Prenatal Alcohol Exposure Is Associated with Altered Subcellular Distribution of Glucocorticoid and Mineralocorticoid Receptors in the Adolescent Mouse Hippocampal Formation

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Background: Accumulating evidence indicates that several of the long-term consequences of prenatal alcohol exposure (PAE) are the result of changes in the development and function of cortico-limbic structures, including the hippocampal formation. The glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) are key regulators of hippocampal formation development, structure, and functioning and, thus, are potential mediators of PAE’s effects on this brain region. In the present studies, we assessed the impact of PAE on components of corticosteroid signaling pathways in the mouse hippocampal formation.

Methods: Throughout pregnancy, mouse dams were offered either 10% (w/v) ethanol sweetened with 0.066% (w/v) saccharin (SAC) or 0.066% (w/v) SAC alone using a limited (4-hour) access, drinking-in-the-dark paradigm. The hippocampal formation was isolated from naive postnatal day 40 to 50 offspring, and subcellular fractions were prepared. Using immunoblotting techniques, we measured the levels of GR, MR, 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), and the FK506-binding proteins 51 (FKBP51, FKBP5) and 52 (FKBP52, FKBP4). Finally, we determined the effect of PAE on context discrimination, a hippocampal-dependent learning/memory task.

Results: PAE was associated with reduced MR and elevated GR nuclear localization in the hippocampal formation, whereas cytosolic levels of both receptors were not significantly altered. FKBP51 levels were reduced, while FKBP52 levels were unaltered, and 11β-HSD1 levels were increased in post-nuclear fractions isolated from PAE mouse hippocampal formation. These neurochemical alterations were associated with reduced context discrimination.

Conclusions: The data support a model in which PAE leads to increased nuclear localization of GRs secondary to reductions in FKBP51 and increases in 11β-HSD1 levels in the adolescent mouse hippocampal formation. Persistent dysregulation of GR subcellular distribution is predicted to damage the hippocampal formation and may underlie many of the effects of PAE on hippocampal-dependent functioning.

Key Words: Prenatal, Alcohol, Glucocorticoid, Mineralocorticoid, 11β-HSD1, Hippocampus.

In both humans and laboratory animal models, prenatal alcohol exposure (PAE) exerts a multitude of dose-dependent effects on the developing central nervous system. Many of these effects, or their secondary consequences, persist throughout the life span of the offspring, causing a range of physical, behavioral, cognitive, and social dysfunctions that are collectively termed fetal alcohol spectrum disorders (Streissguth and O’Malley, 2000). While several brain regions are impacted by in utero alcohol exposure, the damaging effects of PAE on hippocampal formation structure and functioning are particularly pronounced (Valenzuela et al., 2012). Corticosteroids (corticosterone in rodents and cortisol in humans), acting via binding to the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), are key regulators of hippocampal formation development, structure, and functioning (McEwen, 2012); thus, GRs and MRs are potential mediators of many of the effects of PAE on this brain region. Whereas the GR is widely distributed throughout the rodent brain, the MR is restricted to limbic structures, primarily the septal–hippocampal complex, and some cortical areas (Reul and de Kloet, 1986).

The GR and MR are primarily localized to the cytoplasm of the cell in the absence of ligand and translocate to the nucleus upon binding of an agonist (Nishi, 2012). Trafficking of the MR and GR between the cytosolic and nuclear compartments is regulated by several chaperone and co-chaperone proteins, including the FK506-binding proteins 51 (FKBP51, FKBP5) and 52 (FKBP52, FKBP4), and
in intracellular level of corticosteroid (Gaglianina et al., 2010; Nishi et al., 2001; Vandevyver et al., 2012). 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), which, in the hippocampus, acts as a reductase, converting the inactive 11-dehydrocorticosterone to its active form, corticosterone (Sooy et al., 2010), has been shown to be an important regulator of hippocampal formation corticosteroid levels (Yau et al., 2001, 2011). Within the nucleus, the GR–ligand complex and MR–ligand complex regulate gene transcription via transactivation and transrepression (De Bosscher et al., 2008). Datson and colleagues (2001) reported that there is limited overlap of the genes that the MR and GR regulate in the hippocampal formation.

