CHAPTER

The Social Environment and Epigenetics in Psychiatry

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CHAPTER OUTLINE

KEY CONCEPTS

- Phenotypic variations may be caused by differences in long-term programming of gene function rather than variation in gene sequences *per se*, and studies of the basis for inter-individual phenotypic diversity should consider epigenetic variations in addition to genetic sequence polymorphisms.
- DNA methylation and chromatin structure are found in a dynamic balance throughout life that is maintained and defined by sequence-specific factors that deliver DNA methylation and histone modification to genes.

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- The fact that histone modifications and DNA methylation are potentially reversible processes suggests that psychosocial, pharmacological and social interventions may be used to treat mental illness.
- Epigenetic programming in the brain of rodents by maternal care early in life is a highly stable yet reversible process that results in long-term changes in gene expression.
- In our studies of social adversity in humans in early life, we found that aberrant DNA methylation of the glucocorticoid receptor promoter leads to decreased transcription of the gene and that this effect was associated with a history of early childhood abuse or neglect in humans.
- Microarray studies of gene expression programming by maternal care in rodents and childhood abuse or neglect in humans indicate that the epigenetic response to early-life experiences involves coding and non-coding regions of the genome and a number of genes related to stress and neuroplasticity.
- Several studies in rodents and humans indicate that genes related to neuroplasticity and stress are epigenetically labile in response to social factors in adulthood, outside of sensitive periods of development.
- Novel high-throughput technologies for epigenetic analysis that have enabled epigenetic studies of human populations and epigenetic changes throughout the genome must be matched by equally powerful analyses of phenotypes for an understanding of epigenetic contributions to behavior.

ABBREVIATIONS

5-mC 5-Methylcytosine
BIS-seq Bisulfite sequencing
CRF Corticotropin-releasing factor
DNMT DNA methyltransferase
GAD67 Glutamate decarboxylase 67
GC Glucocorticoid
GR Glucocorticoid receptor
HATs Histone acetyltransferases
HDAC Histone deacetylase
HDACi HDAC inhibitor
HPA Hypothalamic–pituitary–adrenal
LTP Long-term potentiation
MeDIP-seq Methylated DNA immunoprecipitation sequencing
miRNA microRNA
NGFI-A Nerve growth factor inducible A
PBMC Peripheral blood mononuclear cell
SAM *S*-adenosylmethionine
SES Socioeconomic status
TSA Trichostatin A
TSST Trier Social Stress Test

Introduction: social factors, genes, and gene expression programming

Social factors, particularly those that are encountered early in life such as parental care, can have profound effects on neurobiological trajectories and long-term consequences for mental health. It has been largely accepted that normal brain development depends upon a complex interplay between genetic and environmental factors. The field of behavioral epigenetics has provided a paradigm in which to examine novel mechanisms by which experiences can become "biologically embedded," influencing the development and maintenance of behavioral adaptations to environmental challenges.

It was once thought that patterns of gene expression are programmed such that, in the absence of pathology, they are largely resistant to variations in the environment during cellular development and differentiation. However, accumulating evidence now indicates that many different cell types execute distinct patterns of gene expression that are highly responsive to physiological and environmental cues

during development. To understand the relationship between environmental variation and variation in gene expression, a number of experimental rodent models have been developed to assess the influence of duration and timing of environmental exposures on behavioral outcomes. In addition, recent technological advances have enabled studies in human populations on unprecedented scales, enabling subphenotyping of large cohorts and the use of endophenotypes in human populations in conjunction with molecular assessments related to gene function, albeit typically in peripheral tissues. This research has provided evidence that a key component of the influence of environmental factors on gene expression programming lies in molecular mechanisms that operate "above the genome," termed epigenetic.

In this chapter, we will discuss several examples of approaches to rodent models and human cohort studies that have been applied to examine the influence of social factors on behavioral and mental health outcomes. We will highlight discoveries in the field that relate to epigenetic mechanisms that mediate risk and resilience to environmental challenges, with a particular focus on neurodevelopment. First, we will review basic epigenetic mechanisms and their known role in gene regulation. Next, we will discuss how epigenetic differences contribute to inter-individual variation in behavior. We will then highlight studies in animals and humans of the role of social factors in early life in mediating inter-individual differences in behavior via epigenetic mechanisms. We will complement this discussion with examples of these linkages outside of sensitive periods. Finally, we will discuss critical issues in the field for future studies of the role of the social environment and epigenetics in psychiatry.

