Published in final edited form as:

Neurotoxicol Teratol. 2018 ; 66: 63–79. doi:10.1016/j.ntt.2017.12.009.

Epigenetic mechanisms in alcohol- and adversity-induced developmental origins of neurobehavioral functioning

KE Boschen¹, SM Keller², TL Roth², and AY Klintsova²

¹Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill NC 27599

²Department of Psychological and Brain Sciences, University of Delaware, Newark DE 19716

Abstract

The long-term effects of developmental alcohol and stress exposure are well documented in both humans and non-human animal models. Damage to the brain and attendant life-long impairments in cognition and increased risk for psychiatric disorders are debilitating consequences of developmental exposure to alcohol and/or psychosocial stress. Here we discuss evidence for a role of epigenetic mechanisms in mediating these consequences. While we highlight some of the common ways in which stress or alcohol impact the epigenome, we point out that little is understood of the epigenome's response to experiencing both stress and alcohol exposure, though stress is a contributing factor as to why women drink during pregnancy. Advancing our understanding of this relationship is of critical concern not just for the health and well-being of individuals directly exposed to these teratogens, but for generations to come.

Keywords

epigenetics; prenatal alcohol exposure; early-life stress; intervention

Introduction

Adverse experiences during fetal development and early postnatal life can significantly affect the physiological and behavioral trajectory of offspring. Prenatal alcohol and toxic stress exposure are two of the most common agents resulting in disturbed development of offspring and often act concomitantly (CDC, 2017; DiPietro, 2012; Driscoll et al., 1990; Watson et al., 1999). Many women turn to substance use as a way to cope with stress during pregnancy (Hanna et al., 1994; Skagerström et al., 2011; Yali and Lobel, 1999). Understanding the biological basis of the significant physical and behavioral consequences of developmental alcohol and/or stress exposure is important for developing care guidelines for pregnant women and parents as well as for developing therapeutic interventions for

*Address correspondence to: Anna Klintsova, Ph.D. or Tania L. Roth, Ph.D., Department of Psychological and Brain Sciences, University of Delaware, 108 Wolf Hall, Newark, DE 19716, Phone: (302) 831-2271, Fax: (302) 831-3645, aklintsova@psych.udel.edu or troth@psych.udel.edu.

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.

affected offspring. Epigenetic modifications to chromatin represent mechanistic pathways through which early teratogen exposure can affect brain and behavioral development. Widely studied in developmental biology, epigenetics refer to events that alter gene activity without directly impacting the DNA sequence. For this review, epigenetics includes DNA methylation, histone acetylation and methylation, posttranscriptional regulation of gene expression via microRNAs, and multigenerational (referring to intergenerational and/or transgenerational) effects. These events are important during development to direct cell proliferation, differentiation, and neural patterning (Guibert and Weber, 2013; Monk et al., 1987), as well as in the postnatal and adult brains, where *de novo* epigenetic modifications represent a path through which environmental influences can affect gene activity (Jones and Takai, 2001).

Changes to chromatin structure can occur at many levels. DNA methylation, the addition of methyl groups to cytosines in DNA, is one of the most studied epigenetic modifications in terms of developmental alcohol or stress exposure. Methylation often represses gene transcription (Jones and Takai, 2001) (as depicted in Figure 1), though this effect is dependent on cytosine location in the genome (Guibert and Weber, 2013). Methyl groups are added to DNA via DNA methyltransferases (DNMT1, 3A, DNMT3B, and DNMT3L), which are differentially expressed throughout development (Okano et al., 1999). DNMT1 is typically associated with maintenance of methyl marks carried through replication or “cell memory,” while DNMT3A and 3B are essential for *de novo* methylation (Okano, 1999). DNMT3L has been less well-studied, but has been to act through suppression of inherited maternal methylation marks and stimulation of DNMT3A activity (Bourc'his et al., 2001; Hata et al., 2002). Active demethylation of DNA can occur through hydroxymethylation, with the formation of 5-hydroxymethylcytosine (5-hmC) catalyzed by the ten-eleven translocation methylcytosine dioxygenase (TET) family of enzymes (Guibert and Weber, 2013). It should be noted that the majority of studies discussed in this review do not distinguish between 5mC and 5hmC. Methylation patterns are highly dynamic across development and methylation is a critical part of stage-dependent gene regulation (Guibert and Weber, 2013; Monk et al., 2016; Monk et al., 1987). Thus, disruption of methylation patterns during gestation or postnatally by teratogens would have lasting ramifications on ongoing developmental processes. In addition, environmentally-driven alterations to methylation status can remain stable across the lifespan and even be perpetuated across generations (Laird, 2003; Meaney and Szyf, 2005).

Other chromatin modifications can alter gene activity, including histone acetylation and trimethylation (Figure 1). Histone acetylation loosens chromatin to make DNA more accessible to transcription factors (Grunstein, 1997; Struhl, 1998). In terms of histone methylation, downstream effects on gene regulation depend largely on the specific amino acid modified. For example, trimethylation of Histone 3 lysine 4 (H3K4me3) is associated with activated transcriptional activity, while trimethylation of other lysine residues is associated with transcriptional repression. Beyond modifications to individual amino acids, chromatin accessibility is controlled through complex combinations of modifications to histone tails (Jenuwein and Allis, 2001). Specialized protein domains recognize each combination and are directed to alter chromatin organization. Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), are additional pathways through which prenatal alcohol

exposure (PAE) and stress can alter protein synthesis (Figure 1). Mature miRNAs are fragments of RNA cleaved from primary miRNA (pri-miRNA) by the enzyme Dicer (He and Hannon, 2004). These mature miRNAs silence gene expression either by preventing translation of mRNA into protein or by speeding up degradation of the mRNA. Other ncRNAs have important biological roles as well, including RNA splicing and DNA replication (Mercer et al., 2009).

This review discusses the growing body of evidence that both PAE and developmental stress exposure affect an individual's cognitive development and risk for psychopathology in part through changes to the epigenome. The primary focus of this review is on animal models of PAE or gestational or postnatal stress as most epigenetic research has utilized these models. Where appropriate, the translational relevance of animal data is discussed. Areas where the effects of developmental alcohol and stress intersect will be discussed as well. These areas of overlap include discussion of epigenetic modification of genes involved in neural development and hypothalamic–pituitary–adrenal (HPA) axis regulation, molecules involved in the regulation of epigenetic marks, and miRNA expression. In addition, we discuss important areas for further research, including multigenerational effects of alcohol and stress as a way in which teratogen exposure could impact the health and well-being of several generations, and possible pharmacological, nutritional, and behavioral interventions to rescue negative outcomes of developmental alcohol or stress exposure, specifically in relation to their effect on the epigenome.

Prenatal Alcohol Exposure

One of the most widely used teratogenic substances is alcohol, and the National Institute on Alcohol Abuse and Alcoholism estimates that 1 in 8 women drink alcohol while pregnant (2015). PAE leads to an array of adverse physical, cognitive, and behavioral outcomes, with Fetal Alcohol Spectrums Disorders (FASD) recognized as the leading preventable cause of developmental disability in the United States (CDC, 2017). Heavy alcohol exposure results in characteristic craniofacial and eye malformations, which are present alongside low birth weight and growth retardation and classified as Fetal Alcohol Syndrome (FAS). PAE also alters neurobehavioral outcomes, with patients exhibiting memory (Mattson et al., 1999; Rasmussen and Bisanz, 2011; Rasmussen et al., 2010), executive functioning (Bertrand and Consortium, 2009; Connor et al., 2000), and social functioning deficits (Irner et al., 2012; Stevens et al., 2012), as well as increased impulsivity (Franklin et al., 2008) and risk of incarceration (Streissguth et al., 2004).

A myriad of contributing factors dictate the type and severity of alcohol-induced damage, including the dose and pattern of alcohol exposure, the developmental stage of the embryo or fetus, and individual differences in genetics and metabolism. The role of epigenetic modifications in risk and resilience to the teratogenic effects of PAE is still largely unknown, with emerging data consistent with the notion that PAE produces epigenetic changes that serve as a mediator of alcohol-induced damage. Epigenetic modifications also represent an avenue through which alcohol exposure could have a lasting negative impact on an individual throughout the lifespan.

Animal models of FASD have played a critical role in both classifying alcohol-related birth defects and understanding mechanisms of alcohol teratogenesis. The use of animal models allows for variables such as timing, dose, and method of exposure to be manipulated while controlling other environmental factors. The vast majority of studies use rodent models and administer alcohol either to the pregnant dam (modeling exposure during the first two trimesters of human pregnancy) or directly to the pups early in the postnatal period (modeling third trimester-equivalent exposure). Common routes of administration for alcohol include i.p. injection (of the dam prenatally or, less commonly, to the pups postnatally), intragastric gavage (either of the dam in prenatal models or of the pups in postnatal models), voluntary drinking (prenatal only), liquid diet (prenatal only), or placement of the dam and/or pups into a vapor chamber. The experimental question often leads to the use of a specific species or exposure paradigm; the models used for each study discussed in this review will be described as necessary. More detailed explanations of rodent models of FASD can be found elsewhere (Boschen and Klintsova, 2017; Gil-Mohapel et al., 2010). One strength of animal models is the ability to investigate the time course of epigenetic modifications in different organ types and brain regions following alcohol exposure during development.

Changes to the epigenome plausibly explain many of the deficits observed following PAE, including altered gene expression patterns, cognitive and behavioral impairments, and increased risk of mental dysfunction later in life; however, the longevity of epigenetic marks following PAE and their causal relation to behavioral outcome has not been fully explored. The following three sections discuss evidence from prenatal, postnatal, and *in vitro* FASD models of alcohol's ability to change DNA methylation, histone acetylation and trimethylation, and where known, their relation to behavioral outcomes.

DNA methylation

DNA methylation is the most commonly studied epigenetic modification as related to developmental alcohol exposure. PAE is associated with alterations in methylation and hydroxymethylation patterns in the developing brain (Chen et al., 2013; Dasmahapatra and Khan, 2016) (Figure 1). Alcohol exposure during mid-to-late gestation disrupted the normal DNA methylation program in the fetal hippocampus through dysregulation of proteins governing methylation and hydroxymethylation, including methyltransferases (Garro et al., 1991), TET1, and methyl-CpG-binding protein (MeCP2) (Chen et al., 2013; Subbanna et al., 2014). The hippocampus of alcohol-exposed fetuses also showed evidence of developmental delay, with fewer cells in the dentate gyrus displaying markers related to mature neuronal status (Chen et al., 2013). However, there was a reduction in the progenitor pool (Ki-67+) as well, indicating the loss of mature neurons could be due to apoptosis or to slower cell cycle progression. Increased time to progress through the cell cycle in combination with apoptosis has been documented in multiple models of FASD and is a contributing factor to the developmental delay in embryonic maturation (Luo and Miller, 1998; Luo et al., 1999; Zhou et al., 2011).

Alcohol could control cell cycle progression and, thus, contribute to delayed embryonic maturation through disruption of highly regulated developmental methylation patterns that

are critical for the precise spatial and temporal expression of developmentally regulated genes. Zhou and colleagues (2011) found that alcohol delayed the DNA methylation program in cultured embryonic cells. Regions that showed the most disruption of DNMT1 activity also displayed significant growth reductions. DNMT1 is responsible for maintaining patterns of methylation and ensuring transmission of methylation marks during replication, making DNMT1 activity critical for cell memory and, potentially, multigenerational effects. Dysregulation of DNMT1, MeCP2, or one-carbon metabolism (Bekdash et al., 2013; Nagre et al., 2015; Ngai et al., 2015) represents one way that developmental alcohol exposure might influence methylation patterns across the lifespan, causing long-term changes to downstream gene expression and, ultimately, behavioral and cognitive impairments. Bekdash et al. (2013) reported long-term increases in expression of DNMT1 and MeCP2 in the hypothalamus of PAE adult rats. Another study reported decreased MeCP2 expression in the prefrontal cortex and striatum of rodents, which correlated with behavioral abnormalities associated with cortical and striatal dysfunction (Kim et al., 2013). Increased DNMT activity was also reported in the postnatal day (PD) 21 rat hippocampus following perinatal alcohol exposure (Perkins et al., 2013). This study intubated the dam and pups across all three trimesters of development and the intubated control group showed many of the same gene expression changes that were observed in the alcohol-exposed group, making it difficult to parse out the effect of alcohol alone vs. stress caused by the intubation procedure. Intubation stress can be a significant confound in developmental alcohol studies (Boschen et al., 2015), highlighting the need to systematically study the unique and overlapping contributions of stress and alcohol on experimental outcomes.

Developmental alcohol exposure models consistently report widespread cell death in the brain following even a single administration of alcohol, with third trimester-equivalent models demonstrating the most vulnerability. While apoptosis is a naturally-occurring phenomenon in the developing brain (Johnston, 2009; Rakic and Zecevic, 2000), alcohol induces massive waves of apoptosis, particularly in brain regions undergoing significant growth when alcohol is administered (Farber et al., 2010; Ikonomidou et al., 2000; Olney et al., 2002). Recent work substantiates that epigenetics play a role in controlling alcohol-induced cell death. Third trimester-equivalent (PD7) alcohol alters levels of DNMTs (Nagre et al., 2015) in a caspase-3-dependent manner, linking methyltransferase activity with apoptotic processes that could result in long-term regional volumetric or functional deficits in the alcohol-exposed brain. One proposed pathway through which alcohol stimulates apoptosis is induction of oxidative stress pathways. Oxidative stress damages cells and DNA through the production of peroxidases and other free radical molecules. PAE increased levels of reactive oxygen species (ROS) and apoptosis in fetal rat cortical neurons, while antioxidant pretreatment of the cells prevented the apoptosis, meaning ROS production directly contributed to increased alcohol-induced cell death (Ramachandran et al., 2003). Lipid peroxidase was also upregulated throughout the brain for up to 12 weeks following postnatal alcohol exposure (Petkov et al., 1992) and until at least postnatal day (PD) 60 following perinatal exposure (Brocardo et al., 2012). Perinatal alcohol also increased protein oxidation in the hippocampus and cerebellum and increased anxiety- and depressive-like behaviors in the mice. While oxidative stress itself is not an epigenetic modification, recent evidence suggests that postnatal alcohol exposure might regulate oxidative pathways through

long-term methylation and gene expression changes in genes related to oxidative stress in the adult mouse hippocampus (Chater-Diehl et al., 2016). Epigenetic regulation of signaling pathways related to free radical scavenging and peroxisome function could be one avenue through which developmental alcohol exposure produces long-term impairments in cellular processes and behavioral output.

Growth restriction is commonly found in offspring exposed to alcohol during gestation. Insulin-like growth factor 2 (IGF-2), along with other growth-promoting hormones, is important for appropriate age-associated growth during gestation. PAE alters methylation patterns of *Igf2* in the placenta of alcohol-treated dams (Haycock and Ramsay, 2009), embryonic tissue (Downing et al., 2011), and adult brain tissue (Laufer et al., 2013). Downing and colleagues (2011) also demonstrated that *Igf2* expression was downregulated following PAE; gene expression was not directly measured in the other two studies. IGF-2 plays a critical role in embryonic development, as knockdown of *Igf2* results in severe growth retardation and bone growth delay (Baker et al., 1993). Alcohol-treated embryos were observed to be significantly smaller in the study by Haycock and Ramsay (2009), while weight differences were not reported in the other two studies. Brain-derived neurotrophic factor (BDNF) is another growth factor important for cell proliferation, dendritic outgrowth, and synapse formation, particularly in the adult brain. Numerous studies have demonstrated that developmental alcohol exposure causes long-term changes to functional and anatomical measures linked to BDNF signaling, including long-term potentiation (Puglia and Valenzuela, 2010), adult neurogenesis (Boehme et al., 2011; Gil-Mohapel et al., 2010; Hamilton et al., 2011; Klintsova et al., 2007), and dendritic morphology (Berman et al., 1996; Boschen et al., 2016; Hamilton et al., 2015; Hamilton et al., 2010; Redila and Christie, 2006; Whitcher and Klintsova, 2008). Studies have demonstrated that *Bdnf* gene expression is altered following developmental alcohol exposure in a timing and region-specific manner (Caldwell et al., 2008; Feng et al., 2005; Heaton et al., 2000; Heaton et al., 2003), though the diverse models and methods of analysis used in these studies makes it difficult to draw definitive conclusions. Epigenetic modification of *Bdnf* has not been well studied in alcohol exposure models, despite being a significant target of interest in prenatal stress research. *Bdnf* exon 1 hypomethylation was reported 24 h following PD4–9 alcohol exposure in the male rat hippocampus; this reduction in methylation was correlated with increased exon 1-driven *Bdnf* gene expression (Boschen et al., 2015). However, by PD72, methylation status had returned to control levels (Boschen et al., 2016). *Bdnf* and other growth factors that could contribute to either delayed embryonic growth or long-term deficits in neuroplasticity need to be further investigated from an epigenetic perspective.

Rodents exposed to alcohol *in utero* often display increased depressive- and anxiety-like behaviors later in life (Brocardo et al., 2012; Caldwell et al., 2008; Hellemans et al., 2010; Hellemans et al., 2008). Children with FASD also present with psychopathology at a greater rate than their peers (O'Connor and Paley, 2009; Steinhausen and Spohr, 1998). PAE might impact stress reactivity by altering long-term HPA axis functioning through epigenetic regulation. Hypermethylation of the proopiomelanocortin (POMC) gene (Bekdash et al., 2013), a polypeptide that serves as a precursor for pituitary-produced hormones including adrenocorticotrophic hormone, β -endorphin, and met-enkephalin, was reported in the adult rat

hypothalamus. Concomitant reductions in *Pomc* mRNA levels were also observed. Increases in one-carbon metabolism, involved in the production of methyl donors, were also reported in the hypothalamus following PAE (Ngai et al., 2015). Developmentally alcohol exposed rats exhibited a short-term increase in plasma corticosterone levels and overactive corticosterone responses to lipopolysaccharide (LPS) in adulthood, further linking developmental alcohol exposure to alterations in stress responsivity (Bekdash et al., 2013; Boschen et al., 2015; Kim et al., 1999).

