Environmental Stressors and Epigenetic Control of the Hypothalamic-Pituitary-Adrenal Axis

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Key Words

Hypothalamic-pituitary-adrenal axis · Stress · Glucocorticoids · Cortisol · Epigenetics · DNA methylation · Histones · Mood disorders · Anxiety disorders

Abstract

In this review, we provide a brief summary of several key studies that broaden our understanding of stress and its epigenetic control of the function and behavior of the hypothalamic-pituitary-adrenal (HPA) axis. Clinical and animal studies suggest a link among exposure to stress, dysregulation of the HPA axis, and susceptibility to neuropsychiatric illnesses. Recent studies have supported the notion that exposure to glucocorticoids and stress in various forms, durations, and intensities during different periods of development leads to long-lasting maladaptive HPA axis response in the brain. They demonstrate that this maladaptive response is comprised of persistent epigenetic changes in the function of HPA axis-associated genes that govern homeostatic levels of glucocorticoids. Stressors and/or disruption of glucocorticoid dynamics also target genes such as *brain-derived neurotrophic factor (BDNF)* and *tyrosine hydroxylase (TH)* that are important for neuronal function and behavior. While a definitive role for epigenetic mechanisms remains unclear,

these emerging studies implicate glucocorticoid signaling and its ability to alter the epigenetic landscape as one of the key mechanisms that alter the function of the HPA axis and its associated cascades. We also suggest some of the requisite studies and techniques that are important, such as additional candidate gene approaches, genome-wide epigenomic screens, and innovative functional and behavioral studies, in order to further explore and define the relationship between epigenetics and HPA axis biology. Additional studies examining stress-induced epigenetic changes of HPA axis genes, aided by innovative techniques and methodologies, are needed to advance our understanding of this relationship and lead to better preventive, diagnostic, and corrective measures.

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Introduction

Due to high prevalence rates, strong patterns of chronicity, and mental debilitation, psychiatric disorders such as depression are projected to be the second leading cause of disability worldwide by 2020 [1]. Efforts at identifying genetic determinants of major psychiatric disorders began in the 1980s with linkage studies and later with ge-

Fig. 1. Disruption of the HPA axis and behavior by stress or glucocorticoids.

nome-wide association studies (GWAS) [2]. Over the past several years, efforts at identifying epigenetic mechanisms of gene function have also gained traction. Despite high heritability of a majority of psychiatric disorders, some conditions, such as major depressive disorder and posttraumatic stress disorder (PTSD), have a relatively lower degree of heritability and further suggest potential involvement of environmental factors [3–5]. In addition, there have been studies that have linked environmental influences, such as diet and nutrition [6, 7], maternal immune response [8, 9], and stress [10–12], as risk factors to what are otherwise highly heritable disorders.

Stress as a Nongenetic Risk Factor

One of the more prevalent and well-studied environmental influences is stress. Preclinical [13–15], epidemiological [16], and clinical studies [17–19] suggest a strong link among exposure to stress, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and susceptibility to neuropsychiatric illnesses. Studies in humans and animal models have reported that stressors in their various forms, durations, and intensities all place significant burden on the HPA axis and its ability to properly regulate the glucocorticoid dynamics [20, 21]. Specifically, activation of the HPA axis by perception and experience of the stressor typically leads to the production and release of the

glucocorticoid cortisol, which is the neuroendocrine mediator of the ‘fight or flight’ response. However, in cases of exposure to trauma or chronic stress, the homeostatic, negative-feedback regulation of the HPA axis becomes disrupted, leading to aberrant glucocorticoid levels that can persist even in the absence of additional stressors. For instance, clinical studies of subjects suffering from PTSD or those who have experienced childhood traumas have reported abnormal baseline cortisol levels in measurements such as the cortisol awakening response [22, 23] or dysregulation of cortisol response during tests designed to challenge HPA axis function, such as the dexamethasone suppression test (and corticotropin-releasing hormone/dexamethasone) [24–26] or the Trier Social Stress Test [27]. Findings from such studies imply that stressors or traumas that provoke HPA axis function above and beyond the acute ‘fight or flight’ response lead to changes in not only tissue-specific processes that are influenced by glucocorticoid signaling, but also those that directly regulate and mediate the stress response itself (fig. 1).

