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Prenatal Influences on Temperament Development: The Role of Environmental Epigenetics

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Abstract

This review summarizes current knowledge and outlines future directions relevant to questions concerning environmental epigenetics and the processes that contribute to temperament development. Links between prenatal adversity, epigenetic programming, and early manifestations of temperament are important in their own right, also informing our understanding of biological foundations for social-emotional development. In addition, infant temperament attributes represent key etiological factors in the onset of developmental psychopathology, and studies elucidating their prenatal foundations expand our understanding of Developmental Origins of Health and Disease (DOHaD). Prenatal adversity can take many forms, and this overview is focused on the environmental effects of stress, toxicants, substance use/psychotropic medication, and nutrition. Dysregulation associated with ADHD/disruptive disorders was noted in the context of maternal substance use and toxicant exposures during gestation, as well as stress. Although these links can be made based on the existing literature, currently few studies directly connect environmental influences, epigenetic programming and changes in brain development/behavior. The chain of events starting with environmental inputs and resulting in alterations to gene expression, physiology, and behavior of the organism is driven by epigenetics. Epigenetics provides the molecular mechanism of how environmental factors impact development and subsequent health and disease, including early brain and temperament development.

Keywords

Prenatal Adversity; Epigenetics; DNA Methylation; Temperament

INTRODUCTION

Environmental exposures can alter maternal physiology in a manner that results in “programming” effects on the fetus. This programming is understood in terms of adaptations to the prenatal environment, which may or may not be beneficial after birth. That is, the

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course of fetal development is altered as a function of critical environmental conditions in a manner that shapes growth and health outcomes into adulthood. According to Hochberg et al. (2011) and others, offspring faces risk in the form of vulnerability to adversity and/or a lack of resilience, if *in utero* programming is discordant with the postnatal environment. This programming effect was first noted in the context of under-nutrition and is often referred to as “Barker’s hypothesis”. Barker and Osmond (1986) relied on epidemiological data to ascertain that gestational under-nutrition was correlated with adult onset heart disease. Multiple investigations have since identified fetal programming contributions to adult disorders (e.g., (Tobi et al., 2014), and interest in Developmental Origins of Health and Disease (DOHaD; (Wadhwa, Buss, Entringer, & Swanson, 2009)) continues to grow. This research encompasses prenatal effects associated with exposure to stress, toxicants, substance use/psychotropic medication, and nutrition. Programming effects of these environmental factors linked with temperament development will be considered in the present review, focused on the underlying biological mechanisms.

Fetal programming is thought to transform the structure and function of tissues/organs, shaping physiological outcomes associated with adult disease. Biological processes underling these programming effects represent a more recent emphasis, as DOHaD research has become increasingly concerned with the role of epigenetic mechanisms that alter gene expression (Hochberg et al., 2011). Understood to mediate the effects of environmental input on the developing organism by shaping gene expression, epigenetics has been defined as “Molecular factors/processes around the DNA that regulate genome activity independent of DNA sequence, and are mitotically stable” (Skinner, 2014). Epigenetic mechanisms play a critical role in fetal programming, providing the basis for either healthy outcomes or disruption of physical and mental health. Environmental epigenetics encompasses a variety of environmental factors that serve to establish and maintain epigenetic modifications, influencing gene expression and the resulting phenotype. Fetal programming associated with maternal stress, toxicants, substance/psychotropic medication use, and nutrition reflects diverse biological pathways. All these exposures are nonetheless relevant to environmental epigenetics, as epigenetic processes provide conduits for conferring their effects to the gestating organism.

Consequences of fetal programming in humans have been documented most widely for physical health/medical outcomes (e.g., (T. J. Roseboom et al., 2011), with behavioral effects often examined in animal models (Babenko, Kovalchuk, & Metz, 2015). Outcomes have not been typically framed as temperament per se, but rather discussed in terms of stress reactivity, susceptibility to anxiety, or impulsivity, without making links to this overarching theoretical framework. Connecting these disparate findings under the umbrella of the psychobiological model of temperament makes it possible to view these in terms of their implications for social-emotional development and developmental psychopathology, and to provide prevention/intervention related recommendations. Importantly, understanding developmental origins of temperament via fetal programming potentiated through epigenetic mechanisms could make earlier preventative efforts possible. Considering prenatal effects of maternal stress, toxicant exposure, substance/psychotropic medication use, and nutrition in the context of the environmental epigenetics framework provides an opportunity to compare resulting offspring epigenetic and behavioral signatures. These exposures often work in

tandem (e.g., inadequate nutrition coupled with stress), and advances in environmental epigenetics will inform whether similar epigenetic mechanisms, overlapping genomic regions, and/or interacting gene networks, are responsible for compound effects.

This review begins with epigenetic mechanisms critical to current research, and those likely to gain importance going forward. Links between temperament and developmental psychopathology are addressed next. We then turn to environmental factors that shape the intrauterine milieu and fetal development: stress, toxicant, substance use/psychotropic medication, and nutritional exposures. Finally, evidence linking DNA methylation and temperament, and implications for behavioral/emotional health are described, followed by conclusions and recommendations for future research.

I. Epigenetic mechanisms

A number of epigenetic mechanisms have been identified, including DNA methylation, histone modifications, chromatic structure/remodeling, and non-coding RNAs (Figure 1).

a. DNA methylation

DNA methylation occurs when a small (methyl) chemical group is attached to the DNA base cytosine (C) in a CpG sequence. Covalent modification of DNA by methylation of cytosine residues serves to alter transcription and genome activity. DNA methylation is responsible for silencing of transposable elements and pericentromeric repeats to ensure genome integrity (Chen, Tsujimoto, & Li, 2004; Kaneko-Ishino & Ishino, 2010; H. Xu, Bai, Collins, & Ghishan, 1999), inactivation of the X-chromosome (Lock, Takagi, & Martin, 1987; Sado, Okano, Li, & Sasaki, 2004) and genomic imprinting (Feil & Khosla, 1999; Reik, Collick, Norris, Barton, & Surani, 1987). Critical to multiple developmental outcomes, DNA methylation is also emerging as important for temperament development (e.g., Fuemmeler et al., 2016).

b. Histone modifications

Histones represent the chief protein component of chromatin, functioning as beads around which DNA winds, and is compressed into nucleosomes. Histones, and their various modifications, or marks, play an important role in regulating gene expression. Covalent post-translational modifications to histone proteins including methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation impact gene expression by altering chromatin structure and/or recruiting histone modifiers. Whereas some result in transcription activation, others have a repressive effect, effectively shutting down transcription. Lysine acetylation, for example, results in more accessible chromatin and tends to be associated with active transcription. Conversely, deacetylases remove acetyl groups from the histones, allowing the DNA to be wrapped more tightly and repressing transcription activity. Histone methylation effects depend on the location and number of methyl groups involved, potentially leading to activation or repression of gene expression (Barski et al., 2007). Exposure to adverse environments has been shown to result in alterations in histone acetylation and methylation status in animals (Weaver et al., 2004). Specifically, histone

acetylation of the glucocorticoid receptor NR3C1 gene differentiated offspring born to high and low licking and grooming mothers along with DNA methylation.

c. Chromatin structure/remodeling

A number of factors come into play as DNA is packaged into progressively larger units, starting with the assembly of nucleosomes and concluding with mitotic condensation, resulting in chromosomes. A variety of factors (e.g., stage of cell life) affect chromatin state, resulting in either euchromatin or heterochromatin. The euchromatin, “beads-on-a-string” structure makes DNA more accessible, promoting transcription and gene expression. The euchromatin state is associated with activating histone marks and remodeling complex recruitment that facilitate transcription factor binding. Heterochromatin, on the other hand, represents a repressed state with respect to gene expression, and is associated with repressive histone and DNA methylation.

d. Non-coding RNAs

ncRNAs include short microRNAs, PIWI-interacting RNAs and long non-coding RNAs, regulating genes translation/transcription and chromatin stability (Zhou, Hu, & Lai, 2010). ncRNAs can directly bind to promoters and interfere with polymerases by mobilizing transcriptionally repressive complexes conducive to heterochromatin (Zaratiegui, Irvine, & Martienssen, 2007) and through posttranscriptional binding (Filipowicz, Bhattacharyya, & Sonenberg, 2008). ncRNAs are involved in a number of activities, with current evidence suggesting the largest class may be that of chromatin modifiers (Horabin, 2013). Whereas some ncRNA actions are sequence dependent, and thus not epigenetic in nature, examples of sequence independent effects have been reported (Cao, 2014).

Although a number of epigenetic mechanisms have been documented, initial implementation in DOHaD research has focused primarily on methylation. Additional epigenetic processes will likely be implicated in the future, likely working in concert; for example, as histone modifications contribute to chromatin remodeling (Figure 1). Accumulating evidence from animal studies supports the important role of epigenetic mechanisms in shaping physiological maturation and behavioral development, conferring effects of various exposures (e.g., (Skinner, Anway, Savenkova, Gore, & Crews, 2008). The human literature lags behind and is subject to greater methodological challenges. Nonetheless, epigenetic effects have been identified utilizing brain tissue and peripheral cells (Nieratschker et al., 2014; Szyf & Bick, 2013). That is, signatures of environment triggers that lead to epigenetic/molecular changes in the brain can also be identified in peripheral tissues, because of systemic consequences of exposure (Lester, Conrad, & Marsit, 2016; Szyf & Bick, 2013), and afford identification in humans. Moreover, issues around cell-specificity of epigenetic programming can be addressed by comparative studies, wherein human peripheral cell epigenetic signature is compared to a brain tissue signature obtained from animals, with the focus on conserved genomic regions and/or gene networks (Nieratschker et al., 2014). This environmental epigenetics review will focus on stress, toxicant, substance use/pharmaceutical, and nutritional prenatal exposures. Pathways that involve maternal physiology and offspring brain development will be discussed as the foundation for temperament and risk/protection with respect to psychopathology. Epigenetic mechanisms

mediate environmental effects on temperament phenotypes. Effects set in motion during gestation likely contribute to developmental psychopathology by impacting temperament growth and could also play a role as risky temperament profiles transform into symptoms and disorders (e.g., infant fear/behavioral inhibition culminate in childhood social anxiety). Temperament related effects are likely to be especially salient in early childhood, as behavioral attributes “come online”.

II. Temperament development and symptoms of psychopathology

We now turn to temperament research addressing negative emotionality, surgency/extraversion, regulatory capacity/effortful control, and links with developmental psychopathology. The study of temperament has a long-standing history. Childhood research began with the work of Thomas and Chess (1977) focused on “difficult temperament”, which reflects high levels of negative affects, coupled with few manifestations of positive emotions and poor regulation. A number of temperament definitions and frameworks have been proposed, several emphasizing biological underpinnings. The study of behavioral inhibition and exuberance, for example, has linked approach and avoidance tendencies to an asymmetry in frontal brain activation (Hane, Fox, Henderson, & Marshall, 2008). The Polyvagal theory (Porges, 2007) addressed how physiological states support different classes of behavior (e.g., vagal withdrawal supports mobilization, fight and flight) and primary emotions are related to autonomic functioning. Currently, Rothbart’s psychobiological model (M. K. Rothbart & Derryberry, 1981), an integration of existing temperament and personality frameworks with other relevant areas, such as neuroscience and comparative studies, represents the most widely referenced approach.

According to the psychobiological model, temperament represents constitutionally based individual differences in emotional and motor reactivity, as well as self-regulation, demonstrating consistency across situations and relative stability over time (M. K. Rothbart & Derryberry, 1981). Emotional reactivity encompasses fear, anger, sadness, and positive emotions. Processes serving to modulate reactivity, including attentional focus, shifting, and inhibitory control provide the basis for self-regulation. The psychobiological approach identifies unique domains of temperament, mapping these onto underlying neurobehavioral systems, and outlines their developmental pathways and interactions. The term “constitutional” emphasizes the connection between temperament and biology. Historically, individual differences in temperament have been linked to the constitution of the organism, as it was understood at the time. Although “constitutionally-based” has been typically interpreted as genetically driven, accumulating evidence suggests epigenetic processes make important contributions and have been incorporated in the definition of temperament more recently (Putnam & Gartstein, 2016). The psychobiological temperament framework applies across the lifespan, with reactive and regulatory domains developing especially rapidly in early childhood. Temperament attributes are understood to encompass physiological reactivity, including elements of the HPA-axis response and patterns of brain activation. These are generally measured along with behavioral markers, typically ascertained through direct observations, and in humans also via caregiver report (Gartstein, Putnam, Aaron, & Rothbart, 2016).

Structurally, temperament encompasses negative emotionality, surgency/extraversion, and orienting regulation/effortful control. Distress proneness is the first to emerge (e.g., (Putnam, Rothbart, & Gartstein, 2008; M. K. Rothbart, 1989). At the same time negative emotionality has strong conceptual and empirical links to the adult personality trait of neuroticism (Evans & Rothbart, 2007). Fearful reactivity/behavioral inhibition, a component of negative emotionality, tends to occur in the context of relative right-hemisphere dominance (right dorsolateral prefrontal cortex and temporal regions), associated with limbic system (i.e., amygdala, basal ganglia, and hypothalamus) activation (Davidson, 1998; Sutton & Davidson, 1997). HPA-axis functioning alterations marked by higher cortisol were also noted for children demonstrating behavioral inhibition/fear (Perez-Edgar, Schmidt, Henderson, Schulkin, & Fox, 2008).

Surgency in infancy is largely manifested through displaying pleasure (e.g., smiling, laughing) and approach behaviors (Gartstein & Rothbart, 2003; M. K. Rothbart, 1989), incorporating characteristics of activity and enthusiasm (M. K. Rothbart & Ahadi, 1994). Individuals higher in positive affect have the tendency to be engaged, rather than disengaged, with their environment (Lonigan, Phillips, & Hooe, 2003), as approach tendencies are potentiated by increased Behavior Activation System input (J. Gray, 1994; M. K. Rothbart & Hwang, 2005). The corticolimbic-striatal-thalamic network that operates via dopaminergic projections from the ventral tegmental area to the subcortical and frontal cortical regions (Depue & Collins, 1999) favors left frontal regions (Coan & Allen, 2004) and provides the foundation for surgency/extraversion. In infancy, relative left frontal asymmetry was associated with approach behaviors, including positive vocalization and facial expressions of joy (Diaz & Bell, 2012; Hane et al., 2008).

Infant regulatory/attentional skills set the stage for the more flexible, frontally mediated effortful control (e.g., (Gartstein, Bridgett, Young, Panksepp, & Power, 2013). The emergence of effortful control coincides with rapid development of the executive attention system, including the lateral prefrontal cortex and anterior cingulate regions (M. K. Rothbart, Derryberry, & Posner, 1994; Rueda, 2012). This critical milestone involves a shift from reliance on the alerting attention network, supported by parietal lobe areas and governed by the norepinephrine system, to a more flexible executive attention network. Anterior cingulate, basal ganglia, and areas of the prefrontal cortex are included in the executive attention network, modulated by dopamine (M. I. Posner, Rothbart, Sheese, & Voelker, 2012). Whereas the alerting system is responsible for filtering sensory input, the executive network is engaged in conflict resolution and selecting between alternative response options (M.I. Posner & Peterson, 2011).

