbit anti-goat (Vector Laboratories, Burlington, ON, Canada) in PBS for 24 h. Then sections were washed 5×10 min and incubated in ABC complex (ABC Elite Kit; 1:1000; Vector Laboratories) for 4 h. Sections were then washed in 0.175 M sodium acetate buffer 2×10 min. Finally, sections were developed using diaminobenzidine in the presence of nickel (DAB Peroxidase Substrate Kit, Vector), mounted on slides, dried overnight, dehydrated, cleared in xylene and cover slipped with Permount (Fisher). All steps took place at room temperature with the exceptions of the primary and secondary antibody incubations, which took place at 4°C .

2.9 Microscopy

An experimenter who was blind to treatment conditions quantified cells using an Olympus CX22LED brightfield microscope. DCX-expressing cells were exhaustively counted in every 10^{th} section throughout the hippocampus, yielding approximately 12 – 15 sections per rat. Functional distinctions in the hippocampus generally align with anatomical distinctions: the ventral hippocampus is generally more involved in stress, anxiety, and fear and the dorsal hippocampus generally more involved in spatial memory (Degroot and Treit, 2004; Hunsaker and Kesner, 2008; Kjelstrup et al., 2002; Maren and Holt, 2004; Moser et al., 1993; Trivedi and Coover, 2004). Thus, DCX-expressing cells were counted separately for the two regions as previously described (Brummelte and Galea, 2010). The total number of DCX-expressing cells in each region was estimated by multiplying the number counted by 10 as previously described (Brummelte and Galea, 2010; Eadie et al., 2005; Kronenberg et al., 2003). Images of every 10^{th} section were taken using an Olympus BX51 microscope with XM10 camera and cellSens Standard (Olympus). In order to determine volume of the dentate gyrus, areas were traced and calculated from the images using ImageJ (NIH), and the volume was calculated using Cavalieri's principle (Gundersen and Jensen, 1987). The density (cells per mm^3) of DCX-expressing cells was also determined by dividing number of cells counted by volume.

DCX-expressing cells were also classified based on developmental stages as previously described (Plumpe et al., 2006). A total of 50 cells per rat were randomly selected from the dorsal ($n = 25$) and the ventral ($n = 25$) hippocampus; cells had to be relatively isolated (i.e., not clumped or overlapping) and had to lie within the granule cell layer. Cells were classified as being in one of three developmental stages based on morphological attributes:

1) proliferative if they had no processes or short, plump processes; 2) intermediate if they had one thin or medium process; or 3) postmitotic if they had long and branching dendrites reaching the molecular layer (Hamson et al., 2013; Plumpe et al., 2006; Workman et al., 2015). No more than 10 cells were selected per any one section and were balanced throughout left and right hemispheres.

2.10 Data Analyses

Maternal behavior variables were analyzed using a mixed ANOVA with maternal diet (control, pair-fed, alcohol) as the between-subjects factor and day as the within-subjects factor. Because not all dams engaged in negative maternal behaviors, we used a proportion test to determine the proportion of alcohol-treated dams that displayed negative maternal behaviors compared with control and pair-fed dams. Body mass, relative adrenal mass, and corticosterone concentrations were analyzed using two-way ANOVA with diet and reproductive experience (nulliparous, postpartum) as between-subjects factors. The density and total number of DCX-expressing cells were analyzed using a mixed ANOVA with diet and reproductive experience as between-subjects factors and hippocampal region (dorsal, ventral) as the within-subjects factor. The proportion of DCX-expressing cells at the 3 different developmental stages was analyzed using a mixed ANOVA with diet and reproductive experience as between-subjects factors and hippocampal region (dorsal, ventral) and stage of maturity (proliferative, intermediate, postmitotic) as the within-subjects factors. *Post-hoc* analyses were conducted using Newman-Keuls procedure. Consumption of alcohol diet (g/Kg body mass) between nulliparous and postpartum females was analyzed using a *t*-test. Data were analyzed using Statistica (Version 12, Statsoft, Tulsa, OK, USA). All analyses were considered significant where $p \leq 0.05$.

3. Results

3.1 Alcohol consumption during pregnancy reduced total nursing and increased negative maternal behaviors, off-nest behavior, and self-directed behavior, but did not significantly change licking and grooming behavior

Alcohol-treated dams had a significantly lower total nursing frequency (main effect of diet: $F_{2,30} = 4.09$, $p = 0.026$, Figure 1A and B) compared with both control ($p = 0.046$) and pair-fed ($p = 0.02$) dams. Differences in total nursing frequency were not attributable to differences in any particular nursing posture, as diet did not significantly alter arched-back nursing, blanket nursing, or passive nursing ($p > 0.25$, data not shown). Dams, regardless of diet, nursed less over time (main effect of day: $F_{9,270} = 16.2$, $p < 0.001$) but there was no significant interaction between diet and day ($p = 0.72$). Individual nursing behaviors significantly changed over time (main effect of day, for all ANOVA: $F_{9,270} > 21.96$, $p < 0.01$), with passive nursing increasing and blanket and arched-back nursing decreasing (data not shown). Licking and grooming frequency decreased over time (main effect of day: $F_{9,270} = 11.42$, $p < 0.001$), but there was no significant main effect of diet ($p = 0.23$) and no significant interaction between diet and day ($p = 0.25$; Figure 1C and D).

Alcohol consumption during pregnancy did not significantly alter the frequency of negative maternal behaviors (diet: $p = 0.11$; Figure 1E). However, the proportion (0.4) of alcohol-

treated dams engaging in negative maternal behavior was significantly higher than that of control dams (0; $p < 0.01$), but not significantly higher than that of pair-fed dams (0.1; $p = 0.06$; Figure 1F).