This link between stress- or glucocorticoid-induced HPA axis dysregulation and further disruption of glucocorticoid dynamics is believed to have detrimental consequences on mood and behavior. One disease that exemplifies this relationship is Cushing’s disease, where adrenocorticotrophic hormone (ACTH)-secreting pituitary adenomas are immune to glucocorticoid-induced suppression of ACTH and endogenous cortisol release during the

Table 1. Representative stress-/HPA axis-associated genes

Location	Symbol	Name	Function	Significance
Extracellular and cell surface	<i>CRH</i> (or <i>CRF</i>)	Corticotropin-releasing hormone	CRH signaling	Trauma, depression, suicide, and animal models of stress [77–80]
	<i>CRHR1</i>	CRH receptor, Type 1	CRH signaling	
	<i>CRHR2</i>	CRH receptor, Type 2	CRH signaling	
	<i>CRHBP</i>	CRH binding protein	CRH signaling	
Cytoplasmic	<i>FKBP4</i>	FK506 binding protein 4	GC sensitivity	Animal model of stress [39, 81]
	<i>FKBP5</i>	FK506 binding protein 5	GC sensitivity	Depression, PTSD, suicide, bipolar disorder [40, 41, 43, 82]
	<i>HSP70/HSPA1</i>	Heat-shock protein, 70 kDa, 1A,1B, and 1L paralog	GC sensitivity	Stress and schizophrenia [83–86]
	<i>HSP90AA1</i>	Heat-shock protein, 90 kDa	GC sensitivity	
	<i>GR</i>	Glucocorticoid receptor	GC sensitivity	Suicide and depression [87–89]
Nuclear	<i>BAG1</i>	BCL2-associated athanogene 1	GC sensitivity	Manic and depressive symptoms in mice [90, 91]
	<i>BDNF</i>	Brain-derived neurotrophic factor	Target gene	Anxiety and depressive phenotypes [64, 92, 93]
	<i>PER1</i>	Period homolog 1	Target gene	Circadian rhythm in bipolar disorder [94–96]
	<i>SGK1</i>	Serum/GC-regulated kinase 1	Target gene	Animal model of stress [97, 98]
	<i>TH</i>	Tyrosine hydroxylase	Target gene	Neurotransmission and psychosis [49, 99–101]

GC = Glucocorticoid.

dexamethasone suppression test. Remarkably, depression is comorbid in 60–90% of these Cushing's patients [28–30], and resolution of both hypercortisolemia and depressive symptoms by surgical removal of the adenomas suggests a causal role for hypercortisolemia and HPA axis dysregulation in the mood of these patients [29, 31]. Similarly, a landmark epidemiological study that examined hundreds of thousands of patients who were prescribed glucocorticoids (i.e., iatrogenic Cushing's syndrome) for nonpsychiatric disorders found a significant increase in cases of depression, suicide, mania, and anxiety associated with glucocorticoid therapy [32]. Taken together, these studies highlight the importance of stress and its principal *in vivo* agent cortisol in mood regulation and necessitate a closer examination of the processes that govern this relationship.

Epigenetics and the HPA Axis: Case Studies

The following studies using animal models and epigenetic tools have reported associations between behavioral changes relevant to mental illness and genes that are

either targets of or directly regulate HPA axis function (HPA axis genes; table 1).