A growing body of research indicates that the risk for externalizing problems (conduct difficulties, hyperactivity, and impulsivity; (Campbell, 1995; Kovacs & Devlin, 1998) is greatest for highly reactive children with pronounced negative emotionality and/or surgency, coupled with deficits in attention and regulatory functioning (Diaz et al., 2015; Gartstein & Fagot, 2003; Martel, Gremillion, & Roberts, 2012; Muris & Ollendick, 2005). Internalizing difficulties that involve personal distress and are affective in nature (e.g., depression/anxiety) can occur as a function of high negative emotionality and low surgency (Fowles, 1994;

Lonigan et al., 2003; Murray & Kochanska, 2002), with non-temperament pathways also relevant for depressive symptoms (Hammen, 2005).

Thus, early manifestations of temperament are not only important as key components of social-emotional development, but are also significant as early markers of psychopathology risk (Figure 2). A number of explanations for the relationship between temperament and psychopathology have been proposed. Vulnerability and pathoplasty models generally assign temperament a causal role in psychopathology. Vulnerability explanations cast temperament as increasing the probability of symptom onset (e.g., (Perez-Edgar et al., 2008). On the other hand, temperament and psychopathology can have independent etiology in pathoplasty models, wherein temperament (or personality) is viewed as impacting the course and/or severity of disorders (e.g., (De Bolle, De Clercq, De Caluwe, & Verbeke, 2016). Alternative differential susceptibility (Belsky & Pluess, 2009) and biological sensitivity to context (Boyce & Ellis, 2005) models are relevant primarily to negative emotionality, viewed as a plasticity, rather than a vulnerability factor. Thus, the differential susceptibility/biological sensitivity to context models make “for better or for worse” predictions with respect to negative emotionality. There is growing empirical support for the positive response of those high in negative emotionality to good environments and interventions (e.g., (Hentges, Davies, & Cicchetti, 2015), and new evidence that negative affectivity primarily confers adjustment risk (e.g., (Moran et al., 2016). Predictive links between temperament and symptoms were also framed in terms of underlying neurobiology. Whittle, Allen, Lubman, & Yucel (2006) proposed a model focused on six brain structures: amygdala, hippocampus, nucleus accumbens, orbitofrontal cortex, anterior cingulate cortex, and the dorsolateral prefrontal cortex, describing joint effects for negative emotionality, surgency/extraversion, regulation, and related symptoms/disorders. Environmental epigenetics can elucidate biological underpinnings of temperament development, in turn expanding our understanding of developmental psychopathology and DOHaD more broadly, as temperament attributes represent key contributing factors.

Human and animal studies showing connections between prenatal environmental exposures and temperament-related phenotypes suggest these exposures, and the cascades of epigenetic effects that follow, impact how reactivity and regulation “come online”. Studies addressing the impact of maternal stress response and HPA-axis functioning make some of the least ambiguous connections between prenatal exposure and alteration in offspring temperament across species, elucidating epigenetic processes that mediate prenatal environment-postnatal behavior relationships. Evidence linking maternal stress during pregnancy and offspring HPA-axis dysregulation also suggests the latter likely manifests in reactive and regulatory components of temperament, as well as their contributions to symptoms/disorders (Figure 2).

III. Environment effects that shape intrauterine environment and fetal development

As environmental epigenetics concerns itself with a spectrum of exposures capable of exerting developmental effects, we now describe these, and established links with behavioral

phenotypes relevant to temperament. Stress exposure has been one of the most widely studied prenatal adversities. Human and animal studies indicate behavioral consequences for the offspring typically involve fear/anxiety and/or behavioral/emotional dysregulation.

Stress: Mechanisms, Definitions, Critical Developmental Periods, and Epigenetic Effects

a. Stress Response: The Neuroendocrine System—In response to stress, paraventricular nucleus of the hypothalamus produces corticotropin-releasing hormone (CRH). CRH stimulates the anterior pituitary to synthesize proopiomelanocortin (POMC). Adrenocorticotrophic hormone (ACTH), a peptide derived from POMC, is subsequently released into the bloodstream. ACTH induces the adrenal glands to produce cortisol in humans (corticosterone in rodents) - the end product of the HPA axis, often labeled the stress hormone (Charmandari, Tsigos, & Chrousos, 2005). A negative feedback loop is created as cortisol binds to glucocorticoid receptors in the hippocampus. This modulation of the hypothalamic pituitary adrenal (HPA)-axis response results in down-regulation of CRH and ACTH (Deppermann, Storchak, Fallgatter, & Ehli, 2014).

A mechanism wherein information from the environment is conferred to the offspring via maternal stress hormones during gestation appears to be conserved across multiple species (Table 1). For instance, predation risk was shown to cause female fish to produce larger eggs with higher concentrations of cortisol (Giesing, Suski, Warner, & Bell, 2011). Behavioral effects were also noted, as juveniles exposed to prenatal stress formed denser shoals, an effective anti-predator strategy. In hares, in utero predator stress was positively correlated to offspring physiological stress reactivity, compromising subsequent reproductive fitness (Sheriff, Krebs, & Boonstra, 2009, 2010). High maternal cortisol concentrations were linked with non-viable births, smaller offspring size, increased cortisol reactivity, vigilance, and anxiety-like behaviors. Prenatally stress-exposed hares also dispersed more effectively, promoting juvenile survival at a cost of reproductive output, in an example of the “increasing glucocorticoid concentrations to survive at the cost of reproduction” strategy (Sheriff et al., 2009).

Stress-related prenatal effects reflect a “calculation” aimed at improving the odds of survival and are not uniformly maladaptive. These adaptations are of interest precisely because of an attempt to balance the needs of the developing organism: the need for optimal development along a species-typical timeline vs. faster development advantageous for immediate survival. The speeded-up gestation translates into alterations in cellular structure/function. Short-term benefits (i.e., faster gestation and survival) are self-evident, yet adverse health consequences in the long-term are also likely. DOHaD research with humans suggests that gestational adversity, presumably resulting in fetal development shortcuts, translates into compromised health in adulthood, including mental health (e.g., (Froehlich et al., 2009; T. Roseboom, de Rooij, & Painter, 2006).

The range of prenatal adversity is notably broader in humans, including stressful or life-threatening situations and maternal reactivity to these circumstances (e.g., PTSD), signaled to the fetus by maternal HPA-axis response (Babenko et al., 2015). Certain stressors can be considered normative, as these generally occur in the context of daily life. Even routine hassles can become problematic, however, especially in concert with symptoms of

depression and anxiety (Diego et al., 2006). Although majority of maternal cortisol (80%–90%) in the placenta is metabolized, higher concentrations of cortisol can disrupt this process, exposing the fetus to higher levels (Meyer, 1985). Increased maternal HPA-axis activity and resultant cortisol production is thought to downregulate placental corticosteroid 11- β -dehydrogenase isozyme 2 (11 β -HSD-2), which metabolizes cortisol into inactive cortisone (O'Donnell et al., 2012). This reduced ability to increase placental 11 β -HSD-2 with an acute stressor is likely critical to intergenerational transmission. That is, women who do not present with elevated cortisol during pregnancy, demonstrating a “blunted” response to chronic stress (Keeshin, Strawn, Out, Granger, & Putnam, 2014; Yehuda & Seckl, 2011), can nonetheless transmit exceedingly high levels of cortisol to the fetus with 11 β -HSD-2 downregulation.

b. Prenatal Stress Exposure: Evidence of Behavioral/Temperament Risk

1. Human conceptualizations of stress and empirical Studies: Highly conceptual definitions of human stress evolved to include cognitive and allostatic processes, vulnerabilities that result from early experiences and impact adaptation (Maccari et al., 2016), yet research examining prenatal effects of stress has generally relied on unidimensional operational definitions. In fetal programming research, operationalizations of stress include traumatic events (e.g., exposure to interpersonal violence or natural disasters), psychosocial stress (daily hassles/stressful events), physiological stress reactivity (e.g., cortisol concentrations), and symptoms of anxiety and depression (Table 2). Multiple studies rely on broad definitions of stress, for example combining indicators of psychosocial stress and symptoms of psychopathology (e.g., anxiety and depression; (Nieratschker et al., 2014). Thus, the literature does not currently provide the basis for making meaningful distinctions with respect to the type of stressor in terms of consequences for the offspring. Another challenge in examining stress with human populations is that exposures are often confounded, such as psychosocial/socio-demographic stress being accompanied by maternal substance use. Confounding effects are somewhat mitigated in quasi-experimental studies wherein stress response/adaptation data are collected in the aftermath of a disaster (Tees et al., 2010; Yong Ping et al., 2015). This approach takes advantage of events that impact large numbers of individuals in a manner that is random with respect to potential confounds. However, stress in the aftermath of a disastrous event does not impact all affected to the same extent, and individuals subject to prior socio-economic or psychosocial stress likely fare worse.

In one study making use of this quasi-experimental design, women exposed to the World Trade Center attacks during pregnancy were followed, along with their offspring. This research focused on Post-traumatic Stress Disorder (PTSD) and physiological stress reactivity in the wake of this man-made disaster (Yehuda, 2002; Yehuda et al., 2005). Maternal PTSD symptoms were associated with lower infant cortisol concentrations, with most pronounced effects in their third trimester (Yehuda et al., 2005). Toddlers whose mothers were exposed to Iowa Floods during pregnancy demonstrated increased cortisol reactivity to a separation. Higher subjective distress was linked with greater HPA-axis reactivity for girls only, and more pronounced effects were associated with later-term exposure (Yong Ping et al., 2015). Prenatal stress effects were also noted earlier in gestation,

with higher self-reported maternal anxiety at 12–22 weeks predicting child HPA-axis alterations. Female offspring only exhibited depression in association with hypo-reactivity (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008). In the short-term, stress is expected to produce an increased HPA-axis response that translates into higher cortisol concentrations, yet the HPA-axis downregulates after chronic periods of elevated stress (Gunnar & Vazquez, 2001). Child stress reactivity is associated with negative emotionality (e.g., fear/behavioral inhibition; (Perez-Edgar et al., 2008), which can be measured via behavioral and physiological indicators (e.g., cortisol), and has been examined in conjunction with a spectrum of maternal prenatal symptoms.

A number of studies indicated associations between maternal anxiety and depression during pregnancy and infant negative emotionality as well as dysregulation. Mothers with higher prenatal emotional stress were more likely to have infants lower in fearfulness (Mohler, Parzer, Brunner, Wiebel, & Resch, 2006). This diminished fear was interpreted as potential behavioral risk, possibly contributing to externalizing disorders, such as ADHD (Mohler et al., 2006). Maternal anxiety during pregnancy was associated with poor infant self-regulation, likely setting the stage for later behavioral and emotional difficulties (van den Heuvel, Johannes, Henrichs, & Van den Bergh, 2015). In the Davis et al. (2007) study, prenatal anxiety and depression averaged across pregnancy were both significantly correlated with infant negative reactivity. Vedova (2014) obtained somewhat divergent results controlling for socioeconomic and partner/relationship factors in predicting difficult temperament - a construct reflecting high negative emotionality. Prenatal depression and state anxiety in the postnatal periods emerged as independent predictors (Vedova, 2014).

Whereas late-term effects of stress-exposure are thought to interfere with critical steps in fetal HPA-axis development, early gestation effects likely result from cortisol impacting cell migration and interacting with additional pathways (e.g., serotonin signaling pathway) involved in brain development (Beijers, Buitelaar, & de Weerth, 2014). Recent reviews have noted consistent links between exposure to prenatal stress and compromised neurodevelopment (e.g., neurogenesis; (Antonelli, Pallares, Ceccatelli, & Spulber, 2016; Fatima, Srivastav, & Mondal, 2017). Stress-related fetal programming research in humans is limited by its correlational nature and reliance on quasi-experimental designs that inherently lack internal validity afforded by the animal models, with the latter serving to further inform these connections.

2. Animal models of prenatal stress exposure: Investigations of prenatal stress effects in animals provide an opportunity to directly manipulate key parameters (i.e., frequency, severity, duration) of stress induction procedures, such as exposure to novel environments, subcutaneous injections of saline, restraint, and predator proximity. Injections of synthetic glucocorticoids have also been utilized to induce conditions that parallel high levels of physiological stress reactivity. These exposures impact the developing fetus, altering offspring neurobiology and behaviors into adulthood (Table 2).

A widely used restraint stress procedure resulted in anxiety-like behavior (decreased time spent in open arms of the elevated plus maze) for prenatally stressed males, with downregulation of glucocorticoid receptors described as a potential mechanism behind these

effects and long-term HPA-axis alterations (Maccari et al., 2003). Another prenatal stress paradigm involves the use of electric shock as an analogue for human trauma exposure (M. S. Golub, Kaufman, Campbell, Li, & Donald, 2006). Prenatally stress-exposed offspring demonstrated increased duration and frequency of ultrasonic calls (sign of distress) during separation from mothers and spent less time in open arms of the elevated plus maze, compared to controls. Another stress induction manipulation relies on a forced swim test in the physical stress condition, with the psychological stress group observing those experiencing physically stressful conditions. Female, but not male, rat offspring exposed to both stress conditions demonstrated a decreased number of entrances into open arms of the elevated plus maze indicative of anxiety, relative to controls (Nazeri et al., 2015). Abe et al. (2007) induced psychological stress in pregnant rats during the last trimester of gestation by having animals observe an electric shock administration procedure. In comparison with control animals, exposed male offspring showed enhanced emotionality in an open field test, depression-like behavior in a forced swim test, and increased HPA-axis activity (higher basal plasma corticosterone levels). In nonhuman primates, administration of dexamethasone resulted in an exaggerated corticosterone response to a mild stressor (blood sampling) for the offspring of mothers who received the highest dose (de Vries et al., 2007). In this paradigm, there is no environmental exposure to stress per se; however, the synthetic glucocorticoid administration is intended to mimic stress exposure effects, specifically HPA-axis reactivity. Although this approach in a sense provides a “short-cut” eliminating the need for an exposure manipulation, it does not reflect complexities of the physiological stress response, which may also involve dampening of HPA-axis reactivity with prolonged exposure.

