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Epigenetics and the regulation of stress vulnerability and resilience

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Abstract

The human brain has a remarkable capacity to adapt to and learn from a wide range of variations in the environment. However, environmental challenges can also precipitate psychiatric disorders in susceptible individuals. Why any given experience should induce one brain to adapt while another is edged toward psychopathology remains poorly understood. Like all aspects of psychological function, both nature (genetics) and nurture (life experience) sculpt the brain's response to stressful stimuli. Here we review how these two influences intersect at the epigenetic regulation of neuronal gene transcription, and we discuss how the regulation of genomic DNA methylation near key stress-response genes may influence psychological susceptibility or resilience to environmental stressors. Our goal is to offer a perspective on the epigenetics of stress responses that works to bridge the gap between the study of this molecular process in animal models and its potential usefulness for understanding stress vulnerabilities in humans.

Keywords

Epigenetics; Glucocorticoids; Neural plasticity; Psychological stress; Resilience; Vulnerability; DNA methylation

Introduction

Psychological stress is a major contributor to morbidity, mortality, and health-care costs. Psychiatric disorders directly linked etiologically to stress, including unipolar major depressive disorder (MDD) and posttraumatic stress disorder (PTSD), are among the top causes of disability and disease burden. MDD is ranked as the number one worldwide cause of years lived with disability and is projected to be the second leading cause of global disease burden (combined morbidity and mortality) by the year 2020 (Lopez and Murray, 1998). Less evidence exists on the epidemiology of PTSD, but it is estimated that PTSD may affect as much as 10% of the general population and is among the 20 leading causes of

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disease burden (Galea et al., 2005, Freed et al., 2010). Furthermore, psychological stress can precipitate or perpetuate other psychiatric disorders, such as schizophrenia, dementia, and addiction (van Winkel et al., 2008, Tsolaki et al., 2009, Sinha et al., 2011), and can negatively affect the course of several non-psychiatric conditions, including cardiovascular disease, cancer, and HIV infection (Cohen et al., 2007, Kaltsas et al., 2012). Many of these conditions are potentially preventable and/or treatable. Improving the ability to predict which individuals are susceptible to stressors would offer the opportunity for early intervention and could have enormous implications for reducing stress-related disability and health-care costs.

Risk for both MDD and PTSD is determined at least in part by genetics (Kendler and Prescott, 1999, Sartor et al., 2012). Concordance rates for monozygotic and dizygotic twins estimate heritability at about 37% for MDD (Sullivan et al., 2000) and 30-35% for PTSD (Skelton et al., 2012). A few genetic polymorphisms have been associated with vulnerability to stressors. For example, single nucleotide polymorphisms (SNPs) in *FKBP5*, a gene coding for a glucocorticoid receptor chaperone, has been shown to interact with childhood abuse to predict adult PTSD symptoms (Binder et al., 2008), and the 5-HTTLPR polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*) have been reported to moderate the effects of stress on depressive phenotypes in some but not all populations tested (Caspi et al., 2003, Risch et al., 2009, Karg et al., 2011). The heritable component of stress responses can be replicated and studied in laboratory animal models, as distinct mouse strains have been shown to display different behavioral responses to presentation of the same stressors (Uchida et al., 2011, Russo et al., 2012, Scharf and Schmidt, 2012). Nonetheless, large-scale genome-wide association studies have failed to show consistent genome-wide significant loci for MDD (Ripke et al., 2013), and given that twin studies have revealed a substantial role for unique environmental risk in the etiology of stress disorders (Sullivan et al., 2000), interest has increasingly turned toward identifying gene $\times$ environment interactions as a more effective way to predict individual stress vulnerabilities.

One important point of gene $\times$ environment interactions occurs at the level of the epigenetic regulation of gene transcription. The term epigenetics is derived from the Greek prefix “epi,” which means upon or over, and genetics, which refers to the sequence of an individual's DNA. In common parlance, “epigenetic” is often used in reference to a wide variety of phenotypic differences between individuals that are independent of the underlying genetic code, such as when genetically identical twins have dissimilar personalities. By contrast, at the molecular level the word has come to signify a specific set of gene regulatory mechanisms that are mediated by biochemical modifications of genomic DNA along with its associated histone proteins and non-coding RNAs, which together comprise chromatin (Bonasio et al., 2010). Epigenetic transcriptional regulatory mechanisms include DNA methylation, posttranslational modifications of histones, and changes in the position of the secondary structures formed by DNA and histones called nucleosomes, all of which can be collectively referred to as the epigenome. What these forms of chromatin regulation have in common is that they impact gene transcription by altering the accessibility and/or activation state of gene regulatory elements, which are the sequences encoded within genomic DNA that serve as docking sites for transcription factors. The consequences of chromatin regulation for gene transcription are context dependent and determined by both the location and the nature of the epigenetic modifications induced. For example, increasing the accessibility of a gene regulatory element could either increase or decrease transcription of nearby genes depending on whether an activator or a repressor binds at that site, and structural changes that open a gene regulatory element may have no effect on gene expression whatsoever if the regulatory binding protein is not expressed.

When established over the course of cellular differentiation, a primary function of chromatin regulation is to underlie the ability of a single genome to be read out into a multiplicity of distinct cell-type specific gene expression programs. However, many of the same chromatin regulatory factors that operate during development remain expressed in terminally differentiated neurons of the mature brain, and it is becoming increasingly clear that the expression and/or function of many of these factors in neurons is subject to regulation by environmental stimuli including stressors. In postmitotic neurons, chromatin regulation thus provides a mechanism to translate environmental exposures into plasticity of gene expression. Importantly, because structural regulation of chromatin can be very persistent (e.g. the lifelong inactivation of one X chromosome in female cells), the epigenome has the potential to encode a molecular memory of past events that can influence gene expression, neuronal function, and future behaviors. At the level of the behaviors we are discussing here, this molecular mechanism would be relevant if some experience-induced chromatin states were to change patterns of gene expression that alter adaptability to stressors, thus changing the likelihood that an individual will display vulnerability or resilience to future stressors.