In vitro alcohol treatment offers a model to assess alcohol effects on methylation patterns within distinct cell populations. Administration of ethanol to neural stem cells increased the length of the cell cycle, specifically the time spent in the G1 and S phases (Hicks et al., 2010). Interestingly, this increase correlated with hypermethylation of multiple cell cycle-related genes, including cyclin family genes related to G1 and G2 progression. In another study, expression of MeCP2 and global methylation markers was altered by both binge-like and continuous ethanol exposure in neural stem cell culture (Liyanage et al., 2015). These cells displayed altered mature morphology compared to controls, suggesting that the observed global methylation patterns in ethanol-treated cells could affect differentiation and maturation processes. This hypothesis is supported by another study demonstrating that alcohol alters differentiation of neural stem cells by limiting the type of cells produced by the stem cell population (Zhou et al., 2011). Reduced differentiation potential occurred alongside changes to methylation patterns during stem cell progression through the cell cycle. Mukhopadhyay et al. (2013) reported decreased global methylation in cultured mouse embryonic fibroblasts, as well as reduced levels of DNA methyltransferases and MeCP2. Taken together, these studies demonstrate that alcohol affects methylation of genes involved in cell cycle progression, though the direction of these changes could depend on factors including cell type, treatment paradigm, and timing of analysis after exposure, as Liyanage et al. (2015) reported differences in methylation status between alcohol exposure and withdrawal. Cell cycle genes were also associated with increased methylation in a whole embryo culture paradigm (Liu et al., 2009). Most importantly, hypermethylation of chromosomes 10 and X was reported to occur at higher rates in alcohol-exposed embryos displaying physical dysmorphologies related to neural tube defects compared to unaffected embryos, illustrating a link between methylation patterns and alcohol-induced malformations.

Evidence of epigenetic alterations in children with FASD remains scarce. One study reported that children with PAE showed altered methylation status in genes related to protocadherins, glutamatergic synapses, and intercellular signaling, though these results were influenced by other factors, including sex and medications (Laufer et al., 2015). Interestingly, differential methylation was observed in imprinted regions, similar to changes seen to imprinted genes such as *Igf2* and *H19* (a long non-coding RNA) in mouse models of FASD (Laufer et al., 2013). Methylation changes to clustered protocadherin-related genes, important for cell adhesion and determination of neuronal identity, have been reported in mouse models of FASD as well (Chater-Diehl et al., 2016). Portales-Casamar et al. (2016) assessed genome-wide methylation patterns in Canadian children with FASD and found significant changes in 41 genes, with hypermethylation spanning genes associated with neurodevelopmental, mood, and substance abuse disorders. Clustered protocadherin genes

were differentially methylated in this sample, similar to previous human and animal work (Chater-Diehl et al., 2016; Laufer et al., 2015). A factor limiting interpretation of these data is the relatively low increase in methylation (>5%) and its relation to gene expression changes in the FASD population. It is worth noting that similarly sized effects were reported in postmortem brain tissue from individuals with autism (Ladd-Acosta et al., 2014). In addition, both the FASD and autistic samples were possibly confounded by the influence of environmental factors, as the mean age of the FASD sample was ~11 years old and ~21 years old in the autistic sample. It is possible that environmental influences could have removed epigenetic marks or created new ones. While more work needs to be done to fully understand the implications of this data, these data suggest that methylation patterns in children with FASD could contribute to long-term cognitive and behavioral outcomes and interventions that affect the epigenome should be explored as therapeutic agents.

Histone modifications

While DNA methylation is often associated with reduced gene expression, other chromatin modifications (histone acetylation) are often correlated with increased transcriptional activity. The downstream effect of histone trimethylation is dependent on the specific nucleotide residue involved. In addition, it is important to remember that overexpression of genes can be disruptive. For instance, over-expression of cardiac-specific genes following early gestational alcohol exposure was determined to be regulated through Histone 3 acetylation (Zhang et al., 2014), and thought to contribute to the development of heart malformations and congenital heart disease (CHD). *In vitro* analysis of cardiac progenitor cells reached a similar conclusion that altered Histone 3 acetylation patterns following alcohol exposure could lead to CHD. In children with FASD, certain types of heart defects are associated with exposure, including those affecting the ventricles and arteries (Burd et al., 2007; Yang et al., 2015).

Histone 3 modifications often associated with increased gene transcription have been noted in *in vitro* models and in the fetal cortex following gestational day 7 alcohol administration (Veazey et al., 2015). While not all epigenetics modifications were correlated with gene expression, upregulation of mRNA for the genes *Tet1* (involved in hydroxymethylation) and the cell proliferation marker *Ki67* were observed. Conversely, PAE from gestational days 7 – 21 reduced histone marks associated with activation and increased those associated with transcriptional repression in the hypothalamus in adulthood (Bekdash et al., 2013). The length of alcohol exposure, age, and brain region-specific differences in responsiveness to alcohol likely contribute to the opposing findings in these studies. Third trimester-equivalent alcohol exposure altered levels of CREB binding protein, a histone acetyltransferase, and acetylation of Histones 3 and 4 in the developing brain (Guo et al., 2011; Subbanna et al., 2014). P7 alcohol exposure also induced histone methylation and demonstrated that alcohol-induced apoptosis could be reduced through inhibition of the lysine dimethyltransferase G9a (Subbanna et al., 2013). These studies directly link histone modifications to neurodegenerative processes that could contribute to fetal alcohol effects (Figure 1).

The link, however, between histone modifications and gene expression is not always clear. For example, Histone 3 lysine 4 trimethylation, a mark usually associated with

transcriptionally active promoters, was enhanced in the hippocampus of adult mice exposed to alcohol during early gestation (Zhang et al., 2015). Specifically, this mark was associated with the gene *Slc17a6*, which encodes a protein related to vesicular glutamate transport. However, while *Slc17a6* gene expression was concomitantly enhanced, downstream VGLUT2 levels were decreased, suggesting other post-transcriptional modifications, such as microRNAs (miRNAs), could be interrupting the processing of this mRNA into protein. miRNAs are an exciting branch of epigenetic research and will be discussed in relation to overlap with the developmental stress research.

Developmental Stress Exposure

Experiencing psychosocial stress early in life likewise predisposes individuals to cognitive dysfunction, addiction, and increased prevalence rates of psychiatric disorders (Enoch, 2011; Gilbert et al., 2009; Schenkel et al., 2005; Shea et al., 2005). While these stressful experiences occur early, behavioral outcomes often do not manifest until later in life. For example, disorders including posttraumatic stress disorder (PTSD), depression, and schizophrenia that have increased prevalence rates among individuals with a history of early-life stress (Kendall-Tackett, 2002; Schenkel et al., 2005; Weiss et al., 1999) typically emerge during adolescence or adulthood (Adriani and Laviola, 2004; Agid et al., 1999; Costello et al., 2003; Holmes, 2013). Epigenetic mechanisms have emerged as a mediating factor between early stress and its outcomes, both immediate and latent (Kundakovic and Jaric, 2017; Lewis and Olive, 2014; McGowan and Roth, 2015; Roth, 2012, 2013; Roth et al., 2009b; Roth and Sweatt, 2011; Silberman et al., 2016; Tsankova et al., 2007). Common targets of the effects of early-life stress include the prefrontal cortex (Kolb et al., 2017), hippocampus (Fenoglio et al., 2006), amygdala (Cohen et al., 2013), and HPA axis (Essex et al., 2011) (Figure 2). These brain regions are also implicated in cognitive deficits and psychiatric disorders associated with developmental stress (Aihara et al., 2007; Blair, 2008; Drevets et al., 1997; Zierhut et al., 2010). The implications of stress exposure vary as a result of developmental time period during which the stress was incurred (Gee and Casey, 2015; Lupien et al., 2009). Moreover, different forms of early-life stress can elicit different outcomes (Dong et al., 2015; Mychasiuk et al., 2011; Schmidt et al., 2011; St-Cyr and McGowan, 2015). Consequently, the following sections discuss the implications of developmental stress exposure organized by the various models used to study early stress. The same modifications discussed above regarding the effects of developmental alcohol exposure (DNA methylation, histone modifications, and ncRNAs) are described together for each model where appropriate.

Gestational stressors

Stress experienced by the mother while pregnant has consequences for the epigenome and behavior of her offspring. Exposure to maternal glucocorticoids is one potential mechanism by which maternal stress during gestation could induce damaging effects on offspring. In a study that employed restraint stress during pregnancy (day 14–20 of gestation), expression of 11 β -hydroxysteroid dehydrogenase type 2 (*Hsd11 β 2*), which is important in protecting the developing fetus from circulating maternal glucocorticoids, was reduced in the placenta while DNA methylation of *Hsd11 β 2* and *Dnmt3a* expression were increased (Pena et al.,

2012). *Dnmt1* expression was increased in the cortex of these animals (Pena et al., 2012), which could promote increased *Hsd11b2* methylation. This effect has been replicated in humans, as higher self-reported stress during pregnancy was associated with increased methylation of *HSD11B2* as well as FK506 Binding Protein 5 (*FKBP5*), a gene which has an inhibitory effect on glucocorticoid signaling, in the placenta (Monk et al., 2016). Further, increased methylation of both genes was linked with reduced fetal coupling (i.e. correlation between fetal movement and heart rate) (Monk et al., 2016). Reduced expression of *HSD11B2* resulting from increased DNA methylation could create vulnerability to glucocorticoids in the prenatally stressed fetus which could, in turn, enhance the effects of future stress exposure.

Because glucocorticoid receptors (GRs) play a regulatory role in HPA axis functionality and negative feedback (Herman et al., 2012) and altered levels of GRs have been associated with anxiety and depression (Ridder et al., 2005; Yehuda et al., 1993; Yehuda et al., 1991), research has focused on the methylation patterns of *NR3C1*, which codes for the GR protein in humans. Numerous studies have found increased methylation of *NR3C1* and concomitant reduced gene expression with gestational stress exposure. For example, women pregnant during the Tutsi genocide and their children have reduced levels of cortisol, increased mineralocorticoid receptor levels, increased methylation of *NR3C1* in leukocytes, and reduced GR levels (Perroud et al., 2014). Psychosocial stress (i.e. exposure to war) incurred by the mother during gestation was positively correlated with *NR3C1* methylation in the umbilical cord blood of offspring, an effect that was not observed in methylation of the mother's blood suggesting this was reflective of the intrauterine environment (Mulligan et al., 2012). Stress was also negatively correlated with newborn birthweight (Mulligan et al., 2012). A similar effect was observed with intimate partner violence; this form of stress had no effect on *NR3C1* methylation of the mother but altered *NR3C1* methylation in whole blood of offspring (Radtke et al., 2011). Adolescent children whose mother experienced partner violence during pregnancy demonstrated increased methylation of *NR3C1*. Interestingly, methylation was not enhanced in adolescents if their mother experienced intimate partner violence either before or after pregnancy. Taken together, these studies highlight the ability of the gestational environment to influence methylation associated with the HPA axis. While it is possible that the stress incurred by the mother could alter her maternal behavior and in turn affect the offspring's HPA axis, this seems unlikely given that women that experienced the violence before or after pregnancy did not have offspring with altered *NR3C1* methylation (Radtke et al., 2011).

Experiencing psychological stress during pregnancy, such as clinical depression and anxiety, has implications for *NR3C1* methylation of the gestating offspring. Maternal depressed mood during the third trimester of pregnancy was associated with increased *NR3C1* methylation in cord blood mononuclear cells that mediated an enhanced cortisol response to stress in three month old infants (Oberlander et al., 2008). Pregnancy-related anxiety and cortisol levels of the mother were also correlated with *NR3C1* methylation of the cord blood of the infant (Hompes et al., 2013). Depression experienced during pregnancy decreased *Bdnf* methylation in buccal cells collected from infant offspring (Braithwaite et al., 2015). Male, but not female, infants also showed increased *NR3C1* methylation (Braithwaite et al., 2015). Another study found that mothers that reported symptoms of depression during the

second trimester of pregnancy had newborns with reduced promoter methylation of *SLC6A4* in umbilical cord leukocytes, which codes for the serotonin transporter (Devlin et al., 2010). Future studies examining associations of methylation and long-term behavioral outcomes in offspring following exposure to maternal depression and anxiety during gestation are warranted.

Limited access to resources during pregnancy is another form of gestational stress exposure that induces predisposition to later disease states. In 1998, Quebec suffered a severe ice storm that caused roughly five billion dollars' worth of damage and left individuals with no electricity, limited access to water, and no central heat in below 0° Celsius temperatures for up to six weeks. Offspring of women pregnant during this ice storm exhibited widespread alterations in DNA methylation. Teenage offspring exposed to the Quebec ice storm during gestation exhibited altered methylation status of 2,872 CpG sites spanning 1,564 genes in T-cells collected from blood samples (Cao-Lei et al., 2015). Many of the genes differentially methylated were related to immune and metabolic function. The methylation differences in offspring were mediated by their mother's cognitive appraisal (i.e. negative versus positive experience) of their experience during the ice storm. In these children, their mother's objective level of prenatal stress predicted their body mass index (BMI) and adiposity; women who reported greater stress had offspring with a greater BMI and central adiposity. A protective effect of DNA methylation was observed in diabetes-related genes whereby methylation of certain CpG sites negatively mediated the effects of prenatal stress on central adiposity and BMI (Cao-Lei et al., 2016). Because this cohort of offspring is still in their teenage years, future work will be necessary to determine their long-term outcomes. However, the Dutch Hunger Winter of 1944–1945 is another example of resource deprivation during pregnancy that has been studied on a long-term scale. During this time, individuals including pregnant women were rationed only 400–800 calories per day. Individuals with mothers who were exposed to famine during pregnancy have increased prevalence rates of schizophrenia and heart disease (Brown et al., 1995; Painter et al., 2005). In these individuals, methylation of the *IGF2* gene was found to be decreased in whole blood samples collected six decades after stress exposure relative to their non-exposed siblings (Heijmans et al., 2008). This effect was specific to individuals that were exposed to the famine during periconception (Heijmans et al., 2008; Tobi et al., 2009). It is important to note that all of the studies examining the effects of stress on methylation in humans utilized peripheral samples, so it is unclear the extent to which the changes discussed here reflect methylation levels in the brain. However, there is evidence of abuse during childhood changing GR methylation in the brains of suicide completers (Labonte et al., 2012; McGowan et al., 2009).

Restraint stress of pregnant dams is employed as an animal model of schizophrenia. Lending support to the applicability of rodent prenatal stress for studying schizophrenia, multiple studies have found links between epigenetic marks in the postmortem brain of individuals with schizophrenia and rodents subjected to prenatal stress. For example, individuals with schizophrenia have reduced expression of *BDNF*, a gene important in development and synaptic plasticity, and altered levels of epigenetic regulators such as TETs and DNMTs (Dong et al., 2012; Gavin et al., 2012; Jindal et al., 2010; Ruzicka et al., 2007). Adult mice subjected to prenatal stress were found to have increased levels of *Dnmt1* and *Tet1* in the

frontal cortex and hippocampus coinciding with reduced *Bdnf* expression. Reduced *Bdnf* expression corresponded with increased methylation and hydroxymethylation of the *Bdnf* gene (Dong et al., 2015). These same animals showed locomotor hyperactivity and decreased social interaction, both of which are behavioral indices of schizophrenia (Amann et al., 2010). As evidence for a link between these brain and behavioral implications of stress exposure, a positive correlation was found between social approach and *Bdnf* expression (Dong et al., 2015). *Reelin*, a gene implicated in the psychopathology of schizophrenia, was also reduced with a corresponding increase in DNA methylation of the *Reelin* promoter. These animals were hyperactive, had deficits in learning and memory, and displayed increased levels of anxiety as adults (Palacios-García et al., 2015). Another study found that decreased *Reelin* and glutamic acid decarboxylase 67 (GAD67) levels in adult mice with a history of prenatal stress were associated with overexpression of DNMTs in GABAergic neurons within the frontal cortex and hippocampus (Matrisciano et al., 2013). Further, increased methylation, hydroxymethylation, and MeCP2 and DNMT1 binding were observed at the *Reelin* and *Gad67* promoters (Matrisciano et al., 2013). These animals displayed a schizophrenia-like phenotype that was found to be ameliorated by the histone deacetylase inhibitor valproic acid or the antipsychotic clozapine (Matrisciano et al., 2013). Altered GABAergic functioning occurs in schizophrenia and is thought to contribute to both positive and negative symptoms characteristic of the disorder (Guidotti et al., 2005; Guidotti et al., 2000). Taken together, these studies demonstrate the ability for prenatal stress to alter the epigenome and, in turn, produce schizophrenia-like symptoms in rodents.