Alcohol-treated dams had higher frequencies of off-nest behavior (main effect of diet: $F_{2,30} = 4.12$, $p = 0.026$) compared with both control ($p = 0.034$) and pair-fed ($p = 0.025$) dams (Figure 2A and B). In addition, although off nest behavior increased throughout the postpartum period (main effect of day: $F_{9, 270} = 19.4$, $p = 0.001$), there was no significant interaction between diet and day ($p = 0.26$). Alcohol-treated dams had significantly higher frequencies of eating (main effect of diet: $F_{2,30} = 13.87$, $p < 0.001$; Figure 2C and D) and drinking (main effect of diet: $F_{2,30} = 3.1$, $p = 0.031$; Figure 2E and F) compared with both control ($ps < 0.03$) and pair-fed ($p = 0.008$ and $p = 0.047$, respectively) dams.

3.2 Reproductive experience increased body mass but did not significantly alter amount of alcohol-diet consumed

Reproductive experience did not significantly alter amount of alcohol diet consumed ($p = 0.5$) or blood alcohol concentrations (nulliparous: 110.42 ± 20.01 mg/dl, $n = 7$; pregnant: 107.36 ± 32.83 mg/dl, $n = 3$; $p = 0.94$). In pregnant females, alcohol diet and pair feeding attenuated body mass gain compared with control females on days 14 and 21 following the initiation of diet, whereas in nulliparous females, only pair feeding attenuated body mass gain compared with control females on day 14 following initiation of diets (day by experience by diet interaction: $F_{4, 100} = 13.57$, $p < 0.001$; Table 1). At perfusion (22 days following the cessation of alcohol diet), postpartum females were significantly heavier than nulliparous females (main effect of reproductive experience: $F_{1,48} = 24.12$, $p < 0.001$; Figure 3A). There were no other significant main effects or interactions on body mass ($ps > 0.45$).

3.3 Chronic alcohol exposure increased corticosterone concentrations and relative adrenal mass in nulliparous rats only

In nulliparous females, previous alcohol consumption increased corticosterone concentrations compared with those in both control ($p < 0.01$) and pair-fed ($p < 0.05$; diet by experience interaction: $F_{2,41} = 7.69$, $p < 0.01$) groups (Figure 3B). Alcohol consumption also increased relative adrenal mass compared with control ($p = 0.01$) and tended to increase adrenal mass compared with pair feeding, in nulliparous rats only ($p = 0.06$; diet by experience interaction: $F_{2,46} = 3.71$, $p = 0.03$; main effect of reproductive experience: $F_{1,46} = 14.55$, $p < 0.001$; Figure 3C). By contrast, in postpartum rats, previous alcohol consumption suppressed corticosterone concentrations compared with those in control ($p = 0.04$), but not pair-fed ($p = 0.64$; Figure 3B) groups, but did not alter relative adrenal mass ($ps > 0.55$; no significant main effect of diet: $p > 0.22$; Figure 3C). Postpartum rats tended to have smaller absolute adrenal mass compared with nulliparous rats ($p = 0.069$) but there was no significant main effect of diet and no significant interaction between diet and reproductive experience ($ps > 0.23$; data not shown).

3.4 Reproductive experience reduced the total number and density of DCX-expressing cells in the dorsal and ventral dentate gyrus in late postpartum rats, but there were no

long-lasting effects of previous alcohol consumption in either nulliparous or postpartum rats

Reproductive experience significantly reduced total number of DCX-expressing cells in both the dorsal ($p < 0.001$) and the ventral ($p < 0.001$; region by reproductive experience interaction: $F_{1,45} = 6.51$; $p = 0.014$) hippocampus. Additionally, for both nulliparous and postpartum females, the ventral hippocampus contained a greater number of DCX-expressing cells compared with the dorsal hippocampus ($ps < 0.001$). There was no significant main effect of diet on total number of DCX-expressing cells and diet did not interact with region or reproductive experience ($ps > 0.63$; Figure 4).

Postpartum rats also had a lower density of DCX-expressing cells compared with nulliparous rats in both the dorsal ($p < 0.001$) and the ventral ($p < 0.001$; region by reproductive experience interaction: $F_{1,45} = 10.49$, $p = 0.002$; main effect of region: $F_{1,45} = 6.17$, $p = 0.017$) hippocampus (Figure 5A). Additionally, among postpartum rats, the ventral hippocampus contained a higher density of DCX-expressing cells compared with the dorsal hippocampus ($p < 0.001$) but this was not evident in nulliparous rats ($p = 0.69$). There was no significant effect of diet on density of DCX-expressing cells nor did diet interact with region or reproductive experience ($ps > 0.22$). See Figure 5B and C for representative photomicrographs of the dorsal dentate gyrus from nulliparous and postpartum rats, respectively. There were no significant main effects or interactions on hippocampal volume ($ps > 0.43$, Table 2).

3.5 Reproductive experience reduced the proportion of DCX-expressing cells in the postmitotic stage and increased the proportion in the proliferative stage

Regardless of hippocampal region, postpartum rats had a significantly greater proportion of proliferative cells ($p = 0.03$, stage by experience interaction: $F_{2,90} = 7.44$, $p = 0.001$; Figure 6A), and tended to have a greater proportion of intermediate cells ($p = 0.08$; Figure 6B), but had a significantly smaller proportion of postmitotic cells ($p < 0.001$; Figure 6C) compared with nulliparous rats. Postpartum rats also had a greater proportion of postmitotic cells than proliferative cells ($p = 0.004$). In nulliparous rats, by contrast, the proportions were: proliferative < intermediate < postmitotic cells ($ps < 0.048$).

Regardless of reproductive experience, the dorsal hippocampus contained a greater proportion of proliferative cells compared with the ventral hippocampus ($p < 0.001$; Figure 6A) and a smaller proportion of postmitotic cells ($p < 0.001$, stage by region interaction: $F_{2,90} = 35.53$, $p < 0.001$; main effect of stage: $F_{2,90} = 30.57$, $p < 0.001$; Figure 6C). There was no significant difference in the proportion of intermediate cells along the dorsal-ventral axis ($p = 0.31$). In the ventral hippocampus, the proportions were: proliferative < intermediate < cells postmitotic ($ps < 0.001$). The dorsal hippocampus, however, contained equivalent proportions of cells across the 3 developmental stages ($ps > 0.21$). There were no other significant main effects or interactions ($ps > 0.51$). See Figure 6D, E, and F for representative photomicrographs of proliferative, intermediate, and postmitotic DCX-expressing cells, respectively.