Influences of Poor Maternal Care on Neurodevelopment

Stress-mediated epigenetic modifications may be more pronounced during the stress-vulnerable, early-life period where regions implicated in emotionality and stress reactivity such as the hippocampus, amygdala, and the prefrontal cortex are undergoing rapid changes in dendritic density, myelination, and synaptic plasticity [33, 34]. Weaver et al. [35] have reported that good maternal nursing behavior (vs. neglect) is required for proper postnatal epigenetic programming of HPA axis function in adulthood. They examined exon I₇ promoter region of *Nr3c1* (glucocorticoid receptor, *Gr*) and found that CpG dinucleotides that reside within the binding sites for the nerve growth factor-inducible protein NGFI-A are heavily methylated in the pups that experienced poor nursing behavior. This differential methylation state was associated with decreased binding to NGFI-A, decreased *Nr3c1* expression, elevated plasma corticosterone

levels, and anxiety-mediated behaviors [36]. A similar postnatal study using 'stress-abusive' nursing mothers documented lasting DNA methylation changes in *Bdnf*, a target gene of glucocorticoid signaling [37] that encodes an important neurotrophic factor, which was associated with maternal maltreatment when the abused female pups themselves became nursing mothers [38].

Conditions Associated with Anxiety Disorders

Other bodies of work modeling anxiety disorders established additional components of the glucocorticoid receptor (GR)-associated signaling complex as targets of glucocorticoid exposure and traumatic stress. One such gene is *FK506 binding protein-5 (Fkbp5)*, a chaperone protein and primary regulator of intracellular GR-signaling [39], which has been implicated in numerous association studies of depression, bipolar disorder, and PTSD [40–43]. Work by Lee et al. [44] demonstrated that glucocorticoid administration to adolescent animals is capable of inducing loss of DNA methylation and increase in expression of *Fkbp5*. Results showed that methylation alterations observed in the glucocorticoid response element (GRE) persisted into adulthood and were associated with anxiety-like behavior [45]. Another study examined human lymphocytes derived from subjects exposed to childhood trauma and reported loss of DNA methylation in a blood-specific, intronic region of the *FKBP5* gene [46]. Their findings that linked DNA methylation of *FKBP5* and its transcription were consistent with previous studies of this gene in the context of glucocorticoid resistance and hypercortisolemia. Interestingly, a crucial SNP (rs1360780) implicated in several studies of depression and PTSD, and located adjacent to the site of the epigenetic changes, had a moderating effect on DNA methylation and expression, demonstrating a gene-environment interaction [46].

Conditions Associated with Adult-Onset Depression

In adults, social defeat stress has been shown to underlie profound changes in social interactive behavior and reduction of *Bdnf*, both of which become reversed following treatment with the tricyclic antidepressant imipramine [47]. The same psychosocial paradigm produced loss of methylation and increase in expression of the corticotropin-releasing factor (*Crf*) gene in stress-vulnerable mice, with imipramine reversing methylation loss at a potentially important cyclic AMP (cAMP) response element (CRE) [48]. Stress-induced increase in hypothalamic *Crf* is consistent with elevation of plasma corticosterone. Similarly, social isolation stress imposed on

adolescent mutant *Disrupted-in-Schizophrenia (DISC1)* transgenic mice resulted in elevation of plasma corticosterone and hypermethylation of the promoter of the *tyrosine hydroxylase (Th)* gene. In this model of gene-environment interaction, *Th* promoter of neurons that projected to the mesocortical, but not mesolimbic, brain became selectively hypermethylated [49]. In addition, epigenetic and behavioral deficits associated with isolation stress were prevented when the animals were concurrently treated with the GR antagonist mifepristone (RU486). This implicated glucocorticoid signaling in epigenetic control of behavior.

While it is unclear at this time as to how different genes, anatomical regions, and developmental periods contribute to the observed behavioral changes, these studies nonetheless have begun to elucidate crucial mechanisms that govern depression-related behaviors. As the field of stress epigenetics is emerging, many of such studies are needed to address the gap in knowledge and integrate these important findings under a unifying framework.