How mechanisms of gene regulation "above the genome" contribute to inter-individual differences in behavior

Cell-type-specific patterns of epigenetic modifications are not exclusively genetically determined and are to some degree responsive to environmental signaling throughout life. The dynamic nature of epigenetic signaling contrasts with the static nature of the genetic code and provides a mechanism of gene-by-environment interactions that bridge inherited variation with variation originating from environmental and stochastic sources. It should be noted, however, that these sources of variation are not necessarily mutually exclusive, as genetic variation can interact with epigenetic variation [1]. However, in contrast to the relatively static nature of genetic variation, epigenetic variation is potentially amenable to psychosocial or therapeutic intervention. Thus, we and others have argued that a complete understanding of the origins of health and human disease—including psychiatric disorders—requires an integrative understanding of genetic, environmental, and stochastic contributions to epigenetic signaling [2,3]. As we will discuss below, with specific examples, there is accumulating evidence indicating that variation in neuroplasticity and behavior observed in humans and animal models as a function of social factors is associated with changes in gene function via epigenetic mechanisms.

Overview of epigenetic mechanisms

The epigenome consists of the chromatin and its modifications, as well as a covalent modification by methylation of cytosine rings found at the dinucleotide sequence CG [4], although recent evidence has indicated that 5-methylcytosine (5-mC) also occurs in non-CpG contexts in differentiated somatic cells [5,6]. The epigenome determines accessibility of the transcription machinery to DNA. Here, it is

necessary to distinguish between the open and closed configuration of chromatin. Densely packaged chromatin can be visualized microscopically and is termed heterochromatin, whereas open accessible chromatin is termed euchromatin [7–11]. Within heterochromatic regions, genes are inaccessible to the transcriptional machinery and therefore silent, whereas genes within euchromatic regions may be transcribed. Specific modifications to histones create a "histone code" that regulates accessibility of the transcriptional machinery. Another level of epigenetic regulation occurs via the activity of non-coding microRNAs (miRNAs) that regulate gene expression at different levels: silencing of chromatin, degrading mRNA, and inhibiting translation [12]. More details of epigenetic mechanisms of regulation of gene expression are given in Chapter 2 of this book.

Targeting of epigenetic modifications to specific genes

To date, knowledge about how specific environmental factors target select gene sequences remains poor, although we will review one such example below for maternal care. This gap in knowledge remains a significant challenge for the field. Transcription factors and repressors are known to recruit non-specific histone-modifying enzymes to specific genomic loci and target specific genes by recognizing specific *cis*-acting sequences in genes, binding to these sequences and attracting specific chromatin modifying enzymes to genes through protein–protein interactions [13].

A central question regarding gene-specific changes in DNA methylation associated with the environment concerns the targeting of these sequence-specific changes to specific loci in the genome. Because processes mediating DNA methylation and demethylation are non-specific, targeting must be achieved via other mechanisms. There is evidence that chromatin configuration can regulate the accessibility of genes to the DNA methylation machinery. For example, the histone deacetylase inhibitor (HDACi) trichostatin A (TSA), which leads to hyperacetylated chromatin, also leads to active DNA demethylation [14]. A change in histone acetylation is normally caused by transcription factors that recruit histone acetyltransferases (HATs), which may cause histone acetylation and facilitate demethylation. Examples of histone-modifying enzymes shown to interact with DNA methyltransferase 1 (DNMT1) are histone deacetylase 1 (HDAC1), HDAC2, the histone methyltransferases SUV3–9, and EZH2, a member of the multiprotein polycomb complex PRC2 that methylates H3 histone at the K27 residue [15–18]. DNMT3a was also shown to interact with EZH2, which targets the DNA methylation–histone modification multiprotein complexes to specific sequences in DNA [18].

