Epigenetic Mechanisms of Depression and Antidepressants Action

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Abstract

Epigenetic mechanisms, which control chromatin structure and function, mediate changes in gene expression that occur in response to diverse stimuli. Recent research has established that environmental events and behavioral experience induce epigenetic changes at particular gene loci that help shape neuronal plasticity and function, and hence behavior, and that some of these changes can be very stable and even persist for a lifetime. Increasing evidence supports the hypothesis that aberrations in chromatin remodeling and subsequent effects on gene expression within limbic brain regions contribute to the pathogenesis of depression and other stress-related disorders such as post-traumatic stress disorder and other anxiety syndromes. Likewise, the gradually developing but persistent therapeutic effects of antidepressant medications may be achieved in part via epigenetic mechanisms. This review discusses recent advances in understanding epigenetic regulation of stress-related disorders and focuses on three distinct aspects of stress-induced epigenetic pathology: the effects of stress and antidepressant treatment during adulthood, the life-long effects of early life stress on subsequent stress vulnerability, and the possible trans-generational transmission of stress-induced abnormalities.

Introduction

The importance of environmental influences in shaping brain structure and function has highlighted the profoundly plastic nature of the brain. In fact, all organisms continuously face stressful events and changes in their environment that can affect their homeostasis. Stress responses are reactions to these external challenges and include changes in the central nervous system (CNS) and in various peripheral organs that aim to reinstate the initial homeostasis (1). The perception of a stressful situation activates a large number of neuronal circuits, in particular, the hypothalamic-pituitary-adrenal (HPA) axis, the locus coeruleus, and autonomic noradrenergic centers in the brainstem. These and many other initial stress responses target numerous limbic brain regions, such as prefrontal cortex, hippocampus, amygdala, and ventral striatum (also termed nucleus accumbens or NAc) (Figure 1) (2, 3). These changes in the CNS directly affect learning and memory, alertness, arousal, and perhaps basal anxiety, and promote adaptive behavioral responses to subsequent stresses (4).

The inability to regulate and terminate these stress responses can result in many forms of dysregulation, as illustrated by sustained elevations of glucocorticoid levels, which profoundly affect neuroendocrine systems including immune responses, metabolism, and reproduction (5). Indeed, stressful experiences have been shown to increase the prevalence of a wide array of health impairments, including coronary artery disease, chronic pulmonary disease, and certain cancers (6, 7). Stress can also have negative consequences on behavioral adaptations and predispose some individuals to depressive- or anxiety-related disorders (8).

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However, there is a strong inter-individual variability in susceptibility to stress, such that most individuals are resilient: they can maintain normal physiological and psychological function despite being subjected to horrendous stress (9, 10). Under the right conditions, prior exposure to moderate levels of stress might promote future resilience, a phenomenon referred to as "stress inoculation" (11). Although stress-related disorders such as depression, post-traumatic stress disorder (PTSD), and other anxiety syndromes are partly hereditary, a majority of the risk appears to be non-genetic. The central hypothesis in the field, therefore, is that exposure to a range of stressful stimuli in combination with an individual's genetic constitution determine his or her initial coping strategy, maladaptive *vs.* resilient behavioral responses, and ultimate response to treatment (12, 13).

Stress-induced changes in gene expression within the brain's limbic circuitry have long been posited to mediate this interplay between genes and environment (14). Altered expression of several candidate genes has received a great deal of attention. Induction of brain derived neurotrophic factor (BDNF) in hippocampus is important in the efficacy of antidepressant treatment and its repression may contribute to the pathophysiology of depression in animal models (15). In contrast, sustained induction of BDNF in the NAc, which persists for at least 1 month after chronic social defeat stress, mediates depression-like behavioral abnormalities, whereas reversal of this effect induces an antidepressant-like response (9, 16). Other examples include suppression of the WNT/DVL/GSK3β/β-catenin signaling cascade in several limbic brain regions in response to chronic stress, with opposite changes seen upon antidepressant treatment (17–20), and the long-lasting down-regulation of glucocorticoid receptors (GR) in hippocampus resulting from a deficiency in maternal care (21). More recently, genome-wide measures of gene expression have been used to take a more open-ended view of alterations that occur in limbic regions after chronic stress or antidepressant treatment (9, 16, 22–29). This work has also extended to the study of resilience in chronic stress models to identify specific genes whose altered expression actively opposes the development of stress-induced abnormalities (9, 30, 31).

In these various studies, the fact that chronic stress or antidepressant treatment can induce altered expression of certain genes that persist for weeks after the last exposure suggests the involvement of epigenetic mechanisms, which have been implicated in mediating highly stable changes in gene expression during development and in adult tissues. Epigenetics, in its most general sense, is the study of the regulation of a gene's transcriptional potential in the absence of changes to the DNA sequence. The burgeoning field of epigenetics has been concerned with heritable changes in transcriptional potential as well as the role of equivalent mechanisms in controlling transcriptional regulation during the lifetime of a single organism. Numerous mechanisms underlying epigenetic regulation have been described to date, including direct modification of DNA's transcriptional potential by methylation of cytosine bases, and indirect regulation of DNA's accessibility through several types of chemical modification of the proteins involved in chromatin structure, including histones and many non-histone proteins (32). These mechanisms determine the accessibility of a gene to the transcriptional machinery and ultimately the ability to produce mRNA transcripts (Figure 2). An additional mode of epigenetic regulation is mediated by several types of non-coding RNAs (ncRNAs), including small noncoding RNAs (sncRNAs) and long noncoding RNAs (lncRNAs), which influence gene expression at multiple levels, for example, by modifying chromatin structure at specific genes, RNA splicing, and mRNA stability (33).

Much of what we know about epigenetic mechanisms comes from developmental biology, where chromatin changes govern the acquisition and stability of cellular phenotypes throughout differentiation (34, 35). Such cellular differentiation is accomplished by complex epigenetic regulation of individual genes via cues from surrounding cells and influences from the organism's environment. In contrast, many cell types in the adult organism no

longer divide, yet their phenotype, including their transcriptional potential, changes dramatically in response to environmental challenges. In the brain, there are many thousands of cell types with distinct phenotypes that are subjected to experience-dependent phenotypic modification throughout the lifetime. There is growing evidence that epigenetic mechanisms play a key role in this plasticity, thereby mediating stable functional changes in the brain in response to environmental exposures (36–41). By stably changing diverse aspects of neuronal function, epigenetic modifications have been implicated in learning and memory (42–44), social and maternal behaviors (45, 46), and pathological behaviors, such as drug addiction (47, 48), depression (26) and PTSD (49). Such epigenetic modifications could underlie the longlasting behavioral abnormalities seen in these disorders as well as the extraordinary inter-individual variability in vulnerability to adversity. In fact, epigenetics might partly explain the difficulty in identifying the specific genetic variations that contribute to these syndromes, the significant discordance between monozygotic twins, and the differential gender susceptibility to certain psychiatric diseases (50, 51). The study of epigenetic regulation thus opens new doors to understanding normal behavioral traits as well as disease etiology in humans. Although best demonstrated in cancer and stem cell biology, pathological epigenetic marks have been observed in psychiatric disorders, for example, in postmortem tissue of depressed patients (26, 52, 53), and individuals that committed suicide (54). However, careful interpretation is required since these epigenetic marks could represent the cause of the disability or a mark of the disease state, or both.

In this review, we discuss recent progress toward understanding epigenetic regulation by stress during early life and adulthood in the brain's limbic circuitry, and how such mechanisms might contribute to susceptibility versus resilience to stress-related disorders and to their reversal by antidepressant treatments. We also address recent insight into possible epigenetic transmission of behavioral adaptations to stress across generations.

Overview of Epigenetic Mechanisms

DNA Methylation

Cytosine bases can be covalently modified by methylation at the 5' position, resulting in a methyl group projecting into the major groove of DNA (55). In mammals, this predominantly occurs in the palindromic sequence 5'-CpG-3' (and occasionally 5'-CpNpG-3') and does not interfere with normal hydrogen bonding with complementary guanine bases. Approximately 3% of all cytosines in the human genome are methylated (56), and proper cytosine methylation (5mC) is required for cell differentiation, genetic imprinting, suppression of repetitive elements, and X-chromosomal inactivation (57). The location of CpG bases in mammalian DNA is uneven, occurring at high concentrations in select regions termed CpG islands. CpG islands overlap with the promoters of 50–60% of human genes and are typically methylated to a much lower extent than CpG dinucleotides found outside of islands (58).

Although methylated cytosines can both prevent and promote the binding of various proteins to DNA (59), CpG methylation in promoter regions is generally considered to repress gene transcription (see below). DNA methylation is catalyzed by DNA methyltransferases (DNMTs), a family of enzymes that include DNMT1, DNMT2, DNMT3a, and DNMT3b (60). These enzymes play distinct roles, with DNMT1 maintaining methylation patterns during DNA replication, while DNMT3a and DNMT3b appear to catalyze *de novo* methylation of previously unmethylated double-strand DNA (55, 61). Several DNA demethylation pathways have been proposed, such as those mediated by methyl CpG binding domain protein 2 (MBD2) (62), growth arrest and DNA-damage-inducible protein 45 alpha (GADD45a) (63), and GADD45b (64). Recent studies indicate DNA oxidation/repair pathways may drive active DNA demethylation in nondividing neurons. Also, ten-

eleven-translocation (TET) enzymes have been shown to oxidize 5mC into 5′-hydroxymethylcystosine (5hmC), which can be further oxidized into 5-formolcystosine and 5-carboxylcystosine (65–68). TET family hydroxylase-induced oxidation-deamination thus represents a new candidate for the long-sought DNA demethylase in brain (69). Interestingly, all three forms of 5mC derivatives seem to be enriched in brain, and 5hmC displays a developmentally programmed acquisition in neuronal cells (70).