4. Discussion

In the present study we show that chronic alcohol consumption during pregnancy altered maternal behavior by reducing the time spent nursing and increasing self-directed, off nest, and negative maternal behaviors, without altering licking and grooming behavior. Furthermore, corticosterone concentrations were increased in nulliparous, but decreased in postpartum females, whereas relative adrenal mass was increased in nulliparous and unchanged in postpartum rats after a 21-day period without alcohol that followed a 21-day period of alcohol consumption. Moreover, after 21 days without alcohol exposure, regardless of previous diet, reproductive experience significantly reduced neurogenesis. That is, 21 days after parturition (i.e., at weaning), postpartum female rats had a reduced proportion of more mature, postmitotic DCX-expressing cells, and an increased proportion of less mature, proliferative DCX-expressing cells compared with nulliparous rats. Collectively, these data suggest that alcohol consumption for 21 days was not sufficient to alter hippocampal neurogenesis following a 21-day period without alcohol exposure, whereas HPA axis function was decreased in postpartum but increased in nulliparous rats. Thus, pregnancy and lactation may buffer long-term effects of alcohol on HPA axis disruption. These data are also consistent with the idea that HPA responses to stress are typically suppressed in the postpartum period and that this hyporesponsiveness extends to the effects of alcohol on the HPA axis.

4.1 Alcohol consumption during pregnancy impairs maternal care

Our data indicate that alcohol consumption during pregnancy yielded subtle but significant disruptions in maternal care throughout the postpartum period, a time when females were no longer consuming alcohol. Indeed, alcohol consumption, but not pair-feeding, during pregnancy reduced total nursing behaviors, without selectively reducing any particular nursing posture. Further, alcohol consumption increased self-directed behaviors such as eating, drinking, and off nest behavior and increased 'negative' maternal behaviors such as stepping on or dragging pups. These findings are highly relevant to our understanding of effects of alcohol on mother-infant interactions, as this is the first study to perform extensive observations of maternal behavior in dams that had consumed alcohol during gestation, in the home cage setting, without disturbing the mother or offspring. Additionally, this comprehensive maternal behavior profile also clarifies some of the previous conflicting results in the literature: some studies found that alcohol consumption during gestation did not alter maternal behavior (Anandam et al., 1980; Ewart and Cutler, 1979) whereas others found that it negatively affected pup retrieval (Abel, 1978; Ness and Franchina, 1990). Pup retrieval paradigms involve temporary removal of the dam from the nest and this disruption may be stressful and in turn, impact dams that had previously consumed alcohol to a greater extent than control dams, which may, at least in part, explain the differences among studies. Additionally, alcohol in combination with nicotine during gestation increased time away from pups and reduce maternal touching and sniffing of pups (McMurray et al., 2008). It is possible that deficits in maternal behavior of alcohol-treated dams could manifest from changes in pups' behavior towards the mother. Rat pups prenatally exposed to alcohol have a longer latency to nipple attach and reduced ultrasonic vocalizations (Chen et al., 1982; Kehoe and Shoemaker, 1991; Rockwood and Riley, 1990), which could limit alcohol-

exposed pups' ability to elicit the same levels of maternal care as controls. This notion is further supported by cross-fostering experiments in which control mothers also reduced maternal behavior when caring for alcohol-treated pups (Subramanian, 1992).

Importantly, alcohol consumption during pregnancy did not change the frequency of pup licking and grooming compared with that of control dams. Offspring that receive low levels of licking, grooming and arched-back nursing have increased stress-evoked HPA responses compared with offspring that received high levels of licking, grooming and arched-back nursing (Liu et al., 1997). Importantly, rats exposed to alcohol *in utero* have greater HPA responses to stress compared with control and pair-fed rats (Hellemans et al., 2010; Hellemans et al., 2008; Weinberg et al., 2008). Our finding that alcohol did not significantly alter licking, grooming or arched-back nursing in dams indicates that long-term changes in HPA and behavioral function of offspring prenatally exposed to alcohol are likely mediated by effects of alcohol itself.

In clinical studies, analysis of the specific effects of alcohol consumption during pregnancy on maternal care is extremely difficult, if not impossible, due to the difficulty in controlling for confounding factors, including: pattern, dose, and timing of maternal alcohol consumption; alcohol consumption prior to, during and following pregnancy; polydrug use; maternal age; maternal mental health history; and socioeconomic status (Knudsen et al., 2015; Olson et al., 2001). Nevertheless, our maternal behavior data corroborate the very few clinical studies that focus on maternal care (O'Connor and Paley, 2006; Pearson et al., 2012), and indicate that maternal behaviors are likely to be altered in mothers who consume alcohol during pregnancy.

4.2 Prior alcohol exposure potentiates corticosterone responses and increases adrenal mass in nulliparous, but not postpartum rats

Alcohol-treated nulliparous rats had higher corticosterone concentrations at perfusion, 21 days after the alcohol exposure period, compared with both control and pair-fed nulliparous rats. Additionally, among nulliparous rats, alcohol-treated females had larger relative adrenal mass compared with control females. By contrast, postpartum females had lower corticosterone concentrations than controls and no change in adrenal mass. These findings suggest that reproductive experience protected against or blocked the long-term effects of alcohol on adrenal size and output. These data are consistent with prior research indicating that stress-induced HPA activity is typically suppressed during the postpartum period (Atkinson and Waddell, 1995; Lightman, 1992). These data are also consistent with previous work indicating that although gestating and lactating females generally have higher basal corticosterone concentrations (Allolio et al., 1990; Neumann et al., 1998; Ogle and Kitay, 1977), they have reduced stress-induced corticosterone and ACTH responses when compared with naïve females (Lightman, 1992; Lightman et al., 2001; Neumann et al., 1998; Toufexis et al., 1999). The finding that postpartum females in the pair-fed condition were similar to females in the alcohol condition in their corticosterone levels suggests that reduced food intake during pregnancy may have played some role in reducing postpartum CORT concentrations, possibly through metabolic effects of restricted feeding. Collectively, our data suggest that chronic alcohol consumption potentiates long-term HPA axis activity

in nulliparous rats but that the postpartum period, in which no further alcohol consumption occurs, may facilitate recovery of alcohol-induced changes in the HPA axis.