Thus, prenatal stress has been shown to significantly alter offspring development across human and animal studies, with a range of physiological and behavioral effects. Whereas animal studies suggest primarily anxiety-like behavioral effects, human outcomes were more varied, with some indication of risk for externalizing symptoms (Mohler et al., 2006). Prominent late-gestation effects were reported (e.g., Yehuda et al., 2005) and suggest disruption of fetal HPA-axis development, yet early gestational impact of stress was noted as well (Y. Golub et al., 2016). Crews (2010) argued neuronal and behavioral plasticity, or the genotype’s ability to produce variable phenotypes in response to environmental inputs, peaks during embryonic development. Thus, early and later-term gestational exposure to stress appears to impact behavioral phenotypes related to temperament, albeit via different developmental pathways. Sex differences were documented (e.g., Nazeri et al., 2015); however, a consistent pattern has not emerged. Further study is required in part because females have not always been included. Epigenetic mechanisms are critical to connections between prenatal stress and subsequent behavioral/temperament development. We now turn to the emerging literature demonstrating epigenetic processes play a key role in the intergenerational transmission of stress-related risk, conferring environmental adversity to the offspring.

c. Prenatal Stress Exposure: Epigenetic Effects

1. Human studies: Consistent with the environmental epigenetics perspective, multiple studies indicate that epigenetic processes, DNA methylation in particular, provide a critical

bridge between prenatal stress exposure, offspring brain development, and shifts in physiology/behavior relevant to temperament. Maternal depression and anxiety during pregnancy were associated with increased infant methylation of NR3C1 (the glucocorticoid receptor gene), which predicted higher infant salivary cortisol stress response (Oberlander et al., 2008). Maternal pregnancy related anxiety was also positively associated with infant NR3C1 methylation (Hompes et al., 2013). There is some indication of sex differences in the NR3C1 stress-related methylation shifts. Methylation levels were elevated as a function of prenatal stress for female, but not male infants, and increased methylation was positively associated with fearfulness for females only (Ostlund et al., 2016). Methylation of SLC6A4 (the serotonin transporter gene) was also affected by NICU-related stress. Increased methylation of the SLC6A4 gene was noted for preterm, compared to full-term infants. SLC6A4 methylation status was negatively correlated with mother-report of infant duration of orienting and approach for the preterm infants only (Montirosso et al., 2016). However, NR3C1 and SLC6A4 are not the only genomic conduits of prenatal stress effects, and a recent review implicated altered expression of 31 genes in prenatal origins of anxiety-like behavior (Nieto, Patriquin, Nielsen, & Kosten, 2016). Perhaps in the most definitive study to date, Nieratschker et al. (2014) examined the impact of prenatal stress on methylation in human infants, monkeys, and rats, utilizing a genome-wide approach and identifying conserved differentially methylated genes. Across species, 30 genes were associated with differential methylation as a result of prenatal stress. MORC1 was emphasized as a candidate genetic marker of prenatal stress, due to its prior associations with Major Depressive Disorder (MDD; (Nieratschker et al., 2014)). Thus, most human research to date addressed methylation of candidate genes, and the only genome-wide study did not make any connections with behavioral outcomes, which remains to be accomplished in future investigations.

2. Animal studies: In animal studies, DNA methylation changes explained the influence of maternal prenatal stress on the density of glucocorticoid receptors in the fetal brain (hippocampus in particular), thought to alter sensitivity to stress throughout the life of the offspring (Babenko et al., 2015; Weaver et al., 2004). Restraint stress during gestation resulted in altered CRH DNA methylation in the hypothalamus and increased anxiety-like behavior (e.g., decreased entries into open maze arm; (L. Xu, Sun, Gao, Cai, & Shi, 2014). Restraint stress during the last ten days of gestation also resulted in anxiety-like behavior for adult offspring and altered RLN expression. Specifically, higher levels of DNA methylation in the promoter region were associated with decreasing mRNA expression in the cortex following exposure. The latter is significant because RLN is responsible for protein Reelin, which regulates brain development through cytoskeleton modifications. Thus, DNA methylation appears to provide a critical bridge between prenatal stress, offspring brain development, and shifts in physiology/behavior relevant to temperament.

Toxicant exposure: Pesticides and Environmental Contaminants

A similar picture emerges for toxicant in utero programming, wherein epigenetic processes link exposure to temperament outcomes. A variety of environmental contaminants, such as lead, DDT, polychlorinated biphenyls (PCBs), and bisphenol A (BPA) have been investigated. As noted in a recent review, multiple animal studies and a more limited human

literature suggest that prenatal toxicant exposure alters neurodevelopment, setting the stage for potentially risky temperament and symptoms/behavior problems (Antonelli et al., 2016).

a. Endocrine Disruptors (BPA, Vinclozolin, DDT, DES, PCB): Developmental Effects—Endocrine disruptors are exogenous substances capable of dysregulating the endocrine system primarily by binding to hormone receptors, disrupting cell signaling pathways, and inhibiting hormone synthesis. These contaminants interfere with physical development, especially of the reproductive and the central nervous systems, which translates into alterations in emotional functioning, attentional and behavioral regulation components of temperament. Epigenetic mechanisms are critical to the profound effects of endocrine disruptors on developmental cascades, as exposure to these toxic agents introduces changes in gene expression (Manikkam, Guerrero-Bosagna, Tracey, Haque, & Skinner, 2012). Existing studies with humans indicate consistent links between gestational endocrine disruptor exposure and behavioral shifts, albeit typically without addressing epigenetics.

1. Human studies: Prenatal BPA exposure and child behavior problems were linked, controlling for postnatal BPA effects, with a sex-dependent pattern of results (Perera et al., 2012). Boys with greater exposure received elevated emotionally reactive and aggressive ratings (Table 3). Among girls, higher exposure was associated with significantly lower anxious/depressed and aggressive scores. Casas et al. (2015) examined programming effects of prenatal BPA exposure and reported increased ADHD symptoms at 4 years-of-age. Stronger associations were observed for boys, compared to girls, with greater 3rd, rather than with 1st trimester, effects. These results suggests sex-specific mechanisms of BPA exposure may in part be determined by the timing of prenatal effects.

Dichlorodiphenyltrichloroethane (DDT) is arguably the most widely used agricultural insecticide, with adverse health effects. Technical grade DDT includes a number of impurities and its breakdown product dichlorodiphenyldichloroethylene (DDE) is able to cross the placenta and pass into breast milk (You et al., 1999). We located one study linking DDE exposure and emotional/behavioral indicators. Sioen et al. (2013) reported a significant positive association between prenatal DDE exposure and overall emotional/behavioral difficulties for 7–8 year-old girls, but not boys.

Diethylstilbestrol (DES), an estrogenic drug, was wrongly administered to millions of pregnant women (until 1971 in the US) in an effort to prevent spontaneous abortions and promote healthy development (Swan & vom Saal, 2001). In humans, adverse effects of DES exposure were documented for the women as well their offspring (Giusti, Iwamoto, & Hatch, 1995; Greenberg et al., 1984). Most relevant with respect to temperament, adult offspring exposed to DES during most of gestation were more likely to experience symptoms of depression and anxiety, along with other psychiatric concerns, compared to those not exposed *in utero* (Vessey, Fairweather, Norman-Smith, & Buckley, 1983).

PCBs are the most common among endocrine disruptors (Casati, Sendra, Sabilia, & Celotti, 2015) because of their extensive use in dielectric and coolant fluids, no longer produced due to their toxicity and persistence. Chronic exposure to low levels of PCBs is of particular

concern given evidence of cumulative endocrine, metabolic and behavioral effects (Casati et al., 2015; Colciago et al., 2009; Stewart et al., 2008). Disruption of neurodevelopment for prenatally exposed offspring was associated with manifestations relevant to temperament: decreased novelty preference in infancy (Boucher, Bramoullé, Djebbari, & Fortin, 2014) and higher ADHD problems for school-age children (Sagiv et al., 2010).

Although most often examined in the context of physical development and cognitive functioning, associations with behavioral changes reflective of temperament were also noted for all of the considered contaminants.

2. Animal studies: The animal literature speaks to similar links between toxicant exposures and behavioral/temperament outcomes, indicating that prenatal effects can be transgenerational in nature. BPA exposure induced largely sexually dimorphic behavioral changes in young adult mice (Table 3). Chasing behavior in males was reduced to levels similar to females. Anxiety-like behaviors in an open field increased in females, decreasing in males, with elevations in aggression noted for both sexes as a result of prenatal BPA exposure (Kundakovic et al., 2013).

André and Markowski (2006) examined learning behavior following perinatal exposure to vinclozolin, an endocrine disruptor with antiandrogenic effects. Acquisition of a reinforced response was not affected. However, males exposed to vinclozolin perinatally did not demonstrate any response extinction once food was no longer available, suggesting an alteration in sensitivity to reward/approach. In addition, Colbert et al. (2005) demonstrated an effect of perinatal vinclozolin exposure on social behaviors. Males exposed during gestation and lactation demonstrated an increase in social play marked by more frequent initiation behavior, interpreted as a sign of hyperactivity and developmental delay.

Behavioral consequences of DES exposure were characterized in animal studies investigating developmental effects, also using this compound as a positive control. (Palanza, Parmigiani, Liu, & vom Saal, 1999) reported that prenatal exposure to DDT and DES increased the frequency of same-sex aggression for male and female mice, at low doses only. There was a difference in DDT and DES effects, as males exposed to the lowest DDT dose only exhibited less intense attacks than controls.

PCB exposure in rodents has been largely studied perinatally, with animals affected during lactation as well as gestation, because this timeframe corresponds to a critical brain sex differentiation period (Colciago et al., 2009). Perinatal PCB exposure was shown to result in a dimorphic pattern of hypothalamic testosterone activating enzyme expression. A learning deficit on a passive avoidance test was also noted, as exposed males failed to develop an increased latency to enter an area associated with a previously delivered foot shock (Colciago et al., 2009). This apparent decrease in sensitivity to cues of danger represents an aspect of fearful reactivity and reflects PCB effects on temperament development.

Prenatal endocrine disruptor exposure resulted in temperament development alterations, including increased anxiety-like and playful/aggressive behavior. There is also indication of sex differences. Learning paradigms are not directly applicable to temperament; however,

sensitivity to cues of reward vs. punishment can be a function of relative approach and avoidance activation. Both neurobehavioral systems appear compromised by prenatal exposure to androgen disruptors. Before we consider epigenetic mediation of environmental toxicant *in utero* effects, other contaminant exposures should be noted.

b. Lead and Mercury Prenatal Exposure: Developmental Effects

1. Human studies: In humans, prenatal exposure effects of lead have been demonstrated into adolescence, with more frequent/severe behavior problems, delinquent and antisocial behaviors, considering multiple covariates (Dietrich, Ris, Succop, Berger, & Bornschein, 2001). Sioen et al. (2013) reported a significant positive association between prenatal lead exposure and hyperactivity for 7–8 year-old children, controlling for other contaminants. Prenatal lead exposure effects included increased activity and deficits in focused attention at 12 months, as well as greater behavioral difficulties in the toddler period (Vermeir & Viaene, 2002–2007). Lead exposure was associated with compound effects, acting in concert with prenatal substance use. Specifically, the risk for ADHD increased for children prenatally exposed to nicotine along with lead (Froehlich et al., 2009).

Thus, *in utero* lead exposure in humans has been well characterized in terms of contributing to impulsivity/approach aspects of temperament and difficulties with attention/regulation.

2. Animal studies: Animal studies offer more conclusive links, examining prenatal mercury as well as other contaminant exposures, noting potential sex differences and potency of low-dose exposures. Perinatal methylmercury (derived from mercury) exposure has been studied primarily in tandem with PCB, as these contaminants are typically traced to the same source (Newland & Paletz, 2000). Learning-related effects of prenatal exposure have been the main focus of research, with some findings relevant to temperament (Table 3). Specifically, prenatal mercury exposure in rats resulted in elevated rates of responding in an operant learning procedure, even when selenium intended to ameliorate adverse effects of methylmercury was administered to the animals. High rates of responding during reinforcement omission trials indicated prenatal mercury exposure enhanced the efficacy of reinforcement (Reed & Newland, 2007), potentially leading to manifestations of impulsivity and/or reward dependency relevant to temperament. Male, but not female, mice demonstrated decreased spontaneous motor activity, yet increased amphetamine-induced motor activity, and altered dopamine levels. With respect to prenatal lead exposure, related sequelae were consistently more notable in animals assigned to the low-dose condition (Leasure et al., 2008).

The range of prenatal contaminant effects is extensive, with a number of behavioral consequences relevant to temperament. Animal studies demonstrate fearful/anxious behaviors, disruptions of social behavior and reinforcement learning in exposed animals, with human research showing an increased risk for ADHD and behavior problems more broadly (Dietrich et al., 2001; Kundakovic et al., 2013; Piedrafita, Erceg, Cauli, & Felipo, 2008). This pattern of results implicates reactivity (e.g., reward/approach orientation vs. fearfulness/behavioral inhibition) and regulation (i.e., deficits in emotional/behavioral control), indicating temperament is impacted by maternal contact with toxicants during

gestation. There is evidence that male offspring may be more affected (Andre & Markowski, 2006; Colciago et al., 2009), and lower concentrations of contaminants, as opposed to higher levels of exposure, may be more detrimental in some instances (Leasure et al., 2008; Palanza, Parmigiani, & vom Saal, 2001). Importantly, critical shifts in the epigenome were shown to occur as a function of prenatal environmental contaminant exposure.

c. Environmental Contaminants: Epigenetic Effects

1. Human studies: A recent review considered 23 studies linking environmental contaminant exposure and offspring DNA methylation changes to Autism Spectrum Disorders (Keil & Lein, 2016). Consistent with the environmental epigenetics framework, methylation changes were described as mediating environmental risk and conferring these effects onto behavioral phenotypes via alterations in gene expression and brain development. Shifts in the methylome and brain development that result from *in utero* contaminant exposures and contribute to risk for ASD can also be expected to affect temperament.

2. Animal studies: Animal studies provide additional insights into epigenetics bridging toxicant exposure in the womb and behavioral effects. Studies using BPA and vinclozolin provide evidence of transgenerational inheritance via permanent alterations in the epigenome of the germ line (Anway & Skinner, 2008). Skinner et al. (2008) reported a sexually dimorphic disruption of transcription in the hippocampus and amygdala, and associated behavioral effects. Third generation female rats from the vinclozolin-exposed line exhibited increased anxiety-like behaviors, whereas male rats displayed a decrease. Crews et al. (2007) examined rat mate preference in a task that engaged multiple brain regions including amygdala, hippocampus, cingulate cortex, and anterior hypothalamus. Third generation vinclozolin lineage males were less attractive to females, reflecting a differential mate preference. This study offers evidence of epigenetic transgenerational inheritance with evolutionary implications, as exposure effects translated into methylome shifts, in turn producing changes critical to reproductive success.

Luo et al. (2014) investigated histone modifications as the mediator of lead-related effects in rats, linking prenatal exposure to ADHD-like behaviors. A dose-response relationship was observed, as increased lead exposure resulted in greater locomotor activity - an analogue of human hyperactivity. Histone acetylation was significantly increased in the hippocampus as a result of chronic lead exposure, supporting the role of this epigenetic mechanism as a mediator between toxicant exposure and offspring symptoms. Thus, the Luo et al. (2014) investigation suggests prenatal lead exposure alters a temperamental phenotype associated with developmental psychopathology, namely activity level, via an epigenetic mechanism. Together, these studies are compelling in pointing to epigenetic mechanisms as the primary vehicle of environmental exposure effects, as changes in the epigenome alter gene expression, with subsequent developmental shifts often related to temperament.