Weinberg and colleagues have reported that, in rats, PAE does not alter hippocampal formation mRNA levels (Glavas et al., 2007) or cytosolic protein levels (Kim et al., 1999) of the GR and MR. However, Nammi and colleagues (2007) have reported that PAE is associated with increased 11β-HSD1 enzyme levels and activity in liver and adipose tissue of postnatal day (PD) 7 and 3-month-old rats. If PAE were associated with increased 11β-HSD1 levels in the hippocampal formation, nuclear trafficking of GR and MR may be increased, secondary to elevations of intracellular corticosterone. This, in turn, could alter gene regulation and hippocampal formation functioning. We are unaware of any studies that have analyzed the effects of PAE on hippocampal formation 11β-HSD1 levels or on nuclear levels of the GR and MR in this brain region. We hypothesized that PAE is associated with elevated hippocampal formation 11β-HSD1 levels and increased association of the GR and MR with the nuclear fraction. Further, as trafficking of the GR and MR is regulated by FKBP51 and FKBP52, and no studies have been reported regarding the impact of PAE on these proteins, we assessed their levels in the hippocampal formation of PAE mice.

In the present studies, we focused on the effects of PAE that are observed during adolescence, a period during which several psychiatric disorders first appear or become exacerbated and during which adversity can exert long-term consequences (Andersen, 2003; Kessler et al., 2010). In broadest terms, adolescence in rodents extends from approximately PD 28 to PD 60 (Brenhouse and Andersen, 2011; Spear, 2000). It is important to identify the effects of PAE on adolescent biological and behavioral processes to develop effective strategies for treating the long-term impact of PAE.

We report that GR levels were elevated and MR levels were reduced in a nuclear fraction isolated from the hippocampal formation of PD 40 to 50 male mice that were exposed to alcohol prenatally. These changes were associated with an increase in cellular 11β-HSD1 levels and a reduction in FKBP51 levels. To determine whether these changes may impact hippocampal functioning, we measured hypothalamic corticotropin-releasing hormone (CRH) levels, which have been reported to be under inhibitory control from the hippocampal formation (Herman et al., 2005), and found that they were significantly elevated in PAE mice. Further, PAE mice displayed impaired ability to discriminate similar contexts, which has been shown to be dependent on hippocampal formation functioning (Sahay et al., 2011). These studies add to a growing body of evidence demonstrating that prenatal exposure to moderate levels of alcohol is associated with persistent changes in hippocampal formation neurochemistry and functioning.

**MATERIALS AND METHODS**

**Animals**

All procedures were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee and adhered to practices recommended in “Recognition and Alleviation of Pain and Distress in Laboratory Animals” (Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, Washington, DC, 2002). In all studies, naïve 40 to 50-day-old male mice were used; only 1 animal was used from a litter to control for litter effects. For the immunoblotting studies, 5 to 7 different litters were tested per prenatal condition, and for the context discrimination test, 10 different litters per prenatal condition were used. All mice were naïve at the time of tissue collection or behavioral testing; none of the animals used in the behavioral studies were used for the immunoblotting studies. All animals were maintained on a reverse 12-hour dark/12-hour light schedule (lights off at 08:00 hours). All procedures were performed during the dark cycle. Dams and male breeders were individually housed, and offspring were group housed in cages of 2 to 5 same-sex littersmates.