Studies administering chronic, unpredictable stressors to pregnant dams have contributed to the finding that the long-term implications of exposure to stress *in utero* are sex-specific. Chronic, unpredictable stressors have applicability to human populations, as in daily life humans encounter a variety of unpredictable challenges. The Bale laboratory exposes pregnant mice to a variety of stressors, including loud noises, predator odor, cage changes, and wet bedding at unpredictable times. Offspring exposed to this exhibit reduced corticotrophin-releasing factor (*Crf*) methylation in the hypothalamus and amygdala and increased *GR* methylation in adulthood (Mueller and Bale, 2008). Behaviorally, the prenatally stressed animals demonstrate reduced hedonic behavior (decreased sucrose preference), increased depressive-like behavior (increased immobility in the forced swim test) and increased anxiety-like behavior. Intriguingly, these effects were specific to male offspring (Mueller and Bale, 2008). In another study employing prenatal exposure to unpredictable and variable maternal stress, offspring showed increased *Bdnf* exon IV methylation in the medial prefrontal cortex of males and reduced length of telomeres in both sexes in adulthood (Blaze et al., 2017). Another report found deficits in spatial memory and reduced H3 acetylation in both males and females with a history of prenatal chronic, variable stress (Benoit et al., 2015). However, only stress-exposed females had higher *Dnmt1* levels and increased basal corticosterone (Benoit et al., 2015). Mice exposed to predator odor, an ethologically relevant stressor for mice, during the second half of gestation demonstrated an increased corticosterone response and increased avoidance behavior to predator odor exposures in adulthood. This corresponded with increased expression of corticotrophin-releasing factor receptor 1 (*Crr1*) in the amygdala and reduced *Bdnf* expression and methylation in the hippocampus of adult female animals (St-Cyr and

McGowan, 2015). It is unclear why these studies have found divergent outcomes regarding sex-specificity while other reports have not found sex differences in the outcomes of stress exposure (Mychasiuk et al., 2011). While the reason underlying the impact of sex on early-life stress is unknown, there are baseline differences in the expression of epigenetic regulators between the sexes (Auger et al., 2011; Nugent et al., 2015). Differences in the levels of epigenetic regulators at baseline or differences in circulating hormones between the sexes could contribute to these discrepancies; however, more work is needed to fully understand the differential impact of stress on the sexes.

The intensity and duration of the prenatal stressor is another factor that mediates the long-term impacts of prenatal stress, as opposite effects have been observed in the epigenome and behavior of animals incurring mild versus high prenatal stress. For example, both male and female offspring experiencing mild prenatal stress exhibited increased global methylation, while offspring exposed to high stress showed reduced global methylation in the hippocampus at PD21 (Mychasiuk et al., 2011). Mild stress reduced locomotor activity, while high stress increased locomotor activity. Timing of the gestational stressor is also critical in determining the effects the stressor will have on offspring, as neural systems develop at different gestational time points leaving different system vulnerable at different times of gestation. Multiple studies have demonstrated time-specific effects of prenatal exposure to stress (Heijmans et al., 2008; Mueller and Bale, 2008; Tobi et al., 2009).

Maternal separation

Infant rodents are completely dependent on their mothers for survival during their first few weeks of life. Dams provide their offspring with warmth, food, and help void them of waste via anogenital licking. In addition, the first two weeks of life comprise the stress hyporesponsive period during which time rodent pups display blunted stress responses if their mother is present (Levine, 1994). Therefore, experiencing periods of separation from the mother is a salient postnatal stressor that elicits long-term effects on the HPA axis. For example, daily maternal separation for the first 10 days of life resulted in increased expression and reduced DNA methylation of *Pomc*, a precursor to the hormone ACTH that is released by the pituitary during HPA axis activation (Wu et al., 2014). Daily separation for 360 minutes daily for the first three weeks of life also resulted in reduced *Pomc* expression in the hypothalamus, while increased expression of *Dnmt1* was observed in the pituitary (Todkar et al., 2015). Within the paraventricular nucleus (PVN), adult animals subjected to maternal separation showed hypomethylation and increased expression of arginine vasopressin (*Avp*) (Murgatroyd et al., 2009), a gene that has been implicated in the pathology of anxiety and depression (Neumann and Landgraf, 2012). Behaviorally, these animals showed a hyperactive stress response and altered passive stress coping (Murgatroyd et al., 2009). Another group exposed mice to a 24 hour maternal separation on postnatal day nine and found altered methylation of *Avp* and *Nr3c1* within the hippocampus of adult animals. These changes in DNA methylation were paralleled by changes in anxiety-like behavior and an increased corticosterone response to stress (Kember et al., 2012). Together, these studies demonstrate the capacity for stress during the early postnatal period to elicit long-term effects on DNA methylation that may mediate altered stress responsivity across the lifespan.

Maternal separation has been used as an animal model of depression, as exposure to early-life maternal separation elicits a depressive-like phenotype in adulthood (Vetulani, 2013). Rat pups that experienced three hours of daily maternal separation from postnatal days one through 21 had reduced expression of *Bdnf* that corresponded with reduced H3 and H4 acetylation and increased MeCP2 binding at the *Bdnf* exon IV promoter within the hippocampus (Seo et al., 2016). Behaviorally, stressed animals showed increased immobility in a forced swimming test (Seo et al., 2016). These brain and behavioral outcomes were potentiated by experiencing a second stressor in adulthood (Seo et al., 2016). Similarly, *GR* expression was reduced with a corresponding decrease in H3 acetylation at the *GR* promoter and this was exacerbated by a subsequent stress incurred in adulthood (Park et al., 2017). In both studies, the implications of maternal separation on adult behavior and neurobiology were rescued by treatment with the antidepressant escitalopram (Park et al., 2017; Seo et al., 2016). These studies highlight the ability for a second stressor to potentiate the effects of early-life stress and could offer a potential mechanism through which early stress could predispose an individual to depression.

Experiencing early-life stress can influence vulnerability to drug addiction in adulthood, and epigenetic marks resulting from stress could contribute to enhanced drug seeking behavior. Rats exposed to daily maternal separation exhibited increased locomotor sensitization to cocaine as adults, indicative of an increased responsivity to the drug. This corresponded with increased DNMT levels in the nucleus accumbens, a brain region involved in the rewarding aspects of addiction (Anier et al., 2014; Cornish and Kalivas, 2000). Further, these animals showed global hypomethylation and hypermethylation of protein phosphatase 1 (*PPI*) and adenosine A_{2A} receptor (*A_{2A}R*) genes in the nucleus accumbens. This corresponded with reduced expression of *A_{2A}R* and *PPI* (Anier et al., 2014). Because behavioral sensitization to cocaine has been shown to induce hypermethylation and reduced expression of *A_{2A}R* and *PPI*, this study elucidated a potential route through which early-stress could predispose an individual to addiction (Anier et al., 2010). Another study determined that animals with a history of maternal separation began self-administering methamphetamine earlier and self-administered a greater quantity of methamphetamine relative to control animals. This paralleled decreased MeCP2 levels in the nucleus accumbens core (Lewis et al., 2013). Collectively, these studies highlight global and gene-specific alterations resulting from maternal separation that could underlie enhanced drug seeking behavior.

Consistent with cognitive deficits observed in human populations with a history of early-stress, maternal separation models have likewise found cognitive deficits in rodents and these deficits likely have epigenetic underpinnings. For example, infants exposed to maternal separation demonstrate memory deficits in the Morris water maze test as adults (Wang et al., 2014). This behavioral deficit was seen alongside decreased methylation and increased H3 acetylation of the *Crf* promoter in the hippocampus (Wang et al., 2014). Blocking CRF signaling rescued memory impairments, suggesting that the epigenetic alterations to *Crf* were responsible for the stress-induced memory impairments. Further linking epigenetic marking of *Crf* and memory impairments, environmental enrichment ameliorated both of these effects of stress (Wang et al., 2014). Memory deficits in novel-object recognition have likewise been found in rodents with a history of maternal separation (Moreno Gudiño et al., 2017). This deficit was rescued by consuming a diet rich in the methyl-donor choline in

periadolescence. Another study exploring recognition memory deficits resulting from maternal separation found that administration of a histone deacetylase inhibitor rescued memory deficits and also increased H3 acetylation and expression of *Bdnf* (Albuquerque Filho et al., 2017). These data show consistent deficits in memory resulting from maternal separation and demonstrate the capacity for later-life interventions to ameliorate developmental stress outcomes via alterations to the epigenome.

Aversive Parental Care Including Maltreatment

Caregiver maltreatment, including neglect and abuse, is incurred by 10–15% of the population in the United States (Jud et al., 2016; Kessler et al., 1997). Thus, studying its implications and attempting to develop interventions to help individuals with a maltreatment history is an important avenue of research. Numerous rodent models involving maternal resource deprivation have been utilized to explore the implications of maltreatment on the brain and behavior (Walker et al., 2017). In our lab, we use a model of maternal maltreatment that capitalizes on resource deprivation. Pups are exposed to a dam that has limited nesting resources and consequently performs aversive behaviors toward the pups including stepping on, dropping, dragging, and roughly handling them. This manipulation is performed for 30 minutes daily for the first postnatal week of life. We employ a within litter design whereby 1/3 of the litter is exposed to the maltreatment condition, 1/3 of the litter remains in the home cage with the biological dam, and the remaining 1/3 of the litter is cross-fostered to a nurturing, non-biological dam. Using this model, our lab has uncovered a number of epigenetic alterations to the *Bdnf* gene in animals exposed to maltreatment. Because *Bdnf* is critical in development and its expression is altered in numerous disorders including depression and schizophrenia, *Bdnf* has emerged as a possible candidate neurobiological link between developmental stress and adult outcomes (Roth and Sweatt, 2011).

The prefrontal cortex, a brain region implicated in the psychopathology of bipolar disorder and schizophrenia, is one target of maltreatment (Chai et al., 2011). We have uncovered altered DNA methylation at the *Bdnf* gene in both whole (Roth et al., 2009a) and medial prefrontal cortex across the lifespan in maltreated animals (Blaze et al., 2013). Methylation of *Reelin* was altered 24 hours following the final bout of maltreatment, however, methylation normalized by adolescence (Blaze et al., 2013). Increased *Bdnf* methylation was found to be specific to neuronal, rather than glial, cells (Blaze and Roth, 2017). Levels of the epigenetic regulators *Dnmt1*, *Dnmt3a*, *MeCP2*, *Gadd45b*, and *Hdac1* were all found to be altered in the medial prefrontal cortex of maltreated male adult rats, while only *Gadd45b* expression was altered in females (Blaze and Roth, 2013). Females also displayed reduced Histone 3 lysine 9/14 acetylation at the *Bdnf* exon IV promoter (Blaze et al., 2015). Alterations in both gene specific (i.e. *Bdnf*) and global methylation have been found in the amygdala and dorsal and ventral subregions of the hippocampus of maltreated animals (Doherty et al., 2016; Roth et al., 2014). Other gene targets of maltreatment include neuropeptide Y and the oxytocin receptor, which have both been found to be altered in the amygdala of adolescent animals (Hill et al., 2014).

There is a dynamic nature to the epigenetic alterations we have observed across the lifespan. For example, at exon *IV* of the *Bdnf* gene in the medial prefrontal cortex, female rodents with a history of maltreatment had less methylation in adolescence but higher methylation in adulthood (Blaze et al., 2013). However, in the whole prefrontal cortex, *Bdnf* exon *IX* methylation was increased 24 hours after the final day of maltreatment exposure (i.e. PD8) and this increase persisted into adulthood. Outcomes of maltreatment are also sex-specific; in the amygdala, adult females have increased methylation of *Bdnf* exons *I* and *IV* while males have reduced methylation at both exons (Roth et al., 2014). This demonstrates that although DNA methylation changes resulting from postnatal stress can be long lasting, they can also change over time and the changes are highly dependent upon sex, gene locus, and brain region. Further work is needed to identify reasons underlying temporal, regional, and sex differences in methylation.

Being subjected to abuse and/or neglect by the caregiver has likewise been linked with epigenetic alterations in human populations. For example, the *NR3C1* 1_F exon promoter exhibited enhanced levels of methylation and decreased levels of expression and transcription factor binding in the hippocampus of suicide victims with a history of childhood abuse relative to suicide victims that had not experienced abuse during childhood (McGowan et al., 2009). This finding was extended to additional splice variants of the *NR3C1* gene (i.e. 1_B, 1_C, and 1_H), which were found to be downregulated in suicide-completers with an abuse history. Further, DNA methylation at these promoter regions was correlated with transcript expression (Labonte et al., 2012). In peripheral blood, levels of methylation at the *NR3C1* gene were found to be positively correlated with the amount of maltreatment sustained during childhood (Perroud et al., 2011). Because of the role GRs play in terminating HPA axis activity, reduced GR numbers could intensify the effects of stress and, in turn, increase the risk for stress-induced pathology in adulthood. The ribosomal RNA gene promoter was likewise found to be hypermethylated in hippocampus, but not cerebellum, of suicide-completers with a history of childhood abuse (McGowan et al., 2008).

Studies examining peripheral DNA methylation have also identified epigenetic signatures of childhood maltreatment. In candidate gene studies examining methylation of the gene that encodes the serotonin transporter (i.e. *SLC6A4*), individuals that experienced abuse during childhood demonstrate increased methylation of the *SLC6A4* promoter and reduced *SLC6A4* expression (Beach et al., 2010; Vijayendran et al., 2012). The serotonin transporter is important for emotional regulation (Hariri and Holmes, 2006), and reduced *SLC6A4* expression has been linked with suicidal behavior, particularly in individuals that experienced childhood maltreatment (Roy et al., 2007). Genome-wide investigation revealed that children experiencing severe maltreatment had differential methylation at 2,868 CpG sites (Yang et al., 2013). Some of the genes exhibiting differential methylation were involved in neural communication, immune response, and brain development. These data show the ability for childhood abuse to alter methylation at multiple gene loci in the brain and periphery.

Data exist to support the notion that DNA methylation resulting from childhood maltreatment mediates negative outcomes of maltreatment. For example, in the peripheral

blood of adults with a diagnosis of PTSD, individuals with a history of childhood abuse demonstrated a specific profile of gene expression which was non-overlapping from that of individuals with a PTSD diagnosis never exposed to abuse (Mehta et al., 2013). These changes in gene expression were associated with altered patterns of DNA methylation at these same gene loci (Mehta et al., 2013). Within individuals with a diagnosis of borderline personality disorder, maltreatment experienced during childhood was positively correlated with *BDNF* methylation (Perroud et al., 2013). As evidence for increased *BDNF* methylation contributing to borderline personality disorder symptoms, individuals who responded positively to intensive dialectical behavior therapy exhibited reduced *BDNF* methylation after completing their treatment regimen relative to their methylation levels prior to treatment (Perroud et al., 2013). Further, single nucleotide polymorphisms (SNPs) have been shown to mediate the relationship between child abuse and adult outcomes. Demethylation of *FKBP5* in carriers of the *FKBP5* rs1360780 risk allele was found to mediate the relationship between childhood abuse and increased PTSD prevalence in adulthood (Klengel et al., 2013). These data lend support to the idea that maltreatment during childhood has vast implications for the epigenome which likely contribute to behavioral and disease states associated with maltreatment.

Non-nurturing maternal care

Dams exhibit natural variability in quality of maternal care exhibited towards their offspring, with some dams performing high levels of licking and grooming (LG) and others performing low levels of LG towards offspring until PD 8 (Caldji et al., 1998). Experiencing inadequate maternal care (i.e. low levels of LG in rodents) has been linked with lifelong alterations in stress responsivity. Animals that received low levels of LG in infancy reveal increased anxiety-like behavior as measured by the open field test and novelty suppressed feeding. Additionally, these same animals exhibit an enhanced corticosterone response to acute stressors. Similar to humans that experienced abuse during childhood, animals with a history of non-nurturing maternal care have reduced levels of *GR* expression in their hippocampus, which was found to correspond with increased methylation, decreased H3K9 acetylation, and reduced transcription factor binding at the *GR* promoter suggesting that epigenetic programming mediates the effects of infant caregiver experience on adult stress responsivity (Weaver et al., 2004).

Receiving non-nurturing maternal care also has implications for maternal behavior. Female offspring of low-LG dams exhibit low LG toward their own offspring, while female offspring of high-LG dams exhibit high levels of LG toward their offspring. The medial preoptic area (MPOA) is a brain region instrumental in maternal behavior (Fleming et al., 1983; Fleming and Walsh, 1994; Numan, 2012; Pedersen et al., 1994). Females subjected to low LG in infancy demonstrate decreased *ERα* expression within the MPOA from infancy through adulthood (Champagne et al., 2006; Peña et al., 2013). Decreased gene expression corresponded with increased levels of methylation and reduced transcription factor binding at the exon 1b promoter region of *ERα* (Champagne et al., 2006). Cross-fostering experiments have shown that *ERα* expression is under the control of infant caregiver experience, as pups demonstrate *ERα* expression consistent with that of the dam that raised them rather than their biological mother (Champagne et al., 2006). Because *ERα* is a ligand-

activated transcription factor, altering *ERα* expression can have implications for the transcription of other genes containing estrogen response elements (Marino et al., 2006). Indeed, expression of the oxytocin receptor, a gene that contains an estrogen response element, is altered within the MPOA as a result of LG received in infancy (Champagne et al., 2001; Peña et al., 2013). Both estrogen and oxytocin act on the MPOA to promote maternal behavior and pup responsiveness (Numan, 2012; Numan et al., 1977; Pedersen et al., 1994). Therefore, these data highlight gene targets of early-life LG that contribute to the transmission of maternal behavior from mother to offspring.

Male and female rodents receive differential maternal care with male pups receiving more LG than their female littermates (Moore, 1984, 1985; Moore and Chadwick-Dias, 1986; Moore and Morelli, 1979). Therefore, manipulating the sex composition of litters has implications for maternal behavior. Consistent with work from the Meaney laboratory showing a regulatory role of quantity of LG behavior on *GR* methylation, adolescent female offspring raised in a single-sex litter (i.e. all female pups) were found to have increased methylation of the *GR* promoter in the nucleus accumbens relative to females reared in a mixed-litter (Kosten et al., 2014). Methylation of *Oprm1*, which is important in maternal attachment, was increased in the hippocampus in adolescent rats reared in single-sex litters (Hao et al., 2011). Taken together, these data show that altering the quality of maternal care a rodent received in infancy has long-term effects on the methylation status of behaviorally-relevant genes.

Overlap between Developmental Alcohol Exposure and Stress

Developmental alcohol and toxic stress exposure are teratogens that increase an individual's risk for developing behavioral problems and psychiatric disorders later in life (Shea et al., 2005; Streissguth et al., 2004). Many of the brain regions affected by developmental stress are also targets of prenatal alcohol, including the prefrontal cortex, hippocampus, HPA axis, and cerebellum. The susceptibility of the epigenome to developmental alcohol or stress exposure is becoming apparent, with similarities including: 1) altered methylation states of genes involved in neural development, plasticity, and regulation of the HPA axis; 2) altered levels of epigenetic regulators including DNMTs and MeCP2; 3) changes in non-coding RNA expression; and 4) sex-specific consequences.