DNMTs can directly interact with transcription factors, presumably allowing the methylation of promoter regions at specific locations. In a recent study, nearly 80 transcription factors were found to interact with DNMTs (71). This enables gene-specific binding of MBDs. MBDs have been shown to repress gene transcription through recruitment of corepressor complexes that can interfere with the transcriptional machinery or directly regulate chromatin structure (see below). MBDs are essential for normal development and growth, as loss of function mutations of the MBD, methyl CpG binding protein 2 (MeCP2) is associated with Rett's Syndrome (72, 73) and the MBD Np95/ICBP90-like ring finger protein regulates the cell cycle and has been implicated in tumorogenesis (74). Although originally thought to be a very stable modification, it appears that DNA methylation is subject to dynamic regulation in the adult brain (64, 75, 76), and regulation of DNA methylation at specific genes has been demonstrated in multiple cases (77–79).

Histone Modifications

In eukaryotic cells, DNA is densely packed into chromatin through interactions with large protein complexes called nucleosomes. Individual nucleosome cores are composed of an octamer containing four histone dimers, one dimer each of histones H2A, H2B, H3, and H4, around which are wrapped 147 bp of DNA in approximately two superhelical turns (36). Variant histone proteins or post-translational modifications of histones cause changes in chromatin compaction that are correlated with more "open" (euchromatin: transcriptionally permissive) and "closed" (heterochromatin: transcriptionally repressive) states (80). The N-terminal tails of histones are exposed at the surface of the nucleosome and exhibit multiple, reversible covalent modifications that alter the accessibility of DNA to the transcriptional machinery in a regulated fashion (Figure 2). In addition, histones can be substituted with variants, such as the CENP-A variant of H3, which is found specifically in centromeric DNA, and whose tail contains a significantly different set of modifiable residues, which suggests that substitution of this histone may be essential for mitosis (81).

Histone acetylation, which negates the positive charge of lysine residues in the histone tail, is associated with transcriptional activation. This is likely due to a weakening of the interaction with the DNA strand that depends upon the negative charge of lysines (36, 82). In contrast, lack of histone acetylation correlates with gene repression. Histones are acetylated by histone acetyltransferases (HATs), which use acetyl coenzyme A as a cosubstrate, and deacetylated by histone deacetylases (HDACs). HATs comprise a large family of proteins and acetylate multiple lysine residues in the tails of both H3 and H4. HDACs are composed of multiple families of proteins divided into three classes. In the brain, Classes I and II appear to regulate histone deacetylation at most genes, while Class III enzymes (comprising the sirtuin proteins) deacetylate numerous nuclear and cytoplasmic substrates in addition to histones and are implicated in numerous cellular functions (83–86).

Unlike the clear correlation of histone acetylation with transcriptional activation, histone methylation can be associated with both transcriptional activation and repression depending on the particular residue modified and the extent of methylation. Both lysine and arginine residues can be methylated by various histone methyltransferases (HMTs), which use S-adenosylmethionine (SAM) as a cosubstrate. Methylated arginine residues are converted to citrulline by deaminases, while lysines are demethylated by lysine demethylases (87).

Unlike acetylation, methylation does not substantially alter the charge of the target residues, but can dramatically change the steric profile and potential molecular interactions through multivalent addition of mono-, di-, or tri-methyl groups. Examples of the bidirectional effects of histone methylation include trimethylation of lysine 4 of H3 (H3K4me3), which is associated with transcriptional initiation, and di- and trimethylation of lysines 9 and 27 of H3 (H3K9me2/3 and H3K9me2/3), which are associated with transcriptional repression (47). In contrast, H3K36me3 is associated with transcription elongation.

Multiple additional modifications of histone tails are known, including phosphorylation, ubiquitination, sumoylation, and ADP ribosylation, among others (32, 36). For instance, phosphorylation of serine 10 of H3 is associated with increased gene transcription through a complex mechanism involving HAT recruitment and prevention of HMT activity at lysine 9 (47). Modification of one histone can also affect subsequent modification of other histones within the nucleosome, as ubiquitination of H2B appears to be a prerequisite for H3K4 methylation and the resulting initiation of transcription (88). The level of complexity in the nearly infinite possible combinations of various modifications at multiple residues of histone tails, as well as the introduction of histone variant subunits, has led to the proposal of a histone "code" whose "reading" by various histone-interacting proteins is essential for the proper regulation of gene expression (81, 89).

Noncoding RNAs

Many types of ncRNAs are well known, such as ribosomal (rRNA) and transfer (tRNA) RNA. More recently, many additional ncRNAs have been described, divided between the lncRNAs and various short varieties, including micro (miRNA), small nuclear (snoRNA), promoter associated (PASR), Piwi-interacting RNA (piRNA), and transcription initiation (tiRNA) types (33). lncRNAs are generally characterized as being longer than 200 nucleotides; they can be spliced like mRNAs to form functional secondary structures and can act as precursors for various sncRNAs (90). Although only a small number of functional lncRNAs have been characterized to date, they have been shown to control gene expression programs at every level (91). lncRNAs appear to function via multiple epigenetic mechanisms, and can modulate the status of protein-coding genes through the recruitment of chromatin remodeling complexes to specific regions of the genome, thereby regulating the chromatin structure of a single promoter region, a cluster of genes, or an entire chromosome (90). lncRNAs can also serve as an enhancer to regulate gene transcription (92).

miRNAs are the best studied sncRNAs in brain. They are post-transcriptional regulators which can bind to complementary sequences on target mRNAs and lead to translational repression or mRNA degradation (93). It appears that miRNAs can bridge with other epigenetic mechanisms to regulate neural plasticity (94). In addition, sncRNAs can influence epigenetic regulation of gene expression. For instance, sncRNAs bound to Argonaute proteins (and other factors required for inhibitory RNAs or RNAi's, such as Dicer) have been demonstrated to generate H3K9 methylation, and have since been implicated in the process of heterochromatin formation through recruitment of lysine methyltransferases (KMTs) (95). In *C. elegans*, short interfering RNAs (siRNAs) and RNAi's trigger long-term gene silencing in a dominantly heritable fashion, with multiple chromatin remodeling factors required for maintenance of the silent state (96).

Regulation of the Epigenome by Stress and Antidepressant Treatment in Adulthood

There is now considerable evidence that chromatin remodeling is a dynamic process that occurs throughout life in many organs including the brain (34, 97, 98). Patterns of DNA

methylation and histone acetylation gradually change over time in monozygotic twins (99). Such drift might not occur randomly and could represent the effect of the environment and the accumulation of the individual's experience. Chromatin remodeling processes are necessary for learning and memory, and aberrant epigenetic modification can lead to cognitive deficits (100, 101). It is within this context that an increasing number of studies have shown that exposure to stress promotes alterations in various epigenetic marks, in particular, histone acetylation and methylation as well as DNA methylation, in various limbic brain regions (Figures 1 and 2). Additionally, experimental manipulation of the epigenetic machinery within these regions has been demonstrated to potently control mood states and responses to stress and antidepressant treatments.

Regulation of DNA methylation in adulthood

Given that DNA methylation is a relatively stable epigenetic mark in post-mitotic cells, it is likely that it participates in the long-lasting change in gene expression underlying the development of stress-induced abnormalities. Recent reports show changes in CpG methylation levels at gene promoters implicated in HPA axis reactivity and antidepressant treatment. Depressive-like symptoms induced by chronic social defeat stress are accompanied by the sustained upregulation of CRF, which is not observed in mice that are resilient to the stress (31). CRF is expressed by neurons of the paraventricular nucleus (PVN) in the hypothalamus and controls HPA axis activity as well as several other stress responses in the brain. Stress induction of the *Crf* gene is accompanied by a decrease in DNA methylation at its promoter. Chronic imipramine treatment was sufficient to reduce *Crf* mRNA levels and increase DNA methylation at the *Crf* promoter only in socially-defeated mice. A related observation is that conditional knockout of MeCP2 in the PVN, which reduces the repression of certain methylated genes, induces an abnormal physiological stress response (102). This shows that epigenetic regulation of genes is critical for appropriate regulation of the HPA axis. Interestingly, female rats exposed to chronic mild stress show increased DNA methylation at the *Crf* promoter in the PVN, suggesting sex-specific alterations in HPA axis activity (103).

Activation of the HPA axis and the subsequent release of glucocorticoids have a profound impact on hippocampal function because hippocampal neurons express high levels of GR. Therefore, the hippocampus has received a lot of attention for its role in stress-induced behavioral and cognitive deficits and antidepressant responses. One mechanism by which stress affects hippocampal function is through repression of BDNF, as noted above (104). Chronic exposure to predator stress increases methylation of the *Bdnf* promoter in hippocampus (105), possibly contributing to the morphological changes and neuronal impairments reported after chronic stress in animal models and in depressed humans (106, 107).