**Prenatal Alcohol Exposure**

The PAE paradigm has been described previously (Brady et al., 2012). Alcohol consumption (8.04 g ethanol [EtOH]/kg body weight/4 h) was similar to that reported in our recent studies (Brady et al., 2012, 2013), which yielded average blood alcohol concentrations of approximately 90 mg/dl after 4 hours of consumption. Briefly, C57BL/6 female mice (Jackson Laboratory, Bar Harbor, ME) were provided limited access to either EtOH 10% (w/v) or 0.066% (w/v) saccharin (SAC) or 0.066% (w/v) SAC alone for 4 h. Drinking was established prior to mating, maintained throughout gestation, and withdrawn using a step-down procedure over a 6-day period. Offspring were weaned at approximately 23 days of age.

**Isolation and Subcellular Fractionation of Hippocampal Formation Tissue**

Mice were killed by decapitation without anesthesia between 10:00 and 11:00 hours. The hippocampal formation and hypothalamus were rapidly dissected, snap-frozen using liquid nitrogen, and stored at −80°C until preparation of subcellular fractions. Tissue was homogenized as described in Samudio-Ruiz and colleagues (2010). For the GR and MR studies, nuclear (1,000 g, 6 minutes, 4°C) and cytosolic (200,000 g, 30 minutes, 4°C) fractions were prepared as described in Weeber and colleagues (2001) without the intermediate freezing step; for the 11β-HSD1, FKBP51, FKBP52, and CRH studies, a postnuclear (1,000 g, 6 minutes, 4°C) fraction was isolated. Subcellular fractions were snap-frozen using liquid nitrogen and stored at −80°C until analyzed by immunoblotting.

**Immunoblotting**

Immunoblotting was conducted using established protocols (Goggin et al., 2012) with the following changes. GR and MR were assessed in nuclear and cytosolic subcellular fractions using 14 and 12 µg total protein, respectively. FKBP51, FKBP52, 11β-HSD1,
and CRH were evaluated using 30 μg of cellular lysate. Briefly, samples were diluted in NuPAGE® LDS Sample Buffer (#NP0007; Invitrogen, Grand Island, NY) and NuPAGE® Sample Reducing Agent (#NP0004; Invitrogen) and heated for 10 minutes at 70°C. Samples were loaded into NuPAGE 4 to 12% Bis-Tris gels (#NP0336; Invitrogen) and separated by electrophoresis using an Xcell II Blot Module (#E69051; Invitrogen) and MOPS SDS Running Buffer (#NP0001; Invitrogen). Samples were transferred to PVDF membrane (#162-0177; Bio-Rad Laboratories, Hercules, CA) and stained with Coomassie Brilliant Blue R-250 (#161-0400; Bio-Rad) as a loading control, as described by Perrone-Bizzozero and colleagues (1996). Membranes were then blocked for 1 hour in 0.25% (w/v) Tropix® 1-BLOCK™ (#T2015; Applied Biosystems, Grand Island, NY) and incubated overnight in primary antibody directed against GR (1:1,000; Santa Cruz catalog # sc-1004, Santa Cruz Biotechnology, Dallas, TX), MR (1:1,000; Santa Cruz sc-11412), FKBP51 (1:500; Santa Cruz sc-13983), FKBP52 (1:500; Santa Cruz sc-100758), 11β-HSD1 (1:500; Santa Cruz sc-20175), or CRH (1:500; Santa Cruz sc-10718). Unbound antibody was removed with 4 washes in Tris-buffered saline with Tween-20 (TBST) at room temperature followed by incubation with goat anti-rabbit IgG:HRP secondary antibody (#31460, 1:30,000; Pierce, Rockford, IL). Unbound secondary antibody was removed with 4 washes in TBST, and membranes were incubated for 1 minute in Western Lightning Plus ECL (#F-BX57; Phenix Research Products, Candler, NC) and developed in a Kodak D-19 developer (Eastman Kodak Company, Rochester, NY). Immunoreactivities in each lane were quantified using Quantity-One 1-D Analysis Software (Bio-Rad) and corrected to their respective Coomassie stain.