Epigenetic mechanisms and mental health

In the broadest definition, epigenetic mechanisms refer to the combination of mechanisms leading to the long-term programming of gene function without a change in gene sequence. Epigenetic programming occurs primarily during development to generate the complex patterns of gene expression characteristic of complex organisms such as humans. Like gene expression, epigenetic mechanisms are somewhat dynamic in response to environmental exposures, especially though not exclusively during fetal development and early in life. Thus, particular phenotypic variation observed in human populations could be a function of epigenetic differences leading to the long-term programming of gene function as well as the genetic sequence. We and others have argued for this reason that the analysis

of inter-individual phenotypic diversity should take into account epigenetic variations in addition to genetic sequence differences [19]. We and others have also proposed that DNA modifications are maintained in an equilibrium between methylation and demethylation that is maintained as long as this equilibrium of sequence-specific factors engaging particular gene sequences is maintained [3,19]. This process is essential for normal development and the process of tissue-specific cellular differentiation. Physiological or environmental signals, which alter the signaling pathways in the cell, may result in altering this balance by activating or suppressing specific *trans*-acting factors.

Some critical environmental exposure, such as variations in maternal behavior, could alter the progression of epigenetic programming during development postnatally as well as *in utero*. Thus, variation in environmental exposures during these sensitive periods could result in epigenetic and therefore phenotypic differences later in life. Recent data suggest that social exposures early in life also impact the epigenome, resulting in differences in epigenetic programming and as a consequence in behavioral differences later in life [3]. As a result, certain early in life exposures that alter epigenetic programming may lead to later-life psychiatric disorders [3].

It is important to understand the mechanisms driving variations in epigenetic programming in order to identify the behavioral pathologies that result from such mechanisms. Unlike genetic mechanisms, the dynamic nature of epigenetic mechanisms implies that they are potentially reversible and amenable to therapeutic intervention [20]. Because various drugs used in the treatment of psychiatric disorders such as schizophrenia and mood disorders have known epigenetic effects, interventions targeting the epigenetic machinery could have important consequences for normal cognitive function. Once the rules governing the effects of environmental exposures on epigenetic processes are understood, it might be possible to design strategies to prevent and reverse deleterious environmentally driven epigenetic alterations.

The response of epigenetic machinery to environmental signals

Whether or not the DNA methylation at particular genomic loci is reversible has important implications for the possibility that the regulation of these loci by DNA methylation will be responsive to physiological and environmental signals throughout life. Enzymes that mediate changes in DNA methylation, such as DNMTs, are present in neurons [21], and there are data suggesting that DNMT levels in neurons change in certain pathological conditions such as schizophrenia [22]. The presence of DNMT in neurons suggests that the DNA methylation machinery is poised to act even in postmitotic cells, indicating that the DNA methylation is a balance of methylation and demethylation reactions [19]. miRNAs have been linked to behavioral pathologies in humans and regulate gene function through a variety of mechanisms, as has been extensively reviewed elsewhere [23–26]. These mechanisms are currently the subject of intense investigation in animal models as well. For example, altered miRNA expression in adulthood has been linked to early-life stress in rats [27], suggesting that it is responsive to environmental alterations in early life. Environmental or physiological events that interfere with signaling pathways may result in chromatin alterations [28]. Because signal transduction pathways are activated by cell-surface receptors, they may potentially serve as conduits for epigenetic change, linking the environmental trigger at cell-surface receptors with gene-specific chromatin alterations and reprogramming of gene activity [28]. Below, we will discuss an example of such a pathway that leads from maternal behavior to long-term programming of gene expression in the hippocampus.

Social factors associated with epigenetic modifications during sensitive periods of development

The maternal environment exerts a profound mediating role between environmental exposures and the developing offspring. In mammals, this mediation may occur as a result of a direct fetal exposure via the maternal–fetal interface, alterations in maternal physiology pre- and postnatally, or changes in mother–offspring interactions during early postnatal life [3]. A prominent and well-studied feature of maternal effects on neurodevelopment is its influence on the hypothalamic–pituitary–adrenal (HPA) axis, a major regulator of the endocrine response to environmental challenges. The regulation of circulating glucocorticoids (GCs) maintains homeostatic energy balance across the circadian cycle and mediates the physiological and behavioral response to stress. Output from the stress axis begins with sensory input on environmental variation that initiates a cascade of endocrine responses from the hypothalamus, culminating with the release of GCs in the form of cortisol or corticosterone that feed back on a variety of neural circuitry.