Genetic background influences the outcome of stress exposure in animals and humans, and this could be mediated in part by DNA sequence-directed differences in epigenetic modifications in response to stress, a possibility which is just beginning to be explored. Chronic mild stress increases anxiety- and depression-like behaviors in BALB/C mice, effects not seen under the same conditions in C57BL/6 mice. Exposure to stress differentially regulates glial cell-derived neurotrophic factor (GDNF) expression in the NAc in the two strains of mice (108), with BALB/C mice displaying decreased GDNF expression and C57BL/6 mice showing increased expression. The authors report increased DNA methylation and MeCP2 binding at the *Gdnf* promoter in the NAc of both strains after chronic stress, changes associated with increased expression of DNMT1 and DNMT3a. However, the two strains are distinguished by the protein complexes that associate with MeCP2 at the *Gdnf* promoter. In BALB/C mice, MeCP2 reportedly interacts with HDAC2 to decrease H3 acetylation and concomitantly represses *Gdnf* transcription in NAc, whereas

the association of MeCP2 with CREB, a transcriptional activator, is purported to enhance GDNF expression in the NAc of C57Bl/6 mice. The authors propose that these differences are driven by different methylation patterns at the *Gdnf* promoter, although much further work is needed to validate these findings as well as understand their molecular basis. Nevertheless, these observations are consistent with reports that MeCP2 can act as a repressor or activator of gene transcription (109). In the Flinders Sensitive Line (FSL) genetic rodent model of depression, elevations in DNA methylation at the *P11* promoter were reduced by chronic administration of escitalopram, leading to an increase in P11 and a decrease in DNMTs in the prefrontal cortex (110). P11 interacts with serotonin and perhaps other receptors and regulates their function, and decreased levels of P11 have been observed in post-mortem depressed patients and associated with depressive-like behaviors in mice (111, 112). Finally, decreased DNA methylation at the promoter of the serum interleukin 6 (IL-6) gene in peripheral blood mononuclear cells was associated with higher levels of IL-6 in adults with a history of depression (113). IL-6 is part of the inflammatory response system that has been shown to be dysregulated in some depressed individuals (114).

As noted, induction of DNMTs occurs in NAc after chronic social defeat stress (115), and recent evidence demonstrates directly that this adaptation contributes to the development of depressive-like behaviors. Overexpression of DNMT3a in the NAc facilitates vulnerability to the deleterious effects of the stress. This mechanism appears critical for the establishment of maladaptive behaviors because intra-NAc infusion of two DNMT inhibitors, RG108 or Zebularine, reversed the behavioral deficits induced by chronic stress (108, 115). Since DNMTs generally function to repress gene transcription, these data suggest that the development of stress-induced behavioral abnormalities is accompanied in the NAc by the downregulation of genes implicated in reward and motivation. On the other hand, DNA methylation in other brain regions, such as those important for the HPA axis as discussed above, constitutively represses genes that participate in stress responses. Therefore, it is unlikely that drugs that globally affect DNA methylation would be viable therapeutic approaches, although targeting site-specific DNA methylation may be a valuable pathway for development of future therapies.

Regulation of histone acetylation in adult responses to stress

Although considered more labile, some histone post-translational modifications can contribute to the persisting abnormalities of stress-related psychopathology. After chronic social defeat in mice, there is a transient decrease in total cellular levels of H3 acetylation in NAc followed by a more persistent increase which lasts for at least 10 days after the last stress (26). This lasting increase may be mediated via the sustained repression of HDAC2 expression in this brain region. Interestingly, increased H3 acetylation and decreased HDAC2 levels are seen in the NAc of depressed humans. Nevertheless, these adaptations seem to be adaptive, since local infusion of HDAC inhibitors into the NAc, or local inhibition of HDAC2 via overexpression of a dominant negative mutant of the enzyme, exerts potent antidepressant-like actions in several behavioral assays (26, 108). Moreover, local administration of an HDAC inhibitor produced a partly similar pattern of gene expression changes in NAc as seen with systemic fluoxetine treatment (26). Chronic social defeat stress induces more transient increases in global H3 acetylation levels in other brain regions, such as hippocampus, amygdala, and medial prefrontal cortex, and these effects may also be adaptive since HDAC inhibitors are antidepressant-like when administered into these regions as well (116, 117). However, other groups have observed decreased H3 acetylation levels in hippocampus after chronic stress and the reversal of these effects by imipramine treatment (116, 118, 119).

Nevertheless, several groups have demonstrated antidepressant-like effects of HDAC inhibitors upon systemic administration (120–122), with such inhibitors having no effect on

depression-like behaviors in non-stressed mice (123). These studies thus suggest that HDAC inhibitors show some potential as novel antidepressant agents. However, the lack of specificity of the inhibitors used to date, and their limited ability to penetrate the brain, are major caveats that must be considered. Given the lack of brain-specific HDAC isoforms, it seems unlikely that HDAC inhibitors of acceptable safety and specificity can be developed for treatment of depression. Still these data can buttress drug discovery efforts by helping to identify the genes important in depression models, which can then be targeted for drug development. For example, comparison of genes within a given limbic region regulated by fluoxetine versus HDAC inhibitors might allow identification of novel mechanisms that are unique to HDAC inhibition and perhaps capable of better, faster antidepressant responses (26).

Currently, little is known about the specific genes at which stress-induced changes in histone acetylation mediate such regulation of depression-like behavior. Chronic social defeat stress down-regulates *Bdnf* transcription in hippocampus, an effect reversed by antidepressant treatment (120). While the repression may be mediated by enhanced DNA and histone methylation (see below), antidepressant reversal of this effect is associated with increased H3 acetylation at the *Bdnf* promoter (120, 124). This hyperacetylation resulting from antidepressant treatment is mediated by the down-regulation of HDAC5 in hippocampus, which was shown to be necessary for the behavioral effects of chronic antidepressant administration (120). Further work is needed to identify, in a genome-wide manner, the many other genes involved in mediating the antidepressant-like effects of enhanced histone acetylation at the levels of several limbic brain regions.

Regulation of histone methylation in adult responses to stress

Chronic social defeat, coincident with the sustained increase in H3 acetylation in NAc noted above, causes a global decrease in levels of H3K9me2, a repressive mark, mediated via downregulation of the associated HMTs, G9a and G9a-like protein (GLP) (125). These latter adaptations represent stress-induced pathological changes, because conditional knockout of G9a from the NAc, or its pharmacological inhibition, promoted susceptibility to social stress (125). In contrast, G9a overexpression in NAc, which increases H3K9me2 levels, promotes stress resilience and induces antidepressant-like responses (26, 125). One gene that contributes to these effects is Ras, a small GTPase upstream of the ERK signaling cascade. Stress-induced decreases in H3K9me2 at the *Ras* gene in the NAc leads to an increase in its expression and to subsequent activation of the ERK signaling cascade, including activation of CREB, which then induces depression-like behavioral abnormalities (Figure 3). It is interesting that two adaptations associated with gene activation (reduced H3K9me2 and increased H3 acetylation) exert opposite behavioral effects. One explanation is that different genes are affected by these modifications, a possibility that now requires direct investigation with genome-wide methods.

Chronic stress has also been shown to alter levels of histone methylation in hippocampus. Differential effects on global levels of H3K4me3, H3K9me3, and H3K27me3 within distinct hippocampal subfields occur in response to acute versus chronic restraint stress (126). Interestingly, our group found that chronic social defeat stress increases H3K27me3 levels at the *Bdnf* gene promoter in hippocampus in concert with its repression (120). Histone repressive marks are often associated with increased DNA methylation, and this is consistent with increased methylation observed at this gene in response to other forms of stress (105).

The discussion above highlights the need for genome-wide assessments of chromatin changes, which will allow identification of previously unknown mechanisms involved in stress-related disorders and antidepressant action. Such studies are just now getting underway. ChIP-chip analysis (chromatin immunoprecipitation followed by genome-wide

promoter arrays) for H3K9me2 identified numerous genes that display increases or decreases in this chromatin mark one month after chronic social defeat stress (Figure 4) (127). Interestingly, a similar global pattern of H3K9me2 binding was observed after prolonged adult social isolation. Although these two forms of chronic stress are ethologically distinct, they induce some similar behavioral abnormalities (9, 128). Furthermore, imipramine treatment reverses the large majority of these histone methylation changes in NAc induced by chronic social defeat stress, and the pattern of chromatin changes induced by imipramine resembles that seen in resilient mice (127). These novel findings suggest that one mechanism by which antidepressants work is to induce some of the same molecular adaptations that occur naturally in more resilient individuals, and that a new path forward in antidepressant drug discovery is to mimic such natural resilience mechanisms. For example, these data implicated the WNT/DVL/GSK3β/β-catenin pathway in the NAc in mediating susceptibility versus resilience, as well as antidepressant action, findings later validated by direct manipulation of this cascade (20).

Another ChIP-chip study mapped the genome-wide binding of SETDB1, an H3K9 HMT, in mouse prefrontal cortex and identified the NR2B NMDA glutamate receptor subunit as one key target (129). Interestingly, these authors also demonstrated that overexpression of SETDB1 in forebrain exerts antidepressant-like actions, consistent with the observations, noted above, that G9a, another H3K9 HMT, also produces antidepressant-like effects when overexpressed in the NAc (125). The identification of NR2B as one relevant substrate emphasizes the need in the field to further characterize the influence of chronic stress and antidepressant treatments on synaptic plasticity in several key limbic regions (130). Loss of KAP1, a protein implicated in transcriptional repression, also increases stress vulnerability (131), highlighting the large number of chromatin mechanisms that likely control adaptations to chronic stress.

