Journal of Developmental Origins of Health and Disease (2012), **3**(6), 395–408.

doi:10.1017/S2040174412000426

REVIEW

Behavioral epigenetics and the developmental origins of child mental health disorders

B. M. Lester[1,2,3,4]*, C. J. Marsit[5], E. Conradt[1,4], C. Bromer[6] and J. F. Padbury[3,4]

[1]*Brown Center for the Study of Children at Risk at Women and Infants Hospital of Rhode Island, Warren Alpert Medical School of Brown University, Providence, RI, USA*
[2]*Department of Psychiatry and Human Behavior, Warren Alpert Medical School of Brown University, Providence, RI, USA*
[3]*Department of Pediatrics, Warren Alpen Medical School of Brown University, Providence, RI, USA*
[4]*Department of Pediatrics, Women and Infants Hospital of Rhode Island, Providence, RI, USA*
[5]*Departments of Pharmacology & Toxicology and Community & Family Medicine, Geisel School of Medicine at Dartmouth, Hanover, NH, USA*
[6]*Department of Neuroscience, Brown University, Providence, RI, USA*

Advances in understanding the molecular basis of behavior through epigenetic mechanisms could help explain the developmental origins of child mental health disorders. However, the application of epigenetic principles to the study of human behavior is a relatively new endeavor. In this paper we discuss the '*Developmental Origins of Health and Disease*' including the role of fetal programming. We then review epigenetic principles related to fetal programming and the recent application of epigenetics to behavior. We focus on the neuroendocrine system and develop a simple heuristic stress-related model to illustrate how epigenetic changes in placental genes could predispose the infant to neurobehavioral profiles that interact with postnatal environmental factors potentially leading to mental health disorders. We then discuss from an 'Evo-Devo' perspective how some of these behaviors could also be adaptive. We suggest how elucidation of these mechanisms can help to better define risk and protective factors and populations at risk.

Received 1 August 2011; Revised 24 May 2012; Accepted 29 May 2012; First published online 3 July 2012

Key words: epigenetics, fetal programming, human, infant neurobehavior, mental health

Introduction

Advances in understanding the molecular basis of behavior through epigenetic mechanisms could help explain the developmental origins of child mental health disorders. However, the application of epigenetic principles to the study of human behavior is a relatively new endeavor. In this paper we summarize what is now known as the '*Developmental Origins of Health and Disease*' (DOHaD) including the role of fetal programming. We then review epigenetic principles and their recent applications to behavior. Much of the work in DOHaD has involved the neuroendocrine system and this is another area connecting DOHaD with epigenetics. We too focus on the neuroendocrine system and develop a simple heuristic stress-related model to illustrate how epigenetic changes in placental genes could predispose the infant to neurobehavioral profiles that interact with postnatal environmental factors potentially leading to mental health disorders. We then discuss from an 'Evo-Devo' perspective how some of these behaviors could also be adaptive. Elucidation of these mechanisms can help to better define risk and protective factors and populations at risk.

*Address for correspondence: Dr B. Lester, Department of Pediatrics, Brown Center for Children, Women and Infants Hospital, 101 Dudley Street, Providence, RI, 02905 USA.
(E-mails blester@wihri.org)

DOHaD

It is now well established in preclinical, prospective clinical and epidemiological studies that early development can have echoes across the lifetime.[1–3] Early work showed that measures of birth size was related to adult risk of coronary heart disease and other metabolic syndromes including hypertension, stroke, insulin resistance, type 2 diabetes and dyslipidemia.[4–6] The relationship between impaired fetal growth and an increased incidence of cardiovascular disease, hypertension and type 2 diabetes were especially strong in those who became obese in adolescence or adulthood. The disease burden is increased when there is a 'mismatch' between the prenatal and postnatal environments.[7]

The literature on the influence of prenatal stress on offspring suggests that many biological factors acting during prenatal life are associated not only with the development of common adult cardiovascular and metabolic[8–16] disorders but also with neurobehavioral and behavioral disorders.[17–20] Low birth weight is related to mental illness including schizophrenia,[21] depression[18,19,22] and psychological distress.[23–26] However, it is generally accepted that birth size is not at the heart of these disorders, but rather this phenotype acts as a proxy for the quality of the intrauterine environment, which itself reflects factors critical not only to fetal growth but also to adult health.[3] Conversely, adverse events can affect the

fetus and have long-term consequences without affecting birth size. Pregnant women undernourished during famine gave birth to normal-sized infants who, in a nutritionally adequate environment, later became obese.[27,28]

These observations on 'developmental origins' are due, in part, to environmental factors acting early in fetal life, with effects on developing systems that alter structure and function, and likely behavioral expression. These effects are possible because developmental plasticity enables the organism to change or reprogram structure and function in response to environmental cues. The adaptive significance is that plasticity enables a range of phenotypes to develop from a single genotype depending on environmental factors. The biological purpose of this fetal programming is to alter the set points or 'hard-wire' physiological systems to prepare the fetus for optimal adaptation to the postnatal environment. Undernutrition that reduces birth size may be one of many environmental factors that serve as a prenatal signal that reprograms the fetus. For example, programming that hard-wires the fetal neuroendocrine system to cope with stress could result in altered behavior patterns consistent with elevated stress hormones.