DNA methylation of neurodevelopmental and plasticity-related genes

Genes related to neurodevelopment and HPA axis regulation are commonly altered in both developmental stress and alcohol models. Clustered protocadherin genes are important for establishing neuronal identity in the developing brain and can be epigenetically regulated by prenatal alcohol exposure or adverse maternal care (Chater-Diehl et al., 2016; Laufer et al., 2015; Laufer et al., 2013; McGowan et al., 2011). Protocadherin expression has been implicated in neurodevelopmental disorders, including schizophrenia, due to changes in axonal guidance and cortical folding (Gregório et al., 2009; Hirabayashi and Yagi, 2014). Protocadherin genes are also regulated by CCCTC-binding factor (CTCF), a transcription factor downregulated in somatic DNA of offspring following paternal drinking (Knezovich and Ramsay, 2012).

CTCF also regulates transcription of certain imprinted genes, such as paternally imprinted *Igf2* and *H19*. Expression and methylation status of many imprinted genes are affected by developmental alcohol exposure in both humans and rodent models (Laufer et al., 2017; Laufer et al., 2015; Laufer et al., 2013; Portales-Casamar et al., 2016), though additional work is needed to determine the full impact of developmental stress exposure. There is some evidence of overlap, as *Igf2* is differentially methylated and downregulated in rodent models of prenatal alcohol exposure and in children with FASD (Downing et al., 2011; Laufer et al., 2015; Laufer et al., 2013) and decreased methylation of *IGF2* was also found in individuals that had been exposed to famine conditions during periconception (Heijmans et al., 2008). Maternal diet has been shown to also alter methylation of imprinted genes in rodent models (Dunn and Bale, 2011; Zhang et al., 2010).

Epigenetic regulation of growth factors, such as *Igf2* and *Bdnf*, is altered by developmental alcohol exposure (Boschen et al., 2016; Downing et al., 2011; Haycock and Ramsay, 2009; Laufer et al., 2013) and has been extensively researched in relation to early stress exposure (Blaze et al., 2017; Blaze et al., 2015; Braithwaite et al., 2015; Roth et al., 2009a). Notably, growth factors are linked to the development of neuropsychiatric disorders including depression and schizophrenia. Mental health outcomes are poorer in individuals with prenatal exposure to alcohol or to significant stress, such as famine or childhood maltreatment. Alterations in *Pomc* methylation status and expression as well as stress-reactivity have been found in models of either alcohol or early stress exposure (Bekdash et al., 2013; Murgatroyd et al., 2009; Todkar et al., 2016); alongside changes in plasticity-related genes, such as *Bdnf*, these changes could underlie increased prevalence rates for stress-related pathology associated with these types of teratogen exposures (Caldwell et al., 2008).

Another important aspect of stress and alcohol overlap is the dynamic nature of epigenetic alterations to *Bdnf*. In animal models of both FASD and early-life stress the implications of teratogen exposure on the epigenome can vary depending upon age. For example, in an animal model of FASD methylation of *Bdnf* exon I was reduced on PD10, but methylation levels normalized by PD72 (Boschen et al., 2015; Boschen et al., 2016). A similar effect was observed in an animal model of early-life stress, where *Bdnf* exon IV methylation was reduced in the adolescent brain but increased in the adult brain (Blaze et al., 2013). The significance of the dynamic nature of these epigenetic changes remains to be elucidated. Learning and memory processes and normal neural development rely on ever-changing patterns of epigenetic marks, thus it is important to put any epigenetic modifications in the context of normative epigenetic programming and gene activity (Meaney and Ferguson-Smith, 2010; Szulwach et al., 2011). In addition, disruptions to neural development arising from aberrant epigenetic programming early in life could predispose an individual to developing neuropsychiatric disorders in adulthood, even if the epigenetic patterns normalize over time (Ptak and Petronis, 2010).

Expression of epigenetic regulators

Another commonality between the consequences of early-life stress and alcohol are the observed alterations in levels of epigenetic regulators throughout the brain. For example,

DNMT1 was found to be increased across the brain in animals with a history of early-life stress or alcohol exposure (Bekdash et al., 2013; Blaze and Roth, 2013; Dong et al., 2015; Pena et al., 2012). MeCP2, which is important for neurodevelopment, is another epigenetic regulator found to change after exposure to either stress or alcohol. Importantly, alcohol and stress studies have consistently found a reduction in MeCP2 expression in the frontal cortex (Blaze and Roth, 2013; Kim et al., 2013); reduced MeCP2 in the frontal cortex has been linked with developmental disorders (Gonzales and LaSalle, 2010; Nagarajan et al., 2006). These changes to epigenetic regulators could underlie the genome-wide and gene specific alterations observed in DNA methylation and posttranslational histone modifications. There is also evidence that changes to the epigenome resulting from developmental perturbations are potentiated by exposure to further insult (a “second-hit”) later in life (Hellemans et al., 2010; Park et al., 2017; Seo et al., 2016). A second-hit, in the form of either another stressor, alcohol exposure, or infection, could cause an individual with already altered levels of epigenetic regulators to have more significant adverse outcomes, including those in the realm of learning and memory (Guan et al., 2015; Roth et al., 2010).

Non-coding RNAs

Non-coding RNAs represent one epigenetic pathway through which both PAE and developmental stress have been shown to affect protein synthesis and, ultimately, CNS function. Disruption of noncoding RNAs, such as miRNAs, can cause a wide range of cellular or DNA damage, contributing to long-term deficits (Figure 1). miRNAs affect the production of protein products through translational repression or degradation of the mRNA. *In vivo* and *in vitro* models of FASD have identified numerous miRNAs that are impacted by developmental alcohol exposure, including miR-9, miR-20a, miR-21, miR-30, miR-103, miR-151, miR-153, miR-335, and miR-140-3p (Balaraman et al., 2014; Balaraman et al., 2012; Guo et al., 2012; Ignacio et al., 2014; Pappalardo-Carter et al., 2013; Pietrzykowski et al., 2008; Sathyan et al., 2007; Soares et al., 2012; Tal et al., 2012; Wang et al., 2009). In a study that examined the effects of prenatal stress on miRNA expression, dams were administered daily restraint stress and forced swimming from days 12–18 of gestation (Zucchi et al., 2013). This stressor disrupted maternal behavior, as reduced tail chasing behavior was exhibited by dams exposed to stress. In the whole brains of their newborn male offspring, 336 miRNAs were differentially expressed. Notably, some of the same miRNAs were altered by prenatal stress as have been mentioned in the developmental alcohol literature, including miR-9, miR-20a, miR-103, and miR-151.

The downstream effects of miRNA suppression on protein levels and, ultimately, behavioral and cognitive processes, is highly dependent on the specific target mRNAs for each miRNA. Notably, many of the miRNAs target genes altered by alcohol and stress are involved in cell proliferation and apoptotic pathways, including regulation of the Notch and Bax signaling, neurodevelopment, cognitive function, the stress response, hormonal regulation, and brain pathologies (Franklin et al., 2010; Guo et al., 2012; Laufer et al., 2013; Stringer et al., 2013; Tal et al., 2012; Zucchi et al., 2013). Altered levels of miRNAs have been found in individuals with psychiatric illnesses including schizophrenia and bipolar disorder (Hunsberger et al., 2009; Miller and Wahlestedt, 2010; Moreau et al., 2011), suggesting

miRNA disruption is another avenue through which prenatal stress or alcohol exposure could alter behavior and mental health outcomes.

Sex Differences

There is a growing literature demonstrating that the implications of developmental stress or alcohol are sexually divergent. Increased attention and appreciation of sex-specific consequences has emerged in recent years, most recently fueled by the directive from National Institutes of Health in 2014 requiring researchers to address sex differences in all new grant proposals (Clayton and Collins, 2014). The interaction of sex and developmental teratogen exposure is an area of research that has been ignored for too long. Numerous sexually dimorphic changes emerge after stress exposure, including changes to behavior, gene expression, and DNA methylation (Blaze and Roth, 2017; Franklin et al., 2010; Mueller and Bale, 2008; Roth et al., 2014; St-Cyr and McGowan, 2015). Sex-specific changes to anxiety- and depressive-like behaviors have been some of the most consistently reported behavioral effects following developmental stress exposure. Sex-specific outcomes have also been observed in individuals with FASD and in animal models. For example, male children with FASD are diagnosed with comorbid attention deficit-hyperactivity disorder (ADHD) at twice the rate of females with FASD (Herman et al., 2008). In addition, females and males with FASD who had been diagnosed with ADHD had sex-specific differences in executive functioning compared to the sex-matched controls; females with ADHD had worse executive functioning while males with ADHD performed better. In animal studies, male rats prenatally exposed to alcohol show increased anxiety-like behavior, while females displayed more depressive-like behavior and higher levels of corticosterone (Weinberg et al., 2008).

Limited research has been conducted on the influence of sex on epigenetic modifications following developmental alcohol exposure. One study found that the male, but not female, germline transmits methylation changes to the *Pomc* gene resulting from prenatal alcohol exposure (Govorko et al., 2012). Another study using an animal model of alcohol exposure found female specific effects, as female but not male subjects exhibited alterations in methyl metabolism in the hippocampus (Ngai et al., 2015). Current literature supports continued investigation of sex as an important mediator of teratogen-induced epigenetic modifications and long-term health outcomes.

Future Directions: Combining Stress and Alcohol Models and Assessment of Multigenerational Effects and Therapeutics

Despite the considerable work done characterizing epigenetic modifications in models of developmental alcohol or stress, many questions remain. First, compared to the depth of knowledge stemming from the developmental stress literature, FASD researchers are still in the beginning stages of their investigations regarding how *in utero* alcohol alters epigenetic patterns and if changes to the epigenome causally relate to behavioral outcomes and can be used as reliable biomarkers. Many studies that assess epigenetic endpoints do not include analysis of downstream gene or protein expression. These additional measures can help determine the functional consequences of the epigenetic marks. Furthermore, it is unknown

if alterations to epigenetic status in a cell population are always due to *de novo* changes following an exposure to stress or alcohol or if these changes are representative of preexisting marks that distinguish certain cell populations. As PAE and stress can induce apoptosis (Farber et al., 2010; Heaton et al., 2003; Kim et al., 2015), it is possible that the surviving cell population has a certain epigenetic profile that becomes apparent following the widespread cell death. While this alternative is unlikely to always be the case, it is a hypothesis that should be explored.

Secondly, further investigation of the intersection between developmental stress and alcohol exposure are needed, as few studies have combined these exposures despite significant translational relevance. As some pregnant women use alcohol as a means to cope with stress (Hanna et al., 1994; Schneider et al., 2008), understanding the consequences of concomitant prenatal alcohol and stress exposure is an important area of investigation. Though epigenetic modifications were not examined, Schneider and colleagues (1997) reported that rhesus macaques exposed to prenatal alcohol and noise stress had reduced birth weight, impaired motor coordination and response speed, and delayed motor maturity and orientation. Similarly, Alberry and Singh (2016) used a model of prenatal alcohol exposure followed by maternal separation during the neonatal period in mice. Offspring exposed to both ethanol and separation demonstrated hypoactivity and impaired performance on certain behavioral tasks. In most cases, maternal separation increased the severity of behavioral measures negatively affected by prenatal ethanol exposure; however, pairing the timing of the stress with the alcohol exposure might result in a more significant interaction. Interestingly, many of the reported effects were sex-dependent, suggesting an influence of epigenetic programming on experimental outcomes. Use of similar experimental frameworks to these studies in conjunction with an epigenetic lens stands to broaden our understanding of the pathways through which developmental alcohol and stress exposure render brain damage and behavioral impairment, and identify interventions for their rescue. In addition, we have identified two areas of research that hold both intense scientific and clinical interest related to both developmental alcohol and stress exposure: 1) the ability of epigenetic marks to be passed through the germline and affect subsequent generations of offspring; and, 2) the development of therapeutics to address behavioral and cognitive deficiencies.

Multigenerational Effects

One of the most important and fascinating implications of epigenetic modifications is the potential for intergenerational or transgenerational transmission. Intergenerational transmission involves direct exposure of a teratogen on the germline, whereas transgenerational transmission excludes direct exposure (for example F2 generation involving the paternal germline or F3 involving the maternal germline) (Klengel et al., 2016). Both PAE and developmental stress have been shown to have multigenerational effects through epigenetic modifications to the germline (Figure 3). While the literature regarding inter- and transgenerational epigenetic effects associated with PAE is limited, there is evidence that paternal experience with alcohol has an impact on methylation patterns in the germline. For example, males rats exposed to alcohol for 9 weeks had significant changes to methyltransferase levels in their sperm and produced offspring with lower birth weight (Bielawski et al., 2002). While methylation patterns were not directly assessed in the

offspring, numerous reports of behavioral and physical abnormalities have been reported following paternal alcohol exposure (Abel and Lee, 1988; Bielawski and Abel, 1997; Ledig et al., 1998); epigenetic changes carried through the germline are one way to envision how paternal drinking could affect offspring. Knezovich and Ramsay (2012) reported significant reductions in CTCF, a transcription factor sensitive to methylation at its target DNA binding sites and that regulates methylation patterns and chromatin structure related to clustered protocadherins and paternally methylated imprinting control regions of DNA, including *H19* and *RASgrf1*. However, analysis of sperm methylation in males revealed no differences following alcohol exposure, indicating that the methylation changes in the offspring must be due to some other epigenetic mechanism. Further confirming the sensitivity of the male germline to alcohol, transgenerational changes to *Pomc* methylation status and mRNA levels were reported through the F3 male germline following PAE, while the female germline was not affected (Govorko et al., 2012).

Similarly, significant evidence supports that developmental stress can have consequences that are perpetuated across generations. The effects of maternal separation on DNA methylation are not just found in rodents directly exposed to the separation stress, but also in their offspring (Franklin et al., 2010). Cannabinoid receptor-1 (*Cnr1*), *Crf2*, and *MeCP2* were found to be differentially methylated in the sperm of F1 males and the brains of their offspring (i.e. the F2 generation). Anxiety and depressive-like behavior were altered across generations and the direction of the change was dependent upon sex and generation. For example, F1 males spent more time immobile in a forced swim test while females showed reduced levels of immobility in the F1 and F2 generations (Franklin et al., 2010). F2 males did not exhibit altered behavior relative to control males, but F3 males exhibited increased immobility comparable to that of F1 males (Franklin et al., 2010). Multigenerational transmission of epigenetic marks have also been found with prenatal stress (Ward et al., 2013), postnatal stress (Roth et al., 2009a), and preconception stress of both the mother (Zaidan et al., 2013) and father (Mychasiuk et al., 2013). Paternal stress has likewise been demonstrated to have implications for the behavior and epigenome of offspring (Rando, 2012). Males exposed to preconception stress had offspring with impaired learning on a motor coordination task alongside altered levels of global methylation in the frontal cortex and hippocampus (Mychasiuk et al., 2013). Additionally, sperm miRNAs have been suggested as a mechanism of multigenerational epigenetic programming, as sperm miRNA levels are altered following stress and microinjection of these miRNAs into zygotes causes dysregulation of gene expression related to the HPA axis (Rodgers et al., 2013; Rodgers et al., 2015). It is worth noting that the concept of epigenetic transgenerational transmission is controversial, and some argue that alterations in maternal behavior due to the fitness of the offspring or mating patterns of the male could explain the perpetuation of epigenetic marks across generations rather than germline transmission (Whitelaw, 2015). Future work is needed to parse apart the effects of prenatal and postnatal environment versus germline transmission in the perseverance of epigenetic marks between generations.

Therapeutic Strategies

The epigenome remains malleable throughout the lifespan; consequently, outcomes of developmental alcohol or stress exposure are potentially reversible by altering the adult

epigenome. Current evidence supports the efficacy of nutritional, behavioral, and pharmacological strategies as a means to induce epigenetic modifications and normalize brain function in the adult. Otero et al. (2012) reported that choline supplementation during and after postnatal alcohol exposure reduced hypermethylation in the hippocampus and prefrontal cortex. Choline has positive effects on behavioral and neuroanatomical measures in rat models of FASD (Thomas et al., 2007; Thomas et al., 2004; Thomas and Tran, 2012); these data together suggest that one of its mechanisms of action might be through epigenetic processes. Deficits on novel object and place recognition caused by early life stress can also be reversed through choline supplementation during periadolescence (Moreno Gudiño et al., 2017). Supplementation with a methyl-rich diet had beneficial effects on pup mortality rates, prenatal growth, and rate of vertebral and digit malformations in mice prenatally exposed to alcohol, though further work is needed to link this diet with gene-specific methylation changes (Downing et al., 2011). Methyl-rich diet supplementation in adulthood has also been shown to alter epigenetic marks associated with GRs in animals exposed to low licking and grooming during the neonatal period (Weaver et al., 2005).

Aside from pharmacological manipulations, environmental manipulations have likewise been shown to have beneficial effects through altering the epigenome. Exercise and environmental complexity have been used as behavioral interventions and have beneficial effects on neuroplasticity and behavioral measures (Boschen et al., 2016; Gómez-Pinilla et al., 2002; Hamilton et al., 2012; Helfer et al., 2009). In alcohol-exposed rat pups, exercise (voluntary wheel running) was associated with reduced methylation at exons *I* and *IV* of the *Bdnf* gene, as well as significantly increased *Bdnf* exon-specific mRNA levels and hippocampal dendritic complexity (Boschen et al., 2016). Similarly, rodents subjected to stress in infancy have shown a rescue of brain and behavioral outcomes via exercise (Wearick-Silva et al., 2017) and environmental enrichment (Champagne and Meaney, 2007; Wang et al., 2014). Maternal separation during the first two postnatal weeks altered behavioral performance and exon-specific *Bdnf* gene expression in adolescence; however, three weeks of exercise reversed these changes (Wearick-Silva et al., 2017). These results should be interpreted with caution, as control runners showed decreases or no change in *Bdnf* gene expression after running, a result that is contrary to most literature investigating exercise and BDNF.