In this article, we discuss the evidence that epigenetic regulation of chromatin plays a role in determining the adaptive or maladaptive nature of neural and behavioral responses to environmental stressors. Our purpose is not to provide an exhaustive review of all the literature linking chromatin and stress, but rather to offer a perspective on this topic by focusing on selected stress-inducible epigenetic regulatory mechanisms and specific gene targets that contribute directly to the behavioral responses induced by environmental stressors. Given the diverse usage of the terms “stress,” “adaptation,” “vulnerability,” and “resilience,” we first define these terms in the context of animal models and human studies. We then offer an example of how the epigenome influences stress responses by focusing on the evidence for DNA methylation-dependent regulation of genes in the hypothalamic-pituitary-adrenal (HPA) axis, a key regulatory pathway for coordinating organismal stress responses. Finally, we discuss the implications of these data for and the challenges of applying this knowledge to the identification, prevention, and treatment of stress-related psychiatric disorders.

Stress, adaptations, resilience, and vulnerability

The term stress can be used to refer to either a stressful stimulus, the organism's response to a stressful stimulus, or the consequences of this response (Levine, 2005). By contrast, the term stressor has been more consistently used to denote the physical and emotional challenges that threaten organismal homeostasis, and stress response denotes the organism's behavior in the face of such challenges (Chrousos and Gold, 1992, Karatsoreos and McEwen, 2011). Because stressors are physiologically coupled to stress responses, some researchers have advocated for use of the more holistic term allostasis to describe the process by which variations in internal function allow an organism to achieve stability in the face of constantly changing environments (Karatsoreos and McEwen, 2011). However for the purposes of this article, to differentiate environmental effects on the epigenome from the biochemical and behavioral consequences of chromatin regulation, we will use the term stressor to denote a challenge posed by the environment and the term stress response for the molecular, hormonal, neural, and/or behavioral response evoked by such a stressor.

Adaptive and maladaptive responses to stress

Although the terms stressor and stress response commonly carry negative connotations, stress responses can be either adaptive or maladaptive and even similar responses can have different adaptive value in distinct environmental contexts. For example, combat veterans exhibit vigilance and fear responses that are adaptive in the combat zone because they decrease the likelihood of injury and death. However, these same responses become

maladaptive if they generalize to other settings and persist after return to civilian life. Other determinants of the adaptive value of the stress response include the intensity, type, and timing of the stressor. Stressors that occur early in development have greater and more enduring effects than stressors that occur in adulthood, effects that vary depending on stressor intensity, duration and frequency (Hoffmann and Spengler, 2012, Russo et al., 2012). Chronic excessive stress carries a high risk of detrimental consequences, whereas acute intermittent stress may be essential for successful adaptation to changing natural and social environments (McEwen, 2008). Mild to moderate stress may enhance cognitive and emotional learning via the induction of neural plasticity and the adaptive remodeling of neural circuits, and may thus be a necessary component of learning and successful psychotherapy (Cozolino, 2010). The importance of stressor intensity for determining stress response has been further highlighted over the years by the definition of Post-Traumatic Stress Disorder (PTSD) in the Diagnostic and Statistical Manual for Mental Disorders (DSM). In the 4th edition of DSM, a stressful event has been traditionally considered capable of causing PTSD only if it involves the “actual or threatened death or serious injury, or a threat to the physical integrity of self or others” and induces “intense fear, helplessness, or horror” (American Psychiatric Association, 2000). Although this criterion was recently revised in the 5th edition of DSM, intensity remains an important characteristic of traumatic events, which should involve “death, threatened death, actual or threatened serious injury, or actual or threatened sexual violence.” (American Psychiatric Association, 2013).

Yet even when encountering a stressor of high intensity, only a small minority of individuals will develop stress disorders. Whereas more than two thirds of the general population experiences an event meeting the definition of “traumatic” in their lives, only 5-10% of the population develops PTSD (Galea et al., 2005). Similarly, only 20 to 25% of the individuals exposed to intense stressors develop Major Depressive Disorder (MDD) (Cohen et al., 2007). These tendencies of individuals to exhibit adaptive versus maladaptive stress responses are widely described in terms of resilience and vulnerability (or susceptibility) respectively. Traditionally, research studies tend to define vulnerability as the induction of pathological processes upon stressor exposure (e.g. onset of MDD following a divorce), whereas the absence of psychiatric symptoms despite stressor exposure has been viewed as resilience. However, these descriptions risk implying that resilience is a passive process, whereas experimental evidence indicates that resilience can be actively invoked by molecular, hormonal, neural, and/or behavioral mechanisms that either counteract already established vulnerabilities or prevent the expression of vulnerable phenotypes (Russo et al., 2012). Such active mechanisms not only prevent the development of vulnerability states, but may also lead to a state of enhanced functioning following stress. In this sense, resilience may reflect a state of heightened adaptability induced by exposure to previous stressors. This model is supported by studies in rodents and primates showing that, compared with controls, animals exposed to brief intermittent stressors during critical developmental windows - an exposure termed “stress inoculation” - exhibit subsequently enhanced novelty seeking behavior, decreased anxiety, and greater stress tolerance in adulthood (Parker et al., 2004, Russo et al., 2012). In parallel, studies in humans have described the phenomenon of posttraumatic growth (PTG), which is characterized by positive psychological change following exposure to challenging experiences, and which in some cases has been reported to be a more common phenotype than PTSD following trauma exposure (Levine et al., 2008).

Comparing stress in rodents and humans

Rodent models are commonly employed to study the molecular basis of stress responses. We refer the interested reader to comprehensive reviews of these behavioral paradigms published elsewhere (Krishnan and Nestler, 2011, Scharf and Schmidt, 2012). Vulnerability

and resilience are commonly defined in rodents based on the degree to which individual animals with a common genotype or two groups of animals with different genotypes show differential outcomes, such as social avoidance, anhedonia, or metabolic syndrome, following a common exposure to any one of a number of experimentally-delivered stressors, such as chronic social defeat stress, forced swim, or inescapable foot shock (Russo et al., 2012). These paradigms offer a strategy both for elucidating molecular hypotheses of the mechanisms linking stressor exposure with specific stress responses, as well as for testing the efficacy of potential therapeutic drugs.

Nonetheless, there exist inherent limitations in our ability to interpret the emotional connotations of rodent behaviors. Thus it is important to critically evaluate the use of “susceptible” (implying maladaptive) and “resilient” (implying adaptive) to describe behaviors in experimental rodent models with respect to how findings from these studies may be applied to psychiatric disorders. Misinterpretation of the adaptive value of rodent behaviors is particularly problematic when using data from a rodent model to predict stress vulnerabilities in humans. For example, immobility time in the forced swim and tail suspension tests is often considered to be a depressive-like behavior because the ability of drugs to reduce rodent immobility in these paradigms is a strong predictor of their antidepressant efficacy in humans (Cryan et al., 2005). However, immobility in these tests may be also viewed as an active adaptive response to conserve energy until escape becomes possible. Finally, since the animal has never been placed in this environment before and cannot anticipate that it will be removed shortly, a third interpretation is that floating is a passive coping response to a helpless situation. As this example highlights, the risk of anthropomorphizing rodent behavior can be limited by focusing description of rodent behavioral paradigms and their outcomes in terms of the face validity (similar symptoms), construct validity (similar pathophysiological causes), and pharmacological validity (similar drug responses) of each test with respect to the psychiatric disorder of interest.