4.3 Reproductive experience suppresses neurogenesis and alters DCX-expressing cell maturity in the hippocampus at the end of the postpartum period

At the end of the postpartum period, density of DCX-expressing cells was significantly lower in primiparous compared with nulliparous rats in both the dorsal and ventral hippocampus. These data extend previous work demonstrating that under normal conditions, postpartum females had lower rates of cell proliferation up to day 8 postpartum (Darnaude et al., 2007; Leuner et al., 2007; Pawluski and Galea, 2007) but by day 28 postpartum, cell proliferation was no longer suppressed (Leuner et al., 2007). We found previously that survival of new neurons produced in the dentate gyrus on the first day postpartum was reduced during the postpartum period in primiparous, but not multiparous, dams (Pawluski and Galea, 2007). In the present study, the density of DCX-expressing cells, which are between approximately 1 and 21 days old (Brown et al., 2003), was lower at the end of the postpartum period. Further, postpartum rats also had a lower proportion of postmitotic (more mature) DCX-expressing cells and a higher proportion of proliferative (less mature) cells compared with nulliparous rats, regardless of hippocampal region. These data suggest that although hippocampal neurogenesis is suppressed during the postpartum period, neurogenesis in dams begins to recover just prior to weaning, evidenced by the greater proportion of proliferative cells.

The ventral hippocampus contained a lower proportion of proliferating cells and a greater proportion of postmitotic cells compared with the dorsal hippocampus, regardless of reproductive experience or diet. These data may indicate that the production or development of new neurons in the dorsal hippocampus occurs more quickly than in the ventral hippocampus. This idea is consistent with prior research indicating that new neurons in the dorsal hippocampus mature approximately 1 – 2 weeks earlier compared with those in the ventral hippocampus (Snyder et al., 2012), although this prior study was conducted using male rats.

4.4 Prior alcohol consumption did not significantly alter neurogenesis or DCX-expressing cell maturity in the hippocampus in late postpartum or nulliparous females

Surprisingly, prior exposure to an alcohol diet did not significantly alter hippocampal neurogenesis in either postpartum or nulliparous females after 21 days without alcohol exposure. As noted, similar levels of alcohol consumption suppressed neurogenesis in male and female rats (He et al., 2005; Nixon and Crews, 2002). Similarly, female rats consuming alcohol for 2 weeks in a liquid diet had 40% fewer BrdU-positive cells (likely via reduced cell proliferation) in the dentate gyrus compared with females receiving a control diet (Anderson et al., 2012). This prior study found BACs very similar to ours (Anderson: 54.2 – 147.6 mg/dl; our study: 52.08 – 171.03 mg/dl). However, in the Anderson study, the phenotype of these cells was not determined. It is important to note that BrdU labels cells undergoing DNA synthesis (not just dividing cells) and thus BrdU-labeled cells represent neurons, glia, and undifferentiated cells, whereas DCX is expressed only in neurons. It is possible that, in our study, neurogenesis levels recovered once alcohol consumption ceased.

DCX is expressed for up to 21 days in new neurons (Brown et al., 2003) so cells immunopositive for DCX in our experiment were most likely produced after alcohol consumption had ended, although postmitotic neurons were produced closest to the time of alcohol consumption and their division may have occurred during the end of the alcohol consumption period. However, we did not independently assess whether a decrease in neurogenesis occurred immediately after cessation of alcohol consumption. Thus, future experiments could use DCX expression to assess postpartum and nulliparous females directly after the alcohol-consumption period to determine whether alcohol reduces numbers of immature neurons.

Our finding that there was no significant effect of previous diet after 3 weeks without alcohol exposure on neurogenesis or the proportion of cells at particular developmental stages differs from findings in previous studies. For example, adverse effects of chronic alcohol consumption on both female (Pang et al., 2013) and male (Stevenson et al., 2009) mice emerged only after protracted abstinence. In male Sprague-Dawley rats, however, binge alcohol consumption initially reduced BrdU-positive cells, but significantly increased BrdU-positive cells after 7 days of abstinence (Nixon and Crews, 2004), suggesting a rebound of cell proliferation during withdrawal. Similar findings were reported in alcohol-preferring Wistar rats, where hippocampal neurogenesis increased in both males and females during abstinence following 7 weeks of voluntary alcohol consumption (He et al., 2009). These contrasting findings are likely due to differences in time course of alcohol consumption, species, strain, or type of alcohol diet. Nevertheless, collectively these studies suggest that neurogenesis in the hippocampus of females may be resistant to shorter durations (albeit still chronic) of alcohol consumption.

4.5 Conclusions

In sum, alcohol consumption during pregnancy reduced maternal care by increasing time spent away from pups, negative maternal behaviors, and self-directed behaviors. Alcohol consumption increased HPA function in nulliparous, but decreased HPA function in postpartum, rats, suggesting that reproductive experience may buffer, or facilitate recovery of, the HPA axis after chronic alcohol consumption. Surprisingly, previous alcohol intake did not significantly alter hippocampal neurogenesis or stage of maturity of new neurons after a period without alcohol in female rats. However we found that 21 days after parturition, postpartum rats had fewer immature neurons than nulliparous rats but had an increased proportion of the least mature cells, indicating that neurogenesis may begin to recover during the postpartum period. Together, these data contribute to our understanding of the long-term consequences of alcohol exposure in females, and the influence of reproductive status on these consequences, and are important for understanding how females respond to and recover from chronic alcohol consumption. Moreover, these findings have implications for understanding effects of prenatal alcohol exposure on offspring behavioral and HPA function, enabling us to begin to tease apart effects of alcohol from those of altered maternal care.