Increased opportunity for social interaction is an important part of environmental enrichment paradigms (Moreno Gudiño et al., 2017). Rats exposed to developmental alcohol have altered social behavior in adulthood (Boschen et al., 2014; Middleton et al., 2012; Mooney and Varlinskaya, 2011), however, there is evidence that housing alcohol-exposed adolescents with novel, unexposed rats can reverse some of the epigenetic changes caused by alcohol (Middleton et al., 2012). Specifically, the alcohol-induced expression of genes that code miRNAs related to the cell cycle and apoptosis was reversed following adolescent exposure to social novelty. Similarly, adolescent social enrichment increased oxytocin binding and exploratory behavior in offspring cared for by less nurturing mothers (Champagne and Meaney, 2007). Interestingly, these effects were shown to have multigenerational consequences.

Because of data showing that developmental alcohol and stress act through epigenetic pathways, targeted pharmacological treatments that affect the epigenome hold promise for successful intervention. Adult rats exposed to caregiver maltreatment during the first week of postnatal life demonstrated a rescue of maltreatment-induced DNA methylation of the *Bdnf* gene after treatment with the DNMT inhibitor zebularine (Roth et al., 2009a). Antidepressants have been shown to rescue the depressive-like phenotype and epigenetic marks resulting from maternal separation (Ignácio et al., 2017; Park et al., 2017; Seo et al., 2016). In humans, adults that showed a positive response to treatment with the antidepressant citalopram showed normalized levels of BDNF and reduced levels of a repressive histone mark (H3K27me3) at the *BDNF* exon IV promoter (Lopez et al., 2013). While limited work has been done in PAE models, direct regulation of enzymatic activity through histone deacetylase (HDAC) inhibitors are a pharmacological avenue worth exploring. HDACs act to remove acetylation marks from histones, resulting in a more closed chromatin structure and reduced transcriptional activity (Abel and Zukin, 2008). HDAC inhibitors prevent these enzymes from acting, allowing chromatin to remain in a more permissive state. While HDAC inhibitors have proven neuroprotective and neurorestorative in models of stress and aging (Kim et al., 2007; Langley et al., 2005), at the writing of this review, no studies looking at the direct effect of HDAC inhibitors on PAE-induced epigenetic status or cognitive/behavioral correlates could be found. One of the limiting factors for using this approach is that affecting chromatin structure during development could cause secondary effects on the fetus. Discovery of therapeutic treatments safe for delivery during the prenatal and postnatal periods is a critical step in moving efforts forward to help children with FASD or negatively impacted by early life stress or maltreatment.

Conclusions

Epigenetics is a growing and promising scientific field. As our understanding of the mechanisms and relevance of epigenetic marks grows, we can better use these tools to answer important questions about how early teratogen exposure impacts fetal and neonatal development and contributes to long-term health and behavioral outcomes. It is important to answer these questions methodically and with rigor, with careful interpretation of the data in the context of the cell, the organism, and the environment. Prenatal stress and alcohol exposure, while two of the most common teratogens, are usually studied separately. Stress, however, is the primary factor as to why women drink during pregnancy, and many children who are born to alcoholic mothers continue to experience stress after they are born. While this review has highlighted some of the common ways in which stress or alcohol impact the epigenome, little is understood of the epigenome's response to both stress and alcohol exposure and how that translates to altered CNS function and behavior. Understanding how developmental alcohol and stress exposure together damage the brain and produce cognitive and behavioral impairments through epigenetic programming will allow for the development of better-targeted interventions for affected individuals.

Acknowledgments

Work on this article was supported by a grant from The National Institute of General Medical Sciences (P20GM103653 to AYK and TLR) and Eunice Kennedy Shriver National Institute of Child Health and Human Development (R01HD087509 to TLR). We thank Ashlyn Keller for her edits on the manuscript.

References

- Abel EL, Lee JA. Paternal alcohol exposure affects offspring behavior but not body or organ weights in mice. *Alcoholism: Clinical and Experimental Research*. 1988; 12(3):349–355.
- Abel T, Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Current Opinion in Pharmacology*. 2008; 8(1):57–64. [PubMed: 18206423]
- Adriani W, Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology*. 2004; 15(5–6):341–352. [PubMed: 15343057]
- Agid O, Shapira B, Zislin J, Ritsner M, Hanin B, Murad H, Troudart T, Bloch M, Heresco-Levy U, Lerer B. Environment and vulnerability to major psychiatric illness: a case control study of early parental loss in major depression, bipolar disorder and schizophrenia. *Molecular Psychiatry*. 1999; 4(2):163–172. [PubMed: 10208448]
- Aihara M, Ida I, Yuuki N, Oshima A, Kumano H, Takahashi K, Fukuda M, Oriuchi N, Endo K, Matsuda H. HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. *Psychiatry Research: Neuroimaging*. 2007; 155(3):245–256. [PubMed: 17587554]
- Alberry B, Singh SM. Developmental and behavioral consequences of early life maternal separation stress in a mouse model of fetal alcohol spectrum disorder. *Behavioural Brain Research*. 2016; 308:94–103. [PubMed: 27102339]
- Albuquerque Filho MO, De Freitas BS, Garcia RCL, de Freitas Crivelaro PC, Schröder N, de Lima MNM. Dual influences of early-life maternal deprivation on histone deacetylase activity and recognition memory in rats. *Neuroscience*. 2017; 344:360–370. [PubMed: 28089578]
- Alcoholism N.I.o.A.A.a. Fetal Alcohol Exposure Fact Sheet. 2015
- Amann LC, Gandal MJ, Halene TB, Ehrlichman RS, White SL, McCarren HS, Siegel SJ. Mouse behavioral endophenotypes for schizophrenia. *Brain Research Bulletin*. 2010; 83(3):147–161. [PubMed: 20433908]
- Anier K, Malinovskaja K, Aonurm-Helm A, Zharkovsky A, Kalda A. DNA methylation regulates cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology*. 2010; 35(12):2450–2461. [PubMed: 20720536]
- Anier K, Malinovskaja K, Pruus K, Aonurm-Helm A, Zharkovsky A, Kalda A. Maternal separation is associated with DNA methylation and behavioural changes in adult rats. *European Neuropsychopharmacology*. 2014; 24(3):459–468. [PubMed: 23972903]
- Auger AP, Jessen HM, Edelmann MN. Epigenetic organization of brain sex differences and juvenile social play behavior. *Hormones and Behavior*. 2011; 59(3):358–363. [PubMed: 20619265]
- Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*. 1993; 75(1):73–82. [PubMed: 8402902]
- Balaraman S, Lunde ER, Sawant O, Cudd TA, Washburn SE, Miranda RC. Maternal and neonatal plasma microRNA biomarkers for fetal alcohol exposure in an ovine model. *Alcoholism: Clinical and Experimental Research*. 2014; 38(5):1390–1400.
- Balaraman S, Winzer-Serhan UH, Miranda RC. Opposing actions of ethanol and nicotine on microRNAs are mediated by nicotinic acetylcholine receptors in fetal cerebral cortical-derived neural progenitor cells. *Alcoholism: Clinical and Experimental Research*. 2012; 36(10):1669–1677.
- Beach SR, Brody GH, Todorov AA, Gunter TD, Philibert RA. Methylation at SLC6A4 is linked to family history of child abuse: an examination of the Iowa Adoptee sample. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2010; 153(2):710–713.
- Bekdash RA, Zhang C, Sarkar DK. Gestational Choline Supplementation Normalized Fetal Alcohol-Induced Alterations in Histone Modifications, DNA Methylation, and Proopiomelanocortin (POMC) Gene Expression in β -Endorphin-Producing POMC Neurons of the Hypothalamus. *Alcoholism: Clinical and Experimental Research*. 2013; 37(7):1133–1142.
- Benoit JD, Rakic P, Frick KM. Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behavioural Brain Research*. 2015; 281:1–8. [PubMed: 25496779]

- Berman RF, Hannigan JH, Sperry MA, Zajac CS. Prenatal alcohol exposure and the effects of environmental enrichment on hippocampal dendritic spine density. *Alcohol*. 1996; 13(2):209–216. [PubMed: 8814658]
- Bertrand J. Consortium, I.f.C.w.F.A.S.D.R. Interventions for children with fetal alcohol spectrum disorders (FASDs): overview of findings for five innovative research projects. *Research in Developmental Disabilities*. 2009; 30(5):986–1006. [PubMed: 19327965]
- Bielawski DM, Abel EL. Acute treatment of paternal alcohol exposure produces malformations in offspring. *Alcohol*. 1997; 14(4):397–401. [PubMed: 9209556]
- Bielawski DM, Zaher FM, Svinarich DM, Abel EL. Paternal alcohol exposure affects sperm cytosine methyltransferase messenger RNA levels. *Alcoholism: Clinical and Experimental Research*. 2002; 26(3):347–351.
- Blair R. The amygdala and ventromedial prefrontal cortex: functional contributions and dysfunction in psychopathy. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*. 2008; 363(1503):2557–2565. [PubMed: 18434283]
- Blaze J, Asok A, Borrelli K, Tulbert C, Bollinger J, Ronca AE, Roth TL. Intrauterine exposure to maternal stress alters Bdnf IV DNA methylation and telomere length in the brain of adult rat offspring. *International Journal of Developmental Neuroscience*. 2017; 62:56–62. [PubMed: 28330827]
- Blaze J, Asok A, Roth TL. Long-term effects of early-life caregiving experiences on brain-derived neurotrophic factor histone acetylation in the adult rat mPFC. *Stress*. 2015; 18(6):607–615. [PubMed: 26305287]
- Blaze J, Roth TL. Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *International Journal of Developmental Neuroscience*. 2013; 31(8):804–810. [PubMed: 24120634]
- Blaze J, Roth TL. Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Development and Psychopathology*. 2017; 29(2):477–489. [PubMed: 28401839]
- Blaze J, Scheuing L, Roth TL. Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to maltreatment or nurturing care during infancy. *Developmental Neuroscience*. 2013; 35(4):306–316. [PubMed: 23751776]
- Boehme F, Gil-Mohapel J, Cox A, Patten A, Giles E, Brocardo PS, Christie BR. Voluntary exercise induces adult hippocampal neurogenesis and BDNF expression in a rodent model of fetal alcohol spectrum disorders. *European Journal of Neuroscience*. 2011; 33(10):1799–1811. [PubMed: 21535455]
- Boschen K, Criss K, Palamarchouk V, Roth T, Klintsova A. Effects of developmental alcohol exposure vs. intubation stress on BDNF and TrkB expression in the hippocampus and frontal cortex of neonatal rats. *International Journal of Developmental Neuroscience*. 2015; 43:16–24. [PubMed: 25805052]
- Boschen K, Klintsova A. Chapter Nine-Neurotrophins in the Brain: Interaction With Alcohol Exposure During Development. *Vitamins and Hormones*. 2017; 104:197–242. [PubMed: 28215296]
- Boschen K, McKeown S, Roth T, Klintsova A. Impact of exercise and a complex environment on hippocampal dendritic morphology, Bdnf gene expression, and DNA methylation in male rat pups neonatally exposed to alcohol. *Developmental Neurobiology*. 2016; 77(6):708–725. [PubMed: 27597545]
- Boschen KE, Hamilton GF, Delorme JE, Klintsova AY. Activity and social behavior in a complex environment in rats neonatally exposed to alcohol. *Alcohol*. 2014; 48(6):533–541. [PubMed: 25150044]
- Bourc'his D, Xu G-L, Lin C-S, Bollman B, Bestor TH. Dnmt3L and the establishment of maternal genomic imprints. *Science*. 2001; 294(5551):2536–2539. [PubMed: 11719692]
- Braithwaite E, Kundakovic M, Ramchandani P, Murphy S, Champagne F. Maternal prenatal depressive symptoms predict infant NR3C1 1F and BDNF IV DNA methylation. *Epigenetics*. 2015; 10(5):408–417. [PubMed: 25875334]
- Brocardo PS, Boehme F, Patten A, Cox A, Gil-Mohapel J, Christie BR. Anxiety-and depression-like behaviors are accompanied by an increase in oxidative stress in a rat model of fetal alcohol

spectrum disorders: Protective effects of voluntary physical exercise. *Neuropharmacology*. 2012; 62(4):1607–1618. [PubMed: 22019722]

Brown AS, Susser ES, Lin SP, Neugebauer R, Gorman JM. Increased risk of affective disorders in males after second trimester prenatal exposure to the Dutch hunger winter of 1944–45. *The British Journal of Psychiatry*. 1995; 166(5):601–606. [PubMed: 7620744]

Burd L, Deal E, Rios R, Adickes E, Wynne J, Klug MG. Congenital heart defects and fetal alcohol spectrum disorders. *Congenital Heart Disease*. 2007; 2(4):250–255. [PubMed: 18377476]

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences*. 1998; 95(9):5335–5340.

Caldwell KK, Sheema S, Paz RD, Samudio-Ruiz SL, Laughlin MH, Spence NE, Roehlk MJ, Alcon SN, Allan AM. Fetal alcohol spectrum disorder-associated depression: evidence for reductions in the levels of brain-derived neurotrophic factor in a mouse model. *Pharmacology, Biochemistry and Behavior*. 2008; 90(4):614–624.

Cao-Lei L, Dancause KN, Elgbeili G, Laplante DP, Szyf M, King S. Pregnant women's cognitive appraisal of a natural disaster affects their children's BMI and central adiposity via DNA methylation: Project Ice Storm. *Early Human Development*. 2016; 103:189–192. [PubMed: 27718477]

Cao-Lei L, Elgbeili G, Massart R, Laplante D, Szyf M, King S. Pregnant women's cognitive appraisal of a natural disaster affects DNA methylation in their children 13 years later: Project Ice Storm. *Translational Psychiatry*. 2015; 5(2):e515. [PubMed: 25710121]

CDC. Fetal Alcohol Spectrum Disorders (FASDs). 2017. <https://www.cdc.gov/ncbddd/fasd/index.html>

Chai XJ, Whitfield-Gabrieli S, Shinn AK, Gabrieli JD, Castañón AN, McCarthy JM, Cohen BM, Öngür D. Abnormal medial prefrontal cortex resting-state connectivity in bipolar disorder and schizophrenia. *Neuropsychopharmacology*. 2011; 36(10):2009–2017. [PubMed: 21654735]

Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences*. 2001; 98(22):12736–12741.

Champagne FA, Meaney MJ. Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. *Behavioral Neuroscience*. 2007; 121(6):1353. [PubMed: 18085888]

Champagne FA, Weaver IC, Diorio J, Dymov S, Szyf M, Meaney MJ. Maternal care associated with methylation of the estrogen receptor- α 1b promoter and estrogen receptor- α expression in the medial preoptic area of female offspring. *Endocrinology*. 2006; 147(6):2909–2915. [PubMed: 16513834]

Chater-Diehl EJ, Laufer BI, Castellani CA, Alberry BL, Singh SM. Alteration of gene expression, DNA methylation, and histone methylation in free radical scavenging networks in adult mouse hippocampus following fetal alcohol exposure. *PloS One*. 2016; 11(5):e0154836. [PubMed: 27136348]

Chen Y, Ozturk NC, Zhou FC. DNA methylation program in developing hippocampus and its alteration by alcohol. *PloS One*. 2013; 8(3):e60503. [PubMed: 23544149]

Clayton JA, Collins FS. NIH to balance sex in cell and animal studies. *Nature*. 2014; 509(7500):282–283. [PubMed: 24834516]

Cohen MM, Jing D, Yang RR, Tottenham N, Lee FS, Casey B. Early-life stress has persistent effects on amygdala function and development in mice and humans. *Proceedings of the National Academy of Sciences*. 2013; 110(45):18274–18278.

