Epigenetic Effects of Early Developmental Experiences

Kathryn M.A. Gudsnuk, Frances A. Champagne, PhD*

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- Epigenetic pathway • Postnatal maternal care
- DNA methylation

Experiences occurring in early development can exert long-term effects that lead to either heightened or attenuated risk of physical and psychiatric disease. Of particular importance for these outcomes is the quality of the relationship between a parent and an infant. Parental stress, reduced sensitivity of parents to infant cues, and childhood neglect/abuse may lead to a cascade of biological changes that compromise the functioning of infants, leading to effects that persist into adolescence and adulthood. Moreover, genetic and environmental factors may heighten the vulnerability of infants to these effects. For the high-risk neonate, both underlying vulnerability and adverse early-life experiences are characteristic and may ultimately lead to divergent developmental pathways that compromise future health and well-being. The deprivation or reduction in parental contact that is often experienced by these infants during the neonatal period may be a significant factor in determining the severity of these outcome measures. Thus, understanding the mechanisms through which parental care can alter infant development may provide insight into the potential interventions and practices that are critical in promoting healthy children, adolescents, and adults.

During fetal and infant development, the brain is rapidly changing, leading to proliferation and refinement of neural pathways. The sensitive period created by this time of neuronal plasticity creates a window of opportunity during which experiences can exert long-term effects. Although the study of human brain development and the effects of experiences on these processes has been limited by the availability of appropriate methodological tools, decades of work using animal models has provided some valuable insights. Laboratory studies of rodents suggest that adverse experiences occurring during fetal and/or infant development lead to changes in brain architecture and function. These effects are particularly evident when the quality of the parent-infant interaction is affected, either through parental stress or through

Department of Psychology, Columbia University, Room 406 Schermerhorn Hall, 1190 Amsterdam Avenue, New York, NY 10027, USA
* Corresponding author.
E-mail address: fac2105@columbia.edu

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manipulation of the quality and/or quantity of parental care toward infants. More recent approaches to the study of the mechanism of parental effects have determined that in addition to physiologic and neurobiological outcomes, the quality of the parent-infant interactions may induce a molecular change in offspring, which alters the patterns of gene expression present in specific brain regions. These epigenetic effects indicate that the quality of the early-life environment can change the activity of genes, thus illustrating the dynamic interplay between genes and environmental experiences in shaping development.

This article highlights research that has investigated the epigenetic mechanisms which may contribute to the lasting psychobiological impact of early-life adversity. In particular, the authors focus on studies that demonstrate the impact of maternal stress, variation in parental care, parental deprivation, and infant abuse on the epigenetic pathways. Although primarily drawn from work with laboratory rodents, there is increasing evidence for similar effects in human infants, and this new and emerging evidence is explored. The implications of this research are significant and may provide insight into those features of the early environment that may exert profound effects on the developing brain. The authors speculate as to the way in which this research can inform neonatal practices and provide strategies for improving the care of high-risk infants.

EPIGENETIC PATHWAYS: A PRIMER

The pathways through which DNA exerts a biological effect that leads to variation in physiology, metabolism, and behavior are being increasingly explored using modern techniques from molecular biology. An emerging theme from this work is the dynamic ways in which DNA is regulated by a variety of factors, leading to variation in the expression or activity of genes. Within the cell nucleus, DNA is stored in a highly compact and densely coiled configuration, a strategy that is necessary for storing the billions of nucleotide base pairs in the mammalian genome. For genes to have a biological impact, they must be expressed. Gene expression involves the transcription of DNA into messenger RNA, which is then translated into protein (**Fig. 1**A). The transcription of DNA is a very elegant process, and the timing and level of gene expression are critical for the normal process of development. The mechanisms that are capable of altering gene expression without altering gene sequence are called epigenetic, meaning over or above genetic.[1] Epigenetic mechanisms are typically molecular changes to the DNA itself or to the proteins around which the DNA is tightly coiled. In the context of environmentally induced epigenetic changes, most research has focused on posttranslational histone modifications and DNA methylation.