Substance Use and Effects of Psychotropic Medication

Turning to the substance use/psychotropic medication literature, again there is evidence that environmental exposures alter the epigenome, disrupting brain development and inducing temperament risk for later psychopathology. Epigenetic processes are just now being studied

in this context, yet maternal substance use during pregnancy is known to markedly impact offspring development, with public health implications prompting consumption warnings.

a. Substance (Alcohol, Tobacco, Marijuana, Cocaine) Use

1. Human studies: The hazards of prenatal substance use exposures are well documented. There is a wealth of human research linking *in utero* exposures and offspring outcomes, including temperament, with some longitudinal studies following affected children into adulthood.

Alcohol passes through placenta, directly affecting the developing fetus and impacting placental functioning (e.g., Ahluwalia et al., 2000). Fetal brain development effects are considerable, evidenced by high rates of intellectual disabilities associated with maternal alcohol use during pregnancy (1–5% of live births each year; US Centers for Disease Control and Prevention, 2015). The range of effects caused by prenatal alcohol exposure in totality is referred to as Fetal Alcohol Spectrum Disorders (FASD), associated with deficits in intelligence and adaptive functioning, memory, language, attention/executive functions, and motor skills (e.g., Mattson & Riley, 1998). Most relevant for this review, prenatal alcohol exposure was linked with temperament development (Table 4). Haley, Handmaker, and Lowe (2006) evaluated infant HPA-axis response and heart rate indicators in the context of a social stressor – a Still Face paradigm. Percent of prenatal drinking days from conception to pregnancy recognition (extending several weeks into gastrulation) was related to increased infant cortisol reactivity, heart rate, and negative affect, controlling for maternal depression and income. In a subsequent study, maternal weekly binge drinking (5 drinks) during first 6 weeks of pregnancy predicted infant difficult temperament and sleeping problems, controlling for confounds (Alvik, Torgersen, Aalen, & Lindemann, 2011). Prenatally alcohol-exposed infants also exhibited increased emotional withdrawal and lower activity level, especially those later diagnosed with FASD (Molteno, Jacobson, Carter, Dodge, & Jacobson, 2014).

Tobacco use during pregnancy was also implicated in adverse offspring temperament outcomes. Holz et al. (2014) performed functional magnetic resonance imaging (fMRI), examining novelty seeking and lifetime ADHD symptoms for adults prenatally exposed to tobacco. Participants with a history of *in utero* exposure exhibited a weaker response in the anterior cingulate cortex to a conflict task engaging the executive attention system, coupled with novelty seeking/approach tendencies. Liu et al. (2011) also studied adults prenatally exposed to maternal tobacco use and demonstrated increased anger proneness.

The main psychoactive component of cannabis, Δ^9 -tetrahydrocannabinol (THC), is able to cross the placenta during gestation (Hutchings, Gamagaris, Miller, & Fico, 1989). According to a recent review, chronic/heavy cannabis use during pregnancy appears to disrupt brain maturation, predisposing the offspring to neurodevelopmental disorders (Alpar, Di Marzo, & Harkany, 2016). Six and 10 year-old children prenatally exposed to cannabis presented with more frequent/severe externalizing symptoms, impulsivity and hyperactivity in particular (Goldschmidt, Day, & Richardson, 2000). Chronic maternal marijuana use in the first and third trimesters was associated with offspring depression at 10 years-of-age (K. A. Gray, Day, Leech, & Richardson, 2005). Sex differences were noted, as prenatally exposed girls

(but not boys) exhibited higher aggression and inattention at 18 months of age (El Marroun et al., 2011).

Child behavior problems were examined in the aftermath of prenatal cocaine exposure, with mixed results. Several studies reported significant associations between prenatal cocaine exposure and child behavior problems (Bada et al., 2007; Richardson, Goldschmidt, & Willford, 2009), yet others did not find direct links (Delaney-Black et al., 2004; Eiden, Granger, Schuetze, & Veira, 2011).

Thus, prenatal alcohol exposure clearly disrupts neurodevelopment in humans. Nicotine and marijuana programming effects have also been described. Finally, somewhat equivocal findings were reported with respect to maternal cocaine use during pregnancy.

2. Animal studies: Animal studies inform this literature, suggesting similar differences in the level of developmental impact for the substances examined. Prenatal alcohol effects have been widely studied via animal models, intended to serve as analogues for FASD (Table 4). Atalar, Uzbay, and Karakas (2016) demonstrated ADHD-like behaviors in rats following prenatal alcohol exposure throughout gestation. Exposed pups required more sessions to reach criterion relative to the control group on a reversal learning task, because of difficulty inhibiting a previously reinforced response in favor of an unreinforced/inhibited response. Moderate levels of prenatal exposure across pregnancy achieved through voluntary consumption of ethanol resulted in offspring play behavior changes (e.g., increased wrestling) reflective of aggression (Hamilton et al., 2014).

Increased anxiety-like behavior in rodents was demonstrated across multiple studies considering effects of prenatal ethanol exposure. High ethanol doses (delivered in vapor) during the rat equivalent to the human 3^d trimester of pregnancy resulted in anxiety-like behavior (Baculis, Diaz, & Valenzuela, 2015). Moreover, basolateral amygdala, glutamatergic and GABAergic synaptic transmission was implicated in the observed behavioral alteration (Baculis et al., 2015). Maternal alcohol treatment limited to very early stages of pregnancy (equivalent to the 3^d week post-fertilization in humans) also disrupted brain development (Godin, Poizat, Hickey, Maschat, & Humbert, 2010) and HPA-axis functioning in mice, increasing anxiety-related behaviors (Wieczorek, Fish, O'Leary-Moore, Parnell, & Sulik, 2015). Sex differences were noted: males showed elevated corticosterone following an alcohol injection, whereas females displayed a blunted adrenocorticotrophic response. Anxiety-like behavior declined in exposed female mice, whereas males demonstrated heightened anxiety marked by decreased exploration (Wieczorek et al., 2015). Altering the timing of alcohol administration resulted in different brain malformations and variable patterns of behavioral disruption (Murawski, Moore, Thomas, & Riley, 2015). Importantly, animal models suggest that even moderate levels of prenatal exposure lead to behavioral changes that parallel human symptomatology (Valenzuela, Morton, Diaz, & Topper, 2012).

In utero exposure to nicotine produced deleterious behavioral effects in rats. Exposed males showed increased hyperactivity (more frequent entries) and impulsivity (increased anticipatory responses; (Schneider, Bizarro, Asherson, & Stolerman, 2012). Increased

responding and larger doses of self-administered methamphetamine were interpreted as signs of neurobehavioral changes in the exposed offspring' motivational systems linked with reward and vulnerability to substance use (Lacy, Morgan, & Harrod, 2014). Nicotine exposure resulted in anxiety-like and impulsive behaviors, reducing exploration and increasing instant reward choices (Lee, Chung, & Noh, 2016).

Several studies provide evidence of increased hyperactivity and altered emotionality following prenatal cannabinoid exposure, consistent with human research. Exposed rat pups emitted more frequent ultrasonic vocalizations (USVs) indicative of distress and anxiety when removed from the nest (Trezza, Cuomo, & Vanderschuren, 2008). Adult animals also demonstrated anxiety-like behavior, spending more time in the closed arms of the maze, compared to controls (Trezza et al., 2008). Prenatal cannabinoid exposure is thought to alter offspring emotional functioning via serotonergic and dopaminergic systems (Trezza et al., 2012).

Zeskind & Stephens (2004) examined the impact on prenatal cocaine exposure on distress vocalizations in human infants and rat pups. A shift in the acoustic structure of rodent USVs subsequent to *in utero* cocaine exposure paralleled hyperphonation (high-pitched cry sound) observed in humans. Prenatal cocaine exposure effects on social interactions and vocalizations were studied in mice (Kabir, Kennedy, Katzman, Lahvis, & Kosofsky, 2014). Exposed mice emitted more frequent USVs and demonstrated increased interactions with other animals during initial evaluations. These effects were not observed during follow-up testing, thus, may not be long-term (Kabir et al., 2014). Kabir, Katzman, and Kosofsky (2013) evaluated the impact of prenatal cocaine exposure on rodent recall of extinguished cue-conditioned fear using foot shock and observed significant increases in freezing for prenatally exposed mice. According to Kabir et al. (2013), prenatal cocaine exposure could increase the risk for PTSD by disrupting fear cue learning.

These animal studies compliment the human literature, indicating profound neurodevelopmental effects as a result of *in utero* exposure to alcohol. Less prominent disruptions were noted for prenatal cocaine administration, nonetheless temperament alterations increasing the risk for psychopathology were observed. Prenatal cannabinoid and nicotine exposures were also implicated in long-term effects on offspring emotional functioning/temperament, potentially signaling life-long mental health risk.

b. Psychotropic Medication/SSRI Use

1. Human studies: Prescribed psychotropic medications, Selective Serotonin Reuptake Inhibitors (SSRIs) in particular, have also been linked with adverse offspring developmental effects when administered during pregnancy. Prenatal SSRI exposure has been linked with multiple fetal, neonatal and infant behavioral disturbances (Table 4). For example, SSRI-exposed infants displayed decreased physiological and behavioral responses relevant to negative emotionality in the context of a typical neonatal painful event (Oberlander et al., 2002). Altered neonatal cortisol levels were also reported (Pawluski, Brain, Underhill, Hammond, & Oberlander, 2012), controlling for maternal symptoms. In light of these observations, Gur, Kim, and Epperson (2013) suggested that prenatal SSRI exposure could buffers infants from increased HPA reactivity.

Temperament in SSRI-exposed and non-exposed children was compared. Nulman et al. (1997) did not identify significant differences in temperament for children of mothers using SSRI medication during pregnancy (ages 16 to 86 months) and those not exposed. However, temperament was only assessed directly among children younger than 24 months, with behavior problems examined for older participants. Nulman et al. (2002) again failed to identify differences with a very small sample ($n=18$) of SSRI-exposed children, using similarly limited measures of temperament.

2. Animal studies: SSRI exposure has also been evaluated in animals, often with perinatal/neonatal administration (Table 4). Rodent studies demonstrate life-long biobehavioral alterations following perinatal exposure, including disruption of the serotonin signaling pathway development (Maciag et al., 2006) and increased depression-like behavior in adulthood (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Olivier et al., 2011).

Olivier et al. (2011) examined SSRI exposure from mid-gestation till birth. Prenatally exposed animals responded with increased anxiety, demonstrating greater latency to begin feeding in a novel setting and increased avoidance in a conditioned aversion test. Juvenile SSRI treated animals engaged in fewer social play behaviors. Svirsky, Levy, and Avitsur (2016) also noted prenatal SSRI exposure effects on social behavior, with increased aggression for adult males, but not females. Smit-Rigter et al. (2012) provided evidence of a potential mechanism for serotonergic developmental effects, as SSRI exposure influenced dendritic complexity in the cortex, specifically 5-HT receptors linked with anxiety.

McAllister, Kiryanova, and Dyck (2012) reported a decrease in anxiety-like behavior, as perinatally SSRI exposed mice spent less time in the closed arms in the elevated plus maze. Depression-like behavior, reflected in longer latencies to immobility in the forced swim test, declined as a result as exposure as well. However, perinatal SSRI exposure was also associated with increased immobility (Lisboa, Oliveira, Costa, Venancio, & Moreira, 2007). These discrepant results could be attributed to the SSRI delivery differences. SSRIs were administered orally in the McAllister et al. (2012) study and expected to reach the offspring in relatively lower concentrations.

Thus, the totality of human and animal research addressing *in utero* exposure to maternal substance use indicates considerable impact on offspring emotional/behavioral functioning relevant to temperament. Emotional withdrawal and lower activity level in the context of prenatal alcohol exposure (Molteno et al., 2014), as well as novelty seeking and ADHD symptoms for those prenatally exposed to tobacco (Holz et al., 2014), have temperament implications. Cannabinoid exposure during gestation was associated with adverse behavioral effects primarily related to the dopaminergic system (Alpar et al., 2016) involved in approach/positive affectivity and regulation/effortful control. Sex differences were noted, with increased aggression in SSRI-exposed males (Svirsky, Levy, & Avitsur, 2016). Exposure effects vary by substance in terms of strength and consistency. Most detrimental outcomes were reported for prenatal alcohol use, followed by nicotine and marijuana, with less consistent effects for *in utero* exposure to cocaine and SSRIs. Additional data concerning the timing of most pronounced prenatal effects is required, yet maternal alcohol use creates adversities for offspring health/development throughout gestation.

c. Prenatal Substance and Psychotropic Medication Use: Epigenetic Effects

1. Human studies: We now consider the extent to which epigenetic processes are responsible for conferring prenatal substance use exposure risk. Consistent with the environmental epigenetics framework, prenatal maternal substance/medication use has been associated with offspring methylation changes. Portales Casamar et al (2016) examined genome-wide DNA methylation patterns, identifying Differentially Methylation Regions (DMR) in buccal cells of children with FASD and typically developing controls. Of 658 DMR, 41 displayed differences in percent methylation change >5 %. Children were between 5 and 18 years of age, thus possible confounding postnatal variables were introduced. Laufer et al. (2015) also identified DMR associated with FASD in a comparative study, assessing methylation patterns in children (3–6 years of age) and mice prenatally exposed to alcohol. A total of 269 DMR were reported, with 21 involved in regulation of clustered protocadherin genes (*Pcdhs*) responsible for synaptic complexity in the developing brain (Kalmady & Venkatasubramanian, 2009). In their review of the literature, (Kobor & Weinberg, 2011) described critical developmental windows for alcohol exposure epigenetic effects: prior to conception on the paternal side and maternal alcohol consumption between fertilization and implantation.

A number of studies considered the role of prenatal tobacco exposure in altering DNA methylation in humans. Tobacco exposure was linked with a decrease in methylation of *Sat2*, one of the repetitive elements commonly used as a proxy for measuring genomic DNA methylation changes (Flom et al., 2011). Genome-wide methylation differences in cord blood cells of prenatally tobacco exposed and non-exposed infants were identified (Ivorra et al., 2015). Significant differences for 31 CpG sites associated with 25 genes were observed, adjusting for multiple comparisons. Ladd-Acosta et al. (2016) also identified a methylation signature of prenatal tobacco exposure via a machine learning classification model, reliably differentiating exposed and non-exposed groups. Methylation specific to 26 sites was described as a potential biomarker of *in utero* tobacco exposure. Ivorra et al. (2015) concluded that DNA methylation status at birth could serve as a marker of risk associated with prenatal environmental adversity. However, future studies are required to discern clinical significance of these epigenetic findings, as methylation changes identified in humans thus far have not been linked with behavioral alterations.

DNA methylation differences between prenatally exposed to SSRIs and non-exposed neonates were recently reported. In a genome-wide investigation, Gurnot et al. (2015) identified *CYP2E1*, the gene encoding an ethanol-metabolizing enzyme by the same name, as providing the greatest discrimination between SSRI-exposed and non-exposed neonates. The role of prenatal SSRI exposure and *SLC6A4* promoter methylation status in shaping soothability, an early aspect of temperamental regulation, was examined (Gartstein, Hookenson, et al., 2016). For SSRI-exposed infants only, more advanced development of soothability was observed with higher *SLC6A4* methylation levels.