Context Discrimination

Behavioral sessions occurred between 09:00 and 12:00 hours in a room maintained under dim red illumination. Naive, male mice who were 40 days old at the start of training were used in this study. Context discrimination was assessed using a procedure modified from Sahay and colleagues (2011). Animals were exposed daily to each of 2 contexts (Coulburn Habitest fear conditioning chambers; Coulborn Instruments, Whitehall, PA) that contained similar features with slight variations (e.g., floor type, wall cues). Exposures to the 2 contexts were separated by 2 hours. Training in Context A consisted of 2 sequences of a 90-second wait period followed by a 2-second 0.8 mA footshock and a final 60-second wait period; training in Context B was of the same duration, but no shocks were administered. Training/testing was performed for 7 consecutive days. Freezing behavior (lack of deliberate movement) was scored during the 90 seconds of the trial prior to delivery of the first footshock. A discrimination score (freezing Context A – freezing Context B) (freezing Context A + freezing Context B) was calculated using scores from 2 individuals, one of whom was blinded to subject condition.

Data Analysis

Data from the context discrimination studies were analyzed by 1-way repeated-measures analysis of variance using SPSS (v.20; IBM Corporation, Armonk, NY) followed by Tukey test with Bonferroni correction, as needed. Student’s t-tests were used to compare the 2 prenatal conditions for the immunoblotting studies using GraphPad Software (v.4.03; GraphPad Software, San Diego, CA).

RESULTS

PAE Reduced MR and Elevated GR Nuclear Localization

We measured anti-MR and anti-GR immunoreactivities in cytosolic and nuclear subcellular fractions isolated from the hippocampal formation of PD 40 to 50 SAC and PAE mice (Fig. 1). PAE was associated with a reduction in nuclear MR levels (Fig. 1B), t(10) = 2.27, p < 0.05, and an increase in nuclear GR levels (Fig. 1D), t(11) = 2.79, p < 0.05. There were no significant effects of PAE on cytosolic levels of these receptors (Fig. 1A,C), although there was a trend toward an increase in cytosolic levels of the MR (Fig. 1A), t(11) = 1.79, p = 0.10.

The balance between GR and MR levels has been proposed as an important determinant of hippocampal formation-dependent emotional and cognitive functioning (Oitzl et al., 2010). Calculation of GR/(GR + MR) in the nuclear fraction revealed a significant increase in PAE fractions (Fig. 1E), t(9) = 3.83, p < 0.01.

FKBP51 Levels Were Reduced, Whereas FKBP52 Levels Were Unaltered, in PAE Mice

We sought to identify an underlying mechanism(s) that could account for the observed alterations in nuclear GR and MR in the PAE mice. The MR and GR reside in both the cytosolic and nuclear compartments of the cell; trafficking between these compartments is controlled, in part, by FKBP51 and FKBP52 (Vandevyver et al., 2012), which compete with each other for binding to the steroid heterocomplex (Davies et al., 2002; Galigniana et al., 2010). FKBP51 binding favors cytoplasmic localization of the receptor complex, whereas the binding of FKBP52 enables nuclear trafficking of the complex through its association with dynein (Galiniana et al., 2001, 2010). We assessed the levels of FKBP51 and FKBP52, in post-nuclear fractions prepared from SAC and PAE mouse hippocampal formation. Anti-FKBP51 immunoreactivities were reduced (Fig. 2A), t(12) = 3.25, p < 0.01, in PAE compared with SAC mice. In contrast, anti-FKBP52 levels were not significantly different in fractions prepared from SAC and PAE hippocampal formation (Fig. 2B).

PAE Increased the Level of 11β-HSD1

A second mechanism that regulates the distribution of the MR and GR between cytosolic and nuclear compartments is the intracellular concentration of corticosteroid (Nishi et al., 2001). In the hippocampal formation, cellular corticosterone is derived from both the intracellular conversion of 11-dehydrocorticosterone to corticosterone, catalyzed by 11β-HSD1, and from corticosterone in the extracellular fluid (Sooyi et al., 2010; Yau et al., 2001, 2011).