Within the cell nucleus, DNA is wrapped around histone proteins, and this wrapping allows for the compact storage of the genetic material.[2] However, the expression/activation of genes requires that the DNA become liberated from this dense structure and accessible to transcription factors and other enzymes, such as RNA polymerase, that initiate the transcription process (see **Fig. 1**B). One way to achieve this outcome is to modify the histone proteins so that they are less attracted to the DNA. Histone proteins possess extensions or tails that, when wrapped around the DNA, serve to reduce accessibility of the gene sequence. The addition of chemicals to or removal of chemicals from these histone tails can dynamically change the interaction between histones and DNA. For example, histone acetylation is a process whereby an acetyl chemical is added to the histone protein tail. When this occurs, there is typically increased gene expression because of a looser interaction between the histones and the DNA (see **Fig. 1**C). In contrast, removal of acetyl chemicals from histone tails (deacetylation) results in reduced gene expression. There are many chemicals that can be added

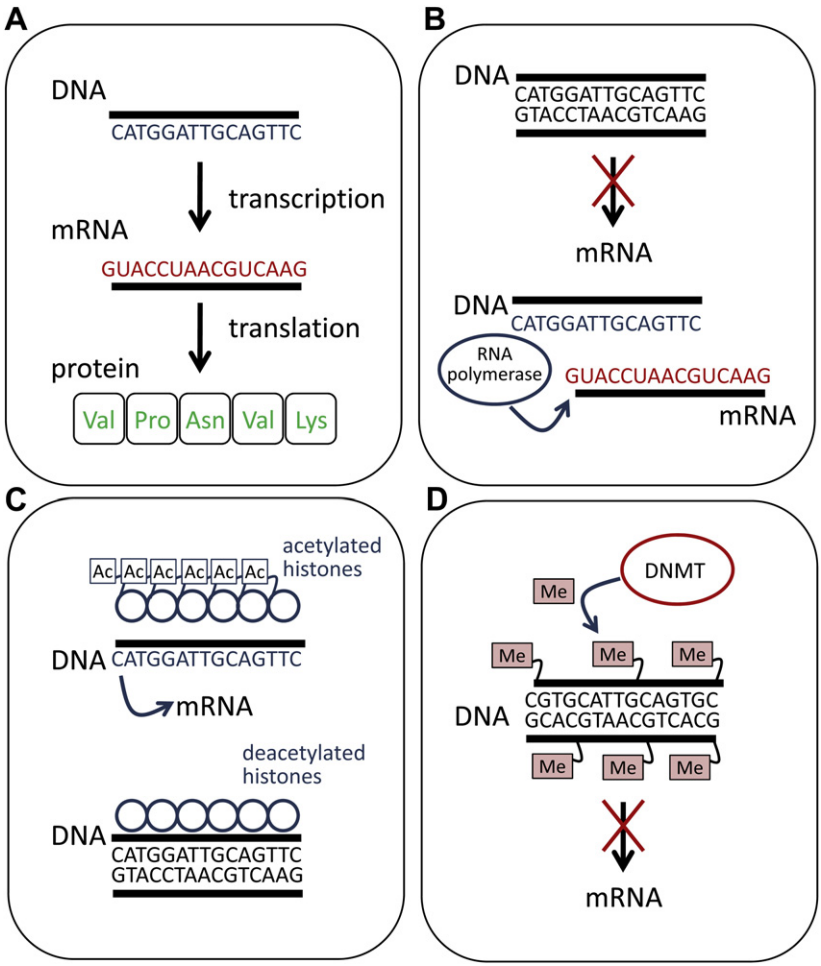

Fig. 1. Gene regulation and epigenetic mechanisms. (*A*) The activity of genes is determined by the level of transcription of DNA to messenger RNA (mRNA). The process of translation involves the use of mRNA to generate a protein consisting of a succession of amino acids. (*B*) When DNA is tightly compact (*top*), there is a suppression of gene expression (ie, no mRNA is produced). When DNA is less compact (*bottom*), enzymes such as RNA polymerase can bind to the DNA and initiate the process of transcription. (*C*) The addition of an acetyl chemical (Ac) to histone proteins can loosen the interactions between histones and DNA and increase the level of gene transcription (*top*). In contrast, deacetylated histones can be found to cluster closely to the DNA and suppress gene expression (*bottom*). (*D*) DNA methylation is a process in which methyl chemicals (Me) are added to cytosines in the DNA sequence by the enzyme DNMT. Methylated DNA is highly compact, and DNA methylation leads to reduced gene expression or gene silencing.

to or removed from the histone tails (a process generally called posttranslational modification) such that histones can undergo phosphorylation, methylation, and ubiquitination and multiple other chemical modifications.[3] Collectively, these modifications are called the histone code.[4] The interpretation of this code is complex, and these histone modifications represent a dynamic and complex strategy for reducing or enhancing gene expression.