Epigenetics and the HPA Axis: Mechanisms

The above studies demonstrate that chronic exposure to stressors or glucocorticoids affects, via uncharacterized signaling, intracellular enzymes such as DNA methyltransferases and/or histone acetyl transferases to transduce environmental stressors into methylation of nucleotide cytosines or acetylation and/or methylation of amino acid lysine. Such modifications in turn can have a lasting influence on gene function by affecting DNA binding of transcription factors or by altering chromatin structure. Emerging evidence points to epigenetic and functional disruption by the direct action of glucocorticoids of two types of stress-associated genes: (1) those that directly govern HPA axis function by modulating intracellular glucocorticoid signaling and sensitivity, and (2) those that cause long-term dysregulation of neuronal processes, such as neurotransmission, that are important for proper mood, emotions, and cognition. For the latter, it is not clear whether the effect is a direct result of the stressor or altered cortisol levels following dysregulation of genes that modulate its signaling. For both types of genes, it is also unclear as to what extent other hormones and neurotransmitters associated with the stress response, in addition to glucocorticoids, may play a role in shaping HPA-axis function.

Epigenetic Alteration of HPA Axis Genes: Direct Influences on Glucocorticoid Signaling and Sensitivity

Few studies demonstrate that stress exposure or glucocorticoid administration epigenetically alters the function of genes that directly modulate glucocorticoid signaling. One of the primary modulators of glucocorticoid signaling is GR itself. Work on maternal nursing behavior has reported high DNA methylation and low histone acetylation of *Gr* exon I₇ associated with poor maternal behavior, at the binding site for the transcription factor NGFI-A [35]. With diminished binding for this protein, GR levels were lower, and plasma glucocorticoid levels were more pronounced following an acute-stressor challenge, suggesting that the quality of maternal behavior, or postnatal stress, can shape HPA axis development. Consistently, there are multiple negative GREs (nGREs) within the intronic regions of *Gr*, and studies have reported a reduction of GR with stress [50] or glucocorticoid treatment [51, 52].

A similar target gene that directly alters glucocorticoid signaling is FKBP5, which binds to GR and co-regulates intracellular glucocorticoid signaling. The role of FKBP5 in glucocorticoid resistance and hypercortisolemia is demonstrated in the New World monkeys, where overexpression of FKBP5 causes reduced affinity of GR for cortisol [53, 54]. Our group reported that glucocorticoid-induced loss of methylation and increase in expression of *Fkbp5* is tissue-specific and dose-dependent [44, 45]. The underlying mechanism of ‘action at a distance’ by these distant intronic GREs has recently been characterized: the examined risk allele adjacent to one of the intronic GREs formed a chromatin loop with the promoter region and allowed the GR/GRE to act as a long-distance enhancer to promote transactivation of *FKBP5* [46]. The overall effect was a more robust transcriptional activity and higher levels of *FKBP5* that in turn limited intracellular GR signaling and promoted glucocorticoid resistance.

Another target of GR signaling that alters HPA-axis function is the gene that encodes the corticotropin-releasing factor (CRF or corticotropin-releasing hormone, CRH). Mice that were susceptible to social defeat stress, as determined by avoidance of social interaction, displayed an increase in activation and decrease in DNA methylation of the *Crf* promoter [48]. The site of methylation change coincided with a CRE, and the authors demonstrate increased response to cAMP when the promoter is hypomethylated. Interestingly, stress-induced behavior and epigenetic alterations became abrogated when imipramine was administered. Knockdown of ex-

pression of *Crf* by introduction of siRNA into the paraventricular nucleus of the hypothalamus (PVN) exhibited the same mitigation of social avoidance behavior, suggesting that CRF-induced plasma glucocorticoid levels can influence depressive behaviors [48]. Similar to *Gr*, regulation of *Crf* is a well-established negative-feedback paradigm, where an nGRE adjacent to the CRE is responsible for promoting stress- or glucocorticoid-induced suppression of transcription and subsequent reduction of ACTH release from the pituitary [55, 56]. More recent studies have also reported elevation of *Crf* mRNA in animals with targeted GR deletions to the PVN [57] and formation of a repressive chromatin complex at the *Crf* promoter following glucocorticoid treatment [58], with another study suggesting that repression via GR signaling may be dependent on treatment duration and independent of the nGRE [59].