These adaptations resulting from fetal programming to 'prepare' for the postnatal environment could be because of epigenetic mechanisms and, further, epigenetic mechanisms could be a factor in the later development of mental health disorders. However, the application of epigenetics to the study of behavior, especially human behavior, is relatively recent. In this article we summarize basic epigenetic principles followed by a description of this new field of behavioral epigenetics. An exhaustive review of all possible epigenetic processes impacting child mental health is beyond the scope of this paper. Thus, we will describe one of many possible epigenetic pathways involving the neuroendocrine system, which could lead to the later development of mental health disorders. Our goal is heuristic, intended to stimulate thinking about this general approach and other possible approaches. For example, although there has been discussion of the fetal/developmental origins of mental health disorders including fetal programming and epigenetics,[26,29,30] the description of specific pathways as described here is novel.

Epigenetic mechanisms

The term 'epigenetics' was introduced by Waddington in 1940[31] as 'the interactions of genes with their environment, by which the genotype gives rise to phenotype and brings the phenotype into being', or ways in which the developmental environment can influence the mature phenotype.[32] Although debate about the definition of epigenetics continues,[33] it is probably safe to describe epigenetics as the inheritance of information based on gene expression control rather than on gene sequence. Epigenetic modifications lead to heritable, yet environmentally susceptible changes in gene expression without altering DNA sequences. This change in gene expression involves acquired, enzymatically catalyzed, covalent modification in DNA or chromatin-associated proteins. Although there are others, the classical and most studied epigenetic mechanisms are DNA cytosine methylation or post-translational modifications to histone proteins involved in chromatin formation.[34–36] Briefly, DNA methylation involves the addition of a methyl group to individual cytosines in the context of CpG dinucleotides, the majority of which exist as 'islands', or regions of DNA with an over-representation of CpGs. Particularly when occurring in gene promoters, this is most often associated with transcriptional gene silencing; in other words, turning off the anticipated activity of the gene. Histones are the proteins in chromatin, which make up the core of the nucleosome, and that enable the DNA to be tightly packed in the cell's nucleus. Protruding from the core nucleosome are tails of these histones that can undergo post-translational modification of specific amino acid residues, including methylation, acetylation, phosphorylation, ubiquitylation and sumoylation. These modifications, in turn, can act as signals to enable or restrict access of regulatory transcription factors to the DNA, altering faithful gene transcription and increasing or decreasing gene expression. However, nucleosomes turn over relatively rapidly and in the absence of DNA replication.[37] Thus, these alterations can allow for a dynamic control of gene expression, more so than that controlled by DNA methylation, which is thought to require DNA replication to remove the methylation marks.

We will argue that fetal programming, operating through epigenetic mechanisms, is altered by the intrauterine environment and could explain some of the origins of mental health disorders. Said another way, 'while genes load the gun, epigenetics pulls the trigger'. Defining the molecular basis of fetal programming sets the stage for the developmental origins of both child mental health and psychiatric disorders but also requires the application of epigenetics to the study of human behavior.

Behavioral epigenetics

There are thousands of studies of epigenetics that have been conducted over the last 40 years; however, the application of epigenetics to the study of behavior is just beginning.[29,38] Lester *et al.*[38] described this new 'discipline' of behavioral epigenetics as the application of the principles of epigenetics to the study of physiologic, genetic, environmental and developmental mechanisms of behavior in human and non-human animals. It typically investigates at the level of chemical changes, gene expression and biological processes that underlie normal and abnormal behavior. This includes how behavior affects and is affected by epigenetic processes. It is interdisciplinary in its approach and draws on sciences such as neuroscience, psychology and psychiatry, genetics, biochemistry and psychopharmacology.

Lester *et al.*[38] conducted a citation search (ISI Web of Knowledge: Science Citation Index) of published articles using the terms 'epigenetics', 'epigenetics and disease' and 'epigenetics and behavior' through June 2011 (Fig. 1).

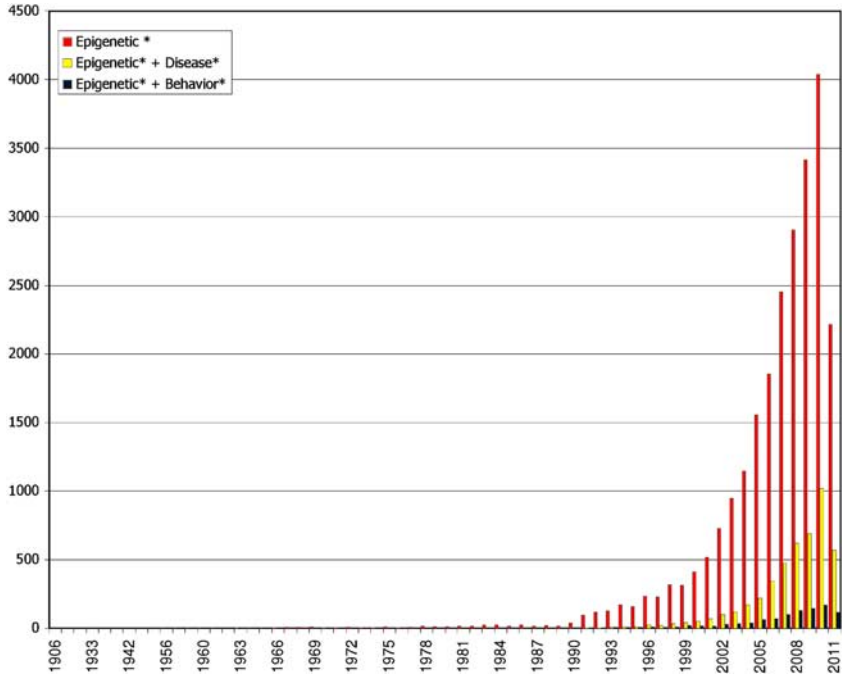

Fig. 1. Epigenetic citations by search terms. Citation search for epigenetic articles by year of publication. The number of published articles using the terms 'epigenetics', 'epigenetics and disease' and 'epigenetics and behavior' indicating the recent emergence of the field of behavioral epigenetics.