A more intractable limitation of conventional rodent models is their inability to recapitulate the intricate relationship between humans and the stressors they encounter throughout their lives in complex social environments. Both genetic and epigenetic factors influence behaviors and thus may also influence in many cases a person's choice of social environment and his/her likelihood of encountering stressors, as well as impact the person's response to those environmental stressors. Furthermore, whereas consistent behavioral responses across a population in multiple stress paradigms is the usual standard required to experimentally implicate a molecular pathway in stress responses, human behavior is far more variable, with distinct resilient and vulnerable phenotypes (defined by symptoms, social functioning and behavior) often co-presenting within the same disorder.

Despite these limitations, rodent behavioral models continue to represent the most efficient approach to elucidating the molecular and cellular mechanisms that underlie the etiopathogenesis of psychiatric disorders. Most importantly, rodent models offer access to brain tissue, which is essential for elucidating the circuit basis of these disorders. In the next section, we review how research from rodent models has helped to provide evidence for a brain circuit plasticity-based theory of the epigenetics of stress responses. We then consider how this knowledge can be used to inform human studies of HPA axis genes in peripheral tissues to improve the identification and treatment of patients with stress disorders.

Stressor-induced changes in neuronal DNA methylation

Methylation of cytosines in the genomic DNA of mammals is a functionally important epigenetic mechanism of transcriptional regulation that is essential for cellular differentiation and organismal development. In most cell types, cytosine methylation occurs

predominantly at CpG dinucleotides, although recent studies have revealed that neurons contain a uniquely large complement of methylated cytosines occurring in the CpH (e.g. CpA, CpC, or CpT dinucleotides) context (Lister et al., 2013, Varley et al., 2013). At a mechanistic level, DNA methylation is a complex epigenetic mark with multiple effects on transcription. Genome-wide sequencing studies have suggested that on average >70% of all CpG dinucleotides across the genome are methylated, meaning there are on the order of at least 35 million individual sites of DNA methylation in a single human cell (Lister et al., 2009). The functional consequences of DNA methylation at any given cytosine depend both on the genomic context of the site and whether methylation recruits or blocks the recruitment of transcriptional regulatory proteins. When found in differentially methylated regions (DMRs), regions of imprinted genes, or in areas of high CpG density, such as CpG islands in gene promoters, methylation is usually associated with gene silencing. This repression can occur either by recruiting the association of methyl-DNA binding proteins that scaffold chromatin repressors, or by inhibiting the association of transcriptional activators or architectural proteins such as CTCF with their regulatory elements (Wang et al., 2012). The functional consequences of low-density cytosine methylation remain less well understood, though it is likely that DNA methylation within promoters and enhancers contributes to developmental regulation of gene expression (Meissner et al., 2008, Mohn et al., 2008), whereas DNA methylation at sites distant from transcription start sites has been found in at least some cases to correlate with gene activation (Varley et al., 2013). Finally, high levels of DNA methylation are found over actively transcribed gene bodies, where its function remains unknown (Hellman and Chess, 2007, Varley et al., 2013).

The persistence of cytosine methylation is often cited as evidence that this chromatin mark could serve as a form of molecular memory on a behavioral timescale. DNA methylation is required for the extremely persistent (e.g. lifelong) repression of gene transcription that characterizes transposon silencing, monoallelic expression of imprinted genes, and X chromosome inactivation (Goll and Bestor, 2005). Moreover, cytosine methylation has an established mechanism of inheritance across cycles of cell division via the activity of the maintenance methyltransferase Dnmt1, which targets hemimethylated cytosines on newly replicated DNA (Goll and Bestor, 2005). However, if DNA methylation were irreversible in postmitotic cells, it would have little relevance for mediating behavioral adaptations to environmental stimuli. Thus, it was the experimental demonstration that DNA methylation patterns in the adult brain are dynamic and subject to regulation by environmental stimuli that spurred the most intense interest in neuronal epigenetics (Meaney and Szyf, 2005, Roth and Sweatt, 2009).

Evidence for rapid, stimulus-dependent regulation of DNA methylation in the adult brain was provided in Miller et al. (2007), who showed that fear conditioning increased or decreased DNA methylation in the hippocampus at single CpG sites in the plasticity-associated *Ppp1ca* and *Reln* genes respectively. These DNA methylation changes were detected within 1 hr of fear conditioning and returned to baseline 24 hr later, revealing an unanticipated lability of this chromatin mark (Miller and Sweatt, 2007). Due to the technical challenges of measuring DNA methylation, most studies, like this one, assay methylation at only a limited number of sites. However, it is likely that stimulus-dependent DNA methylation changes simultaneously at a large number of sites across the genome. For example, using a sampling method to assess DNA methylation across the entire genome, Guo et al. (2011) discovered that 1.4% of >200,000 CpGs measured in hippocampal extracts showed rapid loss or gain of methylation in the 24 hours after a period of synchronous neuronal activation induced by electroconvulsive seizure (Guo et al., 2011). Determining which, if any, of these changes in DNA methylation have biological consequences for neuronal plasticity remains challenging.

Stressors are among the environmental stimuli that can change DNA methylation patterns in the brain, and, as we will review below, various stress paradigms have been shown to decrease methylation of multiple genes encoding intermediaries in the HPA axis. Neural plasticity genes, such as the gene coding for Brain-Derived Neurotrophic Factor (BDNF), are also targets of regulation by DNA methylation. For example, exposure of rat pups to a rodent model of abuse with daily disruptive caregiving during the first postnatal week is associated with increased methylation near *Bdnf* exons IV and IX and reduced *Bdnf* mRNA expression in the prefrontal cortex but not the hippocampus (Roth et al., 2009), whereas exposure of adult rats to predator stress and social instability leads to increased DNA methylation and decreased mRNA expression of *Bdnf* in the hippocampus but not the prefrontal cortex (Roth et al., 2011). The fact that these stressor-induced changes in *Bdnf* DNA methylation are selective for specific brain regions that are physiologically relevant to the environmental exposure suggests they could contribute to the BDNF-dependent brain plasticities underlying behavioral responses to these stressors.