A second epigenetic process of critical importance for development is DNA methylation. The addition of a methyl chemical to cytosines within the DNA strand represents a more stable and enduring epigenetic modification. When cytosines become methylated, there is generally less accessibility to the DNA, and consequently, DNA methylation is thought to be a process that leads to gene silencing (see **Fig. 1**D).[5] Moreover, methylated DNA can attract methyl-binding proteins that cluster around the DNA and attract enzymes that can shift the histone tails into a deacetylated state (further reducing access to the DNA).[6] A feature of DNA methylation that makes this epigenetic mechanism particularly intriguing is the potential heritability of DNA methylation patterns. When cells divide, they reliably copy their DNA material such that each cell within an organism contains the same DNA. A cell's DNA methylation patterns are also copied during the process of cell division. During cellular differentiation, the reliable transmission of DNA methylation patterns from mother to daughter cells is critical.[7,8] The diversity of cell types within one's body is generated through this epigenetic process, leading, for example, to the formation of differentiated neurons, blood cells, and muscle cells, which are identical in genetic information but differ significantly in epigenetic profiles. Thus, the epigenetic character of a cell, by determining the pattern of gene expression, determines the phenotype or character of the cell.

During the fetal and neonatal periods of life, there are rapid changes occurring within the developing organism that require the proliferation and differentiation of cells. The profound importance of epigenetic modifications during this time is highlighted by the effects of disruptions to the enzymes that are necessary for posttranslational histone modifications and DNA methylation. Studies using mice with a targeted deletion of a histone acetyltransferase gene indicate that disruption to this enzyme can be lethal and also associated with deficits in neural tube closing.[9] In the case of DNA methylation, the enzymes that are critical for this process are known as DNA methyltransferases (DNMTs). DNMT1 is a subtype of DNMT classified as a maintenance methyltransferase, which alludes to the role of this enzyme in maintaining the methylation patterns of cells after DNA replication/cell division. In contrast, DNMT3a and DNMT3b are called de novo methyltransferases and are thought to be primarily involved in adding new methyl chemicals to the unmethylated DNA.[10] Deletion of DNMT1 is associated with genome-wide hypomethylation, whereas overexpression of this gene leads to DNA hypermethylation, and both these genetic manipulations induce embryonic lethality.[11,12] Growth and survival are similarly impaired in DNMT3a/DNMT3b mutants.[13] Thus, activation of epigenetic pathways is essential for successful development.

EPIGENETIC PERSPECTIVES OF THE BIOLOGICAL IMPACT OF THE EARLY ENVIRONMENT

Our understanding of the biological significance of epigenetic variation is expanding rapidly. The dynamic, yet potentially stable changes in epigenetic regulation of gene expression that have been observed have raised intriguing questions: Could these mechanisms explain the effect of early-life experiences? Are epigenetic pathways a link between our experiences and the biobehavioral consequences of those experiences? Although initially it was assumed that plasticity of epigenetic modification was limited to the very early embryonic stages of development, this assumption has been challenged by the increasing evidence for environmentally induced epigenetic variation across the life span.[14] Variation in early-life exposure to toxins, nutritional levels, hormones, stress, and social interactions, which have been demonstrated to exert long-term effects on the brain, is also being demonstrated to be associated with histone modifications and changes in DNA methylation across the genome and within

target genes. Although we are still unclear regarding the mechanisms that permit this level of plasticity within epigenetic pathways, the implications of these epigenetic effects are far reaching. The following sections highlight the investigations of the epigenetic effects associated with prenatal maternal stress/distress, postnatal maternal care, and neglect or abuse in early infancy. These studies serve to illustrate the profound impact that parent-offspring interactions can have on molecular, physiologic, neurobiological, and behavioral outcomes.