Acknowledgements

We thank Carmen Chow, Michelle Foisy, Aarthi Gobinath, Dr. Paula Duarte Guterman, Dr. Dwayne Hamson, Stephanie Lieblich, Meighen Roes, Sophia Solomon, Steven Wainwright, and all members of the Weinberg laboratory for assistance. This research was supported by NIH/NIAAA grants R37 AA007789 and R01 AA022460, and NeuroDevNet (Canadian Networks of Centers of Excellence) grant 20R64153 to JW, and Canadian Foundation on Fetal Alcohol Research (CFFAR) grant to CR and JW.

Role of the funding source

This research was supported by NIH/NIAAA grants R37 AA007789 and R01 AA022460, and NeuroDevNet (Canadian Networks of Centers of Excellence) grant 20R64153 to JW, and Canadian Foundation on Fetal Alcohol Research (CFFAR) grant to CR and JW. Funding sources did not contribute to experimental design, collection, analysis or interpretation of data, or decisions regarding submission.

References

- Abel EL. Effects of ethanol on pregnant rats and their offspring. *Psychopharmacology (Berl)*. 1978; 57:5–11. [PubMed: 96469]
- Alloio B, Hoffmann J, Linton EA, Winkelmann W, Kusche M, Schulte HM. Diurnal salivary cortisol patterns during pregnancy and after delivery: relationship to plasma corticotrophin-releasing-hormone. *Clin Endocrinol (Oxf)*. 1990; 33:279–289. [PubMed: 2225483]
- Anandam N, Felegi W, Stern JM. In utero alcohol heightens juvenile reactivity. *Pharmacol Biochem Behav*. 1980; 13:531–535. [PubMed: 7433484]
- Anderson ML, Nokia MS, Govindaraju KP, Shors TJ. Moderate drinking? Alcohol consumption significantly decreases neurogenesis in the adult hippocampus. *Neuroscience*. 2012; 224:202–209. [PubMed: 22906480]
- Atkinson HC, Waddell BJ. The hypothalamic-pituitary-adrenal axis in rat pregnancy and lactation: circadian variation and interrelationship of plasma adrenocorticotropin and corticosterone. *Endocrinology*. 1995; 136:512–520. [PubMed: 7835284]
- Barha, CK.; Lieblich, SE.; Chow, C.; Galea, LA. Previous reproductive experience influences the effects of different forms of estrogen on hippocampus-dependent learning and memory in middle-aged female rats.. Presented at the Society for Behavioral Neuroendocrinology Annual Meeting; Querétaro, Mexico. 2011.
- Barha CK, Pawluski JL, Galea LA. Maternal care affects male and female offspring working memory and stress reactivity. *Physiol Behav*. 2007; 92:939–950. [PubMed: 17716698]
- Becker HC. Effects of alcohol dependence and withdrawal on stress responsiveness and alcohol consumption. *Alcohol research : current reviews*. 2012; 34:448–458. [PubMed: 23584111]
- Beresford TP, Arciniegas DB, Alfors J, Clapp L, Martin B, Du Y, Liu D, Shen D, Davatzikos C. Hippocampus volume loss due to chronic heavy drinking. *Alcohol Clin Exp Res*. 2006; 30:1866–1870. [PubMed: 17067350]
- Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol*. 2003; 467:1–10. [PubMed: 14574675]
- Brummelte S, Galea L. Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience*. 2010; 168:680–690. [PubMed: 20406669]
- Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav*. 2003; 79:359–371. [PubMed: 12954431]
- Chen JS, Driscoll CD, Riley EP. Ontogeny of suckling behavior in rats prenatally exposed to alcohol. *Teratology*. 1982; 26:145–153. [PubMed: 7157191]
- Crews FT, Nixon K. Mechanisms of neurodegeneration and regeneration in alcoholism. *Alcohol*. 2009; 44:115–127. [PubMed: 18940959]
- Crews FT, Nixon K, Wilkie ME. Exercise reverses ethanol inhibition of neural stem cell proliferation. *Alcohol*. 2004; 33:63–71. [PubMed: 15353174]