Ecologists have long recognized that chronic stressors (e.g., predation risk, resource availability, social interactions) play key organizing roles in ecosystems. Indeed, the mechanistic functioning of the HPA axis is highly conserved across vertebrate taxa, underscoring the biological importance of optimal glucocorticoid regulation [29–31]. It is increasingly understood that maternal stressors can induce preparative and adaptive programming in offspring via exposure to maternal GCs. Indeed, a diverse array of stressors can induce relatively permanent changes in the HPA axis of offspring via exposure to maternal stress during pre- and postnatal development: environmental effects on maternal state, predation pressure, quality of the rearing environment, and the unpredictability of the social environment [32]. The relative permanence of such changes in an ecological (natural world) context suggests that the effects of stress on the HPA do not reflect pathology but instead indicate adaptive responses that prepare offspring for environments where stressors are likely to be encountered [32,33].

A large number of human and animal studies have been designed to understand the role of early-life experiences in life-long psychopathology [3]. Human epidemiological studies have indicated that early-life experiences have enduring consequences for health in adulthood—including mental health—as a consequence of establishing long-term health trajectories [34]. For example, early-life low socioeconomic status (SES), a measure of relative financial, educational, and social position, strongly predicts a wide range of mental health problems in adulthood, including schizophrenia and depression [3,35,36]. Likewise, early adverse experiences such as physical abuse or neglect are well-known risk factors for mental health problems later in life [37]. Children who experience parental neglect as a result of institutionalization in early life show profound intellectual impairment and failure to completely catch up, even with social intervention [38]. Childhood physical and sexual abuse also impairs intellectual function and increases the risk of affective disorders and suicide [39]. Environmental experiences during early life have been suggested to exert an enhanced impact on health trajectories in part because early postnatal development is a time of enhanced plasticity [40,41].

Elucidating the biological mechanisms underlying effects of early social experiences on later mental health is challenging in humans for reasons that include limited access to relevant biological material. More is known about the pathways altered by adversity than other forms of early social experience. Studies in animal models have suggested that early-life stress impairs neuroplasticity in brain regions such as the hippocampus and has a lasting impact on endocrine systems underlying the response to psychosocial stressors [35,42]. Laboratory rodent models have been particularly useful in identifying mechanisms of epigenetic regulation in the brain that have then been used to generate hypotheses in humans.

Biomedical studies of humans and laboratory animals indicate a profound effect of parental care early in life on the epigenetic programming of the stress axis and associated behaviors.

Maternal care, epigenetics, and the HPA axis: laboratory animal studies

Research findings by Weaver, Meaney, Szyf, and colleagues in the early 2000s launched epigenetics to the forefront of research on mechanisms leading from maternal behavior to long-term programming of gene expression in the offspring. Earlier work by the Meaney laboratory and others had established that naturally occurring differences in maternal care in the early postnatal period—during the first week of life in the rat—lead to long-term effects on stress and stress-related behavior. The offspring of mothers who naturally exhibit high levels of care show elevated transcript abundance of the glucocorticoid receptor in the hippocampus, enhanced negative feedback sensitivity, and a more modest response to stressors in adulthood [43]. Cross-fostering studies showed that this phenotype is directly attributable to maternal behavior rather than factors related to the prenatal environment, as offspring phenotype was shown to match that of the adoptive mother rather than that of the biological mother [44]. Weaver and colleagues [45] showed that the accompanying change in glucocorticoid receptor (GR) expression was regulated by DNA methylation of the $GR1_7$ splice variant in the hippocampus by inhibition of the binding of NGFI-A, a transcription factor that drives GR expression. $GR1_7$ is one of at least 11 untranslated first exons of the GR gene. Though it is ubiquitously expressed in virtually all cells, levels of expression of GR vary and are controlled in part by tissue-specific expression of GR exon 1 splice variants (this is also true for the human GR exon 1, as will be discussed) [46,47]. In the hippocampus, $GR1_7$ was previously shown to vary in expression as a function of the level of maternal care received [47]. Interestingly, relatively high levels of DNA methylation were maintained among the offspring of low-maternal-care mothers, whereas offspring of high-maternal-care mothers showed demethylation of this promoter during the first week of life, coinciding with emergence of differences in maternal care between the two litter types. The results implied that DNA demethylation (through a yet unknown process) leads to an increased number of GRs and an attenuated response to stress. DNA methylation differences were stable throughout adulthood in these animals, but were reversible by infusion of TSA, a histone deacetylase inhibitor, which also led to increased gene expression in hundreds of other genes [48]. Likewise, lower levels of DNA methylation observed among the offspring of high-maternal-care mothers resembled those of offspring of high-maternal-care mothers given central infusions of the methyl donor *S*-adenosylmethionine (SAM), indicating that enzymes responsible for DNA methylation were poised to act in the adult brain in response to a methyl donor.