PRENATAL MATERNAL STRESS EFFECTS ON EPIGENETIC VARIATION AND INFANT DEVELOPMENT

Stress during pregnancy has been implicated as a significant risk factor for various fetal outcomes and developmental disorders. In humans, the experience of a severe stressor during gestation, such as exposure to a natural disaster or terrorist attack, has been associated with preterm birth, reduced birth weight, and a smaller head circumference.[15,16] Similarly, maternal anxiety and depression have been associated with obstetric complications and preterm birth.[17,18] The long-term consequences of prenatal stress in humans have also been documented and suggest that increased risk of schizophrenia and depression may be linked to this form of early-life adversity.[19] Although it is presumed that fetal exposure to elevated levels of stress hormones in maternal circulation is etiologically relevant to these outcomes, our understanding of the mechanism of prenatal stress effects is drawn primarily from laboratory studies using rodents. In these studies, pregnant rats or mice are exposed to a variety of stressors during gestation, and offspring development, neurobiology, and behavior are assessed. In general, these studies confirmed the short- and long-term effects of maternal stress that have been observed in humans and also indicated that maternal glucocorticoids are a mediating factor for these outcomes.[20,21] In addition, changes in gene expression are associated with exposure to prenatal stress, and these changes in gene activity can persist into adulthood. As such, there is potential for the involvement of epigenetic pathways in these prenatal effects.

In mice, exposure to chronic variable stress during pregnancy has been used to model prenatal adversity and has been found to exert sex-specific long-term neurobiological effects.[22] In particular, this early-life exposure induces changes in the stress response pathways, primarily involving the hypothalamic-pituitary-adrenal (HPA) response to stress. Within the HPA pathways, an external stressor leads to neuronal activation within the hypothalamus, which triggers the release of corticotropin-releasing factor (CRF) and vasopressin. CRF and vasopressin then stimulate the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. The ACTH then stimulates the release of glucocorticoids (cortisol in humans and corticosterone in rodents) from the cortex of the adrenal gland. The glucocorticoids proceed to induce physiologic changes in several tissues, leading to the psychobiological changes we associate with the experience of stress: increased heart rate, blood pressure, and abnormalities in sleep-wake rhythms.[23] Although these short-term effects are often adaptive, increasing alertness and vigilance in response to threat, prolonged exposure to glucocorticoids may induce detrimental effects such as immunosuppression, weight gain, and depressed mood. To prevent the long-term exposure to glucocorticoids, there is a negative feedback loop that acts on the HPA axis involving glucocorticoid receptors (GRs) in the hippocampus. Glucocorticoid stimulation of these receptors triggers an inhibition of hypothalamic and pituitary release of CRF and ACTH, leading to reduced stimulation of glucocorticoid output from the adrenal gland.[24] Thus, there are both stress-enhancing and stress-inhibiting brain regions

and receptor/hormone targets that can be considered when exploring the mechanism through which stress during pregnancy can lead to stress sensitivity in later life.

The epigenetic consequences of chronic variable stress in mice have been examined in several target genes, including CRF and GR. In male pups born to a stressed dam, CRF gene expression is increased and GR gene expression is decreased in adulthood.[22] These changes in gene activity likely account for the increased corticosterone release to stress and increased depression-like behavior that is observed among stressed males. Quantification of DNA methylation within the promoter region of these genes (a region of DNA that is critical for gene regulation) indicates that prenatal stress is associated with decreased DNA methylation of the CRF gene promoter and increased methylation of the GR promoter region in hypothalamic tissue of adult male offspring. The direction of these epigenetic effects coincides well with the notion that increased DNA methylation leads to reduced gene expression. When considering the mechanism of prenatal effects, it is important to consider the functioning of the placenta, a tissue in which dysregulation of gene expression has been found associated with intrauterine growth retardation in humans.[25] In the context of the study of chronic variable stress effects in mice, placental gene expression is altered by gestational stress, with particular effects on DNMT1.[22] Thus, there may be widespread epigenetic variation within the placenta consequent to this early-life exposure.