In contrast to the many citations for 'epigenetics', there are far fewer citations for 'epigenetics and disease' and few citations for 'epigenetics and behavior' (mostly non-human). Using PubMed we identified 178 studies that fit the above description of behavioral epigenetics and grouped the articles into nine categories based on the major construct that was studied. The most studied areas are psychiatric illness (depression, schizophrenia, suicide and bipolar disorder) and substance use (mostly alcohol, cocaine and tobacco). Other areas include learning and memory (memory formation, consolidation, enhancement extinction learning); neurodevelopment (disorders such as Autism, Rett, Prader–Willi and Angelman syndromes, studies of normal development and traumatic brain injury); parenting (maternal care, separation, depression and child abuse); stress [prenatal, hypothalamic–pituitary–adrenal (HPA) responsivity, stress reduction and hippocampal gene expression]; lifestyle (nutrition, exercise); neurodegenerative (disorders such as Alzheimer's, Huntington's) and sexual behavior (male). These are mostly preclinical studies using brain tissue. In human studies other specimens have been used including blood,[39] buccal epithelial cells[40] and in our own work, placenta.[41–44] Epigenetic changes in placental genes enables us to study alterations in the neuroendocrine system related to infant behavior.

Role of the neuroendocrine system

There are few settings in which gene–environment interactions are more profound, critical windows are of a more narrow duration and the latency to onset of effect is shorter than the influence of an adverse intrauterine environment on neuroendocrine and neurobehavioral functioning in the newborn. Neuroendocrine physiology is unique during intrauterine life. Fetal neuroendocrine function is almost exclusively autonomous from that of the mother. There are robust placental mechanisms that create a barrier to passage of maternal hormones and neurotransmitters across the placenta. This includes all of the peptide hormones and releasing factors, all but a trace amount of thyroid hormones and catecholamines and ~10% of the maternal glucocorticoids.[45,46] Although this is a small amount it may be a lot in terms of fetal physiology. There is a very high endogenous secretion rate of catecholamines by the fetus that conditions its capacity for a huge increase in norepinephrine and epinephrine secretion at birth to facilitate postnatal adaptation.[47] The fetus is protected from a hyperadrenergic state *in utero* by robust placental mechanisms for the uptake and degradation of catecholamines. These mechanisms, however, render the fetus exquisitely vulnerable to the effects of catecholamine uptake inhibitors such as cocaine and amphetamines or drugs that increase catecholamine secretion such as nicotine.

Adverse intrauterine exposures including drugs, hypoxia or nutritional stress lead to further increases in the release of fetal catecholamines (epinephrine, norepinephrine and dopamine) and glucocorticoids. These catecholamines in turn alter regulation of the neuroendocrine environment by acting on the HPA axis resulting in altered set points for physiologic, metabolic and behavioral outcomes.[48]

The brain is particularly sensitive to prenatal programming including effects on the HPA axis (for review see

Matthews[46]). Because they are an important feature of the stress response, glucocorticoids have become prominent candidates for mediators of the effects of fetal programming and glucocorticoids can have a major impact on the developing brain.[46] There is evidence that maternal adrenocorticotropic hormone (ACTH) modulates the developing fetal HPA axis.[49] Pregnant dams and sows exposed to stress show increased ACTH and cortisol,[3,50,51] and their offspring exhibit increased cortisol and ACTH levels from the gestational period with exaggerated fear responses persisting in these offspring into adulthood.[48,52,53] Pregnant women treated with a synthetic glucocorticoid, dexamethasone, are more likely to have infants who are more distractible and aggressive at age 3 and 6 years.[54] In addition, excess corticoids may be harmful because they are involved in catabolic activity and cell division in the fetal brain.[55]

One commonly studied proxy for increased exposure to maternal glucocorticoids includes the assessment of maternal antenatal anxiety. In human studies, maternal antenatal anxiety is associated with higher total distress score on a newborn exam, higher basal postnatal cortisol,[56] increased postnatal crying and a difficult tempermament.[57–59] Of relevance to our current model, maternal antenatal anxiety is also related to a high, flattened diurnal cortisol pattern, which, in females only, was significantly associated with greater depressive symptoms.[60] These results persisted even after controlling for concurrent maternal anxiety. Thus, postnatal child HPA-axis functioning, which itself may have been programmed *in-utero*, may mediate the relation between exposure to maternal antenatal anxiety and expression of childhood psychopathology.[26] A flattened or blunted cortisol response has also been related to prenatal cocaine exposure,[61,62] maternal substance use during pregnancy and foster care,[63] suggesting similar perturbations of HPA-axis function in other populations at risk for mental health disorders.

The effects of prenatal stress on adult hippocampal corticosteroid receptor density[18,64–68] may have implications for emotional reactivity. There is a rich literature relating prenatal stress to altered HPA activity and behavior in both animal and human research. Prenatally stressed rats have a high degree of 'emotionality'[69] reflected by decreased locomotion and increased defecation.[13,69–71] They also show less play,[72] more defensive freezing,[73] less movement in an activity wheel[74] and increased vocalizations.[51] Prenatal stress affects their cognitive abilities including operant discrimination,[75] task competence in a water maze[76,77] and memory.[78] In other animal studies, maternal stress during pregnancy results in offspring that are more irritable, anxious and difficult to control.[3,79–82]

