Epigenetic Modifications Mediate Experience-Induced Neuroplasticity; Relevance to the Etiology and Treatment of Posttraumatic Stress Disorder

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Abstract

Covalent chemical modifications, including the acetylation of core histone proteins and methylation of cytosine in or near promoter regions of DNA, influence the efficiency with which genes are transcribed. Chemical modifications that regulate gene expression within postmitotic differentiated neurons can reflect environmental influences, including exposure to stress. These chemical modifications or "marks" may reflect downstream consequences of the transduction of extracellular chemical messengers, such as neurotransmitters, growth factors and hormones, by receptors located at the surface of the neuron or within the cell itself. The ability to decipher the epigenetic code may serve as a record of early childhood adversity that sensitizes to additional epigenetic changes caused by traumatic exposures in adulthood. Further, epigenetic therapeutic interventions may be possible that would attenuate the severity of adverse consequences associated with traumatic exposures in the child and adult. Ideally, targeted epigenetic therapeutic interventions would address stress-induced dysregulation of the hypothalamic-pituitary-adrenal axis and promote expression of therapeutically beneficial neuroplasticity factors.

Introduction

Ultimately, receptor-mediated, activity-dependent signaling pathways that are initiated at the cell surface influence gene expression within the nucleus. Within post-mitotic, terminally differentiated neurons, enduring experience-dependent changes in gene expression underlie changes in synaptic strength within specific circuits, contributing to the plasticity that is necessary for learning and memory, stress responsivity, and substance abuse and psychiatric disorders (Deutsch et al., 2008b; Malan-Muller et al., 2013; Maze et al., 2013; Sun et al., 2013; Sweatt, 2013; Zhang et al., 2013; Zovkic & Sweatt, 2013). Chromatin remodeling affecting the compaction of chromatin and, thereby, the accessibility of transcriptional complexes to

promoter regions of genes, which influences the efficiency of transcription, has emerged as an important mechanism of experience-dependent neuroplasticity. Importantly, the epigenetically determined enduring changes in gene expression do not result from changes or mutations in the sequence of nucleic acids in DNA, rather they often result from covalent modifications of histone proteins (e.g., changes in the acetylation and methylation states of lysine residues) and cytosine residues within the DNA molecule (e.g., methylation or hydroxymethylation), leading to activating or repressive effects on gene expression. With respect to the brain, the definition of epigenetics has broadened to include "chromatin-regulating molecular mechanisms in nondividing neuronal cells" (Zovkic & Sweatt, 2013). Chromatin is the structure that enables approximately 2 meters of double-stranded DNA to be compacted into a nucleus, whose microscopic diameter can be approximately 5 µM. The nucleosome is the repeating unit of chromatin and consists of approximately 147 base pairs of superhelical DNA wrapped around an octamer of four highly conserved core histone proteins: designated H2A, H2B, H3 and H4. The nucleosomes are linked to a fifth histone protein (i.e., H1), which is important for chromatin compaction and its higher-order structure (Deutsch et al., 2008b; Malan-Muller et al., 2013; Maze et al., 2013; Sun et al., 2013; Sweatt, 2013; Zhang et al., 2013; Zovkic & Sweatt, 2013).

Chromatin Remodeling Enzymes

Transcriptional efficiency can also be affected by ATP-dependent chromatin remodeling enzymes that affect the higher-order structure of chromatin (Maze et al., 2013). These enzymes are oligomeric proteins with distinct functional domains, including domains that "read" or recognize distinct "markers" or covalent modifications of histone proteins and a domain with ATPase activity that uses ATP, the biological energy currency, to change the structure of chromatin. ATP-dependent chromatin remodeling complexes can participate in transcriptional activation or repression at a given locus, which is determined by their association with effector proteins, including transcription factors.