Although the translation of this work to the study of human prenatal adversity is challenging because of the limited accessibility to tissues and reduced control over moderating and mediating variables (such as nutrition, smoking, and intensity/timing of stressors), there is increasing evidence for epigenetic variation associated with maternal mood. Depression during pregnancy is associated with increased maternal cortisol level and reduced gestational length and thus shares some common features with stress exposure during pregnancy.[26] Analysis of cord blood samples from infants born to mothers with elevated ratings of depression (using the Hamilton Depression Scale) during the third trimester of pregnancy indicates elevated levels of DNA methylation within the GR promoter region.[27] The degree of DNA methylation within the neonatal GR promoter was found to predict increased salivary cortisol levels in infants at 3 months of age. In a recent study, the persistence of these prenatal effects on GR methylation was highlighted.[28] Children and adolescents (aged 10–19 years) born to women who had experienced stress in the form of intimate partner violence during pregnancy were found to have elevated GR methylation levels in whole blood samples. Although the interpretation of the meaning of these epigenetic changes in blood has yet to be fully elucidated, these findings support the hypothesis of lasting epigenetic variation in response to prenatal adversity.

EPIGENETIC CONSEQUENCES OF POSTNATAL MATERNAL CARE

Variation in the quality and/or quantity of mother-infant interactions has been demonstrated across several species, including humans,[29] and is increasingly being explored as a route through which individual differences in physiology and behavior emerge. This variation can be induced by the quality of the environment, and maternal stress and depression are significant predictors of the quality of postnatal mother-infant interactions.[30,31] In particular, the level of tactile stimulation (gentle touching and stroking) that mothers provide to infants is predicted by maternal mood (eg, depressed mothers provide less tactile stimulation). The tactile context of human caregiving has been demonstrated to influence pain sensitivity, affect, and growth in neonates.[32] Increasing awareness of the advantages of touch for infant development

has led to changes in neonatal practices, such as encouragement of kangaroo care, skin-to-skin contact, and infant massage. These practices may be most advantageous for high-risk preterm infants, and when mothers are involved in the contact, the reciprocal tactile stimulation between mother and infant may contribute to increased maternal responsiveness and infant attachment.[32,33]

The biological impact of mother-infant interactions has been explored in laboratory rodents, and findings suggest that there are natural variations in maternal care in rodents that predict variation in offspring neurobiological and behavioral measures.[34] Rodents provide tactile stimulation to offspring through licking/grooming of pups (LG, a stimulation that can be mimicked by stroking with a paintbrush), and comparison of Long-Evans rat pups that have received low levels of LG with pups that have received high levels of LG has yielded valuable insights into the molecular and epigenetic factors that are shaped by maternal care. Maternal LG has been found to influence HPA reactivity, cognition, and reproductive behavior of offspring.[35] Analysis of the GR levels in the hippocampus of low LG male offspring compared with high LG offspring indicates that increased LG level is associated with increased hippocampal GR level in adulthood.[36] Consistent with this finding, offspring of high LG dams seem to have enhanced negative feedback of the HPA response to stress and have reduced corticosterone and ACTH levels after a stressor. Within the promoter region of the hippocampal GR gene, offspring of low LG dams have increased DNA methylation compared with offspring of high LG dams.[37] This differential methylation is not evident prenatally and emerges during the first week postpartum, a time during which LG behavior is at its peak among high LG dams. Histone acetylation and binding of transcription factors to the GR promoter region were also found to be increased among the offspring of high LG dams.[37] Within the hippocampus of low LG offspring, there are also reduced levels of glutamic acid decarboxylase (GAD1), the rate-limiting enzyme in γ-aminobutyric acid synthesis. Analysis of the GAD1 promoter region suggests that maternal LG induces a decrease in DNA methylation of this gene associated with increases in histone acetylation at this region.[38] Although most of these analyses have been conducted in male offspring, among the female offspring, the experience of low levels of LG during the postnatal period has been found to be associated with decreased transcription of estrogen receptor alpha (ERα) in the medial preoptic area of the hypothalamus (MPOA) and elevated DNA methylation within the promoter region of this gene.[39,40] Maternal effects on ERα DNA methylation and transcription may account for the effects of maternal care on reproductive/maternal behavior of female offspring.[41] Cross-fostering studies confirm that these epigenetic effects on gene expression are associated with the quality of the care received in infancy.