Brain neurotransmitter systems and glucocorticoids interact to modulate both behavior and HPA activity.[83] Disturbances in HPA regulation and brain monoamine levels have been associated with affective and anxiety disorders in humans.[84–87] In human studies, poor health outcomes have been related to prenatal stress including low birth weight, preterm birth and intrauterine growth retardation.[88,89] Moderate to severely stressful life events during mid-gestation are related to low birth weight and small head circumference, suggesting a specific effect on the brain.[55,90] A recent review of the literature linking low birth weight to HPA reactivity suggested that low birth weight, as a proxy for the quality of the intrauterine environment, was associated with greater HPA reactivity in both childhood and adolescence.[90]

In addition to an increased risk of excessive stress reactivity, cardiovascular and metabolic disease, an impaired prenatal environment also affects cognitive and behavioral development. Maternal first-trimester exposure to the stress of war has been associated with an increased risk of the offspring developing schizophrenia in adult life.[91] Similar to the 'emotionality' reported in animal studies, human infants exposed to stress *in utero* show high reactivity, activity and irritability.[92–94] Psychological and behavioral abnormalities have been reported in children exposed to prenatal stress[95–97] including learning and behavior problems[98] and reduced hippocampal volume in both children and adults with histories of child abuse.[99] The effects of prolonged exposure to chronic stress or allostatic load[100] and, concomitantly, prolonged activation of the HPA axis have also been related to physical disease and behavioral disorders into adulthood.[101,102]

A behavioral epigenetic developmental origins model of child mental health disorders

In previous work,[41] we described the effects of prenatal cocaine exposure as a potential stressor producing an adverse intrauterine environment on altered regulation of catecholamines and glucocorticoids. As a stressor, cocaine programs the HPA axis that controls, among other functions, the release of cortisol within the neuroendocrine system and mediates the stress response. This action impacts behavior due, in part, to plasticity of brain neurotransmitter monoamine systems. The cocaine exposure model is but one example of whole classes of stressors that impact and modifies these systems. We have integrated these concepts into a more generic model (Fig. 2) to illustrate how developmental origins could relate to child mental health disorders. Although there are many ways to relate developmental origins to child mental health, we highlight a pathway that involves alterations in the neuroendocrine system based on fetal programming and epigenetic modifications, and subsequent fit between the postnatal environment and infant behavior.

The model shows the impact of an adverse intrauterine environment, which can encompass maternal stress, environmental exposures, nutrition or other adverse conditions, on placental gene expression affecting cortisol and altering the infant's neurobehavioral responsivity to postnatal environmental conditions affecting liability to mental health disorders. We have focused on several candidate genes involved in responsivity and control of the HPA axis in the placenta during gestation, including the norepinephrine transporter (NET),

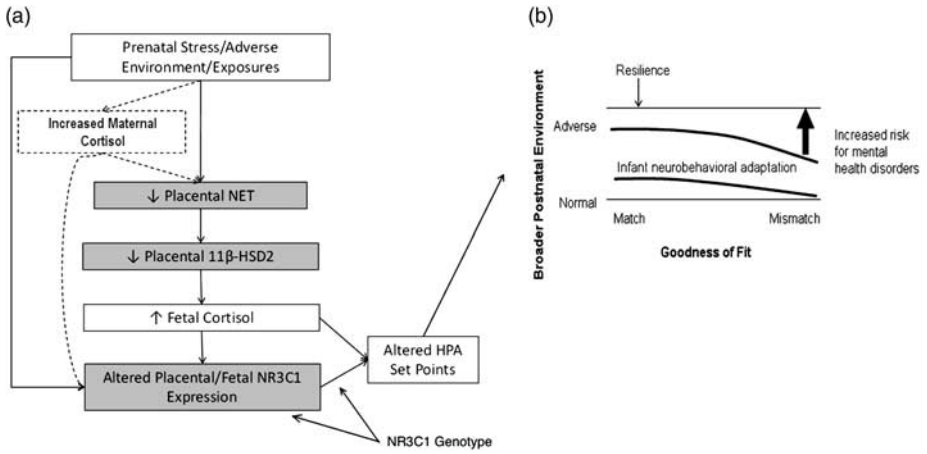

Fig. 2. Developmental Origins Model of child mental health disorders. The model illustrates one of many pathways in which prenatal environmental stressors could impact placental gene expression altering HPA set points and the infant's responsivity to postnatal environmental conditions affecting liability to mental health disorders. Epigenetic effects on key placental genes (shaded in a) increase fetal exposure to cortisol altering the abilities of the infant to respond to the postnatal environment which includes both parenting and broader environmental conditions. The 'Goodness of Fit' (b) or relative match or mismatch between the infant's capacities and the kind of parenting appropriate for the infant's capacities in the context of the degree of adversity of the broader postnatal environment will determine the probability of risk for mental health disorders. This risk is maximized when the infant has restricted behavioral capacities that clash (are a mismatch) with parenting styles in the context of environmental adversity (based on Gluckman and Hanson 2005).

the steroid metabolic enzyme 11beta-hydroxysteroid dehydrogenase-2 (11β-HSD-2) and the glucocorticoid receptor (GR; NR3C1).