A recent study has challenged the idea that $GR1_7$ transcript is regulated by DNA methylation of the NGFI-A response element in rats exposed to stress paradigms that lead to altered NGFI-A levels, although stress does appear to modulate the methylation of other CG sites within the $GR1_7$ promoter [49]. It is likely that DNA methylation of $GR1_7$ gene expression involves the binding of additional transcription factors and/or is context and brain-region specific. It is also likely that the $GR1_7$ is itself part of a response mechanism that involves additional splice variants of GR and perhaps other transcription factors.

We performed a microarray analysis of DNA methylation, H3K9 acetylation, and gene expression in a 7 million base pair region containing the GR gene in the rat hippocampus [50]. We found that epigenetic differences in adulthood that were associated with early maternal care occurred in clustered regions of up to 100 kb but were nonetheless exquisitely patterned, whereby increased transcription

occurred in conjunction with hyperacetylation and hypermethylation of exons and hypomethylation of promoters. We found epigenetic differences in association with altered transcription as a function of maternal care across several GR_1 splice variants. Large epigenetic differences were noted in proximity to the transcription start site of GR, within the first coding exon (exon 2) and within GR introns, suggesting there may be additional regulation of GR via yet-to-be-identified non-coding RNAs within the GR locus. These data were the first to link epigenetic changes across both coding and non-coding regions in the mammalian brain, and they implicate a non-random epigenetic programming across large-scale loci in response to differences in early care. Accumulating evidence indicates that additional genes in neural pathways mediating the stress response are epigenetically regulated by DNA methylation of gene regulatory elements in association with early-life stress—for example, arginine vasopressin in the hypothalamus [51], BDNF in the hippocampus [52], and GAD67 in the prefrontal cortex [53].

These postnatal programming effects appear to derive from environmentally induced alterations of maternal–neonatal interactions involving systems that determine methylation patterns of GR gene promoter sequences and additional loci. It will be important to understand the precise nature of the maternal–neonatal interaction that mediates these changes. For example, there is evidence that artificial stimulation of pups with a paintbrush as a substitute for maternal licking can alter DNA methylation of a promoter region of the estrogen receptor alpha gene in the preoptic area of the hypothalamus [54]. These data have important implications for studies of transgenerational impacts related to maternal care via epigenetic mechanisms, via *behavioral* mechanisms of inheritance rather than gametic inheritance, as maternal behavior is associated with levels of maternal care provided by offspring to their progeny [55].

Parental care, epigenetics, and the HPA axis: human studies

Recent advances in genomics have provided new means to address these questions in large numbers of human subjects in an increasingly comprehensive and powerful manner. In the previous section, we discussed evidence from our studies of widespread but specific epigenetic and transcriptional alterations of the GR gene extending far beyond the GR promoter associated with differences in maternal care [50–53]. Thus, there is mounting evidence that epigenetic mechanisms coordinate widespread changes in gene expression in response to differences in early maternal care or adversity.