The impact of tactile stimulation during infancy on epigenetic pathways has also been explored using supplementary stimulation of rat pups using a paintbrush to provide licking-like stroking. Among artificially reared pups (ie, pups reared in the absence of the mother), the provision of a minimal level of tactile stimulation is necessary for survival and increasing the frequency of this stimulation during the neonatal period can significantly ameliorate the deficits induced by isolation from the mother.[42,43] Tactile stimulation in rodents is thought to influence sexual dimorphism in neuronal circuits, and there is evidence for sex differences in DNA methylation of the ERα promoter region in the MPOA.[44] Males have been demonstrated to have higher levels of ERα DNA methylation in this brain region compared with females. If females are provided with additional tactile stimulation during postnatal days 5 to 7, there are increases in DNA methylation of ERα such that females become indistinguishable from males regarding the methylation of this gene. Previous studies have

demonstrated that male pups are licked more frequently than female pups during the postnatal period,[45] and it may be that this stimulation triggers epigenetic pathways that enhance sex differences in the brain. Enhanced tactile stimulation of pups can also be induced by using a communal nesting paradigm in which multiple females provide care for the neonates.[46] Mouse pups reared in these conditions have been found to have increased histone acetylation at the brain-derived neurotrophic factor (BDNF) gene promoter and, like offspring that have been reared by high LG dams, manifest numerous neurobiological and behavioral effects of this enriched early experience.[47,48] Although maternal care certainly provides stimulation of multiple sensory systems, tactile stimulation provided by mothers to infants can have long-term epigenetic consequences, and these findings complement the growing literature on the hormonal, physiologic, and behavioral effects of human touch.[32]

EPIGENETIC EFFECTS OF ABUSE AND NEGLECT

In light of the impact of natural variations in maternal care on epigenetic pathways, extreme forms of postnatal experience, such as neglect or abuse, would likewise be predicted to induce changes in gene activity. Maternal separation studies on laboratory primates and rodents have been used as a strategy to model early maternal deprivation/neglect and have demonstrated the causal influence of this form of early-life adversity on multiple neuroendocrine and behavioral outcomes. Similar to the effects of prenatal stress and low levels of maternal care, studies using mice in which pups are repeatedly separated from the mother during infancy indicate increased corticosterone secretion in response to stress.[49] Consistent with the role of hypothalamic vasopressin (AVP) within the HPA axis, maternal separation–induced stress sensitivity is associated with increased hypothalamic expression of the AVP gene in adult mice. DNA methylation levels within key regulatory regions of the AVP gene were found to differ between control (normally reared) and maternally separated offspring, with decreased AVP DNA methylation among maternally separated male offspring.[49] This hypomethylation of the AVP gene was also associated with reduced levels of binding of MeCP2 (a methyl-binding protein). Separation-induced effects on epigenetic alterations within the serotonin transporter (5-HTT) gene have likewise been demonstrated. Among rhesus macaques and humans, there is genetic variation within the 5-HTT promoter that has been associated with variation in risk or resilience to stressors such as childhood maltreatment.[50,51] Rhesus macaques that are reared in conditions of maternal separation and possess the risk allele of the 5-HTT gene were found to have increased DNA methylation of 5-HTT gene in blood cells.[52] Thus, there are interactions between genetic variation and epigenetic effects in response to early-life adversity, and these interactions may be an important consideration when predicting the degree of impact of maternal neglect in infancy.