Placental NET and 11β-HSD-2 are pivotal placental genes that program the intrauterine neuroendocrine environment during development. They protect the fetus from excess catecholamines and glucocorticoids, which have harmful effects on the fetus.[103] 11β-HSD-2 in particular converts maternal cortisol to inert cortisone protecting the developing fetus from exposure to maternal cortisol.[104] Lower placental 11β-HSD-2 activity is associated with smaller fetuses in rats[105] and humans.[106–109] Rat pups born to parents who did not express 11β-HSD-2, had significantly lower birth weights, and exhibited more anxiety compared with pups born to parents who expressed 11β-HSD-2.[110] Rare mutations of 11β-HSD-2 are also associated with low birth weight in human infants[111] and increased fetal cortisol levels are associated with intrauterine growth restriction.[112] 11β-HSD-2 modulates the programming effects of prenatal glucocorticoid exposure.[113,114] The HPA axis is highly sensitive to the effects of glucocorticoids on perinatal programming.[3,115–117] High levels of maternal glucocorticoids disrupt intrauterine growth, postnatal HPA-axis function and neurobehavioral outcome. We have shown that placental expression of this key enzyme is potently downregulated at the RNA, protein and functional level by norepinephrine, which is in turn regulated by NET.[118] Downregulation of NET has been associated with an adverse intrauterine environment, maternal/placental disorders such as preeclampsia and exposure to drugs including cocaine and nicotine.[119,120] Reduced placental NET expression from cocaine exposure may lead to increased circulating catecholamines, downregulation of 11β-HSD-2 and chronic fetal hypercortisolism, leading to altered neuroendocrine (HPA axis) activity and dysregulated neurobehavior.

We suggest that the association of prenatal stress and altered fetal development is mediated by effects on 11β-HSD-2. As a result, altered HPA and neurobehavioral reactivity could predispose the child to the development of mental health disorders. Our preliminary findings show decreased 11β-HSD-2 expression in mothers who used cocaine, cigarettes or were depressed during pregnancy and that these changes in placental gene expression were associated with changes in the extent of methylation of placental genomic DNA, specifically within promoter regions, potentially suggesting an epigenetic mechanism responsible for this gene silencing following cocaine and/or nicotine exposure.[41] DNA methylation of the 11β-HSD-2 promoter has been linked to reduced expression of the gene,[121] has been associated with hypertension among individuals treated with glucocorticoids[122] and suggests that excess exposure to glucocorticoids could lead to reduced expression of this gene through DNA methylation. In addition, single nucleotide polymorphisms (SNPs) in the 11β-HSD-2 promoter have been shown to alter transcription factor binding, leading to alterations in gene expression,[123] which further indicates that accessibility of the promoter to transcription factors, mediated through chromatin changes, may be important in the control of expression of this enzyme. Factors that stress the intrauterine environment and trigger this cascade of events can reprogram the HPA axis and are considered risk factors specific to these prenatal effects.

The NR3C1 gene encodes the GR, a nuclear receptor that binds and is activated by cortisol leading to transcription of genes involved in functions related to cortisol stimulation, including stress responses, immune-modulation and energy

metabolism.[124] In addition to its critical role in the cortisol signaling network, GR can also act as a regulator of itself, as well as of other genes in this pathway, including 11β-HSD-2. The promoter region of NR3C1, in addition to multiple copies of the GR-binding site, also contains binding sites of at least 15 different transcription factors. This allows for exquisite tissue-specific regulation and alterations of expression by different physiologic stimuli.[125] The human placenta at term expresses a number of GR mRNA variants, and the level of expression of these variants is altered with the onset of labor and with cortisol exposure, suggesting that stressful events may control its expression.[126] Maternal care has been linked to alterations in methylation of the exon 1–7 promoter region of the NR3C1 gene in animal models.[127–129] In human studies, third trimester stress was associated with increased methylation of the CpG3 site in exon 1F of the NR3C1 promoter (equivalent to the rat exon 1–7), which is thought to alter the binding of the NGF1-A binding site and thus altering GR expression.[39] Rat models have suggested dynamic patterns of expression of GR in late gestation, with near-term rats demonstrating elevated expression of GR in the labyrinth zone of the placenta, the site of maternal fetal exchange, associated with reduced expression of 11β-HSD-2 in this region at this same time.[130]

Interestingly, nutrient restriction, in the ovine model, has been associated with decreased cell proliferation but increased GR expression in 66- and 110-day gestation placentas, coincident with the period of normal maximum placenta growth, and so is thought to be a compensatory adaptation to reduced nutrient availability.[131] Human infants born large for gestational age demonstrated, on average, increased methylation of the exon 1F region in human placenta tissues.[132] Together, these data suggest that expression of the GR in placenta is regulated by the environment and that this regulation, in part, is mediated through epigenetic mechanisms.

In our model, an adverse intrauterine environment and/or prenatal stress alters expression of key placental genes increasing fetal exposure to cortisol and altering HPA set points, which in turn alter the capacity for neuroendocrine and neurobehavioral responses to the postnatal environment. These alterations can be elicited through epigenetic effects with key targets of epigenetic alterations shaded (Fig. 2a). In addition, these 'acquired' effects could be exacerbated in situations where there is already a genetic predisposition for a mental health disorder such that the added epigenetic effects increase the likelihood that the disorder will reach threshold and be expressed. A more sensitive genotype in the presence of epigenetically induced altered gene expression following prenatal stress puts the infant at greater risk for mental health disorders than a more resistant genotype with the same prenatal stress.

The cascade of epigenetic effects *in utero* and their impact on infant behavior shown in the figure has only recently been demonstrated. These studies use a newborn neurobehavioral assessment [NICU (Neonatal Intensive Care Unit) Network Neurobehavioral Scale or NNNS][133] that predicts poorer performance on the 24-month Bayley Psychomotor Developmental Index and cerebral palsy at 2 years,[134,135] as well as behavior problems, school readiness and IQ through $4\frac{1}{2}$ years of age.[136] Thus, epigenetic effects related to neurobehavioral findings on the NNNS could have implications for long-term developmental outcome, including mental health disorders. In a study of 185 healthy newborn infants, DNA methylation of the 11β-HSD-2 promoter region in the placenta was greatest in infants with the lowest birth weight and this increasing methylation was associated with poorer quality of movement on the NNNS.[42] We also studied the extent of DNA methylation of the NR3C1 promoter and a SNP in the promoter region of the NR3C1 gene in these infants.[43] NR3C1 methylation was associated with infant attention and quality of movement on the NNNS. There was also a potential interaction between methylation and genotype on attention suggesting that epigenetic alterations of genetically susceptible infants may further increase the risk of later mental health and developmental problems.