Connor PD, Sampson PD, Bookstein FL, Barr HM, Streissguth AP. Direct and indirect effects of prenatal alcohol damage on executive function. *Developmental Neuropsychology*. 2000; 18(3):331–354. [PubMed: 11385829]

Cornish JL, Kalivas PW. Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *Journal of Neuroscience*. 2000; 20(15):RC89–RC89. [PubMed: 10899176]

Costello EJ, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry*. 2003; 60(8):837–844. [PubMed: 12912767]

- Dasmahapatra AK, Khan IA. Modulation of DNA methylation machineries in Japanese rice fish (*Oryzias latipes*) embryogenesis by ethanol and 5-azacytidine. *Comparative Biochemistry and Physiology Part C. Toxicology & Pharmacology*. 2016; 179:174–183. [PubMed: 26510680]
- Devlin AM, Brain U, Austin J, Oberlander TF. Prenatal exposure to maternal depressed mood and the MTHFR C677T variant affect SLC6A4 methylation in infants at birth. *PLoS One*. 2010; 5(8):e12201. [PubMed: 20808944]
- DiPietro JA. Maternal stress in pregnancy: considerations for fetal development. *Journal of Adolescent Health*. 2012; 51(2):S3–S8.
- Doherty TS, Forster A, Roth TL. Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioural Brain Research*. 2016; 298(Pt A):55–61. [PubMed: 26027495]
- Dong E, Dzitoyeva SG, Matrisciano F, Tuetting P, Grayson DR, Guidotti A. Brain-derived neurotrophic factor epigenetic modifications associated with schizophrenia-like phenotype induced by prenatal stress in mice. *Biological Psychiatry*. 2015; 77(6):589–596. [PubMed: 25444166]
- Dong E, Gavin DP, Chen Y, Davis J. Upregulation of TET1 and downregulation of APOBEC3A and APOBEC3C in the parietal cortex of psychotic patients. *Translational Psychiatry*. 2012; 2(9):e159. [PubMed: 22948384]
- Downing C, Johnson TE, Larson C, Leakey TI, Siegfried RN, Rafferty TM, Cooney CA. Subtle decreases in DNA methylation and gene expression at the mouse *Igf2* locus following prenatal alcohol exposure: effects of a methyl-supplemented diet. *Alcohol*. 2011; 45(1):65–71. [PubMed: 20705422]
- Drevets WC, Price JL, Simpson JR Jr, Todd RD, Reich T, Vannier M, Raichle ME. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature*. 1997; 386(6627):824. [PubMed: 9126739]
- Driscoll CD, Streissguth AP, Riley EP. Prenatal alcohol exposure: comparability of effects in humans and animal models. *Neurotoxicology and teratology*. 1990; 12(3):231–237. [PubMed: 2196422]
- Dunn GA, Bale TL. Maternal high-fat diet effects on third-generation female body size via the paternal lineage. *Endocrinology*. 2011; 152(6):2228–2236. [PubMed: 21447631]
- Enoch M-A. The role of early life stress as a predictor for alcohol and drug dependence. *Psychopharmacology*. 2011; 214(1):17–31. [PubMed: 20596857]
- Essex MJ, Shirtcliff EA, Burk LR, Ruttle PL, Klein MH, Slattery MJ, Kalin NH, Armstrong JM. Influence of early life stress on later hypothalamic–pituitary–adrenal axis functioning and its covariation with mental health symptoms: a study of the allostatic process from childhood into adolescence. *Development and Psychopathology*. 2011; 23(4):1039–1058. [PubMed: 22018080]
- Farber NB, Creeley CE, Olney JW. Alcohol-induced neuroapoptosis in the fetal macaque brain. *Neurobiology of Disease*. 2010; 40(1):200–206. [PubMed: 20580929]
- Feng M-J, Yan S-E, Yan Q-S. Effects of prenatal alcohol exposure on brain-derived neurotrophic factor and its receptor tyrosine kinase B in offspring. *Brain Research*. 2005; 1042(2):125–132. [PubMed: 15854584]
- Fenoglio KA, Brunson KL, Baram TZ. Hippocampal neuroplasticity induced by early-life stress: functional and molecular aspects. *Frontiers in Neuroendocrinology*. 2006; 27(2):180–192. [PubMed: 16603235]
- Fleming AS, Miceli M, Moretto D. Lesions of the medial preoptic area prevent the facilitation of maternal behavior produced by amygdala lesions. *Physiology & Behavior*. 1983; 31(4):503–510. [PubMed: 6657771]
- Fleming AS, Walsh C. Neuropsychology of maternal behavior in the rat: c-fos expression during mother-litter interactions. *Psychoneuroendocrinology*. 1994; 19(5–7):429–443. [PubMed: 7938344]
- Franklin L, Deitz J, Jirikowic T, Astley S. Children with fetal alcohol spectrum disorders: problem behaviors and sensory processing. *American Journal of Occupational Therapy*. 2008; 62(3):265–273. [PubMed: 18557002]
- Franklin TB, Russig H, Weiss IC, Gräff J, Linder N, Michalon A, Vizi S, Mansuy IM. Epigenetic transmission of the impact of early stress across generations. *Biological Psychiatry*. 2010; 68(5):408–415. [PubMed: 20673872]

- Garro AJ, McBeth DL, Lima V, Lieber CS. Ethanol consumption inhibits fetal DNA methylation in mice: implications for the fetal alcohol syndrome. *Alcoholism: Clinical and Experimental Research*. 1991; 15(3):395–398.
- Gavin DP, Sharma RP, Chase KA, Matrisciano F, Dong E, Guidotti A. Growth arrest and DNA-damage-inducible, beta (GADD45b)-mediated DNA demethylation in major psychosis. *Neuropsychopharmacology*. 2012; 37(2):531–542. [PubMed: 22048458]
- Gee DG, Casey B. The impact of developmental timing for stress and recovery. *Neurobiology of Stress*. 2015; 1:184–194. [PubMed: 25798454]
- Gil-Mohapel J, Boehme F, Kainer L, Christie BR. Hippocampal cell loss and neurogenesis after fetal alcohol exposure: insights from different rodent models. *Brain Research Reviews*. 2010; 64(2): 283–303. [PubMed: 20471420]
- Gilbert R, Widom CS, Browne K, Fergusson D, Webb E, Janson S. Burden and consequences of child maltreatment in high-income countries. *The Lancet*. 2009; 373(9657):68–81.
- Gómez-Pinilla F, Ying Z, Roy RR, Molteni R, Edgerton VR. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *Journal of Neurophysiology*. 2002; 88(5): 2187–2195. [PubMed: 12424260]
- Gonzales ML, LaSalle JM. The role of MeCP2 in brain development and neurodevelopmental disorders. *Current Psychiatry Reports*. 2010; 12(2):127–134. [PubMed: 20425298]
- Govorko D, Bekdash RA, Zhang C, Sarkar DK. Male germline transmits fetal alcohol adverse effect on hypothalamic proopiomelanocortin gene across generations. *Biological Psychiatry*. 2012; 72(5): 378–388. [PubMed: 22622000]
- Gregório SP, Sallet PC, Do K-A, Lin E, Gattaz WF, Dias-Neto E. Polymorphisms in genes involved in neurodevelopment may be associated with altered brain morphology in schizophrenia: preliminary evidence. *Psychiatry Research*. 2009; 165(1):1–9. [PubMed: 19054571]
- Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature*. 1997; 389(6649): 349. [PubMed: 9311776]
- Guan J-S, Xie H, Ding X. The role of epigenetic regulation in learning and memory. *Experimental Neurology*. 2015; 268:30–36. [PubMed: 24837316]
- Guibert S, Weber M. Functions of DNA methylation and hydroxymethylation in mammalian development. *Current Topics in Developmental Biology*. 2013; 104:47–83. [PubMed: 23587238]
- Guidotti A, Auta J, Davis JM, Dong E, Grayson DR, Veldic M, Zhang X, Costa E. GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. *Psychopharmacology*. 2005; 180(2):191–205. [PubMed: 15864560]
- Guidotti A, Auta J, Davis JM, Gerevini VD, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Archives of General Psychiatry*. 2000; 57(11):1061–1069. [PubMed: 11074872]
- Guo W, Crossey EL, Zhang L, Zucca S, George OL, Valenzuela CF, Zhao X. Alcohol exposure decreases CREB binding protein expression and histone acetylation in the developing cerebellum. *PloS One*. 2011; 6(5):e19351. [PubMed: 21655322]
- Guo Y, Chen Y, Carreon S, Qiang M. Chronic intermittent ethanol exposure and its removal induce a different miRNA expression pattern in primary cortical neuronal cultures. *Alcoholism, Clinical and Experimental Research*. 2012; 36(6):1058–1066.
- Hamilton G, Murawski N, Cyr SS, Jablonski S, Schiffino F, Stanton M, Klintsova A. Neonatal alcohol exposure disrupts hippocampal neurogenesis and contextual fear conditioning in adult rats. *Brain Research*. 2011; 1412:88–101. [PubMed: 21816390]
- Hamilton GF, Boschen KE, Goodlett CR, Greenough WT, Klintsova AY. Housing in environmental complexity following wheel running augments survival of newly generated hippocampal neurons in a rat model of binge alcohol exposure during the third trimester equivalent. *Alcoholism: Clinical and Experimental Research*. 2012; 36(7):1196–1204.
- Hamilton GF, Criss KJ, Klintsova AY. Voluntary exercise partially reverses neonatal alcohol-induced deficits in mPFC layer II/III dendritic morphology of male adolescent rats. *Synapse*. 2015; 69(8): 405–415. [PubMed: 25967699]

- Hamilton GF, Whitcher LT, Klintsova AY. Postnatal binge-like alcohol exposure decreases dendritic complexity while increasing the density of mature spines in mPFC Layer II/III pyramidal neurons. *Synapse*. 2010; 64(2):127–135. [PubMed: 19771589]
- Hanna EZ, Faden VB, Dufour MC. The motivational correlates of drinking, smoking, and illicit drug use during pregnancy. *Journal of Substance Abuse*. 1994; 6(2):155–167. [PubMed: 7804015]
- Hao Y, Huang W, Nielsen DA, Kosten TA. Litter gender composition and sex affect maternal behavior and DNA methylation levels of the *oprm1* gene in rat offspring. *Frontiers in Psychiatry*. 2011; 2:21. [PubMed: 21629839]
- Hariri AR, Holmes A. Genetics of emotional regulation: the role of the serotonin transporter in neural function. *Trends in Cognitive Sciences*. 2006; 10(4):182–191. [PubMed: 16530463]
- Hata K, Okano M, Lei H, Li E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development*. 2002; 129(8):1983–1993. [PubMed: 11934864]
- Haycock PC, Ramsay M. Exposure of mouse embryos to ethanol during preimplantation development: effect on DNA methylation in the h19 imprinting control region. *Biology of Reproduction*. 2009; 81(4):618–627. [PubMed: 19279321]
- He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nature reviews. Genetics*. 2004; 5(8):631.
- Heaton MB, Mitchell JJ, Paiva M, Walker DW. Ethanol-induced alterations in the expression of neurotrophic factors in the developing rat central nervous system. *Developmental Brain Research*. 2000; 121(1):97–107. [PubMed: 10837897]
- Heaton MB, Paiva M, Madorsky I, Mayer J, Moore DB. Effects of ethanol on neurotrophic factors, apoptosis-related proteins, endogenous antioxidants, and reactive oxygen species in neonatal striatum: relationship to periods of vulnerability. *Developmental Brain Research*. 2003; 140(2):237–252. [PubMed: 12586429]
- Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey L. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences*. 2008; 105(44):17046–17049.
- Helfer JL, Goodlett CR, Greenough WT, Klintsova AY. The effects of exercise on adolescent hippocampal neurogenesis in a rat model of binge alcohol exposure during the brain growth spurt. *Brain Research*. 2009; 1294:1–11. [PubMed: 19647724]
- Helleman KG, Sliwowska JH, Verma P, Weinberg J. Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. *Neuroscience & Biobehavioral Reviews*. 2010; 34(6):791–807. [PubMed: 19545588]
- Helleman KG, Verma P, Yoon E, Yu W, Weinberg J. Prenatal alcohol exposure increases vulnerability to stress and anxiety-like disorders in adulthood. *Annals of the New York Academy of Sciences*. 2008; 1144(1):154–175. [PubMed: 19076375]
- Herman J, McKlveen J, Solomon M, Carvalho-Netto E, Myers B. Neural regulation of the stress response: glucocorticoid feedback mechanisms. *Brazilian Journal of Medical and Biological Research*. 2012; 45(4):292–298. [PubMed: 22450375]
- Herman LE, Acosta MC, Chang P-N. Gender and attention deficits in children diagnosed with a Fetal Alcohol Spectrum Disorder. *Canadian Journal of Clinical Pharmacology*. 2008; 15(3):e411–419. [PubMed: 18953085]
- Hicks SD, Middleton FA, Miller MW. Ethanol-induced methylation of cell cycle genes in neural stem cells. *Journal of Neurochemistry*. 2010; 114(6):1767–1780. [PubMed: 20626555]
- Hill KT, Warren M, Roth TL. The influence of infant-caregiver experiences on amygdala Bdnf, OXTr, and NPY expression in developing and adult male and female rats. *Behavioural Brain Research*. 2014; 272:175–180. [PubMed: 25011012]
- Hirabayashi, T., Yagi, T. *Protocadherins in neurological diseases*, Cell Adhesion Molecules. Springer; 2014. p. 293-314.
- Holmes MR. The sleeper effect of intimate partner violence exposure: long-term consequences on young children's aggressive behavior. *Journal of Child Psychology and Psychiatry*. 2013; 54(9):986–995. [PubMed: 23550816]

- Hompes T, Izzi B, Gellens E, Morreels M, Fieuws S, Pexsters A, Schops G, Dom M, Van Bree R, Freson K. Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. *Journal of Psychiatric Research*. 2013; 47(7):880–891. [PubMed: 23566423]
- Hunsberger JG, Austin DR, Chen G, Manji HK. MicroRNAs in mental health: from biological underpinnings to potential therapies. *Neuromolecular Medicine*. 2009; 11(3):173–182. [PubMed: 19544012]
- Ignacio C, Mooney SM, Middleton FA. Effects of Acute Prenatal Exposure to Ethanol on microRNA Expression are Ameliorated by Social Enrichment. *Frontiers in Pediatrics*. 2014; 2:103. [PubMed: 25309888]
- Ignácio ZM, Réus GZ, Abelaira HM, Maciel AL, de Moura AB, Matos D, Demo JP, da Silva JB, Gava FF, Valvassori SS. Quetiapine treatment reverses depressive-like behavior and reduces DNA methyltransferase activity induced by maternal deprivation. *Behavioural Brain Research*. 2017; 320:225–232.
- Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovská V, Hörster F, Tenkova T. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science*. 2000; 287(5455):1056–1060. [PubMed: 10669420]
- Irner TB, Teasdale TW, Olofsson M. Cognitive and social development in preschool children born to women using substances. *Journal of Addictive Diseases*. 2012; 31(1):29–44. [PubMed: 22356667]
- Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293(5532):1074–1080. [PubMed: 11498575]
- Jindal RD, Pillai AK, Mahadik SP, Eklund K, Montrose DM, Keshavan MS. Decreased BDNF in patients with antipsychotic naive first episode schizophrenia. *Schizophrenia Research*. 2010; 119(1):47–51. [PubMed: 20096541]
- Johnston MV. Plasticity in the developing brain: implications for rehabilitation. *Developmental Disabilities Research Reviews*. 2009; 15(2):94–101. [PubMed: 19489084]
- Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science*. 2001; 293(5532):1068–1070. [PubMed: 11498573]
- Jud A, Fegert JM, Finkelhor D. On the incidence and prevalence of child maltreatment: a research agenda. *Child and Adolescent Psychiatry and Mental Health*. 2016; 10(1):17. [PubMed: 27303442]
- Kember R, Dempster E, Lee T, Schalkwyk LC, Mill J, Fernandes C. Maternal separation is associated with strain-specific responses to stress and epigenetic alterations to Nr3c1, Avp, and Nr4a1 in mouse. *Brain and Behavior*. 2012; 2(4):455–467. [PubMed: 22950049]
- Kendall-Tackett K. The health effects of childhood abuse: four pathways by which abuse can influence health. *Child Abuse & Neglect*. 2002; 26(6):715–729. [PubMed: 12201164]
- Kessler RC, Davis CG, Kendler KS. Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. *Psychological Medicine*. 1997; 27(5):1101–1119. [PubMed: 9300515]
- Kim CK, Turnbull AV, Lee SY, Rivier CL. Effects of prenatal exposure to alcohol on the release of adrenocorticotrophic hormone, corticosterone, and proinflammatory cytokines. *Alcoholism: Clinical and Experimental Research*. 1999; 23(1):52–59.
- Kim HJ, Rowe M, Ren M, Hong J-S, Chen P-S, Chuang D-M. Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: multiple mechanisms of action. *Journal of Pharmacology and Experimental Therapeutics*. 2007; 321(3):892–901. [PubMed: 17371805]
- Kim P, Park JH, Choi CS, Choi I, Joo SH, Kim MK, Kim SY, Kim KC, Park SH, Kwon KJ. Effects of ethanol exposure during early pregnancy in hyperactive, inattentive and impulsive behaviors and MeCP2 expression in rodent offspring. *Neurochemical Research*. 2013; 38(3):620–631. [PubMed: 23283698]
- Kim T-W, Ji E-S, Kim T-W, Lee S-W, Lee C-Y, Lee S-J. Postnatal treadmill exercise attenuates prenatal stress-induced apoptosis through enhancing serotonin expression in aged-offspring rats. *Journal of Exercise Rehabilitation*. 2015; 11(1):12. [PubMed: 25830139]