Environmental stressors regulate the writers and erasers of cytosine methylation

Despite these correlations, it is not trivial to determine whether stimulus-dependent changes in DNA methylation are regulatory (i.e. causative for changes in gene transcription and subsequent behavioral plasticity) or merely an epiphenomenon reflecting changes in gene activation. One clue that stressors may act to directly regulate DNA methylation comes from the analysis of the enzymes that regulate the methylation and demethylation of cytosines. In mammals, methyl groups are added to cytosines by a small group of three enzymatically active DNA methyltransferases – Dnmt1, Dnmt3a, and Dnmt3b (Goll and Bestor, 2005). All three methyltransferases remain expressed in post-mitotic, fully differentiated neurons of the mature brain where they could contribute to stimulus-dependent changes in DNA methylation patterns (Feng et al., 2010). Active demethylation of DNA can occur by at least two potentially non-exclusive enzymatic processes – base excision repair (BER) and oxidation (Niehrs and Schafer, 2012, Pastor et al., 2013). BER is mediated by a complex of proteins including the Gadd45 family of scaffolds, the Aid/APOBEC family of cytosine deamidases and the methyl-DNA binding protein Mbd4, whereas oxidation of 5-methyl cytosine (5mC) to 5-hydroxymethyl cytosine (5hmC) is mediated by the Tet family of proteins (Tet1-3).

Several of these DNA methylation regulatory proteins are subject to stressor-dependent changes in expression (**Figure 1**). For example, LaPlant et al (2010) showed that 10 days of chronic social defeat stress in mice drove a small (10-15%) but significant increase in expression of *Dnmt3a* mRNA in the NAc that persisted for at least 10 days following the final defeat. Viral-mediated overexpression of Dnmt3a attenuated the social interaction response in a submaximal defeat stress paradigm and reduced the latency to immobility in a forced swim test, both of which represent pro-depressive-like behaviors; thus, these data suggest that the induced expression of *Dnmt3a* in the NAc after chronic social defeat stress contributes to the development of depressive-like behaviors in response to this stressor (LaPlant et al., 2010). Potential gene targets of social defeat stress-induced Dnmt3a in the NAc have not yet been identified, however a study from an independent group linked chronic social defeat stress-induced *decreases* in mRNA expression of a different methyltransferase, *Dnmt3b*, with reduced methylation of the promoter of the gene coding for Corticotrophin-Releasing Factor (*Crf*) in the paraventricular nucleus (PVN) of the hypothalamus (Elliott et al., 2010). This stressor simultaneously induced expression of *Gadd45b* in the PVN, raising the possibility that active DNA demethylation along with depressed methyltransferase activity could contribute to stressor-induced changes in promoter methylation status. CRF secreted from neurons of the PVN is a key regulator of the HPA axis and exposure to chronic social defeat stress in this study was associated with

increased PVN *Crf* mRNA expression only in mice that showed stressor-induced social avoidance, again suggesting that the stressor-induced regulation of DNA methylation in this context was pro-depressive.

Changes in DNA methylation have also been associated with resilience in a different experimental stressor paradigm called chronic ultra mild stress (CUMS). The CUMS paradigm utilizes chronic exposure to a series of mild environmental and social stressors (e.g. small cage, paired housing, light exposure, cage tilt, etc) that over time induce depressive-like behaviors (Uchida et al., 2011). Distinct genetic strains of mice differ in the degree to which they show CUMS-induced depressive-like behaviors, suggesting that this paradigm can be used to identify molecular mechanisms of resilience. Exposure to CUMS for six weeks was found to induce mRNA expression of both *Dnmt1* and *Dnmt3a* but not *Dnmt3b* in the ventral striatum of a susceptible mouse strain. One target of DNA methylation-dependent regulation that correlated with behavior in this study is the promoter of the gene coding for Glial-Derived Neurotrophic Factor (GDNF). Expression of GDNF in the ventral striatum of mice was shown to inversely correlate with depressive-like behaviors, and overexpression of GDNF in the ventral striatum of the susceptible strain improved social interaction after CUMS (Uchida et al., 2011). Interestingly, both susceptible and stress-resilient mouse strains showed increased methylation of the *Gdnf* promoter after CUMS, but in the susceptible strain this increased methylation was associated with decreased *Gdnf* expression whereas in the resistant strain it was associated with increased expression of *Gdnf*. This difference between the two strains appears to arise from the distinct complexes of proteins recruited to the sites of promoter methylation. In the susceptible strain the transcriptional co-repressor Hdac2 was recruited in response to increased promoter methylation, whereas in the resistant strain the transcriptional activator CREB became bound.

Stressor-dependent regulation of the methyl-cytosine reader MeCP2

How could the same promoter DNA methylation event in two mouse strains induce opposite effects on gene regulation? Unlike the enzymes that serve as “writers” and “erasers” of DNA methylation, which regulate the amount and distribution of this mark, the methyl-DNA binding “reader” proteins transduce DNA methylation into the local recruitment of transcriptional regulatory complexes. Thus these proteins provide a compelling substrate for encoding functional specificity upon methylated DNA (Fatemi and Wade, 2006). Among the small family of methyl-DNA binding domain (MBD) proteins, methyl-CpG binding protein 2 (MeCP2) is of particular importance in the brain, because loss-of-function mutations in *MECP2* cause the severe human neurodevelopmental disorder Rett Syndrome (Chahrour and Zoghbi, 2007). MeCP2 is widely bound across the neuronal genome in a pattern that closely mirrors the distribution of methylated cytosines (Skene et al., 2010). Once bound to methylated DNA, MeCP2 acts as a scaffold to recruit the binding of multiple chromatin regulators including histone deacetylases, histone methyltransferases, the NCoR co-repressor complex, and the SWI/SNF family helicase Atrx (Nan et al., 1998, Fuks et al., 2003, Nan et al., 2007, Baker et al., 2013, Lyst et al., 2013). If MeCP2 were able to differentially nucleate the formation of distinct protein complexes under different sets of cellular conditions, this could provide a mechanism to confer differential transcriptional specificities upon common DNA methylation events. Consistent with this possibility, recent studies have shown that phosphorylation of MeCP2 can regulate its protein-protein interactions (Gonzales et al., 2012, Ebert et al., 2013). MeCP2 is a target of stimulus-regulated phosphorylation at multiple sites, including Ser80, Ser86, Ser274, Thr308, and Ser421 (Zhou et al., 2006, Tao et al., 2009, Ebert et al., 2013). The biochemical consequences of most of these phosphorylation events remain to be fully understood, however phosphorylation of MeCP2 at Thr308 induced by the activation of neuronal

intracellular calcium signaling pathways has been shown to disrupt the association of MeCP2 with the NCoR repressor complex, providing a mechanism to reduce DNA methylation-dependent repression of a target gene independent of changes in DNA methylation itself (Ebert et al., 2013).