Thus, we assessed 11β-HSD1 levels as a predictor of cellular corticosterone levels. Multiple glycosylation sites have been identified in 11β-HSD1 and immunoblotting studies commonly identify 2 to 4 forms of the enzyme (Blum et al., 2000; Goggin et al., 2012). The glycosylated and nonglycosylated forms of the enzyme all possess reductase activity (Blum et al., 2000), and, thus, we pooled the immunoreactivities measured for each of the 3 bands that were detected in...
the hippocampal formation postnuclear fraction (see representative immunoblot in Fig. 3). We observed a significant increase, \( t(10) = 2.98, p = 0.014 \), in \( 11\beta\)-HSD1 in the PAE hippocampal formation (Fig. 3).

**CRH Protein Levels Were Elevated in PAE Mouse Hypothalamus**

The hippocampal formation exerts inhibitory control over the hypothalamic–pituitary–adrenal (HPA) axis (Herman et al., 2005). Using a rat PAE model in which dams consume a liquid diet containing alcohol (36% EtOH-derived calories) and achieve maternal blood alcohol concentrations of 135 to 155 g/dl, Weinberg and colleagues found that CRH mRNA levels were elevated in the paraventricular nucleus of the hypothalamus (Gabriel et al., 2005), indicating that there is altered central regulation of the HPA axis in these animals. We determined whether the observed changes in hippocampal formation GR and MR localization may exert effects on CRH levels in PAE mouse hypothalamus (Fig. 4). Anti-CRH immunoreactivity associated with PAE hypothalamic tissue lysates was significantly increased relative to levels present in SAC preparations, \( t(12) = 3.74, p = 0.003 \).

**PAE Mice Displayed a Deficit in Context Discrimination**

As noted above, the relative levels of GR and MR have been proposed to regulate hippocampal formation function. Gass and colleagues (2000) showed that the GR/MR ratio is important for neurogenesis in the adult hippocampus, while Mayer and colleagues (2006) reported that the GR antagonist, mifepristone, reverses the decrease in adult neurogenesis that occurs following chronic corticosterone exposure. Because there is substantial evidence that adult neurogenesis...
is disrupted by moderate PAE (Choi et al., 2005), we chose to examine context discrimination, a pattern separation task involving the ability to discriminate between 2 similar contexts, which is dependent upon hippocampal neurogenesis (Sahay et al., 2011). Additionally, we recently reported that PAE mice display deficits in a delayed nonmatch to place radial arm maze task (Brady et al., 2012), another form of pattern separation that is also dependent on the hippocampal formation.

PAE mice had a significant delay in context discrimination learning (Fig. 5). A significant effect of prenatal treatment, \( F(1, 18) = 28.31, p < 0.0001 \), and an effect of the day of run, \( F(1, 18) = 320.0, p < 0.0001 \), were obtained. No interaction between day and prenatal condition was found. These results indicate that both groups learned the task as the trial days progressed, but the PAE mice were slower in forming the discrimination.

DISCUSSION

GR levels were increased and MR levels were decreased, resulting in an increase in the calculated GR/(GR + MR)
ratio, in nuclear fractions prepared from PAE mouse hippocampal formation. These changes were associated with elevated 11\(\beta\)-HSD1 and decreased FKBP51 levels in PAE mice. In contrast, PAE did not lead to changes in cytosolic GR or MR levels, nor were cellular FKBP52 levels altered, in the hippocampal formation. These findings are depicted in the model shown in Fig. 6.

The observed increase in 11\(\beta\)-HSD1 in the PAE mouse hippocampal formation is consistent with the study of Nammi and colleagues (2007), who reported that 11\(\beta\)-HSD1 enzyme levels and activity were increased in liver and adipose tissue of PD 7 and 3-month-old rats that were exposed prenatally to alcohol. Further, the lack of an effect of PAE on hippocampal formation cytosolic GR and MR levels agrees with the study of Kim and colleagues (1999), who reported that GR and MR levels were not altered in a cytosolic fraction prepared from the hippocampal formation of PAE rats.