Epigenetic Alteration of Genes That Influence Neuronal Processes

In addition to HPA axis genes that directly alter plasma glucocorticoid levels, some are directly involved in the regulation of neuron function and neurotransmission. For example, work by Tsankova et al. [47] focused on epigenetic control of hippocampal *Bdnf*, a neurotrophin gene necessary for cell survival and neuroprotection [60, 61]. Regulation of splice variants of *Bdnf* III and IV implicated histone-mediated mechanisms, where social defeat stress has caused methylation of histone H3 at lysine residue 27, and subsequent reduction in transcription was then rescued by inhibition of histone deacetylases with imipramine [47]. Another report showed a robust increase in methylation as a potential mechanism for reduction of *Bdnf* mRNA in the maltreated pups [38]. Similar to the *Crf* promoter, the methylated site within exon IV of *Bdnf* includes a CRE, providing the means by which methylation of DNA can interfere with binding of the CREB transcription factor [38]. In both instances, it is unclear as to how these epigenetic changes occur, although it is presumed to be by glucocorticoid signaling, as both genes are direct targets [62–64]. In addition, Niwa et al. [49] showed that epigenetic downregulation of the *Th* gene in the mesocortical dopaminergic neurons is mediated by GR, as demonstrated by the reversal of stress-induced phenotypes, including behavior, with the glucocorticoid antagonist RU486.

Table 2. Summary of requisite knowledge and technical refinements

Molecular mechanisms	<ul style="list-style-type: none">• Identification and characterization of proteins and enzymes that co-regulate HPA axis genes with GR• Comprehensive list of glucocorticoid targets by GR-seq, ChIP-seq, or methylation arrays of brain tissues
Animal models	<ul style="list-style-type: none">• Knock-out and transgenic animals to study the role of HPA axis genes on behavior• Refinement of techniques for target cell-type enrichment• Identification of epigenetic marks in peripheral tissues that serve as a proxy for those in the brain• Tools to site-specifically alter the epigenome
Human studies	<ul style="list-style-type: none">• Comprehensive list of glucocorticoid targets by GR-seq, ChIP-seq, or methylation arrays of brain tissues• Refinement of techniques for target cell-type enrichment of postmortem brain tissues• Validation of peripheral biomarkers• Gene-environment interactions• Development of drugs that target specific epigenetic enzymes or epigenomic loci

Future Direction of Epigenetics of Stress and HPA Axis Biology: From Basic Science to Clinical Applications

Despite the innovative methods employed by the case studies, additional tools and more comprehensive experiments are necessary to clearly establish the role of epigenetics in stress-induced alterations and subsequent vulnerability to psychiatric illnesses (table 2).

Molecular Studies of Basic Mechanisms

Basic science experiments elucidating the underlying mechanism of stress- or glucocorticoid-induced epigenetics are necessary. One of the first tasks of such experiments is to identify specific methyltransferase and demethylase activities for DNA and histones. Given the ability of GR to act as both an activator and repressor of transcription, through occupation of GREs or nGREs, respectively, along with its capacity as a transrepressor through DNA-independent, tethering mechanisms [65, 66], conditions under which epigenetic changes occur and where inter-study differences arise, such as specific tissues or types of stressors, need to be identified and rigorously replicated. In addition, it is important to