Regulation of miRNAs in adult responses to stress

Additional forms of epigenetic regulation are likely involved in stress and antidepressant action. Chronic administration of fluoxetine reportedly increases levels of miRNA-16 (miR-16), which targets the serotonin transporter (SERT) transcript in serotoninergic neurons and decreases SERT expression (132). More surprisingly, fluoxetine was found to down-regulate miR-16 levels in noradrenergic neurons of the locus coeruleus and to thereby enable certain serotonergic phenotypes in those cells. Further work is needed to validate this adaptation and explore its contribution to the antidepressant effects of fluoxetine. In addition, constitutive and ubiquitous manipulations of SIRT1, a Class III HDAC as noted earlier, has recently been shown to affects basal levels of anxiety, with increased SIRT1 activity associated with increased anxiety-like behaviors (133). SIRT1 has also been shown to decrease the expression of miR-134 in hippocampus, thereby enhancing CREB and BDNF levels (134), which could contribute to its effects on anxiety and stress responses. These early reports highlight the need for additional studies of miRNAs in stress and antidepressant action.

Epigenetic Regulation by Early-Life Stress

During development, the brain is sensitive to environmental changes, such as parental care and stressful events. Cognitive stimulation can lead to broad effects on brain structure and function, hence influencing cognitive and affective function in children, adolescents, and young adults (6, 7). Maltreatment, neglect, and trauma during childhood are known to increase the risk of depressive, anxiety, and substance abuse disorders as well as the risk of becoming an abusive parent (135–139). In contrast, more mild forms of stress may promote resilience (10, 11). Such bidirectional regulation during early life of susceptibility to stress-related illness in adulthood is likely mediated by stable alterations in chromatin structure of

specific genes in brain. Longitudinal studies revealed changes in DNA methylation in buccal epithelial cells that were associated with early life adversity (140), although how such changes relate to brain function remains unknown. Nevertheless, animal models have clearly identified epigenetic rep rogramming of gene expression as a stable mechanism by which early life experience, in particular stress and maternal care, affect the individual's responses to stress later in life (141).

One of the most commonly used procedures for inducing early life stress in rodents is periodic maternal separation during early postnatal life. It is characterized by long-term physiological and behavioral alterations, including elevated glucocorticoid levels and hyperactivity of the HPA axis to subsequent stresses (1, 142, 143). Murgatroyd *et al.* reported that arginine vasopressin (AVP) undergoes increased expression in the PVN with maternal separation. In addition to its role in water homeostasis, AVP is a key regulator of the HPA axis (144). AVP is secreted by PVN neurons and, in conjunction with CRF, regulates the release of adrenocorticotropin hormone (145). Early life stress activates calcium/calmodulin-dependent protein kinase II (CaMKII) and downstream phosphorylation of MeCP2 at serine438 in the PVN, which leads to the release of MeCP2 from *the Avp* gene enhancer (144). This is associated with activation of the *Avp* gene and increased HPA axis stress reactivity, both of which are sustained into adulthood. However, the life-long *Avp* gene induction is not accompanied by persistent CaMKII activation and MeCP2-S438 phosphorylation. Rather, kinase activation and MeCP2 phosphorylation are followed by *Avp* gene enhancer demethylation. Presumably, both CaMKII activation and MeCP2 phosphorylation exert broad transcriptional effects beyond the *Avp* gene, which remains a focus for future investigations. Similarly, newborn rats exposed to stressed caretakers for the first postnatal week display significant increases in DNA methylation at regulatory regions (exons IV and IX) of the *Bdnf* gene in prefrontal cortex and hippocampus, and persistent BDNF downregulation, in prefrontal cortex but not hippocampus (146). Such early life regulation of BDNF expression might influence stress vulnerability later in life (147).

Though the causal role of DNA methylation in transcriptional regulation during postnatal brain development has been challenged (148), these studies of early life stress demonstrate that DNA methylation is an ideal mechanism, at least from a heuristic point of view, for life-long changes in transcriptional potential. Early life stress can also modify histone acetylation levels through changes in HDACs. Maternal separation in BALB/C mice decreases HDACs levels in the adult forebrain, leading to a concomitant increase in acetylated H4 lysine 12 (149). Activation of HDACs and the resulting decrease in acetylated H4 worsen the effect of stress, suggesting that the observed reduction in HDAC expression is a positive adaptive process.

In rodents, the style of maternal care has long-term effects on the behavioral and endocrine responses to stress in offspring (142). Maternal care is measured by the frequency of pup licking and grooming (LG) behavior, which is the major source of tactile stimulation for the neonatal rat that regulates endocrine, cardiovascular, and behavioral responses (150). Variation in maternal behavior is a naturally occurring phenomenon, which can have long-term effects on neural systems that regulate learning and memory, neuroplasticity, and emotional and stress responses (150). In fact, differential levels of maternal care profoundly influence hippocampal glucocorticoid receptor (GR) levels, hence affecting the regulation of the HPA axis by stress. As adults, the offspring of high LG mothers show increased hippocampal GR expression and therefore enhanced negative feedback regulation by glucocorticoids in comparison to adult animals reared by low LG mothers (21). This range of GR expression levels has been traced inversely to levels of methylation at the *GR* gene promoter (Figure 5). Lower levels of methylation enhance the accessibility of the promoter to transcription factors and chromatin regulatory proteins such as CREB-binding protein

(CBP) (151, 152). Increased binding of CBP, a HAT that acetylates specific sites of H3 and H4, leads to increased histone acetylation at the *GR* promoter in high LG offspring (153, 154). All effects of high LG rearing could be reversed in the adult offspring by intracerebroventricular infusion of L-methionine, which acts as a methyl group donor (155). Similarly, infusion of the HDAC inhibitor trichostatin A (TSA) reverses the changes induced by low LG rearing (151). These findings once again demonstrate the interplay between DNA methylation and histone modifications, and show that changes in the epigenome established by the environment during early development may be reversed by pharmacological approaches in adults– emphasizing the plasticity of chromatin regulation in the adult brain. Although low LG rats show a heightened response to stress, they show greater contextual learning under stressful conditions, suggesting that the long-term behavioral adaptations induced by maternal care have some adaptive value (156, 157), i.e., that increased stress responsiveness might prepare the individual to survive in more aversive environments. McGowan et al (158) demonstrated the clinical relevance of these observations in rats: they found increased methylation of the *GR* promoter and reduced GR expression in the hippocampus of suicide victims with a life history of childhood abuse.

Other behaviors are also dependent on mother-infant interactions. Maternal LG behavior affects female offspring in their own expression of maternal care. Indeed, maternal care is a matrilineally transmitted behavior that is mediated in part by long-term alteration of oxytocin expression in the hypothalamic medial preoptic area (MPOA) and its regulation by estrogen (157). Oxytocin plays a central role in social bonding and maternal care. In female offspring of low LG mothers, there is decreased expression of estrogen receptor-alpha (ERα) through increased methylation at the *ERα* gene promoter. This reduces the ability of estrogen to activate oxytocin receptor expression in the MPOA (45), hence reducing the ability of oxytocin itself to promote maternal care. This epigenetic regulation of ERα expression within the MPOA emerges during the first postnatal week and is maintained into adulthood. Cross-fostering studies reveal a direct relationship between the maternal behavior and the behavioral and physiological development of the pup. In contrast, female offspring of high LG animals exhibit reduced methylation at the *ERα* promoter and increased ERα expression. Consequently, these females have increased estrogen sensitivity, and greater oxytocin function, which drives LG behavior. Later changes in the social environment can reverse these behavioral adaptations (159), suggesting that these epigenetic marks are subjected to modification throughout life.

A major need in the field is to extend these studies of AVP, GR, and ERα to identify the presumably many additional genes whose methylation might further contribute to these behavioral phenotypes. By using a high density tiling array, the adult rat chromosome 18, which contains the *GR* gene, was examined for DNA methylation, H3K9 acetylation, and transcriptional changes under differential LG mothering (160). The study demonstrated global epigenomic differences in adult brain, which span gene promoters, coding regions, and gene ends as well as intergenic regions. The data suggest that early life maternal care has a broad effect on transcription, with hundreds of genes implicated (45, 160, 161). Among these genes are those encoding several protocadherin isoforms as well as rRNA, and hypermethylation of the *rRNA* promoter was observed in the hippocampus of humans who died by suicide with a history of early childhood neglect/abuse (162). Further work is needed to characterize the functional consequences of these and the many other observed promoter modifications on life-long stress vulnerability.

Early life stress also encompasses environmental exposures during pregnancy. There is an extensive literature on potential effects of prenatal stress on the development of depression or other psychiatric syndromes (163–165), which would be expected to have an epigenetic basis. In humans, depressed mood during the third trimester of pregnancy induced an

increased DNA methylation at the *GR* promoter in blood leukocytes of the offspring (166). This effect might relate to the finding that offspring of holocaust survivors with PTSD showed lower 24-hour urinary cortisol excretion than offspring of survivors without PTSD (167, 168). These early observations are intriguing and define more mechanistic experiments that are now being carried out in rodent models, as discussed further below under *Possible Heritability of Behavioral Adaptations to Stress*.