We are unaware of any reported studies on the effects of PAE on FKBP51 and FKBP52.

The MR and GR are transported between the cytosolic and nuclear compartments of the cell (Vandevyver et al., 2012). An increase in nuclear GR levels in the hippocampal formation of PAE mice indicates that the trafficking of cytosolic GRs to the nucleus is increased and/or the export of the GR from the nucleus is decreased in these animals. As depicted in Fig. 6, an increase in 11\(\beta\)-HSD1 and a decrease in FKBP51 are consistent with increased translocation of cytosolic GR into the nucleus. It is also possible that nuclear export of the GR, which is dependent on the nuclear export signal present in the GR DNA-binding domain and is controlled by the nuclear export receptor, calreticulin (Holaska et al., 2001), is reduced. Future studies will be designed to assess the effect of PAE on this pathway. An additional, unexplored mechanism that may contribute to the increase in nuclear GR levels is an effect of PAE on the GR nuclear retention signal (Carrigan et al., 2007). In contrast to the GR, MR levels were reduced in the nuclear fraction of PAE mouse hippocampal formation. As the observed increase in 11\(\beta\)-HSD1 and decrease in FKBP51 are predicted to increase nuclear import of the MR, the observed decrease in nuclear MR levels is, thus, likely to be the result of increased nuclear export of the receptor (Fig. 6). Similar to nuclear export of the GR, export of the MR is dependent on a nuclear export signal in the receptor’s DNA-binding domain (Vandevyver et al., 2012). Future studies will be developed to determine the effect of PAE on nuclear export of the MR.

The relative levels of the GR and MR impact behavior and cognitive functions that are dependent on the hippocampal formation (Oitzl et al., 2010). Therefore, the finding prepared from the hippocampal formation of PAE rats.

Fig. 5. Context discrimination for 10 prenatal alcohol exposure (PAE) and 10 saccharin (SAC) control animals. Mice were 40 days old at the start of the procedure. The discrimination score (freezing Context A – freezing Context B) was calculated. Freezing behavior (lack of deliberate movement) was scored during the first 90 seconds of the trials (the initial 90-second wait period of the conditioning program). Average discrimination score ± SEM, n = 10, is plotted on the y-axis and the day of testing on the x-axis. Day 1 is not presented because it is the first time the subjects experience the conditioning chamber and no significant freezing occurred.

Fig. 6. Model depicting the distributions and relative levels of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), and regulators of their distributions, in cells of the hippocampal formation of saccharin (SAC) control and prenatal alcohol exposure (PAE) adolescent, male mice. The distributions of GRs and MRs between the cytosolic (cyto) and nuclear compartments within a cell are shown. 11\(\beta\)-hydroxysteroid dehydrogenase 1 (11\(\beta\)-HSD1) catalyzes the intracellular formation of corticosterone, which, when bound to the GR or MR, promotes translocation of the receptor from the cytosol to the nucleus. FK506-binding protein 51 (FKBP51) opposes translocation of GRs and MRs from the cytosol to the nucleus, as depicted by the dotted and blunt line end. The roles of other chaperone and co-chaperone proteins, including FKBP52, are not shown. Nuclear levels of the receptors are controlled by both import and export mechanisms.
of an altered GR/(GR + MR) ratio could underlie the observed impairment in context discrimination, as well as previously identified deficits in delay and trace fear conditioning and performance in a delay nonmatch to place radial arm maze task in PAE mice (Brady et al., 2012). An increase in the nuclear GR/(GR + MR) ratio is predicted to alter the pattern of genes that are transcribed by these receptors. The GR and MR form homodimers which regulate overlapping, as well as distinct, sets of genes (Datson et al., 2001). In addition, nuclear GR and MR form heterodimers which display increased DNA-binding affinity and transcriptional control compared with homodimers of GR or MR alone (Ou et al., 2001; Trapp and Holsboer, 1996). GR–MR heterodimers may regulate the expression of different sets of genes than those controlled by GR homodimers and MR homodimers (Nishi, 2011; Trapp and Holsboer, 1996). Thus, the presence of increased GR and decreased MR in PAE mouse hippocampal formation is predicted to lead to increased GR homodimer and decreased GR–MR heterodimer formation and, thereby, alter gene expression profiles. This, in turn, is predicted to have consequences on hippocampal functioning.