Childhood abuse is a significant predictor of long-term physical and psychiatric disorder and a major public health concern. Similar to maternal neglect, the experience of maternal abuse can be modeled in laboratory rodents to determine the causal impact of this experience on the developing brain. Among laboratory rats, reduction in nesting materials provided to lactating dams during the postnatal period significantly increases the incidence of aggressive encounters between pups and dams (such as stepping on pups, aggressive grooming, and transporting of pups by a limb) and decreases the frequency of nurturing behaviors such as LG.[53,54] When pups are exposed to caregivers (nonbiological mothers) that are provided with limited nesting materials and placed in an unfamiliar environment, there is increased exposure of pups to these aggressive behaviors. The epigenetic consequence of these

experiences has been determined for the BDNF gene in the prefrontal cortex.[55] Postnatal maltreatment predicts reduced expression of BDNF in the prefrontal cortex in adulthood. Among nonabused offspring, there are very low levels of DNA methylation within the BDNF gene promoter, whereas abused offspring have elevated levels of this repressive epigenetic modification. BDNF plays a critical role in brain development and neuronal plasticity.[56] Thus, reductions in BDNF achieved through epigenetic silencing of this gene may account for the profound effect of childhood maltreatment and lead to heightened risk of psychopathology among abused children.

The relevance of these epigenetic effects for childhood abuse in humans has been highlighted in a study that analyzed postmortem brain tissue.[57] Gene expression and DNA methylation analyses were conducted with brain tissue from suicide victims with or without a documented history of childhood abuse and a control non-suicide comparison group. GR expression in the hippocampus was found to be equivalent among controls and suicide victims without a history of abuse, whereas a history of abuse predicted reduced GR expression. DNA methylation analysis of this gene indicated that, consistent with the gene expression findings, DNA methylation within the GR promoter region was low and equivalent between controls and nonabused suicide victims, whereas childhood abuse was associated with increased DNA methylation and decreased transcription factor binding within the GR gene promoter. The consistency of these findings with the rodent work is clear and suggests that epigenetic mechanisms may play a general role across species in encoding information regarding the experiences of an individual (**Fig. 2**).

Although no direct relationship can be established between the literature on child abuse and neglect and early experiences of high-risk neonates, it is conceivable that the nature of the infant's early birth or congenital problems, painful and unpredictable procedures, inconsistency with pain alleviating and soothing interventions, and separation from the regulating processes of the mother contribute to early changes in brain with potential epigenetic consequences. Moreover, children who have disabilities, similar to those of preterm infants with a documented risk for later developmental delay, are reported to be referred to child protective services more frequently than children who do not have disabilities.[58–60]

EPIGENETICS AND PLASTICITY: IMPLICATIONS FOR INTERVENTION

Although we have much to learn about the processes through which our experiences can shape gene activity, studies of epigenetic variation in response to the quality of the prenatal and postnatal environment illustrate the potential plasticity of these mechanisms. Moreover, the responsivity of these pathways to variation in parental stress or postnatal mother-infant interactions suggests that interventions focused on moderating these aspects of the environment could have a significant impact on our biology. Studies on rodents suggest that maternal stress during gestation can lead to significant reductions in maternal care toward offspring,[61] and the occurrence of this phenomenon in humans is suggested by the effects of maternal stress and depressed mood on the frequency and quality of mother-infant interactions. Thus, an intervention targeted at alleviating maternal stress or mood may have significant downstream consequences for infant development. Increasing parental sensitivity to infant cues and promoting increased caregiver contact with infants may also be an effective strategy for intervention.[62] Studies of severely neglected infants suggest that adoption into foster families can ameliorate deficits in functioning.[63,64] These findings are complemented by animal studies in which enrichment during juvenile development can ameliorate the deficits associated with prenatal or postnatal adversity.[65–67]