Evidence that stressors can act via MeCP2 to regulate stress responses comes from two studies that have linked inducible MeCP2 phosphorylation with stressor-induced neural adaptations (**Figure 1**). In the first study, periodic infant-mother separation was used to induce early life stress (ELS) in postnatal mice (Murgatroyd et al., 2009). 10 days of ELS was sufficient to induce increased expression of the mRNA encoding the HPA axis factor arginine vasopressin (AVP) in the PVN of the hypothalamus for up to 1 year. This increase in *Avp* mRNA expression was associated with decreased CpG methylation of an intragenic *Avp* enhancer and decreased association of MeCP2 with this enhancer, raising the possibility that the persistent change in *Avp* expression arose as a result of an MeCP2- and DNA methylation-dependent process. Interestingly, phosphorylation of MeCP2 at Ser421 (called Ser439 in this paper with respect to its position in the mouse MeCP2 protein) was substantially elevated in the PVN of 10d old mice that were exposed to ELS compared with age-matched controls. This study further showed that CaMKII-mediated phosphorylation of MeCP2 at Ser421 reduces its association with the *Avp* enhancer and that CaMKII relieves MeCP2-dependent repression of *Avp* in a manner that depends on the phosphorylation of MeCP2 at Ser421. On the basis of these data the authors propose that environmental stressors may act via the regulation of MeCP2 to effect long-lasting changes in gene regulation in response to ELS.

Evidence that Ser421 phosphorylation of MeCP2 is functionally required for neural adaptations to stressors came in a study from our lab, which examined depressive-like behaviors and antidepressant responses in MeCP2 Ser421Ala knockin mice (Cohen et al., 2011, Hutchinson et al., 2012b). We found that treating mice with the antidepressants citalopram and imipramine induced Ser421 phosphorylation of MeCP2 in specific parts of the mesolimbic system (Hutchinson et al., 2012a, Hutchinson et al., 2012b). We hypothesized that the slow, but persistent, ability of antidepressants to reverse stress-induced depressive-like behaviors might require the activation of phosphoSer421-MeCP2-dependent gene transcription. To address this question, we subjected MeCP2 Ser421Ala knockin mice and their wildtype littermates to chronic social defeat stress. In this paradigm, 10d of repeated social defeat induces a persistent social avoidance that can be ameliorated by chronic but not acute imipramine treatment (Berton et al., 2006). Both MeCP2 Ser421Ala knockin mice and their wildtype littermates showed social avoidance after 10d of defeat. However, whereas the MeCP2 wildtype mice increased their social interaction times after chronic imipramine, the MeCP2 Ser421Ala knockin mice did not, suggesting that MeCP2 phosphorylation is required to mediate the behavioral plasticities induced by this antidepressant therapy (Hutchinson et al., 2012b). Interestingly, only chronic but not acute imipramine induced MeCP2 Ser421 phosphorylation in neurons of the lateral habenula, which is a brain region strongly implicated in the regulation of stress-induced depressive-like behaviors via its ability to directly modulate the firing of dopaminergic and serotonergic neurons in brainstem nuclei (Hikosaka, 2010, Li et al., 2011). This context- and circuit-specific regulation of MeCP2 phosphorylation provides an important piece of evidence linking phospho-Ser421 MeCP2 with behavioral responses to stressors. We propose that future studies of MeCP2-dependent transcriptional regulation in the lateral habenula may elucidate new molecular mechanisms of the neural adaptations that underlie antidepressant action.

Epigenetic regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis

The context- and circuit-specificity of stressor-induced DNA methylation-dependent regulation of gene transcription is a key piece of evidence supporting the potential relevance of these changes for the alterations in brain function that underlie stress-induced behaviors. However, these findings present a major challenge to the study of DNA methylation and stress in humans. If postmortem brain tissue is available, it is unlikely to have been obtained in temporal correlation with stressor exposures or stress responses, limiting the study of how chromatin regulation in these tissues relates to the behavioral context. Furthermore, human epigenetic studies often rely on biochemical analysis of peripheral blood cells (PBCs). PBCs can be easily and repeatedly obtained from human subjects and are excellent source material both for genetics and for studying the epigenetic regulation of immune cell function. However, these cells have a very limited ability to report the cell type- and brain region-specific epigenetic regulations of gene transcription that underlie the adaptive versus maladaptive nature of behavioral responses to environmental stressors. Nonetheless, given the limitations of modeling many behavioral aspects of human stress responses in laboratory animals, there is a significant need to find ways in which the study of epigenetic regulation of gene expression in human cells and tissues can enhance understanding of stress vulnerability and resilience. Here we highlight one promising area – the epigenetic regulation of HPA axis genes – both because the regulation of these genes is physiologically relevant in peripheral tissues, and because their peripheral regulation has the potential to provide a read-out of central neural adaptations in HPA axis regulation integrated over time.

Epigenetic homeostasis of the HPA axis

The HPA axis plays a central role in coordinating physiological responses to stress in organs throughout the body. Detection of environmental stressors by the limbic system induces neurons in the paraventricular nucleus (PVN) of the hypothalamus to produce CRF and arginine vasopressin (AVP). CRF and AVP act centrally in the brain to trigger secretion of adrenocorticotropin hormone (ACTH), which in turn leads to peripheral secretion of glucocorticoids from the adrenal glands into the blood stream (Chrousos and Gold, 1992). Circulating glucocorticoids reach and exert effects on essentially every organ, including the brain, via activation of the glucocorticoid receptor (GR) (Nicolaidis et al., 2010). The GR is a ligand-dependent transcription factor. In the absence of its ligand, the GR forms a cytoplasmic multiprotein complex with protein folding chaperones, including heat shock proteins and FK506 binding proteins (FKBPs), which regulate GR activity. Following ligation by glucocorticoids, the GR dissociates from this complex and translocates to the nucleus, where it regulates transcription of glucocorticoid-responsive genes. The specific pattern of transcriptional regulation is distinct for each body organ and cell type depending on the cell-type specific accessibility of GR binding sites in the genome (Lu and Cidlowski, 2005). In the brain, a key consequence of glucocorticoid-dependent GR activation is the rapid inhibition of genes encoding intermediates in the HPA axis itself (Russell et al., 2010), thus creating a negative feedback loop to restrain stressor-induced HPA activity.