Histone Modifications

A major mechanism of chromatin remodeling involves covalent modifications of core histone proteins, which occur primarily on highly conserved basic amino acid sequences (especially lysine residues) located at the amino and carboxy terminal tails (Deutsch et al., 2008b; Maze et al., 2013; Sun et al., 2013). These posttranslational modifications include acetylation,

methylation, phosphorylation, and ubiquitination, among other types of covalently attached groups. In general, the acetylation of lysine residues in the N-terminal tail region by histone acetyltransferases (HATs), which comprise a large class of "writers," results in gene activation; the specific acetyl-lysine modifications are referred to as bromodomains and recognized by specific "readers." Histone acetylation results in a reduction or neutralization of the positive charge of core histone proteins that relaxes the electrostatic affinity between the positively-charged histones and negatively charged DNA. Acetylation is the most studied posttranslational covalent modification of histone proteins.

Because of the strength of carbon-carbon bonds, the turnover kinetics of histone methylation is slow and these "marks" can assume multiple valence states (i.e., mono, di, and trimethylated forms) that can serve as the basis of lifelong "epigenetic memory;" methylation occurs at lysine and arginine residues. However, contrary to the former belief that histone methylation "marks" were stable and lasted indefinitely, it is now known that these marks, including the valence states, can also reflect dynamic interactions between multiple distinct histone methyltransferases (HMTs) and histone demethylases (HDMs). In contrast to acetylation, which is usually associated with promotion of gene expression, histone methylation can be associated with gene activation or repression, which is determined by the specific basic amino acid residues that are methylated, interactions with other histone "marks," and the valence state of the methylated amino acid. Thus, methylation of lysine residues 4 or 36 on H3 are most often associated with the promotion of gene expression, whereas methylation of lysine residues 9 or 27 are commonly associated with gene silencing. Rapid and transient phosphorylation of serine residues in the core H3 histone protein (e.g., the serine 10 residue) has also been shown to result from stimulation of cultured striatal neurons and the hippocampal dentate gyrus by growth factors and neurotransmitters. Interestingly, the affinity of the chaperone "reader" protein 14-3-3 for the phosphorylated H3 histone in the serine 10 position is increased by the presence of an acetyl group on the neighboring lysine in the 14 position, which leads to transcriptional activation. The data support the importance of "readers" that recognize distinct combinations of histone marks (Maze et al., 2013).

The relaxation of compacted chromatin can promote gene expression by enabling the recruitment of the transcriptional machinery (Deutsch et al., 2008b; Maze et al., 2013; Sun et al.,

2013). As mentioned, the histone modifications or "markers" are "read" by specific proteins, which can lead to activation or repression of transcription and gene expression. Importantly, the readers often have multiple binding domains that recognize and bind to different histone marks simultaneously; thus, deciphering the "histone epigenetic code" and understanding the functional output of modified chromatin requires examination of multivalent modifications both within (i.e., intra) and between (i.e., inter) nucleosomes. The histone modifications act in a combinatorial manner to influence gene expression. In addition to their association with transcription complexes, "readers" may have domains or be associated with enzymes that can affect other histone modifications or ATP-dependent chromatin remodeling. As reviewed, chromatin output in post-mitotic, terminally differentiated neurons is a dynamic process that reflects experience-induced changes in histone modifications or "marks." The ability of "readers" to integrate and transduce these "marks" into chromatin output reflects, in part, a dynamic relationship between "writers" (e.g., HATs and HMTs), and "erasers" (e.g., histone deacetylases [HDACs] and HDMs); erasers enzymatically remove specific histone modifications. Ultimately, chromatin output reflects reading of the "histone epigenetic code," whose coordinated components include histone modifications, DNA methylation, noncoding RNAs, and transcription factors.