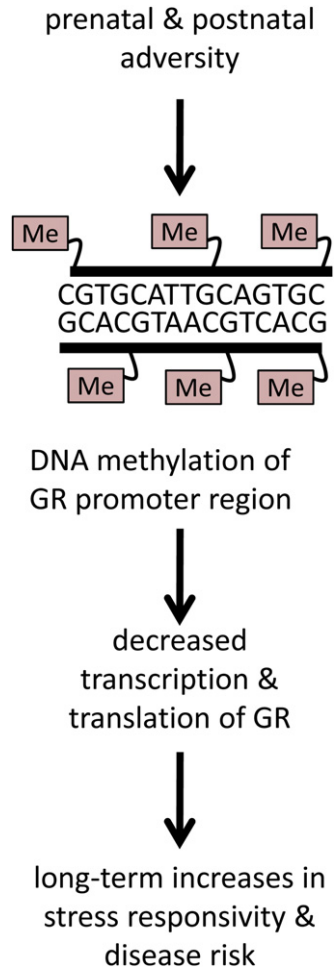

Fig. 2. Summary of the epigenetic effects of adversity on GRs. Studies on humans and rodents provide evidence for prenatal and postnatal adversity (maternal stress, low levels of postnatal infant care, and childhood abuse) on DNA methylation of the GR gene promoter. Increased adversity is associated with increased GR methylation and decreased expression of this gene. Consequently, exposed individuals have a heightened response to stress, leading to increased risk of the disease (physical and psychiatric).

Plasticity is a phenomenon typically associated with the early stages of development, and like most biological processes, changeability of epigenetic modifications may decline over developmental time. However, this phenomenon does not suggest that epigenetic effects are unchangeable in later life. In the case of the epigenetic consequences of variation in maternal LG levels, pharmacologic targeting of the epigenome has been used to reverse the effects of postnatal maternal care on gene expression, physiology, and behavior. Adult males that had been reared by low LG dams were found to have decreased GR promoter DNA methylation, increased hippocampal GR expression, and reduced corticosterone response to stress if treated with a drug that induced histone acetylation.[37] In contrast, if adult offspring of high LG dams are treated with a drug that increases the amount of methyl donors (methyl

chemicals that can potentially methylate the DNA), these offspring are found to have increased GR promoter DNA methylation, decreased GR expression, and heightened stress reactivity.[68] This epigenetic plasticity is likely not limited to pharmacologic interventions, and the change in epigenetic profiles over time and in response to early- and later-life experiences will certainly be an interesting phenomenon to explore in both human and animal studies.

FUTURE DIRECTIONS IN THE STUDY OF EPIGENETICS AND INFANT DEVELOPMENT

For the high-risk neonate, issues regarding parental stress and deprivation of parental contact are very meaningful. Although the biological effects of nutritional or sensory deprivation may be well established, the quality of the early social/tactile environment can likewise have a lasting effect. Continued efforts to minimize the separation between mothers and infants during the postnatal period and to enhance the quality of mother-infant interactions when they occur are likely to ameliorate some of the effects of the early-life adversity to which these infants are exposed. An emerging theme within studies of epigenetic effects of the environment concerns the implications of these effects for subsequent generations of offspring. The heritability of DNA methylation patterns is certainly a critical feature of this epigenetic mark in the context of cellular differentiation. Recent studies suggest that environmentally induced epigenetic variation may also be inherited by offspring and grand-offspring generations.[69] For example, maternal separation in mice has been found to induce DNA methylation changes in the male germline, and similar epigenetic variation is observed in the brains of the offspring of these maternally separated males.[70] Epigenetic modifications may also be transmitted over generations through the stable transmission of maternal behavior from one generation to the next.[71] Taken together, these studies suggest that the consequences of perinatal experiences may not be limited to the individual experiencing adversity but may also be observed in the nonexposed offspring of these individuals. A multigenerational perspective in future studies of high-risk neonates may prove to be informative of the mechanisms through which infants and their families are influenced by these experiences.

Although the translation of laboratory studies of epigenetic effects has certainly made progress, there is much to be gained from continued study of epigenetic variation in humans. Similar to studies on rhesus macaques, there is evidence for genetic-epigenetic-experience interactions in humans, which predict biobehavioral outcomes,[72,73] and examining these interactions in response to the specific experiences of high-risk infants would be particularly informative. Longitudinal studies involving noninvasive measures of epigenetic variation over time would likewise enhance our understanding of the pathways through which the quality of the environment shapes our brain and behavior. As our basic understanding of epigenetic mechanisms expands and techniques for studying these mechanisms become more readily available and feasible to implement, there is increasing promise that an epigenetic perspective can be applied to the study of infant development. This perspective combined with the measures of neurobiological and behavioral function would allow for a depth of understanding of the development of high-risk neonates that spans from molecular to neurobiological and psychological functioning; a depth that may give rise to more refined and targeted interventions to enhance infant and parent well-being.

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