This discussion of epigenetic consequences of early life stress focuses on DNA methylation, which, because of its stability, is an attractive mechanism for mediating life-long behavioral adaptation. However, the notion that DNA methylation changes are subject to dynamic and bidirectional regulation in fully differentiated neurons goes against a prevailing view in the field that it represents a permanent modification during cell differentiation (169). There are thus several reports noted above that *de novo* DNA methylation occurs in postnatal neurons (151), and that DNA demethylation occurs throughout life (75, 76, 151). The notion that there is a wave of *de novo* DNA methylation in neurons early in life is supported by the finding that DNMT expression peaks in neurons during early postnatal life (170). These observations raise the interesting possibility that DNA methylation serves a crucial function in mediating the critical time windows for activity-dependent synaptic formation and refinement, and associated behavioral adaptations, long known to exist in early life (171).

There are normally tens to hundreds of CpG dinucleotides in a given gene's regulatory and coding regions, and the combination of "on" (CpG demethylation) and "off" (CpG methylation) signals at each CpG site produces a complex "methylome" for each gene. Since methylation changes at distinct regions of a gene might exert different functional effects, measures of total DNA methylation do not provide an accurate measure of that gene's transcriptional potential. We are still learning about the functional consequences of different patterns of DNA methylation at neural genes, and much further work is needed to translate the methylome, acting in concert with the newly defined 5hmC, as well as large numbers of other forms of chromatin modifications, into understanding the gene-specific epigenetic effects of early life stress.

Possible Heritability of Behavioral Adaptations to Stress

Our discussion to this point has focused on a role for epigenetic mechanisms in mediating stable, in some cases life-long, neural and behavioral adaptations in response to environmental challenges. Far more provocative is the idea of the transmission of parental adaptations to environmental challenges to offspring when the young do not experience the challenges themselves (Figure 6). Inheritance of acquired traits might offer an advantage to the population by increasing the fitness of the species, and enabling adaptation to variations in the environment at a faster rate than natural selection would allow. Such non-genetic inheritance of acquired behavioral traits is reminiscent of the ideas put forth by Jean-Baptiste Lamarck in his 1809 book, *Philosophie Zoologique*, which have been widely discredited. Rather, the trans-generational transmission of behavioral traits and psychiatric disorders in humans is believed to be mediated via genetic mechanisms working in concert with parenting and culture. Nevertheless, recent advances in epigenetics have renewed interest in possible non-genetic, non-behavioral transmission of behavioral experience via epigenetic mechanisms through the germline. Such use of the term epigenetics, to be distinguished from epigenetic mechanisms occurring within a single organism, continues to cause confusion in the field (172).

Several groups have begun to explore the transmission of stress-related pathologies to subsequent generations via epigenetic mechanisms. Franklin et al. (173) showed that most of the behavioral alterations induced by early life maternal separation are expressed by the F1

offspring of males subjected to maternal separation. These male F1 offspring, when bred with normal females, transmitted their behavioral abnormalities almost exclusively to female offspring for 2 generations (F2 and F3). Maternal separation was reported to alter DNA methylation levels in the promoter regions of the genes encoding MeCP2, the CRF2 receptor, and the cannabinoid receptor-1 (CB1) in the germline of the separated males. Similar DNA methylation patterns were observed in the cerebral cortex of female offspring. No differences in maternal care were observed, suggesting a paternal epigenetic transmission of stress-related behavior, but in a sexually dimorphic manner. In a model of early life stress with impaired maternal care, abnormal DNA methylation and expression patterns of BDNF in the prefrontal cortex are induced for up to two generations (146). Such effects on methylation patterns are not totally reversed by cross-fostering, suggesting that some of these effects are not the result of postnatal experiences. However, females with a history of early life maltreatment show increased anxiety while pregnant, which could influence fetus development. Although prenatal and postnatal effects cannot be ruled out, transgenerational perpetuation of DNA methylation changes could be attributable to epigenetic gametic inheritance as well. Prenatal stress during the early gestation stage leads to dysmasculinized male offspring with altered stress responsiveness (174, 175). This effect was transmitted to the F2 male generation and was associated with reduction in miRNA levels in the brains of male neonates (176). These miRNAs were sensitive to testosterone levels and may play a role in organizing the brain's sexual dimorphism.

While these studies support the robust transmission of behavioral experience through the germline, different effects were obtained in a model of adult exposure to stress. Dietz et al. (177) first showed the transmission of dramatic depression- and anxiety-like behavioral abnormalities induced in adult male mice by chronic social defeat stress to both their male and female offspring. Abnormalities in peripheral biomarkers of chronic stress (plasma levels of BDNF, VEGF, and corticosterone) were also transmitted to the offspring. However, the vast majority of these abnormalities were not observed in offspring of socially-defeated males generated by *in vitro* fertilization; only subtle behavioral effects were apparent. These data suggest that the primary mode of transmission of the effects of chronic stress may not be epigenetic, but behavioral, perhaps through female mice adjusting their reproductive investment depending on interactions with an impaired male, a known phenomenon (178). On the other hand, it is possible that the process of in vitro fertilization corrupted any epigenetic marks in the sperm of defeated males. The discrepancies between this study of adult mice, and those involving early life stress, raise the possibility that epigenetic mechanisms may be more important when they occur during development.

There are several reports of the trans-generational epigenetic transmission of other behavioral experiences, such as diet, drugs of abuse, and chemical toxins. Exposure to cocaine in adult male mice mated with naïve females resulted in offspring that displayed deficits in sustained attention and spatial working memory performance (179). This is associated with a decrease in DNMT1 and an increase in DNMT3 levels in spermatozoa, which might cause alterations in the DNA methylation of certain genes. Maternal high-fat diet increased body size and reduced insulin sensitivity in offspring that persisted across multiple generations (180). At the third generation, this phenotype was only transmitted to female offspring through the paternal lineage, suggesting a stable germline-based trans-generational mode of inheritance (181). This effect might be the consequence of the regulation of paternally imprinted genes that influence developmental changes in growth and body size and result in paternal transmission specifically to daughters (181). Similarly, exposure to endocrine disruptors (vinclozolin) affects DNA methylation at imprinted genes in the male germline, leading to behavioral and physiological abnormalities that are trans-generationally persistent (182–184).

What are the most plausible mechanisms for such epigenetic heritability? Epigenetic marks such as DNA methylation and histone modifications are extensively erased in primordial germ cells during gametogenesis in mice (E10.5 – E13.5) and immediately after fertilization in early embryogenesis to ensure the totipotency of cells in the early embryo. This epigenetic reprogramming limits the potential in mammals for epigenetic trans-generational inheritance. However, there are a few examples in mammals of epigenetic marks that are resistant to both phases of reprogramming: transgenes, retrotransposons, and heterochromatin at centromeres (172, 178, 185–187). During spermatogenesis, while most of the histones are replaced by protamines, some histones are retained in a locus-specific manner and could play a role in early embryo development (188). If retained after fertilization, these epigenetic modifications could participate in the trans-generational transfer of information. Another possible mechanism of trans-generational phenotypic persistence is through cytoplasmic RNAs, including mRNA, siRNA, piRNA, and miRNA (189). Indeed, RNAs from gametes allow the newly fertilized oocyte to initiate transcription and have been observed in large quantity in human sperm (190).

Only maternal-derived imprinted genes are protected from demethylation after fertilization (191). Therefore, altered DNA methylation of these imprinted genes could not persist trans-generationally unless new methylation sites are induced during critical developmental periods by environmental factors. Genomic imprinting is a form of epigenetic regulation that distinguishes the maternal and paternal genomes, leading to a bias in expression between maternally and paternally transmitted genes. Imprinted genes are silenced through DNA methylation and related chromatin modifications established in the germline (192). Proper expression of imprinted genes is essential for embryonic development (193) and numerous postnatal functions (46, 194–196). It is thus not surprising that genetic abnormalities involving imprinted genes cause developmental disorders such as Prader-Willi and Angelman's syndromes. Besides gene imprinting and X-inactivation, whereby DNA methylation coordinates the random silencing of either X chromosome in females, there is growing evidence that allele-specific methylation occurs across the entire genome and is heterogeneous across tissues and individuals (197, 198). Most of this allele-specific methylation is determined by DNA sequence, hence showing Mendelian inheritance patterns. There is also allele-specific methylation occurring through non-cis events, which might contribute to heritability of complex diseases (199). Indeed, a recent genome-wide screen indicated that over 1,300 loci show parental-origin allelic expression bias with complex regulation throughout brain development in a cell-type and gender-specific manner (200, 201). Moreover, DNA methylation at some non-imprinted genes escapes the post-fertilization DNA methylation reprogramming (202). Therefore, allele-specific methylation could contribute significantly to the heritability of complex etiological disorders, including depression and other stress-related disorders, and explain the significant discordance of psychiatric disorders between monozygotic twins, the difference in gender susceptibility to certain psychiatric diseases, and the largely inconsistent genetic association studies of depression (50, 51).

This discussion highlights the difficulties in defining epigenetic inheritance of stressful experiences. First, it is very challenging to fully control for parental behavior. As noted, maternal behavior may be affected by the behavior of the impregnating male. As well, while cross-fostering can control for post-natal maternal care, it does not control for intra-uterine factors which might also be crucial. Likewise, *in vitro* fertilization is not a perfect test for germline mechanisms, since the epigenetic state of sperm may vary as a function of how and when it is harvested with respect to the stress exposure. There may also be other factors in semen that influence the mother or offspring. Ultimately, to establish epigenetic mechanisms for the trans-generational transmission of stressful experiences, it will be essential to demonstrate epigenetic modifications at specific genes in sperm or egg cells and understand

how those are transmitted into alterations in neural function in adult offspring across several generations. Currently, there are no known mechanisms by which such transmission can occur, although this is a challenge for future research.