DNA Methylation

Within intergenic regions, DNA methylation is responsible for the silencing and suppression of expression of transposable and viral elements, which, if expressed, would be harmful (Moore et al., 2013). DNA methylation is also important for the establishment of imprinting, which is a process whereby genes are preferentially expressed from only one of the two parental chromosomes (Moore et al., 2013). Additionally, DNA methylation is a mechanism regulating the differentiation of cells from pluripotent stem cells during development. Finally, DNA methylation represents a mechanism for transducing experiences into long-lasting changes in gene expression, and serves as a major mechanism of epigenetic regulation. Most commonly, DNA methylation in the promoter region of genes enriched in cytosine-guanine (CpG) dinucleotides (so-called CpG islands) leads to transcriptional silencing. About 70% of gene promoters reside within CpG islands and these CpG islands are conserved between mice and humans, consistent with their functional importance. Specifically, DNA methylation involves the catalytic transfer of a methyl group from S-adenosyl methionine (SAM), the universal methyl

donor, to the carbon-5 position on cytosine. Importantly, the epigenetic marks of 5-methylcytosine and 5-hydroxymethylcytosine cannot be differentiated by the current conventional high-throughput methods for mapping patterns of DNA methylation (i.e., bisulfite sequencing and the use of methylation-sensitive restriction enzymes). 5-Hydroxymethylcytosine influences gene expression, similar to 5-methylcytosine, and is also an intermediate in a pathway that leads to DNA demethylation.

The DNA methyltransferases (DNMTs) that are involved in maintenance and perpetuation of patterns of DNA methylation constitute one of the two broad families of DNMTs (Moore et al., 2013). This group of enzymes includes DNMT1, which recognizes the methylated CpG dinucleotide on the hemi-methylated strand and converts unmethylated CpG on its complementary strand into a methylated CpG. The other broad family of DNMTs is the de novo DNMTs, including DNMT3a and DNMT3b, which methylate previously unmethylated cytosines in CpG dinucleotides. In general, the de novo DNMTs create a new methylation pattern of unmodified or naked DNA, whereas DNMT1 functions during DNA replication to assure that the pattern of DNA methylation is preserved in daughter strands. With respect to de novo DNA methylation, transcription factors are thought to play a regulatory role in determining which regions of DNA are to be methylated. Before or after binding to specific DNA sequences, transcription factors may act by recruiting DNMTs or, alternatively, their binding may protect CpG sites from de novo methylation. In addition to its activity during DNA replication, DNMT1 also "repairs" DNA methylation preserving and maintaining a differentiated cell's pattern of DNA methylation. Recent research on disorders such as schizophrenia and hereditary sensory and autonomic neuropathy type 1 (HSAN1) support an important epigenetic role of DNMT1 in post-mitotic neurons in the adult brain. In fact, because no dramatic changes in DNA methylation patterns in forebrain postmitotic neurons are observed in transgenic knockout mice with absent expression of DNMT1 or DNMT3a, it is thought that these two DNMTs may have overlapping roles in these postmitotic neurons (Moore et al., 2013).

DNA demethylation is an active metabolic process whereby a series of sequential chemical reactions leads to deamination and oxidation of 5-methyl-cytosine to a product that is a substrate for the "base excision repair (BER)" pathway; there is no known enzymatic mechanism for cleaving the strong covalent carbon-carbon bond between cytosine and its methyl group in

the 5-position (Moore et al., 2013). The ultimate product of the processing of 5-methyl-cytosine results in a base-pair mismatch that is subject to BER. As noted, 5-hydroxymethyl-cytosine is an intermediate in one of the DNA demethylation pathways that may also have a role in the epigenetic regulation of gene expression. For example, 5-hydroxymethyl-cytosine interferes with the binding of MeCP2 to methylated DNA; MeCP2 is a methyl-CpG-binding protein that contributes to gene repression. The family of methyl-CpG binding proteins are more highly expressed in brain than other tissues, and may possess a "transcriptional repression domain" that binds to repressor complexes (Moore et al., 2013). In addition to active gene repression, MeCP2 has a probable role in the maintenance of DNA methylation patterns because it binds to DNMT1. MeCP2 is also a substrate for posttranslational phosphorylation induced by neuronal activity, which would release it from promoter regions, making these sequences accessible to active DNA demethylation and, thereby, promoting gene expression. That MeCP2 is a critical methyl-CpG-binding protein is supported by the fact that its mutation results in Rett Syndrome, a neurodevelopmental disorder.