2. Animal studies: Animal studies inform regarding epigenetic mechanisms as bridges between *in utero* substance use-exposure and offspring development, especially with respect to epigenetic shifts impacting gene expression in the brain. Rodent models developed to

understand the etiology of FASD in humans enable links between analogue phenotypes to epigenetic mechanisms. Immediate and long-lasting alterations of DNA methylation and miRNAs, crucial for synaptic plasticity and behavioral/emotional functioning, have been reported. Marjonen et al. (2015) noted shifts in offspring DNA methylation, gene expression, and brain structure following chronic moderate ethanol exposure in early gestation, corresponding to the first three-four weeks of human pregnancy. Gene expression analyses conducted with mouse hippocampal tissue of ethanol-exposed adolescent offspring revealed altered expression of 23 genes and three miRNAs. However, no connections were made between gene expression changes and brain structure/function changes.

Methylation of the insulin-like growth factor II (IGF-II), an imprinted gene with a role in memory consolidation and enhancement, was examined in the context of prenatal cocaine exposure with mice (Zhao et al., 2015). Offspring of mothers administered cocaine during pregnancy demonstrated increased anxiety, along with impaired memory. Hippocampal IGF-II mRNA and protein expressions were significantly decreased, likely contributing to the phenotypic changes (Zhao et al., 2015). Prenatal administration of cocaine to mice was also shown to alter genome-wide DNA methylation in the offspring hippocampal tissue (Novikova et al., 2008). Exposed and non-exposed mice were differentiated by methylation of 492 CpG islands, a number associated with repetitive elements (e.g., LINE, Alu, etc.; (Novikova et al., 2008).

Epigenetic alterations associated with prenatal cannabis exposure have also been considered, albeit not as extensively. DiNieri et al. (2011) examined fetal brain tissue and reported decreased dopamine receptor D2 (DRD2) mRNA expression in the human ventral striatum (nucleus accumbens). DRD2 mRNA levels were negatively associated with maternal report of prenatal cannabis use. Morris, DiNieri, Szutorisz, and Hurd (2011) reviewed contributions of cannabis and tobacco exposures, as these *in utero* effects commonly co-occur in humans. Consistent with the environmental epigenetics perspective, a critical role of epigenetic mechanisms, methylation in particular, was noted in shaping offspring neurodevelopment.

Thus, existing research provides evidence of gene expression changes, modulated via methylation and ncRNA mechanisms sensitive to prenatal substance use. The extent of epigenetic alterations may be related to the level of developmental disruption, with alcohol exposure expected to trigger greater gene expression changes. Multiple downstream behavioral effects relevant to temperament have been noted. However, in humans, connections between prenatal substance use exposure, epigenetic changes, and alterations in temperament development, have not been made in a convincing manner.

Nutrition: Under-nutrition, Over-nutrition, and Micronutrients

1. Human studies—These links were somewhat better established in the context of studies addressing prenatal nutritional exposures, in part because of an opportunity for a natural experiment created by a World War II (WWII) humanitarian disaster. Effects of gestational under-nutrition in humans have been studied most extensively following individuals who were prenatally exposed to famine during the Dutch Hunger Winter (1944–1945): a period of severe food shortage in the western region of the Netherlands during the last winter of WWII (Barua & Junaid, 2015). The Dutch Famine Birth Cohort Study is a

population-based investigation with volunteers prenatally affected by under-nutrition (T. Roseboom et al., 2006). *In utero* under-nutrition was predictive of psychopathology, insofar as the role of exposure in affective disorders was documented based on a large-scale archival study (Table 5). Under-nutrition as a result of famine in mid or late gestation resulted in a higher risk for hospitalization for unipolar and bipolar depression, with a stronger effect observed for men (Brown, van Os, Driessens, Hoek, & Susser, 2000). Famine and under-nutrition exposure early in gestation increased the risk for anxiety in adulthood (de Rooij et al., 2011). Males exposed to famine during early gestation were again affected to a greater extent, self-reporting more frequent/severe symptoms of anxiety and depression. This pattern of results is consistent with the evidence indicating males may be more vulnerable to nutritional deprivation during certain periods of fetal development because of faster growth relative to females (Eriksson, Kajantie, Osmond, Thornburg, & Barker, 2010). Other cohorts exposed to under-nutrition and nutritional interventions have been evaluated, however, behavioral/emotional outcomes were not addressed. Interest in fetal programming related to over-nutrition has grown as a result of the “obesity epidemic”. Connections between excessive prenatal nutrition and behavioral/temperament outcomes are starting to emerge.

Prenatal nutrition characterized as “unhealthy” (high in fat) was predictive of multiple emotional/behavioral problems later in childhood (Jacka et al., 2013). Increased maternal intake of unhealthy foods (e.g., processed meat products) during pregnancy predicted child externalizing problems at 5 years of age, controlling for covariates including childhood diet. Most relevant to this review, Gustafsson, Kuzava, Werner, & Monk (2016) reported associations between maternal prenatal dietary fat intake and offspring temperament. Specifically, maternal total fat intake was linked with infant surgency and regulatory capacity at 4-months-of-age, so that higher fat content translated into lower positive affectivity and regulation indicative of temperament risk. Together, human studies indicate temperament alterations and an increased risk for behavior problems/symptoms as a consequence of prenatal exposure to under and over-nutrition.

Prenatal effects of multiple nutrients have been reported for child neurodevelopment (e.g., B vitamins, zinc, protein, iodine, etc. (Georgieff, 2007) and can be expected to influence temperament as a result. For example, Hernández-Martínez et al. (2011) reported that 1st and 2nd trimesters iron deficiency predicted neonatal autonomous nervous system functioning, including signs of stress reactivity. In a recent review, poor nutrition during pregnancy was described as contributing to deficits in neurocognitive development (e.g., sensory–perceptual skills, memory, learning), especially in the context of maternal distress (Monk, Georgieff, & Osterholm, 2013).

2. Animal studies—Connections between unhealthy *in utero* nutrition and subsequent adverse behavioral/temperament outcomes for the exposed offspring are further supported by animal research. A variety of animal models have been utilized to examine fetal programming associated with nutritional factors (Table 5), as these allow precisely timed diet manipulations inducing nutritional accesses and deficits. Levay et al. (2008) examined effects of moderate maternal calorie restriction in preconception and the perinatal period on adult offspring anxiety-like behaviors. Preconception deprivation (50% caloric intake for 3 days) exerted the strongest effect with respect to most offspring anxiety-like behavior (e.g.,

less exploration in the open field). Non-human primates exposed to maternal under-nutrition during gestation demonstrated greater variability in activity level and less emotional arousal (Keenan et al., 2013). Females, but not males, showed lower levels of attention/persistence, ascertained based on computerized task engagement and performance. Keenan et al. (2013) concluded that prenatal diet interventions could be effective in preventing common behavior problems in children.

Preconceptional/perinatal maternal high-fat diet effects on anxiety-related behaviors, as well as expression of GABAergic, serotonergic, and brain-derived neurotrophic factor (BDNF) receptors involved in anxiety and depression, were examined in mice. Offspring in the high-fat diet condition exhibited increased anxiety-like behaviors, with expression alterations noted in the hippocampus (i.e., increased BDNF and GABA receptors; (Peleg-Raibstein, Luca, & Wolfrum, 2012). Adult offspring exposed to perinatal high-fat diet showed increased expression of corticosterone receptors in the amygdala and inflammatory gene expression in the hippocampus and amygdala. Importantly, these changes were linked with increased anxiety behavior (e.g., less time in the open field; (Sasaki, de Vega, St-Cyr, Pan, & McGowan, 2013). Males subjected to a high-fat diet through gestation and lactation demonstrated increased corticosterone serum levels. Anxiety-related effects were mixed, with offspring from pre-gestation dietary intervention group (mothers returned to a healthy diet) showing partial recovery (Rodriguez et al., 2012). Substance-use related behavioral effects of prenatal high-fat exposure have been documented in rats (Karatayev et al., 2015). Exposed offspring engaged in more extensive nicotine and ethanol self-administration, relevant to temperament because of associated impulsivity/behavioral under-control. This connection between prenatal high-fat diet exposure and offspring deficits in regulation was further supported by a recent review. Sullivan, Nousen, and Chamlou (2014) noted that primary offspring outcomes of perinatal high-fat diet programming involved behavioral/emotional dysregulation, further suggesting serotonergic system suppression as the likely mechanism.

The impact of a calorie-dense perinatal diet (high in saturated fat, refined sugar and cholesterol) on the development of piglets was examined (Clouard, Gerrits, Kemp, Val-Laillet, & Bolhuis, 2016). Prenatal exposure resulted in decreased basal salivary cortisol levels, relative to controls. Animals exposed to the high-fat/sugar/cholesterol diet spent more time walking in their pens, with control group animals inactive longer.

Micronutrient deficiencies have been studied extensively in animals. Iron deficiency during gestation was examined in non-human primates, with exposed offspring demonstrating reduced activity. Ratings of “fearful” affect emerged as the most critical temperament descriptor differentiating prenatally iron-deprived animals from controls (M. S. Golub et al., 2006). In rodents, females exposed to zinc deprivation throughout gestation exhibited higher levels of aggression, relative to females born to typically fed mothers and those exposed to overall under-nutrition (Halas, Reynolds, & Sandstead, 1977). Adult offspring perinatally exposed to vitamin D deficiency engaged in more frequent premature responses on a learning task, relative to control animals, interpreted as impulsivity induced by the deprivation (Turner et al., 2013). Hyper-locomotion in rats following vitamin D deficiency was also reported. Full and late term, but not early gestational deprivation resulted in this

behavioral phenotype (O'Loan et al., 2007). Late gestation was described as the likely 'critical window' for vitamin D deprivation, corresponding to mid-gestation in humans (Clancy, Darlington, & Finlay, 2001). Pan et al. (2014) considered effects of both deficient and excessive perinatal levels of vitamin D, following exposed offspring into adulthood. Exposure to vitamin D deficiency resulted in lower offspring activity and increased anxiety (e.g., fewer open arm entries). Vitamin D excess was also linked with adverse effects, and Pan et al. (2014) suggested high concentrations may be toxic.

Folate-deficiency during gestation was associated with more frequent anxiety-related behavior in the elevated plus maze (Ferguson et al., 2005). At the same time, offspring exposed to high levels of folic acid exhibited increased USVs, anxiety, and hyperactivity-like behaviors (Barua et al., 2014). This pattern of result prompted Barua et al. (2014) to recommend moderation in folic acid supplementation.

Overall, behavioral effects as a result of prenatal under and over-nutrition have been observed across human and animal studies, with some indication of sex differences (de Rooij et al., 2011; Keenan et al., 2013). Both sets of investigations implicated symptoms of anxiety, relevant to temperament as fear/behavioral inhibition represents a prominent risk factor. Moreover, animal studies provide evidence of brain development alterations (e.g., expression of receptors involved in anxiolytic medication effects). Behavioral/emotional dysregulation emerged as another consequence of prenatal nutritional adversity, with temperament-related outcomes implicated in studies addressing prenatal deficiencies/ accesses of micronutrients. Impulsivity and activity level increases were noted, with some indication of a 'critical window' with respect to vitamin D effects. These effects are not surprising, as brain development is altered with under and over-nutrition, in part due to sensitivity to deficits or overabundance of specific nutrients (Georgieff, 2007). Cunha et al. (2015) argued for the "Similarities in the Inequalities" model, as offspring prenatally exposed to both under-nutrition and high-fat conditions demonstrated similar effects (e.g., lower birth weight and decreased activity level), compared to controls. In a recent review (Langley-Evans, 2015) suggested: "a limited number of common mechanisms may link nutritional 'stressors' to development changes that result in later disease". Prenatal nutritional effects are both unique and co-acting with respect to maternal stress and mood, leading to alterations in the offspring dopaminergic and serotonergic systems (Sullivan, Smith, & Grove, 2011). Psychosocial stress is a known contributor to unhealthy eating during pregnancy (Hurley, Caulfield, Sacco, Costigan, & Dipietro, 2005) and can alter the manner in which nutrients are metabolized (e.g., accelerating protein break-down), with further negative consequences for the fetus (Monk et al., 2013). As noted earlier, co-occurrence with additional exposures (e.g., substance use) should be addressed in the future. Connecting these overlapping environment exposures to their epigenetic signatures will be important going forward.

3. Prenatal Nutritional Exposure in Humans and Animals: Epigenetic

Mechanisms—The role of epigenetic processes in conferring effects of prenatal nutrition are already being addressed and will be discussed next. Prenatal under-nutrition exposure research in humans provides further support for the environmental epigenetics framework. Results indicate a variety of epigenetic changes, in some cases linked with disease related

phenotypes, but not behavior/temperament (Heijmans et al., 2008; Tobi et al., 2014). Periconceptional exposure to famine during the Dutch Hunger Winter was associated with a lifelong methylation signature linked with physiological phenotypes. For example, methylation of DMR associated with the INSR (physical growth and insulin signaling) gene was positively correlated with birth weight. CPT1A (fatty acid oxidation) gene methylation was positively correlated with LDL cholesterol levels, after adjusting for multiple covariates (e.g., age, smoking; (Tobi et al., 2014). One study with humans utilized a genome-wide approach, demonstrating that micronutrient supplementation was associated with decreased methylation, interpreted as important to “programming gene activity” later in life (Khulan et al., 2012). Unfortunately, behavioral outcomes relevant to temperament were not examined.

In a mouse model, perinatal exposure to a high-fat diet was linked with DNA hypomethylation along with alterations in dopamine and opioid-related gene expression (Vucetic, Kimmel, Totoki, Hollenbeck, & Reyes, 2010). Micronutrient deficits can also influence gene expression, as perinatal iron deficiency was associated with altered mRNA expression in the brain (Clardy et al., 2006). Konycheva et al. (2011) examined prenatal methyl donor deficiency effects on offspring hippocampal methylation of candidate genes involved in glucocorticoid metabolism and anxiety-related behaviors. Exposed animals demonstrated increased anxiety. However, methylation of candidate genes was not associated with any of these behavioral changes, suggesting the need for a genome-wide strategy. Non-human primates prenatally exposed to a chronic maternal high-fat diet evidenced epigenetically driven serotonin signaling pathway alterations linked with fearful/anxious behavior (Sullivan et al., 2010). Sex differences were noted in responses to threatening novel objects, as female high-fat exposed offspring exhibited increased anxiety, and males demonstrated higher levels of aggression. Overall 78% of animals born to mothers subjected to a chronic high-fat diet exhibited disordered behavior. Consistent with the environmental epigenetics perspective, deficits and accesses in prenatal nutrition were shown to result in epigenetic shifts for the offspring, yet only a limited number of studies demonstrated links between these alternations and behavioral outcomes.