These studies demonstrate small, but significant independent associations between DNA methylation variation in these genes and newborn neurobehavioral effects. The magnitude of the effect likely underscored that it is, of course, unlikely that one or two altered genes or a single epigenetic × genotype interaction could explain complicated phenotypes such as newborn neurobehavior let alone later mental disorders. Likely, there is a coordinated interplay between epigenetic variation across numerous loci arising from the intrauterine environment, in concert with (or potentially influenced by) genetic variation in key pathways, which comprehensively dictates neurobehavior and mental health.

To examine such issues, novel approaches aimed at identifying patterns of coordinated molecular profiles based on genetic and/or epigenetic measures need to be employed. For example, we have utilized a recursively partitioned mixture modeling approach[137] to examine the coordinated patterns of expression of epigenetically regulated imprinted genes in the placenta to identify the association between these profiles and neurobehavior on the NNNS.[44] From these data on the expression of 22 imprinted genes, we identified four classes of gene expression for which each subject can be individually classified (Fig. 3). On the basis of these classifications, we demonstrated that infants with a class 4 profile compared with class 1 had a nearly 1 SD reduction in the NNNS quality of movement summary score, which measures motor control, smoothness and maturity.

These data support the hypothesis that coordinated alterations in many genes may be particularly important for our understanding of neurobehavioral development, and suggest that analytical approaches that facilitate modeling these interactions will be critically important in delineating these relationships. Figure 2 provides a simple example, based on our earlier results on NET, NR3C1 and 11β-HSD-2 in which alterations of these three genes can modify the HPA

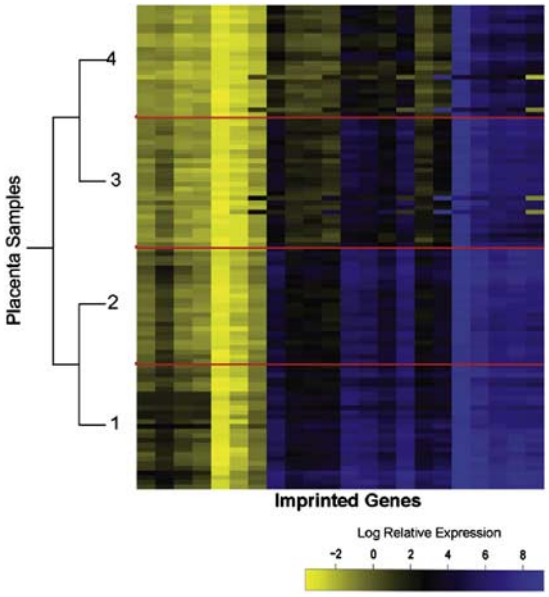

Fig. 3. Heatmap showing four classes of expression signature of the 22 imprinted genes. Gaussian recursively partitioned mixture models, a hierarchical clustering, based on imprinted gene expression identifying 4 distinct classes of expression signature. Samples are in rows, and the expression of the 22 imprinted genes examined is shown by the heatmap in column. The dendrogram depicts the splitting of the samples into 4 classes, which are separated on the heat map by red lines and labeled classes 1, 2, 3 and 4

axis, and the role that the intrauterine environment as well as genotype may play. As these networks grow in complexity, and as novel genes are introduced, the models will need to be expanded to include this novel information. We must also recognize that the genes that will be critical will not be limited to those with already described associations with behavioral or mental health disorders, but may expand to include a variety of other pathways, including those important in fetal development such as imprinted genes and even microRNAs.

The model (Fig. 2b) also shows how the larger (distal) postnatal environment can affect infant behavior altered by changing HPA set points. The postnatal environment can range from 'normal' (even enriched) to adverse including the 'usual suspects' such as poverty, low socioeconomic status, lack of education, poor quality of the home and neighborhood, race and ethnic status, community violence and others. The more immediate (proximal) postnatal environment includes parenting or 'relationship' factors. The infant develops in the context of the caregiving relationship,[138,139] and this relationship lays the foundation for effective methods of coping with stress later in life.[140] When infants are awake, 91% of their time is spent with the primary caregiver, engaged in activities such as feeding and play.[141] The concept of 'goodness of fit' with the parenting or caregiving environment is recognized as one process by which infants may develop problems with coping and self-regulation later in life.

Goodness of fit

The 'goodness of fit' concept has its origin in the field of infant temperament,[142] but has broader applicability as a framework through which to understand developmental processes involving both infant and parent as they dynamically modify each other's behavior through continual positive or negative feedback. A good fit occurs when there is a match between the caregiving environment and the child's behavior, promoting the child's optimal developmental outcome. A poor fit involves a mismatch between child characteristics and the caregiving environment, leading to worse developmental outcome. For example, infant mental and language development was enhanced when mothers were better able to read their babies cry signals.[143] In addition, the combination of infant difficult temperament and dysfunctional family environment was a better predictor of child problem behavior than infant difficult temperament alone.[144] Finally, in intervention work where the caregiving environment is modified to meet the developmental needs of an infant with difficult temperament, better parent/child adaptation ensues.[145]