The GR and MR regulate the expression of several genes that have been shown to play a role in cognitive and emotional processes (Datson et al., 2001), including brain-derived neurotrophic factor and the N-methyl-d-aspartate (NMDA) receptor (NMDAR; Hodes et al., 2012). We have previously shown that hippocampal formation brain-derived neurotrophic factor mRNA levels (Caldwell et al., 2008) and NMDAR subcellular localization (Brady et al., 2013) are altered in PAE mice. Interestingly, glucocorticoids have been reported to negatively regulate FKBP51 expression in the mouse hippocampal formation (Scharf et al., 2011). As FKBP51 levels were elevated in the PAE mouse model, this indicates that regulatory control is altered in these animals.

Wan and colleagues (2005) reported that repeated prenatal exposure to dexamethasone, during the last week of gestation, increased 11β-HSD1 expression in hippocampal tissue isolated from the neonate (PD 1 and PD 7) offspring. Similarly, Shoener and colleagues (2006) found that administration of dexamethasone to pregnant rat dams on gestational days 14 to 19 was associated with elevated 11β-HSD1 mRNA, as well as decreased MR mRNA, in the hippocampus of the offspring, when assessed as adults. Thus, it is possible that the observed increase in 11β-HSD1 in the adolescent hippocampal formation is the result of in utero exposure to stress and/or alcohol, or an influence of early-life experiences. Maternal care has been shown to affect GR expression in the neonatal hippocampal formation (Zhang et al., 2013), but we are unaware of any effect on 11β-HSD1 expression. Additionally, as we have shown that maternal care does not differ significantly between PAE and SAC control moms (Brady et al., 2012), it is more likely that the effects are due to in utero stress and/or an action of alcohol than an alteration in neonatal experiences. Future studies will aim to determine the mechanism for the observed elevation in 11β-HSD1.

Holmes and colleagues (2010) reported that levels of 11β-HSD1 mRNA in mouse hippocampal CA3 pyramidal cells were inversely correlated with spatial learning and spatial memory retention in the hidden platform version of the Morris water maze. These investigators also showed that overexpression of 11β-HSD1 in the forebrain, including the hippocampus, of aged mice was associated with impaired cognitive performance in the Morris water maze task and in a conditioned passive avoidance test. As overexpression in these animals was not associated with alterations in basal or acute stress-induced elevations in plasma corticosterone levels, or with anxiety-like behaviors on an elevated plus maze or in an open field, the results indicate that changes in forebrain 11β-HSD1 alone are sufficient to impact cognitive functioning. Thus, it is possible that the deficit in context discrimination learning in the PAE mice is the result of increased 11β-HSD1 activity in the hippocampal formation.

We also found that PAE was associated with a reduction in FKBP51 levels in the hippocampal formation. Soonhorniyomkij and colleagues (2010) reported that, in mice, reduced hippocampal formation levels of FKBP51 and a reduction in the ratio of FKBP51 to FKBP52 are associated with impaired performance in a single-trial object recognition test. These investigators suggest that reduced levels of FKBP51 lead to imbalances in glucocorticoid signaling, which, in turn, impair learning and memory in the object recognition test. FKBP51 polymorphisms have been identified as risk factors for depression and posttraumatic stress disorder (Storer et al., 2011). Thus, altered GR and MR signaling, secondary to altered levels/functioning of FKBP51, may contribute to the previously observed cognitive and emotional dysfunctions in PAE mice, as well as the impaired contextual discrimination ability observed in the present studies.