In summary, the totality of existing studies suggests important links between environmental exposures, epigenetic alterations, and shifts in behavioral outcomes relevant to temperament (e.g., fear/anxiety). However, connections among these levels of analysis have not been made frequently, especially in humans. In addition, questions remain regarding optimal developmental windows and sex differences in programming effects. In terms of stress, human candidate gene studies suggest methylation of NR3C1 and SLC6A4 are critical to conferring prenatal effects for several temperament outcomes (e.g., infant HPA-axis reactivity; (Montirosso et al., 2016; Oberlander et al., 2008; Ostlund et al., 2016). However, enthusiasm for these as potential markers of exposure effects is dampened by the number of other genes implicated in stress-related methylation shifts, as evidenced by a genome-wide approach (Nieratschker et al., 2014). Toxicant studies provide consistent evidence of environmental exposures translating into epigenetic shifts. These alterations of the epigenome affect developmental cascades relevant to temperament phenotypes (Keil & Lein, 2016; Skinner et al., 2008). Among notable substance-use related findings, Zhao et al. (2015) reported transcription alterations in the hippocampus and increased anxiety-like behavior following prenatal cocaine exposure. Existing prenatal nutrition research indicates

connections between methylation status and temperament, as expected based on the environmental epigenetic framework. Nutrition affects maternal health/physiology – a key determinant of the intrauterine milieu, shaping the progression from environmental exposure to changes in offspring epigenome, brain and temperament development.

IV. DNA Methylation and Temperament: Implications for Behavioral/Emotional Health

The course of prenatal development is altered by exposure to maternal stress, contact with toxicants, substance/psychotropic medication use, and nutrition. These environmental factors impact maternal physiology during gestation and program the offspring. As anticipated by the environmental epigenetics perspective, prenatal exposures appear to confer subsequent behavioral phenotypes via epigenetic mechanisms. Brain development is sensitive to multiple exposures during gestation, and this epigenetically driven fetal programming shapes regions/systems relevant to temperament. Even subtle alterations in brain structure/function during fetal development can become magnified over time, producing life-long deficits (Buss et al., 2012). Development of amygdala and hippocampus, relevant to fear/avoidance and other temperament-related behaviors, was disrupted by prenatal stress (Barros, Myers, Van Driesche, & Tzagoloff, 2006), environmental contaminants (Skinner et al., 2008), maternal alcohol use (Baculis et al., 2015), and a high-fat diet (Sasaki et al., 2013). Prenatal stress effects on the synthesis and transport of dopamine, as well as relevant receptors, were also noted (Antonelli et al., 2016). Development of the dopaminergic system can be disrupted by prenatal lead and cannabinoid exposure, as well as maternal nutritional state (DiNieri et al., 2011; Leasure et al., 2008; Sullivan, Nousen, & Chamlou, 2014). The dopaminergic system plays multiple roles relevant to temperament as it functions to prioritize stimuli or responses, facilitating selection of alternatives in either perceptual-cognitive or motor-behavioral context (Trofimova & Robbins, 2016). The serotonin signaling pathway also pertains to temperament given links to depression and anxiety associated with negative emotionality. Not surprisingly, serotonergic effects were most pronounced in studies examining *in utero* SSRI exposure (Smit-Rigter et al., 2012). Changes in serotonin signaling were also observed in the context of prenatal restraint stress (Miyagawa, Tsuji, Fujimori, Saito, & Takeda, 2011). Compound effects of maternal diet, stress, and mood, as well as sleep and exercise can be expected to shape offspring serotonergic and dopaminergic neurotransmitter systems (Sullivan et al., 2011), thus should be explored in future research. Overall, epigenetically mediated prenatal exposure effects on brain development have considerable implications for temperament, especially stress reactivity, fear/anxiety, impulsivity, and attention/regulation.

Epigenetic mechanisms have been linked with behavioral phenotypes relevant to temperament by multiple investigations. In one such study, genome-wide DNA methylation and mRNA expression analyses were conducted with primate amygdala tissue collected from juveniles exhibiting a fearful temperament (freezing, increased levels of cortisol; (Kalin & Shelton, 2003). Transcriptome analyses distinguished 22 genes with active expression changes, of which two glutamate receptor genes (GRIN1 and GRM5) play a role in fear and anxiety-like behaviors. NR3C1 and SLC6A4 methylation has been linked with

infant duration of orienting, approach, soothability and fear, measured via parent-report (Gartstein, Hookenson, et al., 2016; Montirosso et al., 2016; Ostlund et al., 2016). Methylation of imprinted genes was also associated with infant temperament: higher methylation of MEG3-IG linked with greater surgency and of PEG3 with higher levels of negative emotionality and externalizing problems (Fuemmeler et al., 2016). In adulthood, DNA methylation of OXT (oxytocin structural gene) was associated with sociability and greater superior temporal sulcus activity during social-cognitive functional MRI tasks (Haas et al., 2016). Existing evidence points to the importance of epigenetic processes as mediators of prenatal effects, conferring environmental risk onto temperament-related phenotypes. As indicated by the environmental epigenetics perspective, a variety of environmental adversities impact maternal health/physiology, resulting in a cascade of epigenetic changes that shape CNS maturation and temperament in turn, often translating into similar phenotypes across exposures.

Prenatal exposure to multiple adversities results in temperament profiles marked by high levels of fear/behavioral inhibition and risk for anxiety, with these effects mediated by epigenetic processes. On the animal side, exposure to prenatal stress resulted in a number of offspring “fearful” behaviors, such as higher levels of inhibition in response to foot shock, decreased time spent in the center of an open field and in open arms of the elevated-plus maze, increased defensive withdrawal and conditioned fear (Dickerson, Lally, Gunnell, Birkle, & Salm, 2005; Griffin & Evans, 2003; Ward, Johnson, Salm, & Birkle, 2000). Epigenetic mechanisms were implicated in the risk for increased fear/anxiety observed in the context of prenatal toxicant exposure (e.g., (Skinner et al., 2008) and for humans prenatally exposed to under-nutrition (de Rooij et al., 2011).

With respect to depression, temperament risk primarily involves increased negative emotionality, along with decreased approach/positive affectivity (Lonigan, Carey, & Finch, 1994; Lonigan et al., 2003). This profile resulted from several reviewed exposures shown to operate via epigenetic processes. Alvik et al. (2011) demonstrated that maternal binge drinking in early pregnancy predicted offspring temperament marked by high negative affect, low regulatory capacity and positive affectivity in infancy, which could bode future depressive symptoms. Prenatal high-fat diet was associated with lower infant surgency (Gustafsson, Kuzava, Werner, & Monk, 2016), which could translate into long-term risk for depression given high negative emotionality. Prenatal stress and maternal high-fat diet were shown to disrupt 5-HT neuronal development (Miyagawa et al., 2011; Peleg-Raibstein et al., 2012), likely contributing to temperament risk for depression. *In utero* exposure to SSRIs was associated with offspring serotonergic effects (Oberlander, 2012), although additional research is required to determine their long-term significance.

Tentative conclusions can be drawn regarding disruptive behaviors/disorders. General maternal anxiety during pregnancy was associated with increased infant surgency, negative affectivity, and poorer self-regulation (van den Heuvel et al., 2015), linked with risk for conduct disturbance. Offspring behavioral/emotional dysregulation reported following prenatal trauma-related exposures (i.e., trauma occurrence or maternal PTSD; (Yong Ping et al., 2015) is also consistent with temperament risk for disruptive behaviors. Studies that measure offspring ADHD-like behaviors (Atalar, Uzbay, & Karakas, 2016; Holz et al., 2014;

Luo et al., 2014; Sagiv et al., 2010) suggest multiple prenatal toxicant and maternal substance use effects. Importantly, Luo et al. (2014) demonstrated histone modifications mediated prenatal lead exposure effects on ADHD-like behavior in juvenile rats, serving as a bridge between environmental toxicant exposure and offspring symptomatology.

Biologically speaking, phenotypes are considered beneficial to the extent that these increase survival and subsequent reproductive success. These need not be socially desirable or health promoting in the long-term (Frankenhuis, Panchanathan, & Belsky, 2016). Children who experience adverse fetal programming effects likely present with temperament profiles aimed at increasing their immediate probability of survival (e.g., greater reactivity/fearfulness), which may not align with caregivers' and societal demands/expectations. These temperament trajectories speak to long-term risk, increasing the likelihood of developing psychopathology/behavior problems that typically emerge after the first year of life. Alternatively, these phenotypes may be reflective of increased sensitivity to context, with superior outcomes under optimal environmental conditions. Selection pressures may favor differential plasticity when environmental conditions vary across time, with epigenetic mechanisms explaining "bet-hedging" that leads to varying offspring plasticity (Frankenhuis et al., 2016). As DOHaD research has already demonstrated, environmental epigenetic modifications immediately protective in adverse circumstances (e.g., inadequate prenatal nutrition and stress) result in a spectrum of risk promoting developmental shifts relevant to temperament and mental health in the long-term (Hochberg et al., 2011).

V. Conclusions

Considerable evidence points to the importance of *in utero* environmental exposures in shaping offspring temperament development, with epigenetic mechanisms conferring the risk, as indicated by the environmental epigenetics perspective. Greater understanding of these connections is critical for advancing DOHaD research and specifically for elucidating prenatal origins of developmental psychopathology, which could lead to more effective preventative efforts. Additional study is required to make these connections for eventual use of epigenetic information in targeted interventions.

1) Research that starts with environmental adversities and considers epigenetic alterations as well as brain/behavior effects related to temperament has been limited to date. Investigations that link all components of the developmental progression and levels of analysis are needed, especially with humans. Animal studies provide an opportunity to directly manipulate independent variables related to environmental exposure, ensuring internal validity, yet their external validity with respect to human applications is necessarily limited. Thus, efforts with humans are required, translating animal findings in a manner that provides support for generalizability. Future research could focus on links between epigenetic mechanisms and temperament development in high and low-risk populations. Despite a quasi-experimental design, multiple fetal programming pathways described in this review could be examined. For example, recruiting samples of pregnant women from the same community that differ in terms of psychosocial and/or physiological stress could provide comparisons with respect to effects of the HPA-axis activation. It would be possible to examine other environmental exposures, such as healthy vs. unhealthy prenatal nutrition, in a similar fashion. This

approach could elucidate reliable connections between environmental adversity, maternal health, epigenetic mechanisms, and offspring behavioral outcomes with human participants, given appropriate statistical control of covariates (e.g., postpartum variables).

2) Some conclusions can be made about developmental windows associated with stronger effects; however, additional research is required to clarify these across species for different environmental exposures. Overall, earlier stages of prenatal development afford greater malleability of organ structure and function and are more “open” to environmental influences via epigenetic modifications (Crews, 2010). At the same time, a number of reviewed exposure effects occurred later in gestation (e.g., (Yehuda et al., 2005), presumably because this timing coincides with the maturation of brain regions relevant to temperament. Comparative studies including animal and human participants with parallel exposure windows are likely to be especially informative, given divergent sensitive period findings thus far.

3) Interactions among different *in utero* exposure conditions should be examined more closely in future research, as compound effects are likely relevant to temperament. Epigenetic modifications invoked by different prenatal environmental circumstances may have substantial overlap, leading to diverse developmental and health risks. Comparative studies are likely to inform this effort. Multiple exposures can be manipulated with respect to dose, timing, and duration with animals, in a manner than parallels human prenatal effects. For example, comparative research could address joint effects of prenatal stress and inadequate nutrition, as well as maternal polysubstance use.

4) The pattern emerging from existing studies suggests that males may be more vulnerable to physiological and behavioral effects of prenatal environmental exposures (Andre & Markowski, 2006; Colciago et al., 2009; Eriksson et al., 2010; Leasure et al., 2008). Sex differences are generally predicted in the direction of females exhibiting greater anxiety/depression and males presenting with higher aggression/impulsivity, with a number of studies providing support (Nazeri et al., 2015; Sullivan et al., 2010; Svirsky et al., 2016). However, males born to mothers exposed to psychological stress during pregnancy demonstrated more depression-like behavior (Abe et al., 2007) and females increased in aggression following DET/DES and zinc deficiency *in utero* exposure (Halas et al., 1977; Palanza et al., 1999). Interpretation of sex differences is complicated by the fact that a number of studies were conducted with male animals only (e.g., (Abe et al., 2007; Maccari et al., 2003). Nonetheless, existing evidence suggests fetal programming may have sexually dimorphic effects (Baillargeon, Keenan, & Cao, 2012; Hyde, Mezulis, & Abramson, 2008; Skinner et al., 2008). Thus, offspring sex should be considered as a moderator of links between prenatal exposures, epigenetic shifts, and behavioral outcomes.

5) Potential transgenerational effects linked with epigenetic mechanisms addressed in this review (e.g., (Skinner, 2014) highlight the need for additional study of etiology, as well as preventative efforts. It is critical to understand biological/developmental processes involved in the chain of events that starts with prenatal environmental exposures. Subsequent changes in maternal health/physiology, offspring epigenome and gene expression result in temperament profiles that typically bode risk for symptoms/behavior problems. It will be

important to determine if certain exposures result in epigenetic shifts that afford plasticity, rather than risk. Studies that make connections between methylation signatures of prenatal environmental exposures and temperament profiles linked to risk could facilitate screening and preventative interventions. (Szyf, Tang, Hill, & Musci, 2016) noted that DNA methylation could be used as a biomarker for effects of prenatal exposures on individuals' risk and a measure of treatment progress. Thus, screening/assessment applications could be implemented once sufficient empirical evidence is generated. Links between methylation patterns resulting from *in utero* exposures and risky temperament phenotypes represent the first step to enable targeted selection for preventative treatment. A non-invasive bio-marker (e.g., buccal cell methylation signature) could enable this intervention targeting early in infancy, rather than awaiting behavioral testing at a later time-point. Thus, preventative programs could address parenting/parent-child interactions - modifiable contributors to risk for psychopathology, before maladaptive patterns become the norm. Research that identifies connections between exposures, their epigenetic signatures, and behavioral outcomes is needed to support this preventative approach. Temperamental precursors of symptoms/psychopathology offer advantages relative to the study of full-blown disorders, as these temperament risk factors can be reliably measured in the first year of life. Thus, fetal programming/epigenetic investigations with temperament outcomes will not require lengthy longitudinal efforts, although the latter are important as well. Environmental epigenetics can make a substantial contribution to the study of temperament development, informing our understanding of debilitating mental health symptoms/disorders, as well as efforts aimed at their prevention.