Goodness of fit may explain findings relating HPA reactivity in infants to the quality of the attachment relationship[146,147] and to 'face to face' interaction.[148] In the model (Fig. 2b) it is the 'goodness of fit' between the caregiving environment and infant neurobehavior, in the context of the broader distal environment that determines the infant's relative risk for the development of child mental health disorders. A match is when fetally programmed neurobehavior is compatible with the type of parenting appropriate for that infant. In a mismatch, parenting is not appropriate for the infant's neurobehavior. The probability of developing mental disorders is increased when the infant's capacities (altered by HPA set points) and parenting are a mismatch in the context of postnatal environmental adversity. Several studies have examined this mismatch as it impacts maternal mental health, which in turn can affect infant mental health. For instance, when maternal postnatal experience was negative relative to prenatal expectations of motherhood, mothers demonstrated greater depression symptomatology.[149]

An 'Evo-Devo' perspective

So far, we have described how altered HPA axis and behavioral responsivity could lead to child mental health disorders depending on the fit with the postnatal caregiving environment. However, from an evolutionary perspective there may also be adaptive value to these fetally programmed neuroendocrine and behavioral modifications. This understanding comes from the field of evolutionary developmental biology and is based on the recognition that gene–environment interactions are ubiquitous and extend to the activation of genetic activity by non-genetic influences. In addition to being agents of heredity, genes are now seen as playing a key role in the organization and regulation of development.[150,151] Developmental outcomes are epigenetic not just genetic and this is also true as part of the evolutionary process. The view

of development within evolutionary biology recognizes that changes in evolution reflect changes in development. In addition to the 'traditional' evolutionary processes of random genetic mutation, drift and recombination that produce phenotypic variation and are acted upon by natural selection, epigenetic processes are now seen as contributing to individual ontogeny and adaptation to the more immediate environment.

One of the evolutionary functions of development is the production of variability in developmental patterns to adapt to environmental change. Developmental interactions play a role in evolution as a source of novel variation. What becomes maladaptive may depend on the circumstances. For example, rats and monkeys raised in adversity show increased behavioral hypervigilance and greater stress reactivity, which is similar to children raised in poverty and social upheaval.[152] These behavioral and physiological responses may actually serve to protect these children from an adverse environment by supporting their capacity to detect and respond to threats.[153] Under experimental adversity (frequent separations), mothers become less attentive. Their offspring show maternal behavioral differences but they also grow up to be fearful with highly reactive adrenocortical responses, increased appetite, depression, cognitive deficits and rapid sexual maturation. In humans, we would typically classify these behaviors as psychopathological or maladaptive. Strikingly, the animal work shows that these traits are transmitted to the next generation even when the adverse conditions are no longer present. What these behaviors have in common is that they indicate increased behavioral and physiological variability that could increase the chances that a greater number of offspring will adapt and survive in chaotic and threatening situations in future generations. Offspring with 'normal' behavior and physiology may be less successful in chaotic and threatening conditions.

Behavior belongs to the class of allostatic systems[100] in which the ability to achieve stability through change is vital for survival. In contrast to homeostatic systems that must be maintained within narrow boundaries such as blood pH or body temperature, allostatic systems (including the HPA axis) have broader boundaries that enable us to cope with internal (e.g. infection) and external (e.g. poverty) demands. A narrow behavioral repertoire that could appear 'economical' in the short term by being less negatively affected by postnatal environmental challenges would, over time, be less able to adapt to a wide range of environmental conditions. The sobering thought is that what we label as 'dysregulated' behavior today could be beneficial in a different environment.[150] The increased vigilance and fighting behavior related to cortisol in animal studies could have survival value in hostile environments.

Similarly, although we might be tempted to classify the altered neuroendocrine and behavioral responses in our model as maladaptive, we can also hypothesize that this increased variability could be beneficial for long-term adaptation in a more unpredictable environment. Genetic evolutionary processes operate in the context of prolonged changes in the environment. Genetic variation needs to be maintained to accommodate a wide range of environments. If a given transient environment is unfavorable to phenotypic expression of a certain genotype, fewer genotypes will survive and if this pattern persists over generations, species survival could be threatened. As we have seen, fetal programming because of epigenetic changes enables individuals to adapt to the postnatal environment by increasing the range of phenotypes to a broader spectrum without changing the genotype. This phenotypic variability can therefore allow for a more rapid adaptation to what might be more immediate and transient environmental change, and in fact, the development of epigenetic regulation in fetal programming may be the product of evolution to face more rapidly changing environments than what can be accommodated by spontaneous mutation. For example, low birth weight, a putative marker for a stressful or adverse intrauterine environment, was significantly associated with a negative temperament.[154] Negative temperament, in turn, served as a susceptibility factor such that when raised in environments of support, infants with negative temperaments showed the highest levels of socio-emotional functioning, but when raised in impoverished environments, these infants showed the lowest levels of socio-emotional functioning. Thus, low birth weight served as a marker for a susceptibility factor that enabled children to adapt (perhaps through epigenetic mechanisms) to postnatal demands in a way that may ultimately enhance their reproductive fitness.

The effects of prenatal stress on altering the sensitivity of the HPA axis could be an example of developmental plasticity, wherein, in response to environmental cues, the organism changes structure and function and increases the range of phenotypes that develop from a single genotype. The genome increases variability because prenatal stress is a signal that the fetus has to prepare for a different postnatal environment than the one for which it is programmed. Thus, increasing behavioral variability provides better opportunities for adaptation. Increased aggression or fear in the rat may be adaptive in some environments, but maladaptive in others. If there is a 'good fit' between the phenotype and the postnatal environment, a broader range of genotypes will survive the transient change and maximal genotypic variation will be preserved for long-term adaptation. The fetus could be programmed for a wide range of neuroendocrine and neurobehavioral responses as an adaptation to prepare for a more variable, less predictable, postnatal environment.