identify the complexes of proteins and transcription factors that coordinate the physiological processes in response to environmental factors, as their characterization will broaden our understanding of the epigenetic mechanisms and lead to more target-specific and efficacious drug interventions. Lastly, there is a great need to comprehensively identify stress and glucocorticoid targets in brain tissues. A comparison of a few studies that have used GR-mediated chromatin immunoprecipitation (ChIP) in combination with hybridization on microarray chips (ChIP-chip) or sequencing on high-throughput platforms (ChIP-seq) shows that there is a general lack of common genomic targets of glucocorticoids in different cell types and brain regions [67]. Therefore, unbiased GR- or histone-mediated ChIP experiments and innovative DNA methylation screens [68] performed in different brain regions or populations of neurons of similar function are necessary to identify sets of genes or pathways that are specific for each tissue type. For instance, a recent study that identified genomic targets of GR by ChIP-seq of the hippocampus of rats treated with glucocorticoids [69] is likely to differ in its list of targets from a similar study employing a stress model and investigating the PVN. These proposed studies will shed light on common epigenetic mechanisms that govern the regulation of HPA axis genes and behavior.

Preclinical Animal Models

Animal models will also prove indispensable in understanding HPA axis stimulation and long-term behavioral consequences. First, knock-out and transgenic animals of HPA axis genes that are selected through genomic screens or allelic variations implicated in human studies, such as *Crf*, *Gr*, *Fkbp5*, *Bdnf*, and *Disc1* [48, 49, 70–72], can be generated to demonstrate their causal roles in stress-related behavior. Consequently, similar to human genetic studies, epigenetic studies that incorporate interactions among different epigenomic loci and with genetic variations (gene-environment interactions) are needed to assess stress susceptibility and resilience. Second, refinement of methodologies specific for epigenetics, such as those needed for addressing cellular heterogeneity, can be implemented, for instance, by targeting of specific neuroanatomical subregions such as the PVN [48], dissection of specific neuronal subpopulations by laser-capture microdissection [73], and fluorescence-activated cell sorting of projection-specific dopaminergic neurons [49]. Third, identification of epigenetic correlates of stress exposure between brain regions

and peripheral tissues will be enormously useful for translational research. Lastly, tools to site-specifically manipulate the epigenome, made possible by recent advances in zinc finger targeting of specific enzymatic activities [74–76], can be implemented to mitigate the negative impact of environmental factors.

Human Studies

Foundational knowledge established by basic science research and animal models can be used to strengthen human studies conducted in clinical settings or on post-mortem brain tissues. For instance, epigenomic loci in blood that might serve as proxy for those in brain tissues of animals need to be validated with human specimens to assess their potential in determining cumulative stress exposure or susceptibility to mood disorders. Further, causal relationships between alterations of HPA axis genes and behavioral deficits derived from animal models can provide a stronger argument for pivotal roles of specific genes in diseases. We optimistically state that similar benefits may be obtained in augmenting imaging studies by honing in on specific regions and circuitry implicated in animal studies. Epigenomic studies in humans can also build on top of GWAS to identify gene-environment interactions. Finally, one of the key tenets of epigenetics is the concept of change. As such, development of ‘epigenetic’ medications that target specific enzymatic processes or even specific epigenomic targets to reverse disease phenotype needs to be pursued.

Conclusion

In this article, we have reviewed several studies that provide a potential mechanistic link among stress/glucocorticoid exposure, HPA axis function, and behavior. These studies suggest: (1) chronic exposure to stressors or glucocorticoids causes a persistent disruption of the glucocorticoid dynamics; (2) altered cortisol levels cause deterioration of the HPA axis negative feedback and chronic dysregulation of genes that control glucocorticoid signaling and sensitivity, and (3) persistent disturbances in glucocorticoid signaling can have a negative impact on behavior by epigenetic control of genes that regulate mood and neurotransmission. Finally, these case studies provide a strong motivation to pursue and implement crucial experiments and techniques, such as genome-wide screens and neuron enrichment procedures, respectively, in order to establish a unified framework for a more comprehensive understanding of stress biology.

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