- Darnaudery M, Perez-Martin M, Del Favero F, Gomez-Roldan C, Garcia-Segura LM, Maccari S. Early motherhood in rats is associated with a modification of hippocampal function. *Psychoneuroendocrinology*. 2007; 32:803–812. [PubMed: 17640823]
- Degroot A, Treit D. Anxiety is functionally segregated within the septo hippocampal system. *Brain Res*. 2004; 1001:60–71. [PubMed: 14972654]
- Eadie BD, Redila VA, Christie BR. Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *J Comp Neurol*. 2005; 486:39–47. [PubMed: 15834963]
- Ewart FG, Cutler MG. Effects of ethyl alcohol on behaviour in nursing female mice. *Psychopharmacology (Berl)*. 1979; 66:143–146. [PubMed: 119256]
- Gallo PV, Weinberg J. Corticosterone rhythmicity in the rat: interactive effects of dietary restriction and schedule of feeding. *J Nutr*. 1981; 111:208–218. [PubMed: 7463165]
- Gershon A, Sudheimer K, Tirouvanziam R, Williams LM, O'Hara R. The long-term impact of early adversity on late-life psychiatric disorders. *Current psychiatry reports*. 2013; 15:352. [PubMed: 23443532]
- Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. *J Microsc*. 1987; 147:229–263. [PubMed: 3430576]
- Hamson DK, Wainwright SR, Taylor JR, Jones BA, Watson NV, Galea LA. Androgens increase survival of adult-born neurons in the dentate gyrus by an androgen receptor-dependent mechanism in male rats. *Endocrinology*. 2013; 154:3294–3304. [PubMed: 23782943]
- He J, Nixon K, Shetty AK, Crews FT. Chronic alcohol exposure reduces hippocampal neurogenesis and dendritic growth of newborn neurons. *Eur J Neurosci*. 2005; 21:2711–2720. [PubMed: 15926919]
- He J, Overstreet DH, Crews FT. Abstinence from moderate alcohol self-administration alters progenitor cell proliferation and differentiation in multiple brain regions of male and female P rats. *Alcohol Clin Exp Res*. 2009; 33:129–138. [PubMed: 19076732]
- Hellems KG, Sliwowska JH, Verma P, Weinberg J. Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. *Neurosci Biobehav Rev*. 2010; 34:791–807. [PubMed: 19545588]
- Hellems KG, Verma P, Yoon E, Yu W, Weinberg J. Prenatal alcohol exposure increases vulnerability to stress and anxiety-like disorders in adulthood. *Ann N Y Acad Sci*. 2008; 1144:154–175. [PubMed: 19076375]
- Hellstrom IC, Dhir SK, Diorio JC, Meaney MJ. Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. *Philos Trans R Soc Lond B Biol Sci*. 2012; 367:2495–2510. [PubMed: 22826348]
- Herrera DG, Yague AG, Johnsen-Soriano S, Bosch-Morell F, Collado-Morente L, Muriach M, Romero FJ, Garcia-Verdugo JM. Selective impairment of hippocampal neurogenesis by chronic alcoholism: protective effects of an antioxidant. *Proc Natl Acad Sci U S A*. 2003; 100:7919–7924. [PubMed: 12792022]
- Hofer MA. Early relationships as regulators of infant physiology and behavior. *Acta paediatrica*. 1994; 397:9–18. [PubMed: 7981480]
- Hofer T, Hohenberger A, Hauf P, Aschersleben G. The link between maternal interaction style and infant action understanding. *Infant Behav Dev*. 2008; 31:115–126. [PubMed: 17707913]
- Hunsaker MR, Kesner RP. Dissociations across the dorsal-ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. *Neurobiol Learn Mem*. 2008; 89:61–69. [PubMed: 17931914]
- Kehoe P, Shoemaker W. Opioid-dependent behaviors in infant rats: effects of prenatal exposure to ethanol. *Pharmacol Biochem Behav*. 1991; 39:389–394. [PubMed: 1946579]
- Kim J, Cicchetti D. Longitudinal trajectories of self-system processes and depressive symptoms among maltreated and nonmaltreated children. *Child development*. 2006; 77:624–639. [PubMed: 16686792]
- Kimble DP, Rogers L, Hendrickson CW. Hippocampal lesions disrupt maternal, not sexual, behavior in the albino rat. *J Comp Physiol Psychol*. 1967; 63:401–407. [PubMed: 6064383]

- Kjelstrup KG, Tuvnes FA, Steffenach HA, Murison R, Moser EI, Moser MB. Reduced fear expression after lesions of the ventral hippocampus. *Proc Natl Acad Sci U S A*. 2002; 99:10825–10830. [PubMed: 12149439]
- Knudsen AK, Ystrom E, Skogen JC, Torgersen L. Maternal heavy alcohol use and toddler behavior problems: a fixed effects regression analysis. *European child & adolescent psychiatry*. 2015 doi: 10.1007/s00787-015-0677-5.
- Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, Kempermann G. Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol*. 2003; 467:455–463. [PubMed: 14624480]
- Lan N, Yamashita F, Halpert AG, Ellis L, Yu WK, Viau V, Weinberg J. Prenatal ethanol exposure alters the effects of gonadectomy on hypothalamic-pituitary-adrenal activity in male rats. *J Neuroendocrinol*. 2006; 18:672–684. [PubMed: 16879166]
- Leuner B, Mirescu C, Noiman L, Gould E. Maternal experience inhibits the production of immature neurons in the hippocampus during the postpartum period through elevations in adrenal steroids. *Hippocampus*. 2007; 17:434–442. [PubMed: 17397044]
- Lightman SL. Alterations in hypothalamic-pituitary responsiveness during lactation. *Ann N Y Acad Sci*. 1992; 652:340–346. [PubMed: 1320830]
- Lightman SL, Windle RJ, Wood SA, Kershaw YM, Shanks N, Ingram CD. Peripartum plasticity within the hypothalamo-pituitary-adrenal axis. *Prog Brain Res*. 2001; 133:111–129. [PubMed: 11589125]
- Lindey CM, Meaney MJ, Reader SM. Early maternal care predicts reliance on social learning about food in adult rats. *Dev Psychobiol*. 2013; 55:168–175. [PubMed: 22315158]
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997; 277:1659–1662. [PubMed: 9287218]
- Maren S, Holt WG. Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. *Behav Neurosci*. 2004; 118:97–110. [PubMed: 14979786]
- McMurray MS, Williams SK, Jarrett TM, Cox ET, Fay EE, Overstreet DH, Walker CH, Johns JM. Gestational ethanol and nicotine exposure: effects on maternal behavior, oxytocin, and offspring ethanol intake in the rat. *Neurotoxicology and teratology*. 2008; 30:475–486. [PubMed: 18664381]
- Moser E, Moser MB, Andersen P. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J Neurosci*. 1993; 13:3916–3925. [PubMed: 8366351]
- Murray L, Fiori-Cowley A, Hooper R, Cooper P. The impact of postnatal depression and associated adversity on early mother-infant interactions and later infant outcome. *Child development*. 1996; 67:2512–2526. [PubMed: 9022253]
- Ness JW, Franchina JJ. Effects of prenatal alcohol exposure on rat pups' ability to elicit retrieval behavior from dams. *Dev Psychobiol*. 1990; 23:85–99. [PubMed: 2340959]
- Neumann ID, Johnstone HA, Hatzinger M, Liebsch G, Shipston M, Russell JA, Landgraf R, Douglas AJ. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohipophysial changes. *The Journal of physiology*. 1998; 508(Pt 1):289–300. [PubMed: 9490853]
- Nixon K. Alcohol and adult neurogenesis: roles in neurodegeneration and recovery in chronic alcoholism. *Hippocampus*. 2006; 16:287–295. [PubMed: 16421863]
- Nixon K, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. *J Neurochem*. 2002; 83:1087–1093. [PubMed: 12437579]
- Nixon K, Crews FT. Temporally specific burst in cell proliferation increases hippocampal neurogenesis in protracted abstinence from alcohol. *J Neurosci*. 2004; 24:9714–9722. [PubMed: 15509760]
- O'Connor MJ, Paley B. The relationship of prenatal alcohol exposure and the postnatal environment to child depressive symptoms. *Journal of pediatric psychology*. 2006; 31:50–64. [PubMed: 15802607]