- Klengel T, Dias BG, Ressler KJ. Models of intergenerational and transgenerational transmission of risk for psychopathology in mice. *Neuropsychopharmacology*. 2016; 41(1):219–231. [PubMed: 26283147]
- Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nature Neuroscience*. 2013; 16(1):33–41. [PubMed: 23201972]
- Klintsova AY, Helfer JL, Calizo LH, Dong WK, Goodlett CR, Greenough WT. Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*. 2007; 31(12):2073–2082.
- Knezovich JG, Ramsay M. The effect of preconception paternal alcohol exposure on epigenetic remodeling of the h19 and rasgrf1 imprinting control regions in mouse offspring. *Frontiers in Genetics*. 2012; 3
- Kolb B, Harker A, Mychasiuk R, de Melo SR, Gibb R. Stress and prefrontal cortical plasticity in the developing brain. *Cognitive Development*. 2017; 42:15–26.
- Kosten TA, Huang W, Nielsen DA. Sex and litter effects on anxiety and DNA methylation levels of stress and neurotrophin genes in adolescent rats. *Developmental Psychobiology*. 2014; 56(3): 392–406. [PubMed: 23460384]
- Kundakovic M, Jaric I. The Epigenetic link between prenatal adverse environments and neurodevelopmental disorders. *Genes*. 2017; 8(3):104.
- Labonte B, Yerko V, Gross J, Mechawar N, Meaney MJ, Szyf M, Turecki G. Differential glucocorticoid receptor exon 1 B, 1 C, 1 H expression and methylation in suicide completers with a history of childhood abuse. *Biological Psychiatry*. 2012; 72(1):41–48. [PubMed: 22444201]
- Ladd-Acosta C, Hansen KD, Briem E, Fallin MD, Kaufmann WE, Feinberg AP. Common DNA methylation alterations in multiple brain regions in autism. *Molecular Psychiatry*. 2014; 19(8): 862. [PubMed: 23999529]
- Laird PW. The power and the promise of DNA methylation markers. *Nature Reviews Cancer*. 2003; 3(4):253–266. [PubMed: 12671664]
- Langley B, Gensert JM, Beal MF, Ratan RR. Remodeling chromatin and stress resistance in the central nervous system: histone deacetylase inhibitors as novel and broadly effective neuroprotective agents. *Current Drug Targets-CNS and Neurological Disorders*. 2005; 4(1):41–50. [PubMed: 15723612]
- Laufer BI, Chater-Diehl EJ, Kapalanga J, Singh SM. Long-term alterations to DNA methylation as a biomarker of prenatal alcohol exposure: From mouse models to human children with fetal alcohol spectrum disorders. *Alcohol*. 2017; 60:67–75. [PubMed: 28187949]
- Laufer BI, Kapalanga J, Castellani CA, Diehl EJ, Yan L, Singh SM. Associative DNA methylation changes in children with prenatal alcohol exposure. 2015; 7(8):1259–1274.
- Laufer BI, Mantha K, Kleiber ML, Diehl EJ, Addison SM, Singh SM. Long-lasting alterations to DNA methylation and ncRNAs could underlie the effects of fetal alcohol exposure in mice. *Disease Models & Mechanisms*. 2013; 6(4):977–992. [PubMed: 23580197]
- Ledig M, Misslin R, Vogel E, Holownia A, Copin J, Tholey G. Paternal alcohol exposure: developmental and behavioral effects on the offspring of rats. *Neuropharmacology*. 1998; 37(1): 57–66. [PubMed: 9680259]
- Levine S. The ontogeny of the hypothalamic-pituitary-adrenal axis. The influence of maternal factors. *Annals of the New York Academy of Sciences*. 1994; 746(1):275–288. [PubMed: 7825883]
- Lewis CR, Olive MF. Early life stress interactions with the epigenome: potential mechanisms driving vulnerability towards psychiatric illness. *Behavioural Pharmacology*. 2014; 25(506):341–351. [PubMed: 25003947]
- Lewis CR, Staudinger K, Scheck L, Olive MF. The effects of maternal separation on adult methamphetamine self-administration, extinction, reinstatement, and MeCP2 immunoreactivity in the nucleus accumbens. *Frontiers in Psychiatry*. 2013; 4
- Liu Y, Balaraman Y, Wang G, Nephew KP, Zhou FC. Alcohol exposure alters DNA methylation profiles in mouse embryos at early neurulation. *Epigenetics*. 2009; 4(7):500–511. [PubMed: 20009564]

- Liyanage VRB, Zachariah RM, Davie JR, Rastegar M. Ethanol deregulates Mecp2/MeCP2 in differentiating neural stem cells via interplay between 5-methylcytosine and 5-hydroxymethylcytosine at the Mecp2 regulatory elements. *Experimental Neurology*. 2015; 265:102–117. [PubMed: 25620416]
- Lopez J, Mamdani F, Beaulieu M, Yang J, Berlim M, Ernst C, Turecki G. Epigenetic regulation of BDNF expression according to antidepressant response. *Molecular Psychiatry*. 2013; 18(4):398. [PubMed: 22547115]
- Luo J, Miller MW. Growth factor-mediated neural proliferation: target of ethanol toxicity. *Brain research. Brain Research Reviews*. 1998; 27(2):157–167. [PubMed: 9622617]
- Luo J, West JR, Cook RT, Pantazis NJ. Ethanol Induces Cell Death and Cell Cycle Delay in Cultures of Pheochromocytoma PC12 Cells. *Alcoholism: Clinical and Experimental Research*. 1999; 23(4):644–656.
- Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews. Neuroscience*. 2009; 10(6):434–445. [PubMed: 19401723]
- Marino M, Galluzzo P, Ascenzi P. Estrogen signaling multiple pathways to impact gene transcription. *Current Genomics*. 2006; 7(8):497–508. [PubMed: 18369406]
- Matrisciano F, Tueting P, Dalal I, Kadriu B, Grayson DR, Davis JM, Nicoletti F, Guidotti A. Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. *Neuropharmacology*. 2013; 68:184–194. [PubMed: 22564440]
- Mattson SN, Goodman AM, Caine C, Delis DC, Riley EP. Executive functioning in children with heavy prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*. 1999; 23(11):1808–1815.
- McGowan PO, Roth TL. Epigenetic pathways through which experiences become linked with biology. *Development and Psychopathology*. 2015; 27(2):637–648. [PubMed: 25997776]
- McGowan PO, Sasaki A, D'alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*. 2009; 12(3):342–348. [PubMed: 19234457]
- McGowan PO, Sasaki A, Huang TC, Unterberger A, Suderman M, Ernst C, Meaney MJ, Turecki G, Szyf M. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PLoS One*. 2008; 3(5):e2085. [PubMed: 18461137]
- McGowan PO, Suderman M, Sasaki A, Huang T, Hallett M, Meaney MJ, Szyf M. Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS One*. 2011; 6(2):e14739. [PubMed: 21386994]
- Meaney MJ, Ferguson-Smith AC. Epigenetic regulation of the neural transcriptome: the meaning of the marks. *Nature Neuroscience*. 2010; 13(11):1313–1318. [PubMed: 20975754]
- Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues in Clinical Neuroscience*. 2005; 7(2):103–123. [PubMed: 16262207]
- Mehta D, Klengel T, Conneely KN, Smith AK, Altmann A, Pace TW, Rex-Haffner M, Loeschner A, Gonik M, Mercer KB. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proceedings of the National Academy of Sciences*. 2013; 110(20):8302–8307.
- Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nature reviews. Genetics*. 2009; 10(3):155–159. [PubMed: 19188922]
- Middleton FA, Varlinskaya EI, Mooney SM. Molecular substrates of social avoidance seen following prenatal ethanol exposure and its reversal by social enrichment. *Developmental Neuroscience*. 2012; 34(2–3):115–128. [PubMed: 22572756]
- Miller BH, Wahlestedt C. MicroRNA dysregulation in psychiatric disease. *Brain Research*. 2010; 1338:89–99. [PubMed: 20303342]
- Monk C, Feng T, Lee S, Krupska I, Champagne FA, Tycko B. Distress during pregnancy: epigenetic regulation of placenta glucocorticoid-related genes and fetal neurobehavior. *American Journal of Psychiatry*. 2016; 173(7):705–713. [PubMed: 27013342]

- Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development*. 1987; 99(3):371–382. [PubMed: 3653008]
- Mooney SM, Varlinskaya EI. Acute prenatal exposure to ethanol and social behavior: effects of age, sex, and timing of exposure. *Behavioural Brain Research*. 2011; 216(1):358–364. [PubMed: 20728475]
- Moore CL. Maternal contributions to the development of masculine sexual behavior in laboratory rats. *Developmental Psychobiology*. 1984; 17(4):347–356. [PubMed: 6745497]
- Moore CL. Sex differences in urinary odors produced by young laboratory rats (*Rattus norvegicus*). *Journal of Comparative Psychology*. 1985; 99(3):336–341. [PubMed: 4042618]
- Moore CL, Chadwick-Dias A-M. Behavioral responses of infant rats to maternal licking: variations with age and sex. *Developmental Psychobiology*. 1986; 19(5):427–438. [PubMed: 3758488]
- Moore CL, Morelli GA. Mother rats interact differently with male and female offspring. *Journal of Comparative and Physiological Psychology*. 1979; 93(4):677–684. [PubMed: 479402]
- Moreau MP, Bruse SE, David-Rus R, Buyske S, Brzustowicz LM. Altered microRNA expression profiles in postmortem brain samples from individuals with schizophrenia and bipolar disorder. *Biological Psychiatry*. 2011; 69(2):188–193. [PubMed: 21183010]
- Moreno Gudiño H, Carías Picón D, de Brugada Sauras I. Dietary choline during periadolescence attenuates cognitive damage caused by neonatal maternal separation in male rats. *Nutritional Neuroscience*. 2017; 20(6):327–335. [PubMed: 26714620]
- Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *Journal of Neuroscience*. 2008; 28(36):9055–9065. [PubMed: 18768700]
- Mukhopadhyay P, Rezzoug F, Kaikaus J, Greene RM, Pisano MM. Alcohol modulates expression of DNA methyltransferases and methyl CpG-/CpG domain-binding proteins in murine embryonic fibroblasts. *Reproductive Toxicology*. 2013; 37:40–48. [PubMed: 23395981]
- Mulligan C, D'Errico N, Stees J, Hughes D. Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics*. 2012; 7(8):853–857. [PubMed: 22810058]
- Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmühl Y, Fischer D, Holsboer F, Wotjak CT, Almeida OF, Spengler D. Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nature Neuroscience*. 2009; 12(12):1559–1566. [PubMed: 19898468]
- Mychasiuk R, Harker A, Ilnytskyy S, Gibb R. Paternal stress prior to conception alters DNA methylation and behaviour of developing rat offspring. *Neuroscience*. 2013; 241:100–105. [PubMed: 23531434]
- Mychasiuk R, Ilnytskyy S, Kovalchuk O, Kolb B, Gibb R. Intensity matters: brain, behaviour and the epigenome of prenatally stressed rats. *Neuroscience*. 2011; 180:105–110. [PubMed: 21335068]
- Nagarajan R, Hogart A, Gwye Y, Martin MR, LaSalle JM. Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant MECP2 promoter methylation. *Epigenetics*. 2006; 1(4):172–182.
- Nagre NN, Subbanna S, Shivakumar M, Psychoyos D, Basavarajappa BS. CB1-receptor knockout neonatal mice are protected against ethanol-induced impairments of DNMT1, DNMT3A, and DNA methylation. *Journal of Neurochemistry*. 2015; 132(4):429–442. [PubMed: 25487288]
- Neumann ID, Landgraf R. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. *Trends in Neurosciences*. 2012; 35(11):649–659. [PubMed: 22974560]
- Ngai YF, Sulistyoningrum DC, O'Neill R, Innis SM, Weinberg J, Devlin AM. Prenatal alcohol exposure alters methyl metabolism and programs serotonin transporter and glucocorticoid receptor expression in brain. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2015; 309(5):R613–R622.
- Nugent BM, Wright CL, Shetty AC, Hodes GE, Lenz KM, Mahurkar A, Russo SJ, Devine SE, McCarthy MM. Brain feminization requires active repression of masculinization via DNA methylation. *Nature Neuroscience*. 2015; 18:690–697. [PubMed: 25821913]
- Numan M. Maternal behavior: neural circuits, stimulus valence, and motivational processes. *Parenting*. 2012; 12(2–3):105–114.

- Numan M, Rosenblatt JS, Komisaruk BR. Medial preoptic area and onset of maternal behavior in the rat. *Journal of Comparative and Physiological Psychology*. 1977; 91(1):146–164. [PubMed: 402400]
- O'Connor MJ, Paley B. Psychiatric conditions associated with prenatal alcohol exposure. *Developmental Disabilities Research Reviews*. 2009; 15(3):225–234. [PubMed: 19731386]
- Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 2008; 3(2):97–106. [PubMed: 18536531]
- Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999; 99(3):247–257. [PubMed: 10555141]
- Olney JW, Wozniak DF, Jevtovic-Todorovic V, Farber NB, Bittigau P, Ikonomidou C. Drug-induced apoptotic neurodegeneration in the developing brain. *Brain Pathology*. 2002; 12(4):488–498. [PubMed: 12408236]
- Otero NK, Thomas JD, Saski CA, Xia X, Kelly SJ. Choline supplementation and DNA methylation in the hippocampus and prefrontal cortex of rats exposed to alcohol during development. *Alcoholism: Clinical and Experimental Research*. 2012; 36(10):1701–1709.
- Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reproductive Toxicology*. 2005; 20(3):345–352. [PubMed: 15893910]
- Palacios-García I, Lara-Vásquez A, Montiel JF, Díaz-Véliz GF, Sepúlveda H, Utreras E, Montecino M, González-Billault C, Aboitiz F. Prenatal stress down-regulates Reelin expression by methylation of its promoter and induces adult behavioral impairments in rats. *PLoS One*. 2015; 10(2):e0117680. [PubMed: 25679528]
- Pappalardo-Carter DL, Balaraman S, Sathyan P, Carter ES, Chen WJA, Miranda RC. Suppression and Epigenetic Regulation of MiR-9 Contributes to Ethanol Teratology: Evidence from Zebrafish and Murine Fetal Neural Stem Cell Models. *Alcoholism: Clinical and Experimental Research*. 2013; 37(10):1657–1667.
- Park SW, Lee JG, Seo MK, Ly NN, Lee CH, Cho HY, Choi AJ, Kim G-M, Kim YH. Epigenetic modification of glucocorticoid receptor promoter I 7 in maternally separated and restraint-stressed rats. *Neuroscience Letters*. 2017; 650:38–44. [PubMed: 28414132]
- Pedersen CA, Caldwell JD, Walker C, Ayers G, Mason GA. Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behavioral Neuroscience*. 1994; 108(6):1163. [PubMed: 7893408]
- Pena CJ, Monk C, Champagne FA. Epigenetic effects of prenatal stress on 11 betahydroxysteroid dehydrogenase-2 in the placenta and fetal brain. *PloS One*. 2012; 7(6):e39791. [PubMed: 22761903]
- Peña CJ, Neugut YD, Champagne FA. Developmental timing of the effects of maternal care on gene expression and epigenetic regulation of hormone receptor levels in female rats. *Endocrinology*. 2013; 154(11):4340–4351. [PubMed: 24002038]
- Perkins A, Lehmann C, Lawrence RC, Kelly SJ. Alcohol exposure during development: Impact on the epigenome. *International Journal of Developmental Neuroscience*. 2013; 31(6):391–397. [PubMed: 23542005]
- Perroud N, Paoloni-Giacobino A, Prada P, Olié E, Salzmann A, Nicastro R, Guillaume S, Mouthon D, Stouder C, Dieben K. Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Translational Psychiatry*. 2011; 1(12):e59. [PubMed: 22832351]
- Perroud N, Rutembesa E, Paoloni-Giacobino A, Mutabaruka J, Mutesa L, Stenz L, Malafosse A, Karege F. The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis. *World Journal of Biological Psychiatry*. 2014; 15(4):334–345. [PubMed: 24690014]
- Perroud N, Salzmann A, Prada P, Nicastro R, Hoeppli M, Furrer S, Ardu S, Krejci I, Karege F, Malafosse A. Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Translational Psychiatry*. 2013; 3(1):e207. [PubMed: 23422958]

- Petkov VV, Stoianovski D, Petkov VD, Vyglanova I. Lipid peroxidation changes in the brain in fetal alcohol syndrome. *Biulleten' eksperimental'noi biologii i meditsiny*. 1992; 113(5):500–502.
- Pietrzykowski AZ, Friesen RM, Martin GE, Puig SI, Nowak CL, Wynne PM, Siegelmann HT, Treisman SN. Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. *Neuron*. 2008; 59(2):274–287. [PubMed: 18667155]
- Portales-Casamar E, Lussier AA, Jones MJ, MacIsaac JL, Edgar RD, Mah SM, Barhdadi A, Provost S, Lemieux-Perreault L-P, Cynader MS. DNA methylation signature of human fetal alcohol spectrum disorder. *Epigenetics & Chromatin*. 2016; 9(1):25. [PubMed: 27358653]
- Ptak C, Petronis A. Epigenetic approaches to psychiatric disorders. *Dialogues in Clinical Neuroscience*. 2010; 12(1):25–35. [PubMed: 20373664]
- Puglia MP, Valenzuela CF. Repeated third trimester-equivalent ethanol exposure inhibits long-term potentiation in the hippocampal CA1 region of neonatal rats. *Alcohol*. 2010; 44(3):283–290. [PubMed: 20488644]
- Radtke KM, Ruf M, Gunter HM, Dohrmann K, Schauer M, Meyer A, Elbert T. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Translational Psychiatry*. 2011; 1(7):e21. [PubMed: 22832523]
- Rakic S, Zecevic N. Programmed cell death in the developing human telencephalon. *European Journal of Neuroscience*. 2000; 12(8):2721–2734. [PubMed: 10971615]
- Ramachandran V, Watts LT, Maffi SK, Chen J, Schenker S, Henderson G. Ethanol-induced oxidative stress precedes mitochondrially mediated apoptotic death of cultured fetal cortical neurons. *Journal of Neuroscience Research*. 2003; 74(4):577–588. [PubMed: 14598302]
- Rando OJ. Daddy issues: paternal effects on phenotype. *Cell*. 2012; 151(4):702–708. [PubMed: 23141533]
- Rasmussen C, Bisanz J. The relation between mathematics and working memory in young children with fetal alcohol spectrum disorders. *Journal of Special Education*. 2011; 45(3):184–191.
- Rasmussen C, Soleimani M, Pei J. Executive functioning and working memory deficits on the CANTAB among children with prenatal alcohol exposure. *Journal of population therapeutics and clinical pharmacology*. 2010; 18(1):e44–53.
- Redila V, Christie B. Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus. *Neuroscience*. 2006; 137(4):1299–1307. [PubMed: 16338077]
- Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, Zink M, Hörtnagl H, Flor H, Henn FA. Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. *Journal of Neuroscience*. 2005; 25(26):6243–6250. [PubMed: 15987954]
- Rodgers AB, Morgan CP, Bronson SL, Revello S, Bale TL. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *Journal of Neuroscience*. 2013; 33(21):9003–9012. [PubMed: 23699511]
- Rodgers AB, Morgan CP, Leu NA, Bale TL. Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. *Proceedings of the National Academy of Sciences*. 2015; 112(44):13699–13704.
- Roth TL. Epigenetics of neurobiology and behavior during development and adulthood. *Developmental Psychobiology*. 2012; 54(6):590–597. [PubMed: 22714649]
- Roth TL. Epigenetic mechanisms in the development of behavior: Advances, challenges, and future promises of a new field. *Development and Psychopathology*. 2013; 25(4pt2):1279–1291. [PubMed: 24342840]
- Roth TL, Lubin FD, Funk AJ, Sweatt JD. Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry*. 2009a; 65(9):760–769. [PubMed: 19150054]
- Roth TL, Lubin FD, Sodhi M, Kleinman JE. Epigenetic mechanisms in schizophrenia. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 2009b; 1790(9):869–877. [PubMed: 19559755]
- Roth TL, Matt S, Chen K, Blaze J. Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats exposed to different caregiving environments outside the home. *Developmental Psychobiology*. 2014; 56(8):1755–1763. [PubMed: 24752649]
- Roth TL, Roth ED, Sweatt JD. Epigenetic regulation of genes in learning and memory. *Essays in Biochemistry*. 2010; 48:263–274. [PubMed: 20822498]