HPA axis homeostasis is important for stress resilience and health maintenance, whereas HPA dysregulation has been associated with stress vulnerability and disease phenotypes. In rodents, HPA reactivity is reduced following either high maternal care or brief maternal separation during early life, but in both cases it is associated with phenotypes of decreased fearfulness and anxiety in adulthood (Hoffmann and Spengler, 2012; Levine, 2005; Meaney et al., 2007). By contrast, HPA hyperreactivity is observed in rodents exposed to prenatal stress or prolonged maternal separation during early development, and is related to heightened fearfulness and anxiety in adults (Hoffmann and Spengler, 2012; Levine, 2005; Meaney et al., 2007). In humans, both HPA hyper- and hyporeactivity have been associated with psychiatric disorders. For example, HPA hyperreactivity has been associated with

history of childhood abuse and has been linked to vulnerability to stress and heightened risk for the development of depressive episodes (Heim et al., 2008). On the other hand, excessive suppression of the HPA axis, an effect thought to result from GR hypersensitivity, has been associated with traumatic events and PTSD (Binder, 2009; Morris et al., 2012). Taken together, these findings show that homeostasis of the HPA axis is essential for health maintenance and stress resilience, and these data also suggest that the HPA axis reactivity is programmed by early life stress experiences.

Accumulating evidence suggests that the homeostatic set-point of the HPA axis is influenced by DNA methylation-dependent regulation of multiple genes along the axis (**Figure 2**). The most studied HPA target of epigenetic regulation is the gene encoding the GR (*Nr3c1*) in the hippocampus, and several studies in both rodents and humans suggest that DNA methylation of the *Nr3c1* promoter is tightly linked to stress vulnerability and resilience. Hippocampal *Nr3c1* is upregulated in rats that are either handled or exposed to high maternal care during the first postnatal days, which then mediates enhanced glucocorticoid feedback, long-term decreased HPA axis responsivity, and stress-resilient phenotypes during adulthood (Liu et al., 1997; Meaney et al., 1985). By contrast, early life stress or low maternal care decreases hippocampal *Nr3c1* expression, increases HPA responsivity, and predisposes to stress-vulnerable phenotypes in adults (Meaney et al., 2007; Mueller and Bale, 2008). In both cases, expression of the mRNA encoding the GR is inversely correlated with DNA methylation of CpG residues in the *Nr3c1* promoter that serves as a binding site for the transcription factor nerve growth factor-inducible protein A (NGFI-A). Thus, by driving demethylation of the *Nr3c1* promoter, which then permits NGFI-A dependent GR expression, high maternal care during a critical period in the first week of life is thought to be able to determine the set-point of HPA axis responsivity in adulthood (Mueller and Bale, 2008; Weaver et al., 2004).

Perhaps the most exciting data from these studies is the evidence that pharmacological manipulation of the epigenome in adults appears to be sufficient to reverse the behavioral effects of these early life exposures. For example, administration to adult rats of a histone deacetylase inhibitor, which increased GR expression, was sufficient to reduce the elevated stress responses found in those animals that had received low maternal care as pups. Conversely, administration to adult rats of L-methionine, which increases *Nr3c1* promoter methylation and decreases GR expression, increased stress responses in rats that had received high maternal care. (Weaver et al., 2004; Weaver et al., 2005). This evidence that pharmacological treatment of adults can reverse the early epigenetic programming effects of low maternal care both at the level of gene expression and at the level of behavior has profound implications for the potential of applying our understanding of epigenetic mechanisms of gene transcription to the treatment of stress disorders in humans.

Epigenetics of the HPA axis in humans

Translatability of these findings from rodents to humans is supported by a number of recent studies. *NR3C1* is one of the genes that have been studied in postmortem human brains allowing for a direct comparison of neural regulation between rodent experimental models of environmental stressors and humans. For example, one postmortem study examined the relationship between childhood abuse and methylation of the human *NR3C1* promoter in postmortem brain tissue from suicide victims (McGowan et al., 2009). As compared with non-abused suicide victims or non-suicide controls, suicide victims with history of childhood abuse were found to have both decreased hippocampal GR mRNA expression and increased *NR3C1* promoter methylation. The GR is expressed in peripheral tissues in addition to the brain, and the regulated release of circulating HPA axis regulatory factors from the hypothalamus provides a mechanism for epigenetic plasticity of gene regulation in

the brain to be communicated to these peripheral tissues. If similar epigenetic mechanisms control *NR3C1* in both the brain and periphery tissues, then the *NR3C1* gene may be a promising candidate for identifying epigenetic regulatory events relevant to stress responses. Several studies have suggested that similar to regulation of *NR3C1* in the brain, increased methylation and decreased expression of this gene in PBCs is also a marker of past environmental stressors and maladaptive stress responses. For example, one study found that a history of childhood sexual abuse and its severity positively correlated with the methylation levels of the *NR3C1* promoter as measured in PBCs of adult patients with MDD and borderline personality disorder (Perroud et al., 2011). Another study examined the effects of maternal depression and anxiety in the third trimester of pregnancy on the methylation status of the *NR3C1* promoter and HPA responsiveness of infants. This study found that *NR3C1* promoter methylation in neonatal leucocytes was directly correlated with prenatal exposure to greater levels of maternal depression or anxiety and furthermore predicted higher salivary cortisol levels in response to stressors at three months of age (Oberlander et al., 2008).