- Ogle TF, Kitay JI. Ovarian and adrenal steroids during pregnancy and the oestrous cycle in the rat. *J Endocrinol.* 1977; 74:89–98. [PubMed: 559719]
- Olazabal DE, Pereira M, Agrati D, Ferreira A, Fleming AS, Gonzalez-Mariscal G, Levy F, Lucion AB, Morrell JI, Numan M, Uriarte N. Flexibility and adaptation of the neural substrate that supports maternal behavior in mammals. *Neurosci Biobehav Rev.* 2013; 37:1875–1892. [PubMed: 23608126]
- Olson HC, O'Connor MJ, Fitzgerald HE. Lessons learned from study of the developmental impact of parental alcohol use. *Inf Mental Hlth J.* 2001; 22:271–290.
- Pang TY, Renoir T, Du X, Lawrence AJ, Hannan AJ. Depression-related behaviours displayed by female C57BL/6J mice during abstinence from chronic ethanol consumption are rescued by wheel-running. *Eur J Neurosci.* 2013; 37:1803–1810. [PubMed: 23551162]
- Pawluski JL, Brummelte S, Barha CK, Crozier TM, Galea LA. Effects of steroid hormones on neurogenesis in the hippocampus of the adult female rodent during the estrous cycle, pregnancy, lactation and aging. *Front Neuroendocrinol.* 2009a; 30:343–357. [PubMed: 19361542]
- Pawluski JL, Charlier TD, Lieblich SE, Hammond GL, Galea LA. Reproductive experience alters corticosterone and CBG levels in the rat dam. *Physiol Behav.* 2009b; 96:108–114. [PubMed: 18817795]
- Pawluski JL, Galea LA. Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. *Neuroscience.* 2007; 149:53–67. [PubMed: 17869008]
- Pearson RM, Heron J, Melotti R, Joinson C, Evans J. The impact of alcohol use during pregnancy on maternal responses after birth. *Arch Womens Ment Health.* 2012; 15:433–443. [PubMed: 22915028]
- Plumpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Romer B, Rodriguez GR, Kronenberg G, Kempermann G. Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci.* 2006; 7:77. doi: 10.1186/1471-2202-7-7. [PubMed: 17105671]
- Raineki C, Cortes MR, Belnoue L, Sullivan RM. Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *J Neurosci.* 2012; 32:7758–7765. [PubMed: 22649253]
- Riley EP, Infante MA, Warren KR. Fetal alcohol spectrum disorders: an overview. *Neuropsychology review.* 2011; 21:73–80. [PubMed: 21499711]
- Rockwood GA, Riley EP. Nipple attachment behavior in rat pups exposed to alcohol in utero. *Neurotoxicology and teratology.* 1990; 12:383–389. [PubMed: 2392098]
- Roes, MM.; Van den Brink, C.; Lieblich, SE.; Chow, C.; Galea, LA. Reproductive experience alters the effects of Premarin and surgical menopause on spatial memory and hippocampal plasticity in middle-aged female rats.. Presented at the Society for Neuroscience Annual Meeting.; Washington D.C.. 2014.
- Schneider ML, Moore CF, Adkins MM. The effects of prenatal alcohol exposure on behavior: rodent and primate studies. *Neuropsychology review.* 2011; 21:186–203. [PubMed: 21499982]
- Sliwowska JH, Barker JM, Barha CK, Lan N, Weinberg J, Galea LA. Stress-induced suppression of hippocampal neurogenesis in adult male rats is altered by prenatal ethanol exposure. *Stress.* 2010; 13:301–313. [PubMed: 20536332]
- Snyder JS, Ferrante SC, Cameron HA. Late maturation of adult-born neurons in the temporal dentate gyrus. *PLoS One.* 2012; 7:e48757. [PubMed: 23144957]
- Stevenson JR, Schroeder JP, Nixon K, Besheer J, Crews FT, Hodge CW. Abstinence following alcohol drinking produces depression-like behavior and reduced hippocampal neurogenesis in mice. *Neuropsychopharmacology.* 2009; 34:1209–1222. [PubMed: 18563059]
- Subramanian MG. Lactation and prolactin release in foster dams suckling prenatally ethanol exposed pups. *Alcohol Clin Exp Res.* 1992; 16:891–894. [PubMed: 1443427]
- Taupin P. BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. *Brain Res Rev.* 2007; 53:198–214. [PubMed: 17020783]
- Terlecki LJ, Sainsbury RS. Effects of fimbria lesions on maternal behavior in the rat. *Physiol Behav.* 1978; 21:89–97. [PubMed: 567818]

- Toufexis DJ, Tesolin S, Huang N, Walker C. Altered pituitary sensitivity to corticotropin-releasing factor and arginine vasopressin participates in the stress hyporesponsiveness of lactation in the rat. *J Neuroendocrinol.* 1999; 11:757–764. [PubMed: 10520124]
- Trivedi MA, Coover GD. Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. *Neurobiol Learn Mem.* 2004; 81:172–184. [PubMed: 15082019]
- Uban KA, Sliwowska JH, Lieblich S, Ellis LA, Yu WK, Weinberg J, Galea LA. Prenatal alcohol exposure reduces the proportion of newly produced neurons and glia in the dentate gyrus of the hippocampus in female rats. *Horm Behav.* 2010; 58:835–843. [PubMed: 20736015]
- Valenzuela CF, Morton RA, Diaz MR, Topper L. Does moderate drinking harm the fetal brain? Insights from animal models. *Trends Neurosci.* 2012; 35:284–292. [PubMed: 22402065]
- Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci.* 2004; 7:847–854. [PubMed: 15220929]
- Weinberg J, Bezio S. Alcohol-induced changes in pituitary-adrenal activity during pregnancy. *Alcohol Clin Exp Res.* 1987; 11:274–280. [PubMed: 3307493]
- Weinberg J, Sliwowska JH, Lan N, Hellemans KG. Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. *J Neuroendocrinol.* 2008; 20:470–488. [PubMed: 18266938]
- Workman JL, Chan MY, Galea LA. Prior high corticosterone exposure reduces activation of immature neurons in the ventral hippocampus in response to spatial and nonspatial memory. *Hippocampus.* 2015; 25:329–344. [PubMed: 25269789]

Highlights

- Chronic alcohol consumption during pregnancy reduced maternal care.
- Prior alcohol consumption increased HPA axis function in nulliparous females only.
- Primiparity reduced neurogenesis and altered development of immature neurons.
- Chronic alcohol did not alter neurogenesis after a period of abstinence.