- Roth TL, Sweatt JD. Epigenetic marking of the BDNF gene by early-life adverse experiences. *Hormones and Behavior*. 2011; 59(3):315–320. [PubMed: 20483357]
- Roy A, Xian-Zhang H, Janal MN, Goldman D. Interaction between childhood trauma and serotonin transporter gene variation in suicide. *Neuropsychopharmacology*. 2007; 32(9):2046. [PubMed: 17356577]
- Ruzicka W, Zhubi A, Veldic M, Grayson D, Costa E, Guidotti A. Selective epigenetic alteration of layer I GABAergic neurons isolated from prefrontal cortex of schizophrenia patients using laser-assisted microdissection. *Molecular Psychiatry*. 2007; 12(4):385–397. [PubMed: 17264840]
- Sathyan P, Golden HB, Miranda RC. Competing interactions between micro-RNAs determine neural progenitor survival and proliferation after ethanol exposure: evidence from an ex vivo model of the fetal cerebral cortical neuroepithelium. *Journal of Neuroscience*. 2007; 27(32):8546–8557. [PubMed: 17687032]
- Schenkel LS, Spaulding WD, DiLillo D, Silverstein SM. Histories of childhood maltreatment in schizophrenia: relationships with premorbid functioning, symptomatology, and cognitive deficits. *Schizophrenia Research*. 2005; 76(2):273–286. [PubMed: 15949659]
- Schmidt MV, Wang X-D, Meijer OC. Early life stress paradigms in rodents: potential animal models of depression? *Psychopharmacology*. 2011; 214(1):131–140. [PubMed: 21086114]
- Schneider ML, Moore CF, DeJesus OT, Converse AK. Prenatal stress influences on neurobehavior, stress reactivity, and dopaminergic function in rhesus macaques, Primate Models of Children's Health and Developmental Disabilities. Elsevier; New York: 2008. p. 231-258.
- Schneider ML, Roughton EC, Lubach GR. Moderate alcohol consumption and psychological stress during pregnancy induce attention and neuromotor impairments in primate infants. *Child Development*. 1997; 68(5):747–759. [PubMed: 29106730]
- Seo MK, Ly NN, Lee CH, Cho HY, Choi CM, Lee JG, Lee BJ, Kim G-M, Yoon BJ, Park SW. Early life stress increases stress vulnerability through BDNF gene epigenetic changes in the rat hippocampus. *Neuropharmacology*. 2016; 105:388–397. [PubMed: 26877199]
- Shea A, Walsh C, MacMillan H, Steiner M. Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress disorder in females. *Psychoneuroendocrinology*. 2005; 30(2):162–178. [PubMed: 15471614]
- Silberman DM, Acosta GB, Zubilete MAZ. Long-term effects of early life stress exposure: role of epigenetic mechanisms. *Pharmacological Research*. 2016; 109:64–73. [PubMed: 26774789]
- Skagerström J, Chang G, Nilsen P. Predictors of drinking during pregnancy: a systematic review. *Journal of Women's Health*. 2011; 20(6):901–913.
- Soares AR, Pereira PM, Ferreira V, Reverendo M, Simões J, Bezerra AR, Moura GR, Santos MA. Ethanol exposure induces up-regulation of specific microRNAs in zebrafish embryos. *Toxicological Sciences*. 2012:kfs068.
- St-Cyr S, McGowan PO. Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Frontiers in Behavioral Neuroscience*. 2015; 9:145. [PubMed: 26082698]
- Steinhausen HC, Spohr HL. Long-term outcome of children with fetal alcohol syndrome: Psychopathology, behavior, and intelligence. *Alcoholism: Clinical and Experimental Research*. 1998; 22(2):334–338.
- Stevens SA, Majors D, Rovet J, Koren G, Fantus E, Nulman I, Desrocher M. Social problem solving in children with fetal alcohol spectrum disorders. *Journal of Population Therapeutics and Clinical Pharmacology*. 2012; 19(1):e99–e110. [PubMed: 22535836]
- Streissguth AP, Bookstein FL, Barr HM, Sampson PD, O'malley K, Young JK. Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. *Journal of Developmental & Behavioral Pediatrics*. 2004; 25(4):228–238. [PubMed: 15308923]
- Stringer RL, Laufer BI, Kleiber ML, Singh SM. Reduced expression of brain cannabinoid receptor 1 (Cnr1) is coupled with an increased complementary micro-RNA (miR-26b) in a mouse model of fetal alcohol spectrum disorders. *Clinical Epigenetics*. 2013; 5(1):14. [PubMed: 23915435]
- Struhl K. Histone acetylation and transcriptional regulatory mechanisms. *Genes & Development*. 1998; 12(5):599–606. [PubMed: 9499396]

- Subbanna S, Nagre NN, Shivakumar M, Umapathy NS, Psychoyos D, Basavarajappa BS. Ethanol induced acetylation of histone at G9a exon1 and G9a-mediated histone H3 dimethylation leads to neurodegeneration in neonatal mice. *Neuroscience*. 2014; 258:422–432. [PubMed: 24300108]
- Subbanna S, Shivakumar M, Umapathy NS, Saito M, Mohan PS, Kumar A, Nixon RA, Verin AD, Psychoyos D, Basavarajappa BS. G9a-mediated histone methylation regulates ethanol-induced neurodegeneration in the neonatal mouse brain. *Neurobiology of Disease*. 2013; 54:475–485. [PubMed: 23396011]
- Szulwach KE, Li X, Li Y, Song C-X, Wu H, Dai Q, Irier H, Upadhyay AK, Gearing M, Levey AI. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. *Nature Neuroscience*. 2011; 14(12):1607–1616. [PubMed: 22037496]
- Tal TL, Franzosa JA, Tilton SC, Philbrick KA, Iwaniec UT, Turner RT, Waters KM, Tanguay RL. MicroRNAs control neurobehavioral development and function in zebrafish. *Faseb J*. 2012; 26(4):1452–1461. [PubMed: 22253472]
- Thomas JD, Biane JS, O'bryan KA, O'Neill TM, Dominguez HD. Choline supplementation following third-trimester-equivalent alcohol exposure attenuates behavioral alterations in rats. *Behavioral Neuroscience*. 2007; 121(1):120. [PubMed: 17324056]
- Thomas JD, Garrison M, O'Neill TM. Perinatal choline supplementation attenuates behavioral alterations associated with neonatal alcohol exposure in rats. *Neurotoxicology and Teratology*. 2004; 26(1):35–45. [PubMed: 15001212]
- Thomas JD, Tran TD. Choline supplementation mitigates trace, but not delay, eyeblink conditioning deficits in rats exposed to alcohol during development. *Hippocampus*. 2012; 22(3):619–630. [PubMed: 21542051]
- Tobi EW, Lumey L, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Human Molecular Genetics*. 2009; 18(21):4046–4053. [PubMed: 19656776]
- Todkar A, Granholm L, Aljumah M, Nilsson KW, Comasco E, Nylander I. HPA axis gene expression and DNA methylation profiles in rats exposed to early life stress, adult voluntary ethanol drinking and single housing. *Frontiers in Molecular Neuroscience*. 2015; 8
- Todkar A, Granholm L, Aljumah M, Nilsson KW, Comasco E, Nylander I. HPA axis gene expression and DNA methylation profiles in rats exposed to early life stress, adult voluntary ethanol drinking and single housing. *Frontiers in Molecular Neuroscience*. 2016; 8:90. [PubMed: 26858597]
- Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience*. 2007; 8(5):355–367. [PubMed: 17453016]
- Veazey KJ, Parnell SE, Miranda RC, Golding MC. Dose-dependent alcohol-induced alterations in chromatin structure persist beyond the window of exposure and correlate with fetal alcohol syndrome birth defects. *Epigenetics & Chromatin*. 2015; 8(1):39. [PubMed: 26421061]
- Vetulani J. Early maternal separation: a rodent model of depression and a prevailing human condition. *Pharmacological Reports*. 2013; 65(6):1451–1461. [PubMed: 24552992]
- Vijayendran M, Beach SR, Plume JM, Brody GH, Philibert RA. Effects of genotype and child abuse on DNA methylation and gene expression at the serotonin transporter. *Frontiers in Psychiatry*. 2012; 3
- Walker C-D, Bath KG, Joels M, Korosi A, Larauche M, Lucassen PJ, Morris MJ, Raineke C, Roth TL, Sullivan RM. Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress*. 2017; 20(5):421–448. [PubMed: 28617197]
- Wang A, Nie W, Li H, Hou Y, Yu Z, Fan Q, Sun R. Epigenetic upregulation of corticotrophin-releasing hormone mediates postnatal maternal separation-induced memory deficiency. *PLoS One*. 2014; 9(4):e94394. [PubMed: 24718660]
- Wang L-L, Zhang Z, Li Q, Yang R, Pei X, Xu Y, Wang J, Zhou S-F, Li Y. Ethanol exposure induces differential microRNA and target gene expression and teratogenic effects which can be suppressed by folic acid supplementation. *Human Reproduction*. 2009; 24(3):562–579. [PubMed: 19091803]

- Ward ID, Zucchi FC, Robbins JC, Falkenberg EA, Olson DM, Benzie K, Metz GA. Transgenerational programming of maternal behaviour by prenatal stress. *BMC Pregnancy and Childbirth*. 2013; 13(Suppl 1):S9. [PubMed: 23446000]
- Watson JB, Mednick SA, Huttunen M, Wang X. Prenatal teratogens and the development of adult mental illness. *Development and Psychopathology*. 1999; 11(3):457–466. [PubMed: 10532619]
- Wearick-Silva LE, Marshall P, Viola TW, Centeno-Silva A, de Azeredo LA, Orso R, Li X, Donadio MV, Bredy TW, Grassi-Oliveira R. Running during adolescence rescues a maternal separation-induced memory impairment in female mice: Potential role of differential exon-specific BDNF expression. *Developmental Psychobiology*. 2017; 59(2):268–274. [PubMed: 27807856]
- Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nature Neuroscience*. 2004; 7(8):847–854. [PubMed: 15220929]
- Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *Journal of Neuroscience*. 2005; 25(47):11045–11054. [PubMed: 16306417]
- Weinberg J, Sliwowska JH, Lan N, Hellemans K. Prenatal alcohol exposure: Foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. *Journal of Neuroendocrinology*. 2008; 20(4):470–488. [PubMed: 18266938]
- Weiss EL, Longhurst JG, Mazure CM. Childhood sexual abuse as a risk factor for depression in women: psychosocial and neurobiological correlates. *American Journal of Psychiatry*. 1999; 156(6):816–828. [PubMed: 10360118]
- Whitcher LT, Klintsova AY. Postnatal binge-like alcohol exposure reduces spine density without affecting dendritic morphology in rat mPFC. *Synapse*. 2008; 62(8):566–573. [PubMed: 18512209]
- Whitelaw E. Disputing Lamarckian epigenetic inheritance in mammals. *Genome Biology*. 2015; 16:60. [PubMed: 25853737]
- Wu Y, Patchev AV, Daniel G, Almeida OF, Spengler D. Early-life stress reduces DNA methylation of the *Pomc* gene in male mice. *Endocrinology*. 2014; 155(5):1751–1762. [PubMed: 24506071]
- Yali AM, Lobel M. Coping and distress in pregnancy: an investigation of medically high risk women. *Journal of Psychosomatic Obstetrics and Gynaecology*. 1999; 20(1):39–52. [PubMed: 10212886]
- Yang B-Z, Zhang H, Ge W, Weder N, Douglas-Palumberi H, Perepletchikova F, Gelernter J, Kaufman J. Child abuse and epigenetic mechanisms of disease risk. *American Journal of Preventive Medicine*. 2013; 44(2):101–107. [PubMed: 23332324]
- Yang J, Qiu H, Qu P, Zhang R, Zeng L, Yan H. Prenatal alcohol exposure and congenital heart defects: a meta-analysis. *PloS One*. 2015; 10(6):e0130681. [PubMed: 26110619]
- Yehuda R, Boissoneau D, Mason JW, Giller EL. Glucocorticoid receptor number and cortisol excretion in mood, anxiety, and psychotic disorders. *Biological Psychiatry*. 1993; 34(1):18–25. [PubMed: 8373936]
- Yehuda R, Giller EL, Southwick SM, Lowy MT, Mason JW. Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. *Biological Psychiatry*. 1991; 30(10):1031–1048. [PubMed: 1661614]
- Zaidan H, Leshem M, Gaisler-Salomon I. Prereproductive stress to female rats alters corticotropin releasing factor type 1 expression in ova and behavior and brain corticotropin releasing factor type 1 expression in offspring. *Biological Psychiatry*. 2013; 74(9):680–687. [PubMed: 23726318]
- Zhang CR, Ho M-F, Vega MCS, Burne TH, Chong S. Prenatal ethanol exposure alters adult hippocampal VGLUT2 expression with concomitant changes in promoter DNA methylation, H3K4 trimethylation and miR-467b-5p levels. *Epigenetics & chromatin*. 2015; 8(1):40. [PubMed: 26421062]
- Zhang S, Rattanaray L, MacLaughlin SM, Cropley JE, Suter CM, Molloy L, Kleemann D, Walker SK, Muhlhausler BS, Morrison JL. Periconceptional undernutrition in normal and overweight ewes leads to increased adrenal growth and epigenetic changes in adrenal IGF2/H19 gene in offspring. *The FASEB Journal*. 2010; 24(8):2772–2782. [PubMed: 20371620]

- Zhang W, Peng C, Zheng M, Gao W, Zhu J, Lv T, Liu L, Liu Z, Li H, Xv Y. Prenatal alcohol exposure causes the over-expression of DHAND and EHAND by increasing histone H3K14 acetylation in C57 BL/6 mice. *Toxicology Letters*. 2014; 228(3):140–146. [PubMed: 24857828]
- Zhou FC, Chen Y, Love A. Cellular DNA methylation program during neurulation and its alteration by alcohol exposure. *Birth Defects Research Part A: Clinical and Molecular Teratology*. 2011; 91(8): 703–715. [PubMed: 21630420]
- Zierhut K, Bogerts B, Schott B, Fenker D, Walter M, Albrecht D, Steiner J, Schütze H, Northoff G, Düzel E. The role of hippocampus dysfunction in deficient memory encoding and positive symptoms in schizophrenia. *Psychiatry Research: Neuroimaging*. 2010; 183(3):187–194. [PubMed: 20702070]
- Zucchi FC, Yao Y, Ward ID, Ilnytsky Y, Olson DM, Benzie K, Kovalchuk I, Kovalchuk O, Metz GA. Maternal stress induces epigenetic signatures of psychiatric and neurological diseases in the offspring. *PLoS One*. 2013; 8(2):e56967. [PubMed: 23451123]

Highlights

Developmental alcohol or stress impacts the epigenome.

Little is known regarding the impact of both developmental alcohol and stress exposure on the epigenome, though it is common to experience both.

The effects of developmental alcohol or stress exposure are not limited to one generation.

Interventions aimed at the changing the epigenome could help rescue negative outcomes associated with developmental alcohol or stress exposure.

Figure 1.

Overview of epigenetic modifications induced by prenatal alcohol exposure (PAE). The presence of permissive transcriptional marks, such as histone acetylation or trimethylation, results in transcription of DNA into mRNA and translation of mRNA into protein. Under control conditions, normal CNS, endocrine, and immune function take place. PAE can remove these pro-transcriptional marks or increase the presence of repressive transcriptional marks, such as DNA methylation. Methyl groups either directly disrupt the ability of transcription factors to bind to DNA or recruit other transcriptional repressor proteins (i.e. MeCP2), reducing gene expression. miRNAs act post-transcriptionally to prevent mRNA from being translated into protein. This is associated with increased cell death and oxidative stress, altered cell cycle progression, disrupted endocrine and hypothalamic–pituitary–adrenal (HPA) axis signaling, and behavioral and cognitive deficits.

Figure 2.

In response to stress, activation of the HPA axis takes place. The paraventricular nucleus (PVN) of the hypothalamus releases corticotropin-releasing factor (CRF), which then prompts the pituitary to release adrenocorticotrophic hormone (ACTH), ultimately resulting in the adrenal cortex releasing glucocorticoids (Cortisol, CORT). Glucocorticoids act on the hippocampus, which provides negative feedback turning off HPA axis activity. Developmental stress induces epigenetic changes that affect many genes involved in HPA axis regulation. Epigenetic changes result in altered HPA axis activity and in turn, help explain behavioral aberrations observed in animals with a history of developmental stress.

Figure 3.

Epigenetic modifications represent an avenue through which adverse environmental conditions can affect generations beyond the animals that were directly exposed via intergenerational and transgenerational transmission. Epigenetic marks can be passed through the paternal germline (F0) following preconception exposure to stress or alcohol, resulting in physiological, behavioral, or altered levels of global methylation in the offspring (F1). Exposure of a fetus (via exposure of the F0 pregnant dam) or neonate (F1) to alcohol or stress affects not only the directly exposed animal but also the developing germline. Altered epigenetic marks can then be transmitted to animals that were never exposed to the stressor or alcohol in the F2 generation and beyond, resulting in changes to methylation associated with multiple genes and epigenetic regulators and persistent behavioral perturbations. AE: Alcohol exposure; ELS: Early life stress.