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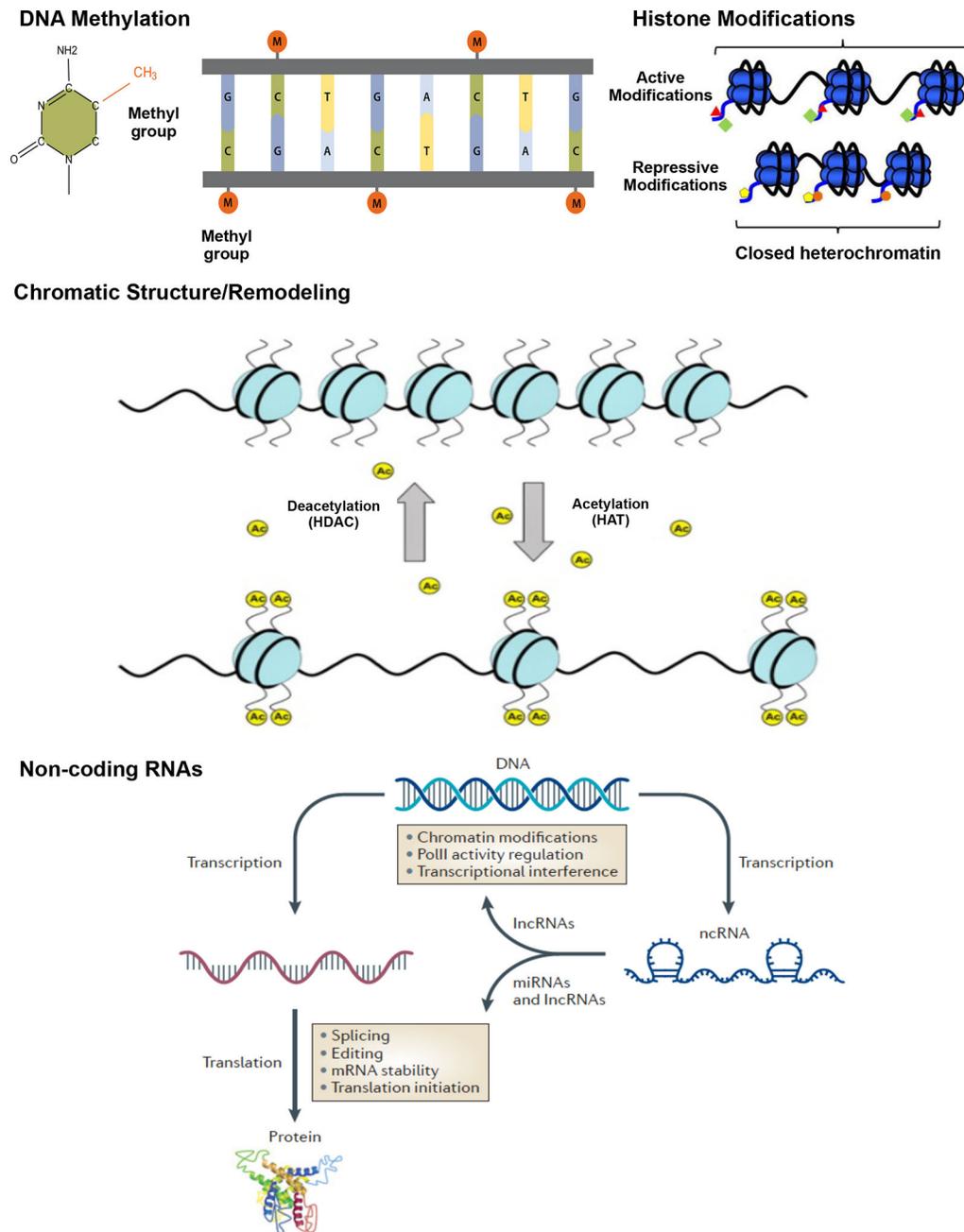


Figure 1. Epigenetic Mechanisms: DNA Methylation, Histone Modification, non-coding RNA. Methylation - DNA Methylation is the addition of a methyl group (M) to the DNA base Cytosine (C) in a CpG sequence. CH₃, the methyl group, is added to a carbon of the cytosine ring via a covalent bond, resulting in 5-hydroxymethyl cytosine. Chromatic Structure/Remodeling – Chromatin structure in heterochromatin states, silencing transcriptional activities, euchromatin HAT catalyzes histone tails leading to active transcription. Histone Modifications - Histone modifications occurs via different enzymes. Active modifications result in permissive effects promoting transcription. Repressive

modification marks serve to halt transcriptional activity in the gene, resulting in closed heterochromatin. Histone deacetylation (HDACs) and acetylation (HAT) represent repressive and permissive modification marks, respectively. Acetylation refers to an introduction of an acetyl functional group (Ac) into a chemical compound, whereas deacetylation reflects the removal of an acetyl group. Non-coding RNAs – Non-coding RNAs perform a variety of functions related to gene expression. Long non-coding RNAs (lnc RNAs) can interfere in transcription and affect chromatin stability, also playing a role in translation. ncRNAs can directly bind to promoters and interfere with polymerase II (PolII), one of three nuclear, DNA-dependent RNA polymerases. Post transcription, Micro RNAs (miRNAs) are able to regulate gene expression by editing, splicing, and affecting stability of mRNA and protein synthesis. This figure is modified from the originals of previous literature (Bagga, 2012; Suter, Takahashi, Grove, & Aagaard, 2013; Wahlestedt, 2013)

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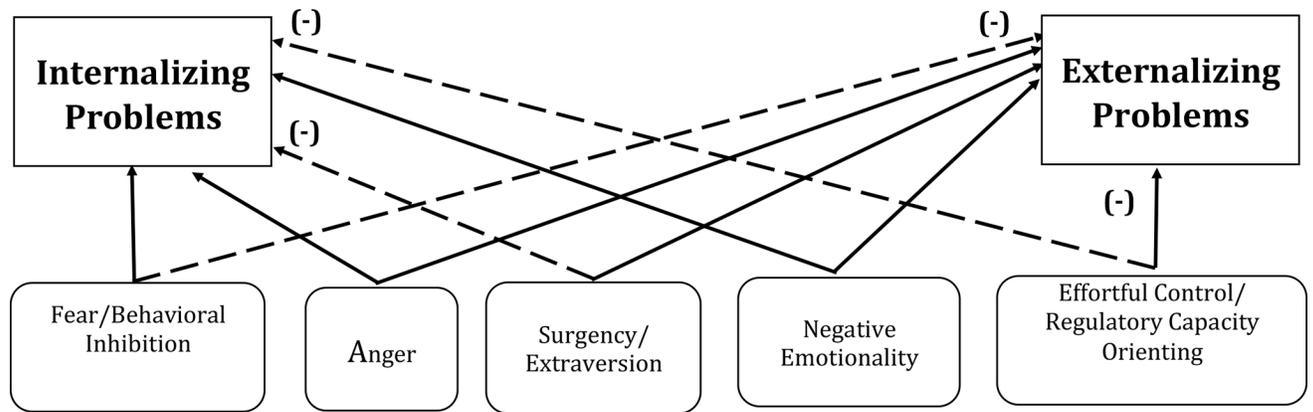


Figure 2. Links between Temperament Attributes and Developmental Psychopathology. The figure depicts most prominent relationships between child temperament traits and broad symptom/behavior problem clusters (internalizing and externalizing). The (-) denotes a negative relationship and (---) denotes a less well established relationship.

Table 1

Maternal Neuroendocrine System Response: Examples of Effects on Offspring Development: Animal and Human Studies. Sample characteristics are provided in a different manner for human and animal studies, due to differences in design and presentation format. The number of participants per condition (n) is indicated for animal experimental investigations, whereas for human correlational studies, the overall number of participants (N) is presented. Additional information concerning numbers of male vs. female participants is also provided when both sexes were included/specified. Developmental timing of exposure indicators varies across studies. For humans, the timing is provided in terms of the number of gestational weeks. In animals these indicators are typically more specific, often presented in terms of the number of days. (↑) denotes increases in offspring physical/structural and functional characteristics and (↓) denotes decreases in offspring physical/structural and functional characteristics.

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(A) Animal Studies				
Predator Exposure				
	↑Egg cortisol concentrations ↑Density of shoaling	n=6–30 fish Measured pre-hatching, in juveniles	Pre-post egg formation: Average 25 days	Giesing et al. (2011)
	↑Fecal cortisol concentrations ↓Body Mass/Length	n=12–14 hares Measured at 30 hours after birth	Late gestation: Last 15 days	Sheriff al. (2009)
	↓Nestling size ↑Longer wing span	n=137–162 (68–92 female) Measured at 2, 8, 14 days	Before and during ovulation: Prior to laying eggs	Coslovsky & Richner (2011)
(B) Human Studies				
Pregnancy anxiety	↓Gray matter volume, prefrontal cortex	N=35 (17 female) Measured at 6 & 9 years	Mid-pregnancy: Measured at 19, 25, 31 weeks	Buss et al. (2010)
	↓Cognitive control/ response inhibition ↓Performance decrement with dual task demands, ↑Alterations in the dorsal prefrontal cortex region activation All effects for 12–20 weeks only	N=49 (20 female) Measured at 17 years	Entire pregnancy: Measured at 12–22, 23–31 and 32–40 weeks	Mennes et al. (2006)

Table 2

Examples of *In Utero* Stress Exposure Effects: Temperament-related Outcomes: Human and Animal Studies. Sample characteristics are provided in a different manner for human and animal studies, due to differences in design and presentation format. For human correlational studies, the overall number of participants (N) is presented, whereas the number of participants per condition (n) is indicated for animal experimental investigations. Additional information concerning numbers of male vs. female participants is provided, when both sexes were included/specified. Developmental timing of exposure indicators vary across studies. For humans, the timing is generally provided in terms of the number of gestational weeks, whereas in animals these indicators are typically more specific, counting embryonic days. The (↑) denotes increases in temperament-related behaviors/displays and/or stress exposure (↓) denotes decreases in temperament-related behaviors/displays and/or stress.

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(A) Human Studies				
Maternal Stress				
911	↓Basal cortisol concentrations, especially with 3 rd trimester exposure	N=38 (male & female) Measured at 12 months	Entire pregnancy: Measured postpartum	Yehuda et al. (2005)
Hurricane Katrina	↑Temperamental difficulty with ↑perinatal PTSD	N=288 (male and female) Measured at 2 and 12 months	Entire pregnancy: Measured at delivery	Tees et al. (2009)
Iowa Floods	↑Cortisol reactivity/separation with ↑maternal prenatal stress	N=94 (48 female) Measure at 29–34 months	Entire pregnancy: Measured within 3 months of delivery	Ping et al. (2015)
Anxiety, Depression	↑Attenuation (flattening) of diurnal cortisol profile with ↑ anxiety at 12–22 weeks	N=58 (29 female) Measured at 14–15 years	Entire pregnancy: Measured at 12–22, 23–32, & 32–40 weeks	van den Bergh et al. (2008)
Emotional Stress, General psychopathology	↓Fearful reactivity to novelty with ↑ emotional stress across pregnancy	N=102 (45 female) Measured at 4 months	Entire pregnancy: Measured at 2 weeks postpartum	Möhler et al. (2006)
Anxiety	↓Regulatory capacity/orienting	N=90 (44 female) Measured at 10 months	Early-mid pregnancy: Measured at 20 weeks	van den Heuvel et al. (2016)
Stress, Anxiety Depression, HPA-axis response	↑Negative emotionality, with ↑ 3d trimester maternal cortisol	N=247 (male and female)	Entire pregnancy: Measured at 18–20, 24–26, and 30–32 weeks	Davis et al. (2007)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
	↑Negative emotionality with ↑ prenatal anxiety/depression scores across pregnancy	Measured at 2 months		
Stress, Anxiety, Depression	↑Temperamental difficulty with prenatal depression, not anxiety	N=107 (male & female) Measured at 3 months	Entire pregnancy: Measured at 28–36 weeks, postpartum	Vedova et al. (2006)
Stress, Anxiety Depression, HPA-axis response	↑Cortisol reactivity/painful event with ↑ maternal cortisol late 2 nd /3d trimester ↓ Behavioral Recovery with ↑ maternal cortisol early 2 nd trimester, ↑stress across pregnancy	N=116 (55 female) Measured at 24 hours after birth	Entire pregnancy: Measures at 15, 19, 25, 31, and 36 weeks	Davis et al. (2011)
Stress, Anxiety, Depression	↑Negative emotionality and fear ↓Falling reactivity/recover from distress	N=282 (126 female) Measured at 6 months	Entire pregnancy: Measured at 14, 24, and 34 weeks	Nolve et al. (2016)
Trauma (lifetime/prenatal), PTSD, depression	↓Behavioral Recovery/social stressor with ↑maternal traumatization (controlling for symptoms)	N=23 (12 females) Measured at 6 months	Majority of pregnancy and the postnatal period: Measured at 6 months	Bosquet Enlow et al. (2009)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
Stress				
Foot shock	↑ Anxiety-like behavior	n=11–28 mice/ condition (5–14 females) Measured at PND9	Mid-gestation: E12	Golub et al. (2016)
Forced swim & observing forced swim	↑Anxiety-like behavior (females)	n=10 rats/ condition (5 females) PND40	Mid/late gestation: E6–16	Nazeri et al. (2015)
Observing foot shock	↑Anxiety/depression-like behavior ↑Basal corticosterone	n=6 rats/ condition (males only) Measured at PND60, 120	Mid/late gestation: E13–20	Abe et al. (2007)
Dexamethasone administration	↑Corticosterone reactivity	n=6–13 monkeys/ condition Measured at 12–14 months	Mid/late-gestation: 22 weeks-birth	deVries et al. (2007)

Abbreviations include (E) - Embryonic day, and (PND) - Postnatal day; (PTSD) - Post Traumatic Stress Disorder.

Table 3

Examples of *In Utero* Toxicant Exposure Effects: Temperament-related Outcomes: Human and Animal Studies. Sample characteristics are provided in a different manner for human and animal studies, due to differences in design and presentation format. For human correlational studies, the overall number of participants (N) is presented, whereas the number of participants per condition (n) is indicated for animal experimental investigations. Additional information concerning numbers of male vs. female participants is provided, when both sexes were included/specified. Developmental timing of exposure indicators vary across studies. For humans, the timing is generally provided in terms of the number of gestational weeks, although in some instances measures are just taken at birth, or not specified beyond a particular trimester or month. For animals, these indicators are more specific, counting embryonic or postnatal days. The (↑) denotes increases in temperament-related behaviors/displays, and (↓) denotes decreases in temperament-related behaviors.