Conclusions

Epigenetic investigations promise to improve our understanding of the mechanisms by which individuals exhibit widely varying responses to adverse life events, both during development and in adulthood. Animal models of stress-related disorders are beginning to reveal specific chromatin modifications that maintain stable patterns of gene expression and thereby mediate the aberrant neuroplasticity associated with these disorders. Equivalent studies have focused on chromatin modifications that contribute to antidepressant responses. Several different types of epigenetic mechanisms have been implicated to date, including stress- or antidepressant-induced changes in DNA methylation, histone acetylation and methylation, and miRNAs. However, the modifications examined to date represent the tip of the iceberg of the diverse and complex range of epigenetic mechanisms that are likely involved in stress and antidepressant action.

Epigenetic mechanisms are regulated throughout life in neurons with some stress-sensitive time windows, such as early life, being particularly important. Although several specific genes have been identified as being permanently regulated (e.g., GR in hippocampus, CRF and AVP in PVN), many other genes probably play a role in stress vulnerability as well. Moreover, in addition to sustained alterations in steady-state mRNA levels of certain genes, it is likely that early life stress also modifies the transcriptional potential of many additional genes through their epigenetic priming or desensitization. Identifying all of the genes that are epigenetically regulated by stress, and important for controlling stress vulnerability across the life cycle, will therefore require the superimposing of several levels of analysis, involving genome-wide analyses of RNA expression along with those of DNA methylation, histone modifications, chromatin remodeling factors, and transcription factors, among many others. Such work will also require the optimization of bioinformatic tools that enable the examination of the vast datasets involved.

This endeavor is further complicated by the large number of brain regions involved in stress-related psychopathology. Most studies to date have focused by necessity (e.g., cost) on single brain regions, despite the knowledge that depression and anxiety disorders involve abnormal functioning of numerous brain circuits. Not only will each brain region have to be investigated independently, but several distinct neuronal and non-neuronal cell types within each region will have to be characterized separately. For example, epigenetic modifications that occur in glutmatergic pyramidal neurons in prefrontal cortex are likely very different from those that occur in several different subtypes of GABAergic interneurons, astrocytes, oligodendrocytes, and so on. Fortunately, tools are emerging that permit the cell type-specific investigation of chromatin modifications in a heterogeneous tissue such as brain (203).

To fully understand epigenetic mechanisms of stress and antidepressant action, the field must transition increasingly to genome-wide approaches, most particularly those utilizing massive parallel sequencing following ChIP (ChIP-seq), RNA isolation (RNA-seq), or selective DNA isolation (e.g., for methylcytosine). These recently developed methodologies provide whole-genome coverage as well as improved sensitivity compared to older ChIP-chip and other microarray approaches. As noted above, the combination of numerous chromatin modifications (204) will allow the delineation of the stress and antidepressant "epigenomes." As our knowledge advances, other types of chromatin modifications and epigenetic regulation that are sensitive to the environment will be indentified, such as

alterations in retrotransposons (205–207). Similar to cocaine exposure (208), stress exposure might increase genomic insertion of retrotransposable elements that regulate the transcriptome (209). A newly recognized form of DNA methylation, 5hmC, is highly abundant in neurons and predominantly localized around the coding region in association with other histone repressive marks, which suggests that it might participate in the epigenetic control of neuronal function (70, 210). These are just some examples of the new lines of investigation that have the potential of revealing fundamentally novel mechanisms controlling stress-related phenomena.

Finally, there has been interest, as noted in this review, in the possible therapeutic applications of drugs aimed at chromatin modifying enzymes (211). HDAC inhibitors exert potent antidepressant-like responses in diverse animal models, as just one example. Whether such drugs offer realistic possibilities for drug discovery remains uncertain, given that most chromatin modifying proteins are broadly expressed throughout the brain and peripheral tissues. Nevertheless, there are many hundreds of known chromatin regulatory proteins; targeting those proteins that are enriched in brain is a viable path forward. Moreover, there is no question that epigenetic characterization of stress models will reveal a far more complete view of the range of proteins and ncRNAs that mediate stress-induced pathology, the reversal of that pathology during antidepressant treatment, and the resistance against developing such pathology in resilient individuals. This knowledge will provide for the first time a comprehensive roadmap for future drug discovery efforts.

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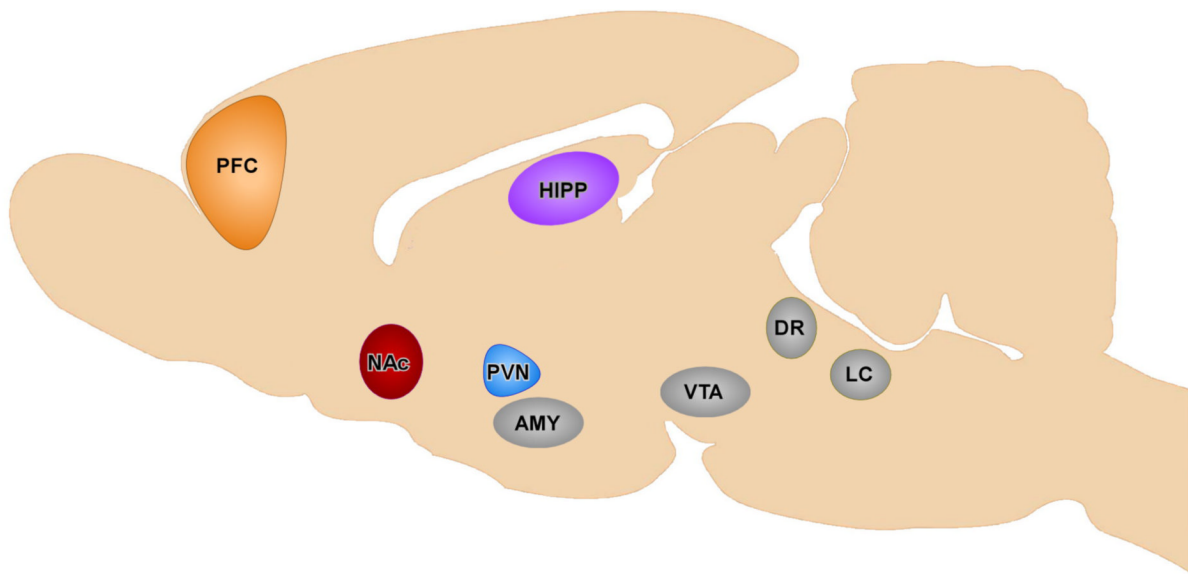
Gene		Region	MeDNA	Associated Mechanisms	Behavioral Paradigm
BDNF	↓	Hipp	↑		Predator stress[105]
BDNF	↓	PFC	↑		Early life stress[146]
CRF	↓ ↑	PVN	↑ ↓		Chronic mild stress (Females)[103] Social defeat (Reversed by Imipramine)[31]
P11	↓ ↑	PFC	↑ ↓		FLS depression model[110] FLS with escitalopram[110]
GDNF	↓ ↑	NAc	↑	↑ MeCP2/HDAC2 ↑ MeCP2/CREB	Social defeat - Sus & Res[108] Social defeat - Sus only[108] Social defeat - Res only[108]
AVP	↑	PVN		↓ MeCP2	Maternal separation[144]
GR	↑	Hipp	↓		High LG (maternal attention)[151]

Figure 1. Effect of stress on DNA methylation and gene expression
Cartoon of sagittal section of rodent brain depicts several inter-connected regions that have been implicated in stress responses (top). Table (bottom) lists the small number of specific genes whose methylation has been shown to date to be altered by stress, the brain regions in which this regulation occurs, and the type of stress involved. Transcriptional mechanisms associated with such altered DNA methylation are listed under Associated Mechanisms. Arrows indicate increase (red) or decrease (green) in gene expression (left) or methylation (right). References are in superscript. Hipp, hippocampus; PFC, prefrontal cortex; PVN, paraventricular nucleus of hypothalamus; NAc, nucleus accumbens; AMY, amygdala; VTA,