Altered DNA methylation of the GR promoter has also been found to be associated with social behaviors and HPA dysfunction. In one of the earliest reports, DNA methylation of GR promoter in infants' cord blood was found to differ as a function of maternal mood during pregnancy and correlate with infants' cortisol response [56]. These data suggest that GR promoter methylation in the brain and in lymphocytes is under epigenetic control as a function of maternal physiology or perhaps differences in maternal care early in the life of the offspring. We examined postmortem brain tissue from adults with well-characterized life histories to investigate the influence of early-life adversity on glucocorticoid receptor DNA methylation in adults with a history of trauma. Our focus was on individuals with a history of severe physical or sexual abuse or neglect during childhood, which is common among suicide victims and is an important risk factor for suicide [3,37]. We examined the GR1F promoter in the hippocampus of human suicide victims and controls [57]. Family dysfunction and childhood adversity are linked to altered HPA stress responses and an increased risk for suicide. The promoter region we examined is upstream of one of several untranslated exon 1 splice variants that are known to regulate tissue-specific expression of GR, akin to the function that the GR exon 1 splice variants serve in the

rodent [46]. The study included three conditions: (1) suicide completers with a history of childhood abuse or severe neglect, (2) suicide completers without a history of childhood abuse or neglect, and (3) individuals who had neither committed suicide nor had a history of childhood abuse or neglect. A fourth group of non-suicide victims with a history of abuse or neglect was not available, partly due to the fact that tissues from such a "control" group are exceedingly rare and were unavailable for our study. In this study, we found that the GR gene was differentially methylated among suicide victims with a history of abuse in childhood, but not among suicide victims with a negative history of childhood abuse, compared with control individuals without a history of suicide. The data suggest that epigenetic processes might mediate the effects of the social environment during childhood on hippocampal gene expression and that stable epigenetic marks such as DNA methylation might then persist into adulthood and influence vulnerability for psychopathology through effects on intermediate levels of function, such as activity of the HPA axis that regulates the stress response. However, it is still unclear whether the epigenetic aberrations were present in the germ line, whether they were introduced during embryogenesis, or whether they were truly changes occurring during early childhood. We also do not yet know the extent to which parental factors *per se* play a role in this phenotype. Despite these important caveats, these data were the first to link the early-life environment to changes in the GR gene in humans. The data parallel those for the rodent study mentioned above, although in a very different context.

We have applied high-throughput approaches to examining DNA methylation, chromatin modifications, and mRNA expression in gene regulatory, coding, intragenic, and intergenic regions in humans in a study that paralleled that described above in rats. We analyzed the GR gene locus by interrogating a 7-MB region containing the GR gene in hippocampi of adult suicide victims who were abused early in life compared with controls using high-throughput DNA microarray [58]. The GR gene locus shows substantial conservation with the same locus in rodents, with an almost identical order or orthologous genes across the locus. Like the study in the rat [50], methylation levels were non-randomly distributed across the locus, indicating that stochastic processes are unlikely to account for the range of variation that we observed in this study. Proximal to the GR gene itself, we found a large region hypermethylated in suicide completers relative to controls within the first coding exon of the GR gene and its proximal promoters, extending previous observations of hypermethylation of the GR1F promoter among suicide victims with a history of abuse [57]. This analysis also revealed differences in DNA methylation in intragenic regions of the GR gene. At this time, we can only speculate that unrecognized non-coding RNAs may reside within this region and affect GR expression. Other differences were discovered within coding regions and the 3′-UTR of the GR gene. These data suggest that GR is epigenetically labile in response to the early-life social environment in both rodents and humans, although the specific alterations that we observed are not identical in both species [58]. Nevertheless, the data indicate that the animal model of parental care may have broad applicability for translational studies aimed at understanding the consequences of epigenetic modification of the GR in humans [3].

Social factors associated with epigenetic modifications outside of sensitive periods of development

Social stress, epigenetics, and the HPA axis: laboratory animal studies

One of the most common paradigms used to model genetic and epigenetic mechanisms in stress-related psychiatric disorders in laboratory animals is chronic social defeat. The paradigm consists of repeated

presentations of an aggressive individual and yields two types of behavioral outcomes: (1) stress-vulnerable individuals who avoid subsequent social contact and show other depressive-like behaviors, and (2) stress-resilient individuals. Interestingly, aspects of the behavioral outcomes associated with social defeat have been shown to be transmitted transgenerationally via the father's sperm [59]. When mice were subjected to social defeat and then bred to non-stressed females or when the defeated father's sperm was used for *in vitro* fertilization, offspring showed a decreased latency to become immobile in a forced swim test, a standard measure of depressive-like behavior. However, there was no reduction in social avoidance among offspring generated by *in vitro* fertilization, in contrast to those bred through standard mating, suggesting that aspects of the father's behavior during mating that may have influenced reproductive success or maternal behavior and physiology are necessary for the expression of avoidance behavior, at least in the experimental context that was examined.