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(A) Human Studies				
BPA				
	↑Emotional reactivity, aggressive (males) ↓Anxiety/depression, aggression (females)	N=198 (111 female) Measured at 3–5 years	Entire pregnancy: Measured at about 34 weeks	Perera et al. (2012)
	↑ADHD at 4 years; stronger effect for males and 3 ^d trimester concentrations	N=438 (206 female) Measured at 1, 4, 7 years	1 st trimester and entire pregnancy: Measured at 12 and 32 weeks	Casas et al. (2015)
DDT				
	↑Total difficulties (emotional, conduct, hyperactivity and peer problems); females only	N=270 (140 female) Measured at 7–8 years	Entire gestation: Measured at birth	Sioen et al. (2013)
DES				
	↑Depression, anxiety	N=660 (315 female) Measured in adulthood	1 st –3 ^d trimesters: Treatment 6–35 weeks	Vessey et al. (1983)
PCB				
	↑ Impulsivity	N=293 (male and female)/n=173 non-exposed; n=40 (lower, middle, and upper exposure groups) Measured at 8, 9.5 years	Entire pregnancy: Measured at birth	Stewart et al. (2005)
	↑ADHD symptoms	N=607 (288 female) Measured at 7–11 years	Entire pregnancy: Measured at birth	Sagiv et al. (2010)
Lead				

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
	↑Anxious/depressed, withdrawn, sleep problems, somatic problems, aggressive and destructive behavior	N=577 (males and females) Measured at 3 years	Entire pregnancy: Measured at birth	Factor-Litvak et al. (1999)
	↑Adolescent delinquent and antisocial behaviors	N=195 (males and female) Measured 15–17 years	Early pregnancy: 1 st trimester	Dietrich et al. (2001)
	↑Hyperactivity, emotional problems (males only)	N=270 (140 female) Measured at 7–8 years	Entire pregnancy: Measured at birth	Sioen et al. (2013)
	↑Activity at 12 months (stronger for males) ↑Inattention, Withdrawal, ↑Total Problems at 24, & 36 months	N=206 (107 females) Measured at 12, 24, & 36 months	Entire gestation: Measured at birth	Vermeir & Viaene (2007)
	↑Disrupted activity level (males only)	n=6–9/condition (males and females) Measured at 1 year	Periconceptional/postnatal: until PND10	Leasure et al. (2008)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
BPA				
	↑Anxiety-like behaviors (females)/↓ males ↓Chasing behavior (males) ↑Aggression	n=16–30 rats/condition (9–14 female) Measured at PND30–40	Entire gestation: administered until birth	Kundakovic et al. (2013)
Vinclozolin				
	↓Response extinction following reinforcement withdrawal (males)	n=14–20 rats/condition (7–10 female) Measured at PND60–80	Perinatal: E14-PND3	André & Markowski (2006)
	↑Immature social play, hyperactivity-like behaviors; effect stronger for males	n=12–22 rats/condition (6–11 female) Measured at PND22, 34	Perinatal: E14-PND3	Colbert et al. (2005)
DES				

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
	↑Aggression	n=16–26 mice/ condition (12–13 female) Measured at PND60– 90	Mid-gestation: E11–17	Palanza et al. (1999)
PCB				
	↑Passive avoidance test deficits (males)	n=10–12 rats/ condition (5–6 female) Measured at PND60	Perinatal: E15–19, PND1–21	Colciago et al. (2009)
Mercury				
	↑Responding during the reinforcement omission trials	n=3–8 rats/condition (males and females) Measured in adulthood	Periconceptional/postnatal: until PND16	Reed et al. (2006)

Abbreviations include: (BPA) - Bisphenol A, (DDT) – Dichlorodiphenyltrichloroethane, (DES) – Diethylstilbestrol, (PCB) - Polychlorinated biphenyls, (ADHD) - Attention Deficit Hyperactivity Disorder, (E) - Embryonic day, and (PND) - Postnatal day.

Table 4

Examples of *In Utero* Substance/Psychotropic Medication Exposure Effects: Temperament-related Outcomes: Human and Animal Studies. Sample characteristics are provided in a different manner for human and animal studies, due to differences in design and presentation format. For human correlational studies, the overall number of participants (N) is typically presented. When indicated, the number of participants in the exposed subsample (n) is also included. The number of participants per condition (n) is always indicated for animal experimental investigations. Additional information concerning numbers of male vs. female participants is provided when both sexes were included/specified. Developmental timing of exposure indicators vary across studies. For humans, the timing is provided in terms of the number of gestational weeks, although in some instances measures are just taken at birth, or not specified beyond a particular trimester or month. In animals these indicators are typically more specific, counting embryonic days or postnatal days. The (↑) denotes increases in temperament-related behaviors/displays, (↓) - denotes decreases in temperament-related behaviors/displays.

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(A) Human Studies				
Alcohol				
	↑ Cortisol reactivity, heart rate, & negative affect	N=55 (21 female) 5–7 months	Entire pregnancy: Measured at about 28 weeks, 6 months postpartum	Haley et al. (2006)
	↑ Difficult temperament	N=1330 (625 female) Measured at 6 months	Early pregnancy (0–6 weeks): Measured at 17 weeks	Alvik et al. (2011)
	↑ Emotional withdrawal, conception exposure only ↓ Activity level, prenatal exposure only	N=144 (67 female)/92 exposed Measured at 6.5 months	Perinatal/postpartum: Measured at about 24 weeks, 1 month postpartum	Molteno et al. (2014)
Nicotine				
	↓ Response in the anterior cingulate cortex during a conflict task engaging executive functions	N=178/38 exposed (104 female) Measured at 25 years	Entire pregnancy: Measured postpartum	Holz et al. (2014)
	↑ Temperamental Anger	N=611/366 exposed (370 female) Measured at 38–48 years	Entire pregnancy: Measured in 3 ^d trimester	Liu et al. (2011)
Cannabis				
	↑ Depression, 1 st and 3 ^d trimester effects	N=633 (317 female) Measured at 10 years	1 st and 3 ^d trimesters: Measured at 3 ^d and 7 th month prenatally	Gray et al. (2005)
	↑ Aggression, females only	N=4077 (2060 female)/88 exposed Measured at 18 months	Early pregnancy: Measured in 1 st trimester	El Marroun et al. (2011)
Cocaine				

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(A) Human Studies				
	↑Behavior problems only for ↑reactive exposed children, with low maternal warmth	N=220 (112 females)/119 exposed Measured at 7, 13, & 18 months	Entire pregnancy: Measured 4–8 weeks postpartum	Eiden et al. (2011)
	↑Hyperactivity, with any prenatal exposure; males only	N=473 (50 females)/204 exposed Measured at 6–7 years	Entire pregnancy: Measured throughout pregnancy & at birth	Delaney- Black et al. (2004)
SSRI				
	↓Motor, social–emotional and adaptive behavior	N=83 (59 females)/31 exposed Measured at 10 months	Entire pregnancy: Measured at 36 weeks	Haley et al. (2013)
	↓Early evening basal cortisol levels	N=76 (42 female)/31 exposed Measured at 3 months	Entire pregnancy: Measured at 2 nd trimester and birth	Oberlander et al. (2008)
	↑Fetal motor activity at 15–19, 27–29 weeks, standard/high doses only	N=263 (136 female)/133 exposed Measured at 15–19, 27– 29, 38 weeks	Entire pregnancy: Measured at 15–19 weeks & birth	Mulder et al. (2011)
	↓Behavioral pain responses ↑Parasympathetic cardiac modulation	N=61 (males and females)/38 exposed Measured at 2 nd day	Entire pregnancy: Measured at 2 nd trimester and birth	Oberlander et al. (2002)
	↑Serum CBG levels, following vaginal delivery only ↑CBG levels linked to ↓Diurnal change in cortisol	N=65 (35 females)/ exposed n=25 Measured at birth	Entire pregnancy: Measured at 2 nd trimester and birth	Pawluski, et al. (2012)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
Alcohol				
	↑ADHD-like behaviors (males)	n=8 rats/ condition (males only) Measured at PND30–PND55	Mid-late gestation: E8–20	Atalar et al. (2016)
	↑ Aggressive play behavior (males)	n=16 rats/ condition (males only) Measured at PND90	Entire gestation: E1–PND1	Hamilton et al (2014)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
	↑ Anxiety-like behavior (males)	n=6 rats/ condition (males only) Measured at PND36–PND50	Late gestation, shortly after birth: 3d trimester equivalent, PND3–5	Baculis et al. (2015)
	↑CORT in restrain stress task (males) ↑ACTH in restrain stress task (females) ↑Anxiety-like behavior (males) ↓Anxiety-like behavior (females)	n=25–34 rats/ condition (12–17 female) Measured at PND60– PND120	Early gestation: E7	Wieczorek et al. (2015)
Nicotine				
	↑Hyperactivity/Impulsivity	n=55–58 (males only) Measured between PND25 and PND50	Entire gestation: 3 weeks before mating till birth	Schneider et al. (2012)
	↑Self-administration of methamphetamine	n=18–19 (10 females) Measured at PND90	Mid-late gestation: E8–E21	Lacy et al. (2014)
	↑Anxiety-like behavior ↑Impulsivity	N=5–6 (about half female) Measured at PND21–22	Perinatal: E15-PND18	Lee et al. (2016)
Cannabis				
	↑USVs/distress (males) ↓Social interaction (males) ↑Anxiety-like behavior (males)	n=12–26 rats/ condition (males only) Measured at PND12, PND35, PND80	Perinatal: E15-PND9	Trezza et al. (2008)
Cocaine				
	↑ Hyperphonation-like quality in USVs	n=34–50 rats/ condition (17–25 female) Measured at PND5	Entire gestation: E1–20	Zeskind et al. (2014)
	↑USVs/distress, PND25 only ↑Social interaction, PND25 & PND35 only	n=18–26 mice/ condition (males only) Measured at PND25, PND35, & PND45	Mid-late gestation: E8–17	Kabir et al. (2014)
	↓Fear extinguishing ↑Freezing response	n=8–9 mice/ condition (males only)	Mid-late gestation: E8–17	Kabir et al. (2013)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
		Measured in adult animals		
	↑Anxiety-like behavior ↓Social play, self-grooming	n=10–12 rats/ condition (males only) Measured at PND28–PND35, PND87– PND177	Mid-late gestation: E11-PND1	Olivier, et al. (2011)
	↑Aggressive behavior (males)	n=20/condition (10 females) Measured at PND28–PND31, PND49–PND52	Entire gestation: E1 until birth	Svirsky et al. (2016)
	↓Anxiety & depression-like behavior (females)	n=16–17/ condition (females only) Measured at PND73–PND83	Perinatal: E15-PND12	McAllister et al. (2012)
	↓Impulsivity (males) ↑Depression-like behavior (females)	n=22–31/ condition (9–14 female) PND30, 70 (females) PND40, 70 (males)	Perinatal: E1-PND 21	Lisboa et al. (2007)

Abbreviations include: (SSRI) - Selective Serotonin Reuptake Inhibitor, (CBG) - corticosteroid-binding globulin, (ADHD) - Attention Deficit Hyperactivity Disorder, (E) - Embryonic day, (PND) - Postnatal day, (CORT) – corticosterone, (ATCH) - adrenocorticotrophic hormone, (USV) - ultrasonic vocalization.

Table 5

Examples of *In Utero* Nutrition Exposure Effects: Temperament-related Outcomes: Human and Animal Studies. Sample characteristics are provided in a different manner for human and animal studies, due to differences in design and presentation format. For human correlational studies, the overall number of participants (N) is typically presented, whereas the number of participants per condition (n) is indicated for animal experimental investigations. Additional information concerning numbers of male vs. female participants is also provided when both sexes were included/specified. Developmental timing of exposure indicators vary across studies. For humans, the timing is provided in terms of the number of gestational weeks. In animals these indicators often refer to whether or not the nutritional exposure was periconceptual, beginning around conception and including the postnatal/lactation period, or starting at pregnancy recognition, with a number of studies providing specific weeks/days. The (↑) denotes increases in temperament-related behaviors/displays, (↓) - denotes decreases in temperament-related behaviors/displays.

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(A) Human Studies				
Under-nutrition				
	↑ Major affective disorder incidence (bipolar & unipolar); 2 nd and 3 ^d trimester effects, stronger for men	N=163,282/41,847 exposed (77,056 female) Measured in adulthood	Entire pregnancy: Measured at 1 st , 2 nd , & 3 ^d trimester estimates from records	Brown et al. (2000)
	↑ Anxiety	N=819 (450 female) Measured at about 58 years	Entire pregnancy: Measured at 1 st , 2 nd , & 3 ^d trimester; estimates from records	de Rooij et al. (2011)
Over-nutrition/High-fat diet				
	↑ Externalizing problems	N=23,020 (male and female) Measured at 1.5, 3, & 5 years	Mid-pregnancy: Measured at 16 to 20 weeks	Jacka et al. (2013)
	↓ Temperamental surgency & regulation	N=48 (24 female) Measured at 4 months	Entire pregnancy: Measured at 13–16, 24–27, and 34–37 weeks	Gustafsson et al. (2016)
Micronutrients				
Iron	↓ Autonomous nervous system functioning, 1 st & 2 nd trimester ↑ Signs of stress	N=216 (109 female) Measured at 48–72 hours postpartum	Entire Pregnancy: Measured at 10–15, 24–27, & 33–34 weeks	Hernández-Martínez et al (2011)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
Under-nutrition				

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
	↑ Anxiety-like behavior (males)	n=5–6 rats/ condition (males only) Measured at PND63–PND70	Periconceptual-Postnatal/lactation	Levay et al. (2009)
	↓Motivation to work for reward ↓Emotional arousal ↑Variable activity level ↓ Persistence/attention (females)	n=7–12 baboons/ condition (3–8 females) Measured at about 3 years	Perinatal: Pregnancy recognition-lactation	Keenan et al. (2013)
Over-nutrition/High fat				
	↑Anxiety-like behavior	n=21–22 mice/ condition (9–10 female) Measured at PND90+	Periconceptual-Postnatal/lactation	Peleg- Reibstein et al. (2012)
	↑Anxiety-like behavior ↓Basal corticosterone & slower return to baseline	n=20–24 rats/ condition (10– 14 females) Measured at PND110	Periconceptual-Postnatal/lactation: 4 weeks prior to mating-PND21	Sasaki et al. (2013)
	↑Gestational corticosterone ↑Anxiety-like behavior	n=6 rats/ condition (males only) Measured at E19, PND80, 110	Periconceptual-Postnatal/lactation	Rodriguez et al. (2012)
	↑Activity level	n=7–8 (female only) piglets/ condition Measured at 4, 5, 6, & 7 weeks	Periconceptual-Postnatal/lactation: 8 weeks prior to conception-8 weeks postpartum	Clouard et al. (2016)
Micronutrients				
Vitamin D	↑Impulsivity	n=12–16 rats/ condition (6–8 female) Measured at 20 weeks	Periconceptual-birth	Turner et al. (2013)
	↑MK-801 induced hyper- locomotion (late and full deprivation only)	n=32 rats (males only) Measured at PND70	Periconceptual-birth: <u>late</u> (conception- birth); <u>full</u> (6 weeks before conception- birth)	O’Loan et al. (2007)
	↑Anxiety (deprivation and excess) PND40 only	n=20–28 rats (10–14 female)	Perinatal: Mating-PND18	Pan et al. (2014)

Exposure	Significant Developmental/ Behavioral Outcome	Sample Characteristics	Developmental Timing of Exposure	Reference
(B) Animal Studies				
	↓Activity (deprivation) PND40 only	Measured at PND35–40, PND100–105		
Iron	↓Spontaneous activity ↓Inhibitory response	n=12–14 monkeys (4–8 females) Measured at 4 months	Prenatal: Entire gestation	Golub et al. (2006)
Folate	↑Anxiety-like behavior	n=40 mice (20 females) Measured at PND63–65, PND68–83	Periconceptional-late gestation: 8 weeks before mating-E18	Ferguson et al. (2005)
Folic Acid	↑USVs/distress ↑Anxiety-like behavior ↑Hyperactivity	N=13–20 (7–9 females) Measured at PND2–6, 2–6 months	Pre and postnatal: Entire gestation – 6 months	Barua et al. (2014)

Abbreviations include: (E) - Embryonic day, and (PND) - Postnatal day, (USV) - ultrasonic vocalization. MK-801 refers to N-methyl-D-aspartate receptor (NMDA-R) antagonist.