In addition to establishing the effects of PAE on the subcellular localization of GRs and MRs, and on proteins that control the distribution of the GR and MR between the cytosolic and nuclear compartments, we sought to determine whether the observed effects were associated with altered hippocampal functioning. As noted above, the hippocampal formation exerts inhibitory control over the HPA axis, with both GRs and MRs having been shown to control HPA axis activity (Herman et al., 2005). Lesions of the fimbria–fornix transsections and lateral fimbria–fornix fiber tracts, which serve as a primary inhibitory communication pathway from the hippocampal formation to the hypothalamus, elevate CRH mRNA in the paraventricular nucleus of the hypothalamus (Herman et al., 1992). Similar to the findings of Gabriel and colleagues (2005), who reported that CRH mRNA levels are elevated in the hypothalamic paraventricular nucleus in PAE rats, we found that hypothalamic CRH protein levels were elevated in PAE mouse hypothalamus, indicating that the hippocampal formation is exerting less inhibitory control over the hypothalamus in PAE animals. However, an equally valid explanation for our data is that glucocorticoid-dependent negative feedback regulation of
the hypothalamus may be reduced, possibly due to reduced GR levels or signaling within the hypothalamus.

The polysynaptic network that projects from the hippocampal formation to the paraventricular nucleus of the hypothalamus appears to play a role in “anticipatory” (psychogenic), rather than “reactive” (physiological), regulation of the HPA axis (Jankord and Herman, 2008). Research on the relationships between our present findings and the regulation of the HPA axis by the hippocampal formation in PAE mice is likely to provide insight into the understanding of the role of altered stress responding in the cognitive and emotional dysfunctions that are observed in these animals.

We also determined the impact of PAE on a hippocampal formation-dependent learning and memory task, context discrimination. Our results reveal a significant effect of both PAE and days of behavioral testing, suggesting that PAE impairs the ability and speed by which exposed animals learn this behavioral task and discern the difference between the contexts. Recent work by Sahay and colleagues (2011) demonstrates that performance in the context discrimination task is dependent on newborn neurons in the dentate gyrus, thus intimately linking this task to intact hippocampal adult neurogenesis. Several studies have investigated the effects of alcohol exposure, both pre- and postnatal, on various components of adult neurogenesis, including proliferation of progenitors, survival, maturation, and integration (Valenzuela et al., 2012). A prenatal exposure paradigm similar to the one employed for the present studies, while not significantly impacting adult neurogenesis compared with controls, does impair the neurogenic response of the hippocampus to enrichment (Choi et al., 2005). We have also shown the same PAE paradigm as used herein yielded animals displaying dentate-gyrus-specific deficits in NMDAR-dependent long-term potentiation (LTP) and reduced GluN2B subunit composition of the NMDAR, which is integral to learning and memory (Brady et al., 2013). Recent work by Hen and colleagues suggests that deletion of GluN2B-containing NMDARs from adult born granule cells in the dentate gyrus induces deficits in context discrimination (Kheirbek et al., 2012). Thus, our previous studies on dentate-gyrus-specific deficits in LTP, altered NMDAR composition, and adult neurogenesis, in concert with the hippocampal changes in GR we provide here, reveal an apparent effect of PAE on the hippocampal formation, which may be responsible for inducing the behavioral deficits that we observe in context discrimination.

Here, we demonstrated that PAE affected the subcellular distribution of the GR and MR in the adolescent mouse hippocampal formation and that these changes were associated with alterations in key components of pathways that control GR and MR localization, as well as impaired context discrimination learning and memory. As adolescence is a dynamic period in brain maturation, neurochemical imbalances present during this period will likely have far-reaching and long-term effects. It will be important to determine the role of the observed changes in various behavioral, neurochemical, and neurophysiologic processes in both adolescence and adults. Additionally, identifying the underlying molecular bases for the changes and interventions that reverse the deficits are critical areas of research.

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