ventral tegmental area; DR, dorsal raphe; LC, locus coeruleus; Sus, susceptible; Res, resilient.
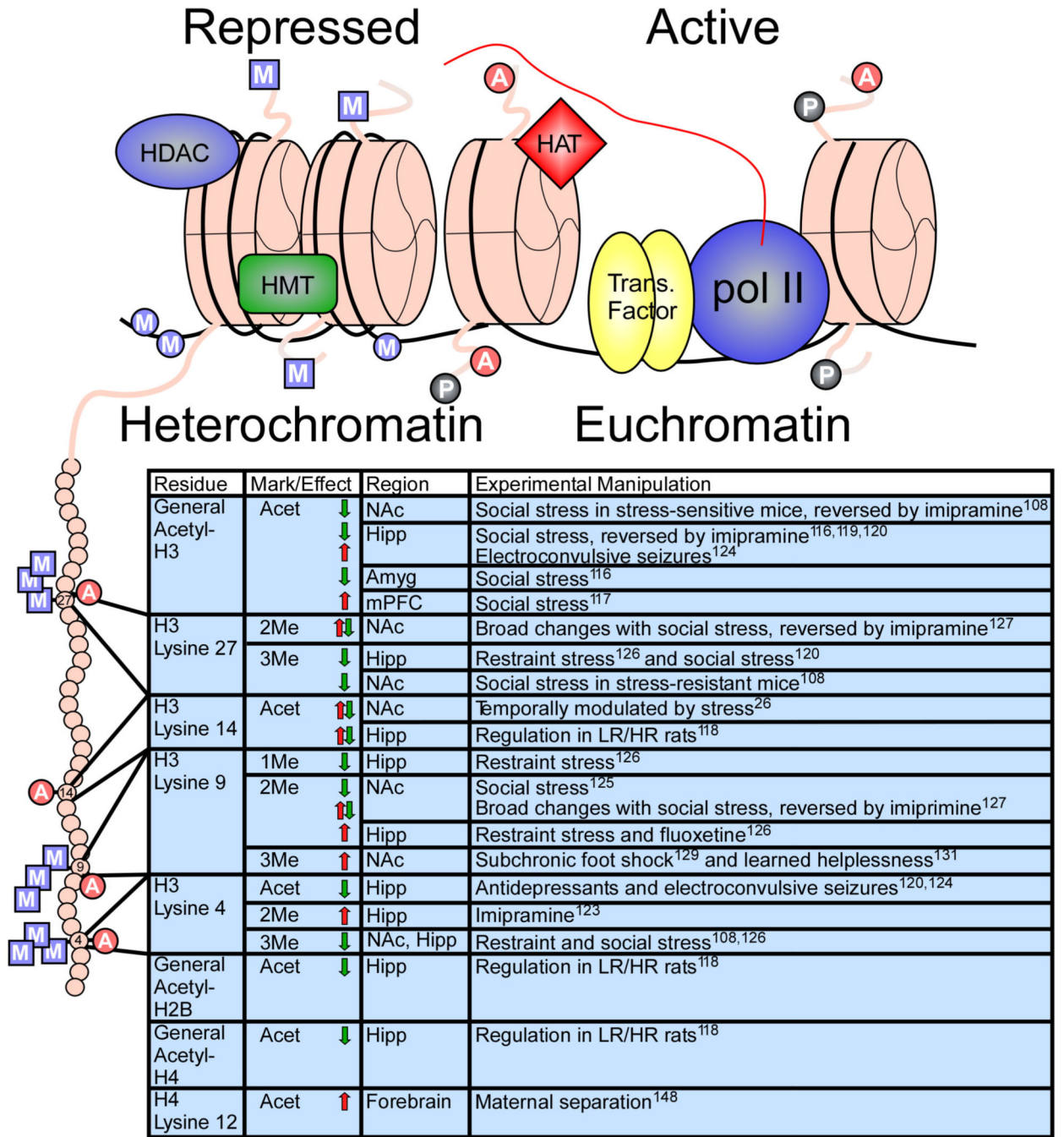
Residue	Mark/Effect		Region	Experimental Manipulation
General Acetyl-H3	Acet	↓	NAc	Social stress in stress-sensitive mice, reversed by imipramine[108]
		↓	Hipp	Social stress, reversed by imipramine[116,119,120]
		↑		Electroconvulsive seizures[124]
		↓	Amyg	Social stress[116]
		↑	mPFC	Social stress[117]
H3 Lysine 27	2Me	↑↓	NAc	Broad changes with social stress, reversed by imipramine[127]
	3Me	↓	Hipp	Restraint stress[126] and social stress[120]
		↓	NAc	Social stress in stress-resistant mice[108]
H3 Lysine 14	Acet	↑↓	NAc	Temporally modulated by stress[26]
		↑↓	Hipp	Regulation in LR/HR rats[118]
H3 Lysine 9	1Me	↓	Hipp	Restraint stress[126]
	2Me	↓	NAc	Social stress[125]
		↑↓		Broad changes with social stress, reversed by imiprimine[127]
		↑	Hipp	Restraint stress and fluoxetine[126]
	3Me	↑	NAc	Subchronic foot shock[129] and learned helplessness[131]
H3 Lysine 4	Acet	↓	Hipp	Antidepressants and electroconvulsive seizures[120,124]
	2Me	↑	Hipp	Imipramine[123]
	3Me	↓	NAc, Hipp	Restraint and social stress[108,126]
General Acetyl-H2B	Acet	↓	Hipp	Regulation in LR/HR rats[118]
General Acetyl-H4	Acet	↓	Hipp	Regulation in LR/HR rats[118]
H4 Lysine 12	Acet	↑	Forebrain	Maternal separation[148]

Figure 2. Chromatin modifications regulated by stress or antidepressant treatment
Illustration (top) indicates histone octomers (pink) in heterochromatin (left) and euchromatin (right), along with associated proteins and histone tail/DNA modifications. A, acetylation; P, phosphorylation; square M, histone methylation; circle M, DNA methylation. Table (bottom) lists histone tail modifications of specific residues—depicted on the expanded histone tail illustration (left)—that are regulated by various stress paradigms or antidepressant treatments within the indicated brain regions. Arrows indicate an increase (red) or decrease (green) in specific modifications. References are in superscript. HDAC, histone deacetylase; HMT, histone methyltransferase; HAT, histone acetyltransferase; Pol II, RNA polymerase II; Trans. Factor, transcription factor.
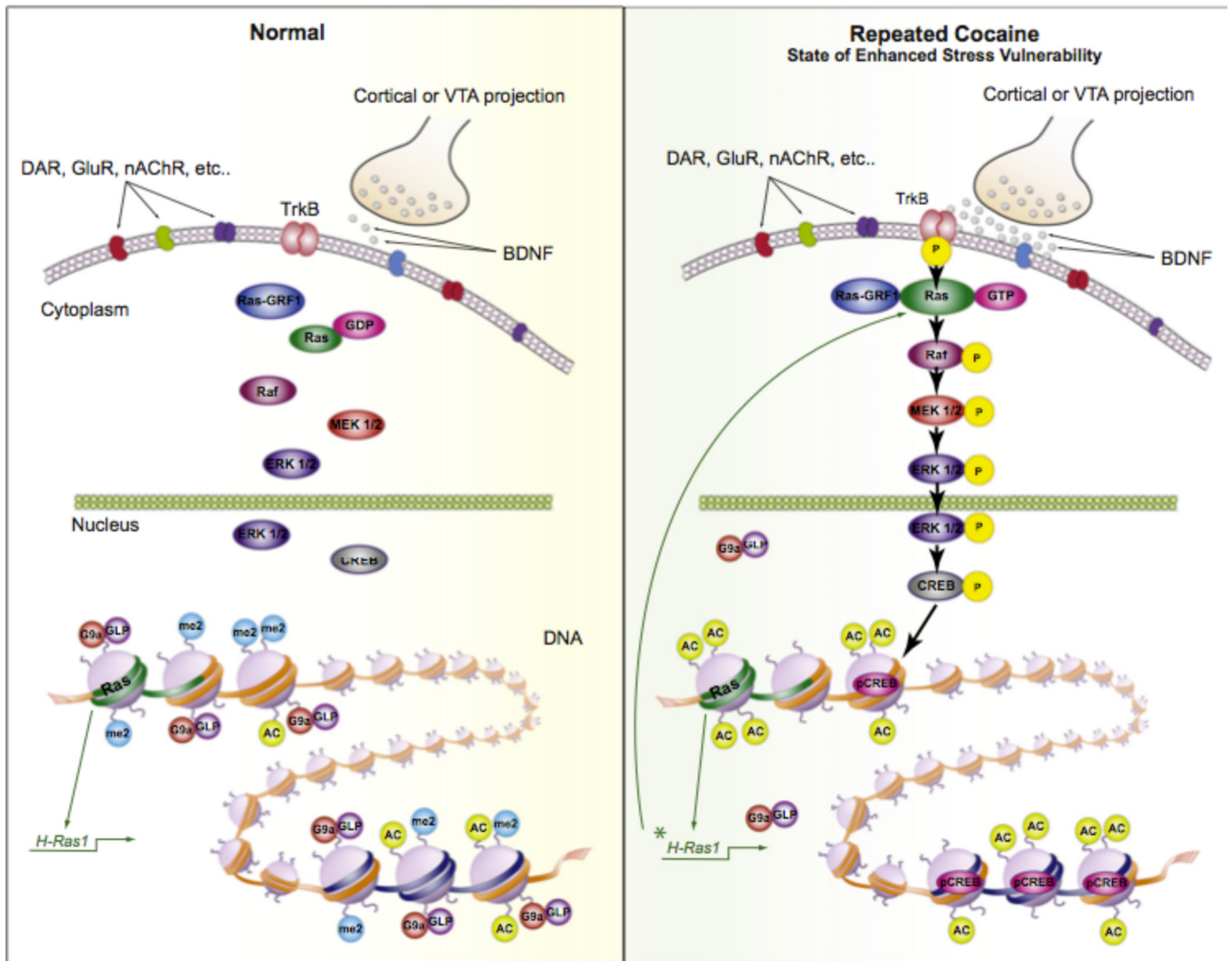
Figure 3. Enhanced Vulnerability to stress via cocaine-Induced priming of BDNF signaling in NAc