Figure 1.

Mean + SEM frequency of all nursing behaviors (arched-back, blanket, and passive) for each day (A) and averaged across the postpartum period (B). Alcohol consumption during pregnancy reduced nursing frequency compared with both pair-fed and control rats ($\dagger$ P s < 0.046). Mean + SEM frequency of licking and grooming for each day (C) and averaged across the postpartum period (D). Diet did not significantly alter licking and grooming. Mean + SEM frequency negative maternal behaviors for each day (E) and the proportion of dams in each group engaging in negative maternal behaviors at any point during the

postpartum period (F). Alcohol consumption during pregnancy significantly increased the proportion of dams displaying negative maternal behaviors compared with control and tended to increased compared with pair-fed rats ($p < 0.01$, $p = 0.06$, respectively).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

Mean + SEM frequency of off-nest behavior for each day (A) and averaged across the postpartum period (B). Alcohol consumption during pregnancy increased off-nest behavior compared with both pair-fed and control rats ($\dagger P_s < 0.035$). Mean + SEM frequency of eating for each day (C) and averaged across the postpartum period (D). Alcohol consumption during pregnancy increased frequency of eating compared with control and pair-fed diets ($\dagger P_s < 0.03$). Mean $\pm$ SEM frequency of drinking for each day (E) and averaged across the postpartum period (F). Alcohol consumption during pregnancy

significantly increased the frequency of drinking compared with control and pair-fed diets ($\ddagger$ $P_s < 0.047$).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

A) Mean + SEM grams body mass at perfusion. Postpartum females were significantly heavier than nulliparous females (* $p < 0.001$). B) Mean + SEM corticosterone concentrations (ng/ml) after 21 days of no alcohol exposure. In nulliparous females, alcohol consumption significantly increased corticosterone concentrations compared with both control diet and pair feeding ($\ddagger$ p s < 0.05). In postpartum females alcohol diet significantly reduced corticosterone concentrations compared with control diet ($p = 0.04$) but not pair feeding ($p = 0.64$). C) Mean + SEM relative adrenal mass (mg/g body mass). In nulliparous females, alcohol increased relative adrenal mass compared with control ($p = 0.01$) and tended to increase compared with pair feeding ($p = 0.06$) but had no significant effect in postpartum females.

Figure 4.

Mean + SEM total number of DCX-expressing cells. Reproductive experience significantly reduced the density of DCX-expressing cells in both the dorsal and ventral dentate gyrus compared with nulliparous rats (§ $p < 0.001$). Also, the ventral dentate gyrus contained more DCX-expressing cells compared with the dorsal dentate gyrus in both nulliparous and postpartum females († $p < 0.001$).

Figure 5.

A) Mean + SEM number of DCX-expressing cells per mm³. Reproductive experience significantly reduced the density of DCX-expressing cells in both the dorsal and ventral dentate gyrus compared with nulliparous rats (§ ps < 0.001). Also, in postpartum females, the ventral dentate gyrus had a higher density of DCX-expressing cells compared with the dorsal dentate gyrus († p < 0.001). Photomicrographs depicting the suprapyramidal blade of the dorsal dentate gyrus from B) a nulliparous female and C) a late postpartum female. Arrows indicate examples of cells expressing DCX. Note, arrows may not indicate all cells as some may be overlapping or in a different plane of focus. Scale bars = 100 μm.

Figure 6.

A) Mean + SEM percent of DCX-expressing cells in the proliferative stage. Postpartum females had a greater proportion of proliferative cells compared with nulliparous females (* $p = 0.03$). The ventral hippocampus also contained a lower proportion of proliferative cells compared with the dorsal hippocampus († $p < 0.001$). B) Neither diet nor reproductive experience significantly altered DCX-expressing cells in the intermediate stage. C) Postpartum females had a lower proportion of postmitotic cells compared with nulliparous females (* $p < 0.001$). The ventral hippocampus also contained a greater proportion of postmitotic cells compared with the dorsal hippocampus († $p < 0.001$). Photomicrographs depicting DCX-expressing cells at D) a proliferative stage, E) an intermediate stage, and F) a postmitotic stage. All scale bars = 50 μm . Note that in photomicrograph F, scale is slightly smaller than D and E to depict extent of dendritic tree.

Table 1

Mean ± SEM percent gained each week following the initiation of diets.

	Nulliparous				Pregnant			
	Control	Pair fed	Alcohol	Control	Pair Fed	Alcohol	Control	Alcohol
Day 7	4.87 ± 0.93	1.45 ± 0.87	2.59 ± 0.77	8.67 ± 0.46	4.74 ± 0.79	4.35 ± 0.98		
Day 14	11.53 ± 1.3	6.14 ± 1.45*	7.43 ± 1.3	24.29 ± 0.81	16.11 ± 0.89†	16.03 ± 1.2†		
Day 21	15.24 ± 1.15	12.11 ± 1.78	14.85 ± 1.69	58.22 ± 1.56	41.97 ± 1.69‡	40.53 ± 2.11‡		

Table 2

Mean $\pm$ SEM volume (mm³) of the dentate gyrus.

	Control	Pair fed	Ethanol
Nulliparous	4.03 $\pm$ 0.23	4.06 $\pm$ 0.21	3.76 $\pm$ 0.23
Postpartum	3.95 $\pm$ 0.16	3.87 $\pm$ 0.19	4.09 $\pm$ 0.18