In addition to the *NR3C1* gene, experimental evidence indicates that additional targets of epigenetic regulatory pathways relevant to HPA axis homeostasis are the chaperone molecules involved in intracellular glucocorticoid signaling. The most studied member of this family is the FK506 binding protein 51 (FKBP51/FKBP5), which is encoded by the *FKBP5* gene. Glucocorticoids induce expression of FKBP5, which in turn modulates GR sensitivity via an ultra-short negative feedback loop. As a result, increased FKBP5 activity is thought to lead to GR resistance and slower recovery of stress-induced increases in HPA activity (Binder, 2009). Four SNPs in the *FKBP5* gene were found in one study to interact with the severity of child abuse as a predictor of adult PTSD, suggesting a role for FKBP5 in the gene $\times$ environment interactions relevant to stress vulnerabilities (Binder et al., 2008). GR induces the expression of FKBP5 via its association with and regulation of glucocorticoid response elements (GREs) in distal enhancers that associate with the FKBP5 promoter through a chromatin looping mechanism. One of the disease-associated *FKBP5* SNPs impairs the association of intron 7 GREs with the *FKBP5* promoter, implicating this chromatin interaction in stress vulnerabilities (Klengel et al., 2013). Furthermore, a history of childhood trauma in subjects with this allele was found to correlate with reduced methylation, as measured in PBCs, of CpG dinucleotides in and around the GREs in intron 7 of the *FKBP5* gene (Klengel et al., 2013). Decreased methylation of this intronic enhancer is associated with higher induction of FKBP5 expression upon GR activation, leading through the FKBP5-dependent feedback regulation of GR to increased GR receptor resistance and a higher HPA axis set-point. These results highlight the fact that stress-induced epigenetic modifications of genes in the HPA axis may be moderated by genetic polymorphisms and by the transcriptional state of stress-related molecules during critical developmental periods. Notably, a study in mice showed that the DNA methylation levels of *Fkbp5* as measured in peripheral blood correlate with the 4-week total glucocorticoid burden independent of any disease-associated alleles (Lee et al., 2011). Although the biological relevance of this study for human psychiatric patients is limited by use of corticosterone administration as opposed to chronic stress exposure in the study, these findings suggest that *Fkbp5* methylation levels in peripheral blood may be a promising biomarker for measurement of stress burden. Future studies will need to determine whether *Fkbp5* methylation also correlates broadly with levels of chronic stress and whether this finding holds true in humans.

Conclusions, directions and challenges for future research

In this review, we have discussed how exposure to psychosocial stressors can induce lasting epigenetic modifications that carry the potential to shape stress responses. Over the past decade, a wealth of studies have revealed that stressors are just one of many environmental

exposures that can impact the extent, type, and genome-wide distribution of epigenetic marks in the brain. The concept that stress vulnerability and/or resilience may result from cumulative, environmentally-induced epigenetic marks that persist over time has important implications for our understanding of the pathophysiology of psychiatric disorders. For example, studies in humans suggest a “kindling hypothesis” for the relationship between stressors and MDD, wherein the requirement for potent environmental stressors progressively diminishes with repeated depressive episodes (Kendler et al., 2000). Although the mechanisms of this kindling-like effect have not yet been elucidated, it is plausible that stressor-induced epigenetic marks may underlie changes in stress vulnerability over the course of the disorder. If this were the case, then pharmacological treatments directed at manipulating the epigenome might become useful tools to impact the course of stress disorders. However a vast gulf remains between establishing that stressors can differentially affect stress responses via the epigenome and determining to what degree this gene regulatory mechanism actually underlies stress vulnerability. We see three major challenges to bridging this gap: 1) developing improved methods to experimentally demonstrate the functional importance of specific epigenetic marks for both gene transcription and stress responses, 2) characterizing the mechanisms of epigenetic regulation in vivo, and 3) translating concepts, experimental paradigms, and results between model animal and human studies.

Emerging methodologies for the study of the epigenome

The fact that environmental stressors can induce changes in DNA methylation that are both closely correlated with changes in expression of stress-responsive genes and restricted to physiologically relevant brain regions argue in favor of their functional importance for behavior. Still, in most cases whether identified changes in DNA methylation or other chromatin marks mediate changes in gene expression or whether they merely reflect those changes remains unknown. In the case of DNA methylation, one of the most significant limitations to understanding its functional importance has simply been the challenge of measuring this chromatin mark. The very first studies characterizing DNA methylation in neurons genome-wide are only now beginning to appear in the literature (Lister et al., 2013, Varley et al., 2013). As understanding of the distribution and regulation of this mark grows it is likely that new functionally meaningful models for DNA methylation will emerge. For example, already the evidence that DNA methylation is highly concentrated over both silenced gene promoters and active gene bodies suggests that the consequences of methylation are context-specific. In addition the recent observation that neurons alone among the differentiated mammalian cell types studied to date possess significant methylation of cytosines outside the CpG context raises the possibility that neurons use unique mechanisms of chromatin regulation that cannot be discovered in other cell types. With affordable next-generation sequencing now available to a broad range of researchers, it can be expected that the speed of chromatin data in the literature will continue to accelerate, providing a strong foundation for the development of new hypotheses of epigenetic functions.

Beyond mapping DNA methylation and other chromatin marks, the expansion of the molecular toolkit for experimentally manipulating the epigenome foretells an upcoming period of rapid advances in functional genomics. One of the most exciting among these developments is the CRISPR methodology, which co-opts bacterial proteins that recognize DNA sequences in order to experimentally recruit chromatin regulatory enzymes to specific gene regulatory elements (Gaj et al., 2013, Mali et al., 2013a). The CRISPR system utilizes the *Streptococcus thermophilus* protein Cas9, which is part of an innate immunity system these bacteria use against attack by bacteriophage. Cas9 has two functional domains, one of which recruits the protein to a specific DNA sequence using an RNA guide, while the other

possesses nuclease activity. In early 2013 multiple research groups published methods harnessing the use of Cas9 for the site-specific editing of the mammalian genome (Cho et al., 2012, Cong et al., 2013, Hwang et al., 2013, Jiang et al., 2013, Mali et al., 2013b). Shortly thereafter several research groups deleted the nuclease domain of Cas9 or replaced it with artificial transcription factors to artificially regulate the site-specific activation of gene expression from specific genomic sites (Cheng et al., 2013, Gilbert et al., 2013, Perez-Pinera et al., 2013, Qi et al., 2013). Given the ease, speed, and low cost of developing guide RNAs to direct Cas9 to any genomic site, and the potential to use the modular structure of Cas9 to experimentally recruit epigenetic modifiers to gene regulatory elements, CRISPR is very likely to lead to rapid new understanding of how epigenetic regulation of the genome impacts gene expression, cellular function, and ultimately, organismal behaviors.