Transgenerational impacts of social experiences have been studied using paradigms involving disrupting social hierarchies and environmental enrichment in laboratory mice. For example, when hierarchies are disrupted by repeatedly changing a rodent's cage mate, anxiety is increased in both mothers and female offspring [60]. Interestingly, disrupted social interaction and increased anxiety are also observed among second- and third-generation female offspring as a result of transmission via the paternal line [60]. Strategies whereby mice are exposed to an enriched environment, such as novel objects or exercise, have also shown that the enriched environment influenced anxiety and social behavior. In one study, adolescent females that were exposed to an enriched environment showed increased social interaction and enhanced learning and long-term potentiation (LTP) in offspring [61]. A recent study that has garnered considerable interest indicates that environmental information regarding fearful experiences may be transmitted transgenerationally in a manner that is independent of social transmission. The authors of this study showed that behavioral sensitivity to a novel odor (but not other odors) that was paired with shock in a fear-conditioning experiment could be transmitted to first- and second-generation offspring [62]. *In vitro* methods were used to demonstrate paternal transmission via the gametes, implicating an epigenetic mechanism. The authors also reported that a specific olfactory receptor (Olfr151), known to be sensitive to the odor used in the fear-conditioning experiment, showed differences in methylation as a function of conditioning in both the father and the offspring. Additional work is needed to understand how such a mechanism is transmitted via the gametes to direct epigenetic change at this specific olfactory receptor. Nevertheless, the results are exciting because they demonstrate the potential involvement of epigenetic processes in transmitting (or perhaps forecasting) information learned in one generation to additional generations.

Studies of specific genes that are altered as a function of social experience using a social defeat paradigm have documented altered hippocampal BDNF transcription with repeated encounters with an aggressive individual in stress-vulnerable individuals. These changes in gene expression occur together with epigenetic changes in the *BDNF* gene. Among defeated individuals, one study found lower transcript abundance of specific BDNF transcripts and increased levels of repressive histone marks on the *BDNF* gene with social defeat stress [63]. In a study of the contribution of individual differences to social defeat stress vulnerability, epigenetic changes that include increased histone acetylation and activation of BDNF VI transcripts were associated with a stress-resilient phenotype [64]. In the hippocampus, medial prefrontal cortex, and dorsal raphe nucleus, the acetylation of histones and the expression of histone-modifying enzymes are also correlated with behavioral outcomes associated with chronic social defeat stress [65]. Other groups have shown that genes associated with the regulation of the HPA axis are epigenetically modified by this form of social stress. Mice that spent less time in a

social interaction zone after social defeat display long-term demethylation of the corticotropin-releasing factor (CRF) gene in the hypothalamus. This change in methylation occurs in the context of overexpression of CRF, an overactive HPA axis, and behavior characteristic of social avoidance [66]. However, mice that are stress resilient do not show these epigenetic changes and spend more time in the social interaction zone after exposure to the same social defeat procedure.

Social stress, epigenetics, and the HPA axis: human studies

In contrast to the studies described above in rodents, to date fewer studies have examined the role of epigenetic modifications in association with the social environment in humans. Recent work has indicated that mildly stressful social experiences can lead to rapid epigenetic modifications in the human genome. A study using the Trier Social Stress Test (TSST) has found short-term increases in DNA methylation of the oxytocin receptor gene in peripheral blood [67]. DNA methylation status of the oxytocin receptor gene is also associated with individual variability in neural responses within brain regions supporting social perception [68]. In another study, the TSST showed gender-specific differences in methylation of the GR gene, with greater methylation of GR among females compared with males and a corresponding decrease in salivary cortisol during administration of the test [69]. Greater stress has also been found in association with lower methylation of the catechol-*O*-methyltransferase Val^{158} allele and more inefficient prefrontal activity [70].