Repeated cocaine increases vulnerability to the depressive-like effects of social defeat stress via priming BDNF signaling through Ras induction in NAc. Under control conditions (left view), BDNF activation of TrkB signaling is limited. However, after repeated cocaine (right view), BDNF-TrkB signaling is elevated in NAc, causing enhanced phosphorylation and activity of downstream-signaling mediators including CREB. This cocaine-initiated maladaptive response occurs not only through increased BDNF signaling in NAc but also through increased Ras expression as a result of decreased G9a binding, and decreased H3K9me2, at the H-Ras1 gene promoter. Chronic stress is associated with similar adaptations in this brain region. Ras also appears to be a target for CREB, creating a positive feed-forward loop, promoting CREB activation and Ras expression as well as depressive-like behavior. From 125.
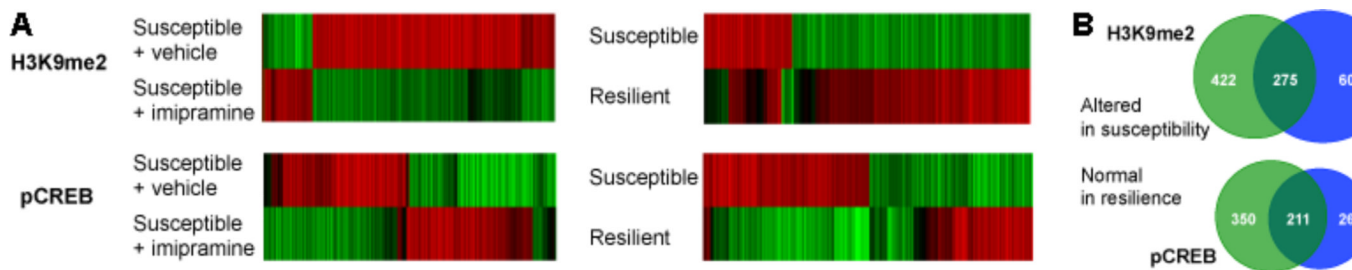
Figure 4. Regulation of H3K9me2 and phospho-CREB (pCREB) binding in NAc after chronic social defeat stress
A. The heatmaps show that, for both marks, the large majority of changes (red, up; green, down) seen in susceptible mice are not seen after chronic imipramine; as well, most changes observed in susceptible mice are not seen in resilient mice and *vice versa*. Note the lower bars in each heatmap are normalized to the upper bars, meaning that green would depict reversal of a red change, not a change in the opposite direction. **B.** The Venn diagrams show the appreciable overlap between apparent mechanisms of imipramine and of resilience. From 127.
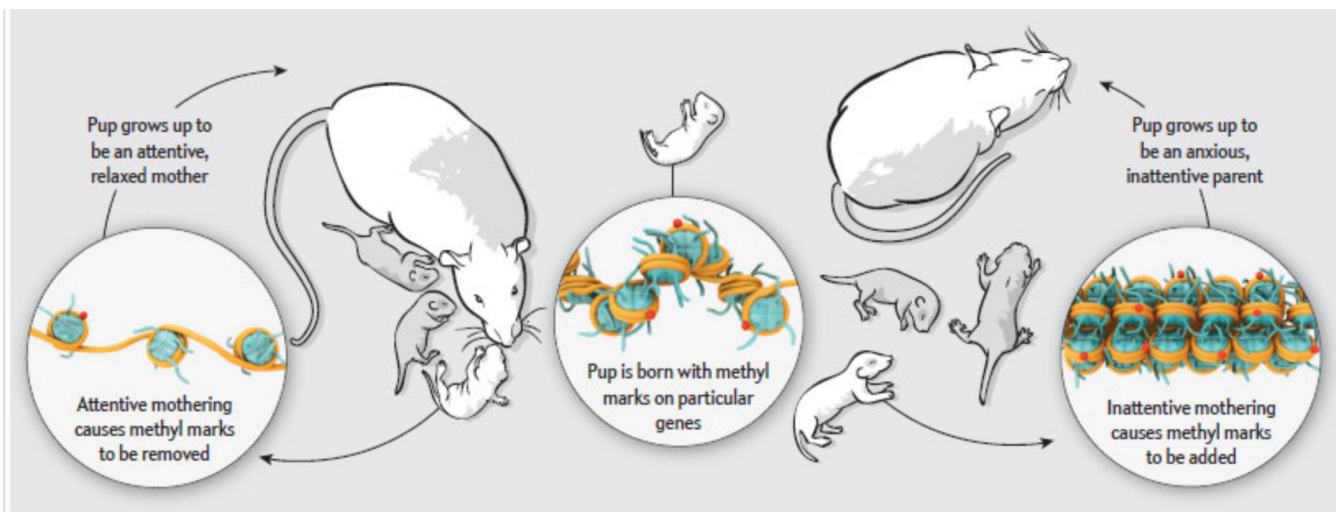
Figure 5. Long-term epigenetic effect of maternal care
Studies in rats have shown that epigenetics can influence maternal behavior and that this effect can be passed from one generation to the next by acting on the pup's brain alone, without altering germ cells. When pups are born, genes involved in regulating the animals' responses to stress are differentially methylated, which enhances sensitivity to stress. If the pups are raised by a mother that displays high levels of grooming behavior, many of these methyl marks are removed, leaving the animals less responsive to stress. When these pups mature, they, too, will be more attentive mothers. If the pups, however, are raised by a mother that displays low levels of grooming, their genes will gain methyl marks. They grow up to be more stress responsive and display lower grooming levels toward their own pups. From 212.
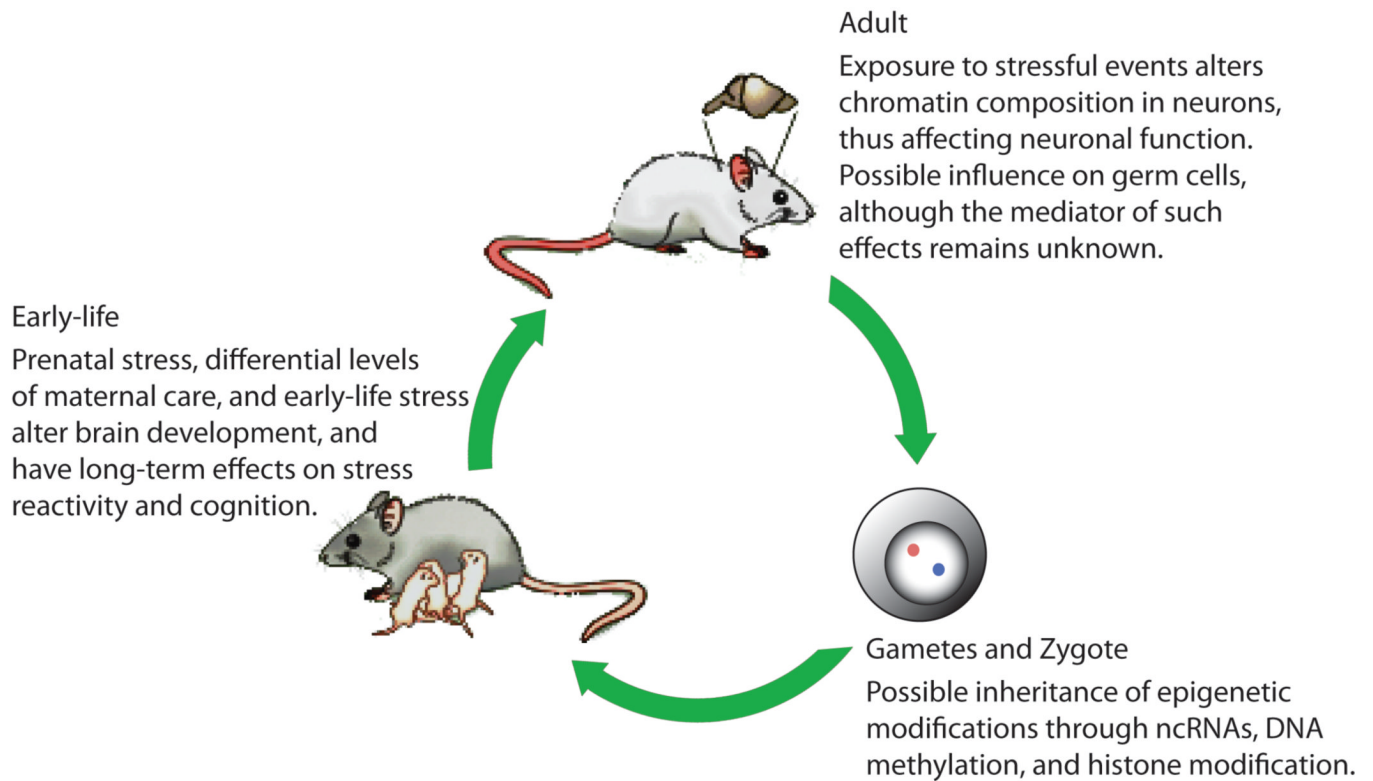
Adult
Exposure to stressful events alters chromatin composition in neurons, thus affecting neuronal function. Possible influence on germ cells, although the mediator of such effects remains unknown.

Early-life
Prenatal stress, differential levels of maternal care, and early-life stress alter brain development, and have long-term effects on stress reactivity and cognition.

Gametes and Zygote
Possible inheritance of epigenetic modifications through ncRNAs, DNA methylation, and histone modification.

Figure 6. Contribution of epigenetic modifications throughout life cycle
Environmental challenges, such as stress, have been associated with several short- and long-term chromatin modifications in the brain affecting neural development and function. Gametes and primordial germ cells are also sensitive to stress and might carry epigenetic transformation through subsequent generations. The development of germ cells is characterized by the genome-wide demethylation of DNA in gametes and the establishment of parental chromatin marks required for genomic imprinting. Following fertilization, there is another genome-wide DNA demethylation phase. However, some epigenetic marks escape such reprogramming (imprinted genes, DNA methylation at specific location, some histone post-translational modifications). Moreover, thousands of non-coding RNAs present in the gametes could transmit their information and potentially affect the cellular phenotype in a non-genomic manner. Modified with permission from 41.