In addition, how the fetus is reprogrammed in response to an environmental signal obviously depends on the nature of the environmental signal. If the signal is poor nutrition, the reprogramming involves changing the set points of metabolic pathways. Here we have the case of a specific insult that triggers specific and directional effects. Our model is different because we are proposing a stress model and the stress signal is non-specific. In the same way that it makes sense for poor nutrition to affect metabolic pathways, it makes sense for

stress signals to affect the stress (HPA) system. However, unlike poor nutrition, where the fetus has a specific environment to prepare for, the non-specific nature of stress may not enable the fetus to prepare for a specific environment. The reprogramming has to enable the fetus to adapt to a wide variety of postnatal environmental conditions, that is, a more unpredictable environment. Thus, rather than altering HPA set points in a specific direction, the alterations would more likely be bi-directional resulting in increased HPA variability. This would enable the fetus to prepare for a more unpredictable postnatal environment and increase the number of infants who could match with the postnatal environment. Similarly, we would expect to see analogous changes in infant neurobehavior; that prenatal stress would increase neurobehavioral variability, some infants will have a broader range of capacities than are typical, whereas others would have a more restricted range of capacities providing more opportunities to match with the postnatal environment.

In the model (Fig. 2b) the infant's responsivity is reprogrammed to allow for a range of predictable environments. The curved lines show the range of infant neurobehavioral adaptation. With a wider range of neurobehavioral adaptation, there is a very low probability for developing mental health disorders, as there are ample opportunities for matching with parenting behavior in the normal broader postnatal environment. However, the range of infant neurobehavioral adaptation is diminished by a poor fit with the parenting environment and further diminished by environmental adversity leading to high risk for developing mental health disorders. Epigenetic processes could operate both prenatally and postnatally affecting behavioral phenotypic expression and the dynamics of this system as we 'drill down' to the molecular level. We might also expect there to be a 'cost' to this HPA and neurobehavioral reprogramming such that it might be effective in the short term but wear down these systems faster if the reprogramming had not occurred, increasing allostatic load and perhaps leading to earlier onset and/or more pronounced manifestations of mental health disorders.

Of course this is one possible pathway. Different stressors or combinations of stressors will have different effects and multiple effects. Poor nutrition can affect metabolic pathways and also be a stressor. Poor nutrition can affect glucocorticoid regulation of body weight and stress can affect birth weight. A single insult such as prenatal cocaine exposure could affect the hippocampus directly as a teratogen and could also be a stressor. The total number of stressors may be important and there may be a threshold for the number of stressors, depending, in part on what they are.

Risk and protective factors

Risk and protective factors can be identified at several levels in this model. Prenatal stress includes a wide array of risk factors and could range from a poor reproductive and/or nutritional history to a more immediate event (e.g. maternal ingestion of a teratogen such as cocaine). These prenatal risk factors, alone and in combination, would create an at-risk intrauterine environment, setting off the cascade of prenatal events described in the model. Genetic risk factors include more sensitive genotypes with, for example, the SNP profile related to mental health disorders. Genetic protective factors include the resistant genotype absent in the SNP profiles. Developmental timing could be a risk factor if an insult occurs during a critical period. Epigenetic modification of specific fetal tissues can confer both risk and protection. Earlier, we mentioned epigenetic changes related to negative behavioral outcomes in both animal and human models. However, epigenetics can also be protective through gene expression control. DNA methylation, for example, can offset the effects of a deleterious polymorphism. The fact that epigenetic changes can be reversible and transgenerational suggests not only that protective factors can be 'programmed' but also that therapeutic interventions may support such positive changes.

Infant behavior (and later temperament and personality) has been described as a risk or protective factor and in our model would relate to variability in neurobehavioral adaptation. Although increased behavioral variability could be a risk factor, it could also be adaptive depending on environmental context. The match between infant and parent is an example of a protective factor that is important for resilience. Thus, the locus of resilience in our model is the upper left quadrant (Fig. 2b) where the child is growing up in an adverse environment, but there is a good fit between child behavior and parenting quality lowering the risk for developing mental health disorders. In addition, other protective factors have been identified including child temperament and personality characteristics, peer relationships and extracurricular activities (e.g. sports), all of which can contribute to resilience.

Populations at risk

The application of modern biology and the developmental origins perspective has lead to the identification of new populations at risk for child mental health disorders as well as a different understanding or redefinition of populations already known to be at risk. Categories for risk include the possibility that the impacts of famine, nutrition, poverty, war or dislocation of populations may take their progeny several generations to recover from and reach their full potential.[155] Thus, some of the current impacts of risk may actually be fallout from a prior generation, exacerbated by new impacts within the generation and placing the next generation in increasing peril. Behaviors, phenotypes, illnesses and other traits once thought to be inter-generational or familial non-genetic heritable traits, that is, learned through caregiving and socialization or resulting from lifestyle, exposures or other experiences, may be epigenetically inheritable or may be the result of epigenetic modifications induced by nutrition, parenting practices and other experiences of the fetus, infant and young child.[71,156,157] Such potentials, although speculative, seem

reasonable and force new consideration of both the origin of traits once thought to be influenced solely by the child's environment and the therapeutic approaches built on these prior understandings. They also suggest that new approaches to understanding these mechanisms, their inheritance and their importance need to be developed and applied, including the growth of the field of human behavioral epigenetics.

Acknowledgments

Grant support: 1 R01 MH094609-01 (Marsit, PI) NIMH. The project described was also supported by Award Number F32DA032175 (Conradt, PI) from the National Institute on Drug Abuse. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health.

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