Conclusion

A more complete understanding of the role of epigenetic mechanisms in perinatal programming will be afforded by studies that address several basic questions. First, in what contexts is the epigenome labile in response to early environment? Are there indeed critical time windows for the influence of the environment on epigenetic trajectories? A number of studies have linked early-life events to changes in neuroplasticity that have a lasting impact on endocrine systems mediating the response to stress [71]. It is not always clear, however, which cell types are relevant to the question under study. This is particularly problematic for studies in humans, where access to neural tissue is non-existent or limited. Peripheral cells such as peripheral blood mononuclear cells (PBMCs) offer an avenue to examine the HPA, as PBMCs are sensitive to endocrine modulation of HPA. Whole blood has also been used, but each tissue type is known to be sensitive to differences in constituent cell numbers, which may bias the results [72,73]. However, in studying environmental impacts prospectively in children, it is not often possible to obtain blood samples, and other tissues must be used. The most commonly used tissue in such epigenetic studies are buccal cells from mouth swabs or saliva. Intriguingly, there is some evidence that such tissue is responsive to early-life adversity, although perhaps not via epigenetic changes in GR *per se* [74]. Buccal cells complement studies of adversity in neurons in the sense that they do represent cells with a common embryonic origin. Such studies will provide a valuable means of resampling to examine epigenetic variance over time and with interventions. In animal studies, a goal going forward for translational work will be to identify labile epigenetic regions like the glucocorticoid receptor that can be assessed in brain and blood in order to generate hypotheses and biomarkers that can be examined in humans. Such research stands to offer critical insights into the manner by which the biological embedding occurs during the perinatal period.

Finally, identifying the specific conditions in which psychopathology arises as a function of early-life environment will shed light on the ultimate causes of epigenetic plasticity and the reasons for why some regions of the genome appear to respond to the environment in early life. For example, Barker's hypothesis, the proposal that pathological outcomes result from reduced fetal growth [75], stimulated research on a variety of health-related conditions arising from early environmental exposures [76]. This research revealed that nutrition and parental care can alter health trajectories in a manner consistent with that of an adaptive response, as both early undernutrition and overnutrition can lead to the same pathological outcomes (e.g., metabolic syndrome, cardiovascular disorders) [76,77]. Thus, the range of responses to early adversity suggests instead that pathology may arise as a function of mismatch between the early-life environment and the later-life environment rather than as a consequence of early-life dysfunction. This distinction is potentially important, because it implies that, for animal and human studies, specific environmental conditions may exist in which pathological responses may instead confer an apparently adaptive advantage.

In the studies we reviewed above from our group examining the epigenetic signature of maternal care in rodents and parental care in humans, we performed DNA microarray studies of the epigenome, which remain the most cost-effective means of targeting specific loci for epigenetic analyses. However, the resolution (~200 bp) of these approaches is less than sequencing-based analyses. As the cost of sequencing continues to decrease, it is now becoming feasible to employ sequencing-based single nucleotide resolution epigenetic analyses of DNA methylation (via MeDIP-seq or bisulfite sequencing [BIS-seq]) and chromatin modifications (via ChIP-seq) of the whole genome. These approaches have important limitations, as have been reviewed elsewhere [78]. Whole genome sequencing generates enormous datasets that require novel approaches for analysis. However, there are encouraging signs for a number of standard platforms that analytical methods are becoming standardized and within the reach of scientists without extensive bioinformatics training, including web-based analysis software such as Galaxy (galaxyproject.org). High-throughput studies such as the ones described above in the rat and human open up a number of questions—undoubtedly more than are answered. For data to be interpretable within this new paradigm, it is clear that these technological advances must be coupled with equally powerful phenotypic methods using appropriate cell-types and conditions.

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Glossary

Chromatin Histone proteins associated with the cell's DNA that regulate its accessibility to gene transcription machinery. Chromatin comes in two forms: heterochromatin, where the DNA is tightly coiled and therefore inaccessible to the transcriptional machinery, and euchromatin, where the DNA is more loosely associated with histone proteins.

Epigenome The overall epigenetic state of a cell that serves as an interface between the environment and the genome.

Histone code The specific pattern of histone protein modifications that delineate the parts of the genome to be expressed at a given point in time in a given cell type.

Phenotype Any observable characteristic of an organism, including its behavior. An organism's phenotype is a product of its genetics and its environment.

Psychopathology The manifestation of mental illness in the form of phenotype, including abnormal behavior and physiology.