Mechanisms of stress-dependent epigenetic regulation

Animal models of the epigenetics of stress responses have been most valuable for establishing the molecular and cellular mechanisms by which stressor-induced changes in chromatin regulation impact behavioral stress responses (**Figure 1**). For example we now know that stressor-inducible plasticity of DNA methylation is mediated at least in part through the regulated expression, and likely the regulated function and/or recruitment, of writers and erasers of this chromatin mark. The functional effects of stressor-induced changes in DNA methylation are context-dependent, and they can lead to either increases or decreases in gene expression depending on the position of the chromatin modification with respect to gene regulatory elements and the recruitment or blockade of protein interactions with the underlying DNA sequence. A further layer of functional specificity is conferred by the stressor-dependent recruitment and/or post-translational modification of the readers of DNA methylation, as exemplified by Ser421 phosphorylation of MeCP2. Most importantly, studies in animal models have revealed that stressor-induced changes in brain chromatin are highly selective for the neural circuits that underlie behavioral stress responses. These data provide the strongest causal evidence to date suggesting their direct linkage to the pathophysiology of stress disorders. Using the power of mouse genetics to selectively measure and disrupt epigenetic regulation of gene transcription in these functional circuits will be key in revealing the functional meaning of these marks. One outcome of these studies could well be the discovery that different epigenetic mechanisms in distinct brain regions are at play at different developmental periods and stages of any stress disorder. If this were the case, then the appropriate therapeutic interventions targeting these mechanisms would need to be varied over the course of a long-term disorder like MDD. Examining systematically how epigenetic marks at multiple genes in distinct neural circuits interact, evolve, and accumulate over the timecourse of stress disorders in model animals may cast light on the mechanisms shaping stress vulnerability and guide the development of stage specific, individualized therapeutic interventions.

As we have discussed, epigenetic control mechanisms are involved in multiple regulatory events that maintain homeostasis of the HPA axis. These data suggest a model in which these epigenetic marks may be acquired cumulatively over the course of multiple exposures to environmental stressors and act synergistically to determine the homeostatic set-point of the HPA axis. Consequently, changes in this set-point may confer stress vulnerability or resilience to future stressors. As we note in **Figure 2**, more evidence exists on epigenetic modifications that confer vulnerability and much less on mechanisms of resilience. By contrast, at the behavioral level we have discussed evidence showing that resilience can result from active processes (Russo et al., 2012), which not only prevent the development of disorders but may also lead to states of enhanced functioning following stress. This state of knowledge likely reflects the predilection of research efforts to examine biological correlations with constructs defined by clusters of psychiatric symptoms, rather than

studying positive outcomes as endpoints of interest. Furthermore, although symptom-based definitions of psychiatric disorders form the basis of current psychiatric diagnostic manuals and guide utilization of health care, it should be borne in mind that psychiatric disorders are defined phenomenologically and do not necessarily reflect known underlying pathophysiology. Thus, studies examining dichotomous outcomes, such as the presence or absence of specific behaviors, might not be the most informative when studying a process as nuanced as stress vulnerability and/or resilience. We propose that future studies linking epigenetics and stress responses should focus on active mechanisms that confer resilience and use continuous behavioral stress response measures as outcomes of interest.

Understanding the epigenetics of stress in humans

Ultimately, both researchers and psychiatrists hope that epigenetics findings from animals models will be translatable to the understanding of stress responses and the treatment of stress disorders in humans. Sufficient data have been accumulated to demonstrate that exposure to stressors can change epigenetic marks in humans as well as model animals. However to be useful for treatment or diagnostic purposes, chromatin modifications of specific genes would need to serve as biomarkers – meaning that the epigenetic processes measured could be said to directly reflect the severity of the disease process and/or measure the effect of treatment. A major hurdle to achieving this goal is the fact that gene expression changes underlying mental illnesses occur within the brain, where biochemical analyses of epigenetic marks are not possible in living humans. In the future the development of molecular imaging technologies may overcome this limitation by using methods like positron emission tomography (PET) to monitor the activation of chromatin regulatory enzymes in the intact brain (Wang et al., 2013). However for now, biomarkers detectable in peripheral tissues like blood remain the most tractable targets for study.

Genes of the HPA axis currently offer one of the more promising biomarkers for stress responses in humans. The evidence that similar epigenetic mechanisms regulate the glucocorticoid receptor gene *NR3C1* in both PBCs and the central nervous system suggests that blood samples could provide a biologically meaningful assessment of processes that contribute to stress responses in the brain. Because animal studies have shown that *Nr3c1* methylation predicts behavioral responses to future stress, putatively measuring epigenetic modifications of this or other HPA axis genes in blood samples may represent a useful strategy for identifying individuals at risk of maladaptive stress responses to environmental stressors. In addition, by serving as a read-out of overall HPA axis activity, analysis of epigenetic marks on HPA axis genes in the periphery could be a useful indicator of the efficacy of a wide variety of therapies for stress disorders, whether these drugs directly target chromatin regulatory enzymes or not. Finally, the evidence that at least some of the chromatin marks laid down on HPA axis genes by early life stress can be pharmacologically modified in adult animals raises the intriguing possibility that epigenetic modifiers might be useful modalities for the treatment of stress-related psychiatric disorders in humans. Nonetheless, the safety and potential usefulness of these emerging modalities remain to be determined.

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Highlights

- Presents a comparison of stress response studies in rodents and humans
- Reviews DNA methylation dependent mechanisms of stress responses
- Highlights HPA axis epigenetics as potential biomarkers for stress in humans
- Discusses promising directions for future studies into the epigenetics of stress

Figure 1.

Molecular mechanisms of stressor-induced regulation and their effects on stress responses. Cytosine methylation is mediated by members of the DNA methyl transferase (DNMT) family (“writers”), whereas demethylation is facilitated by proteins including Gadd45b and the Tet family (“erasers”). Proteins like MeCP2 that bind methylated cytosines and recruit transcriptional coactivators are the “readers” of this mark. The boxes show DNA methylation relevant changes that occur following specific stress paradigms. Red boxes represent epigenetic modifications associated with stress vulnerability, whereas green boxes represent modifications associated with stress resilience. To the right of the boxes are transcriptional consequences of these chromatin regulatory events. The lack of known transcriptional consequences is shown with question marks. White circles, unmethylated cytosines, black circles, methylated cytosines. Circled P represents a stimulus-regulated phosphorylation site on MeCP2. NAc, nucleus accumbens, PVN, paraventricular nucleus of the hypothalamus, LHb, lateral habenula.

Figure 2.

Schematic representation of epigenetic regulation of the hypothalamic-pituitary-adrenal (HPA) axis. The left side of the figure depicts the epigenetic modifications thought to confer vulnerability and the right side the ones thought to confer resilience. Stimulatory loops between sites of the axis are shown in blue, whereas inhibitory loops are shown in red. Potential directions for future research are suggested with question marks. GR, glucocorticoid receptor; HIP, hippocampus; PVN, paraventricular nucleus.