Epigenetic Changes Mediate Contextual Fear Conditioning

Contextual fear conditioning in rodents is a form of hippocampal-dependent associative learning in which an aversive unconditioned stimulus, such as foot shock, becomes associated with the context or place (i.e., conditioned stimulus) in which the aversive stimulus was delivered (Zovkic & Sweatt, 2013). Once the long-term association memories in the fear conditioning paradigm become consolidated, freezing behavior, the unconditioned response to the aversive foot shock, is observed or elicited whenever the animals are placed in the context where the foot shock was originally delivered. (i.e., conditioned response). A variety of epigenetic changes have been described in the hippocampus as a result of this fear conditioning procedure. Some of the observed changes in hippocampus are time-dependent, including reversion to baseline levels within 24 hours, reflecting, perhaps, the processes of memory consolidation in hippocampus and downloading of these memories in the cortex for long-term storage and maintenance. The changes in hippocampus associated with contextual fear conditioning included acetylation of H3 histone protein in the CA1 region; moreover, treatment of adult animals with histone deacetylase (HDAC) inhibitors and, thereby, increasing the acetylation status of H3 led to enhanced memory formation. Complementary changes were also observed in DNA methylation at specific gene loci. For example, contextual fear conditioning was associated with an

upregulation in the hippocampal expression of DNMT 3a and 3b, which led to methylation and transcriptional silencing of a memory suppressor gene (i.e., *protein phosphatase 1*). There was also DNA demethylation and transcriptional activation of the genes coding for reelin and brain-derived neurotrophic factor (BDNF), which are two proteins important for experience-induced neuroplasticity. Interestingly, contextual fear conditioning was associated with increased H3 histone acetylation at BDNF promoter regions, supporting the coordinated activity of DNA methylation and histone acetylation in the regulation of BDNF expression. The changes in levels of DNA methylation for *protein phosphatase 1* and *reelin* in hippocampus returned to baseline within 24 hours (Zovkic & Sweatt, 2013). An inability to extinguish learned fear responses is a major etiological theory of PTSD. The persistence of learned fear responses may ultimately reflect enduring epigenetic changes due to covalent modifications of histone tails and DNA.

Environmental-Induced Epigenetic Modifications Mediate Hypothalamic-Pituitary-Adrenal Axis Dysregulation in Response to Childhood Adversity and Adult Trauma Exposures

A major role of the hypothalamic-pituitary-adrenal (HPA) axis is the maintenance of "allostasis," which is the maintenance of stability in spite of stressful or unanticipated environment exigencies (McGowan, 2013). Childhood adversity can impact the ability of the HPA axis to maintain allostasis and is a major risk factor for posttraumatic stress disorder (PTSD). Current research on the epigenetic consequences of early life adversity focuses on identifying labile areas of the genome that are sensitive to modifications by environmental factors and the ontological time course of epigenetic changes (i.e., when during development are epigenetic changes most likely to occur in response to specific types of adversity). Animal models of early life adversity have clearly shown that the quality of maternal care influences offspring response to novel and stressful environments, which are mediated, at least in part, by epigenetic changes in the HPA circuitry of the offspring. Thus, the quality of maternal care in rats influences lifelong changes in the DNA methylation pattern in hippocampus of the untranslated 1_7 splice variant of the glucocorticoid receptor (GR) promoter, as well as the acetylation status of the lysine 9 residue of the H3 histone protein in offspring (Daskalakis et al., 2013; Malan-Muller et al., 2013; McGowan, 2013; Sweatt, 2013; Yehuda & LeDoux, 2007; Yehuda & Bierer, 2009; Yehuda et al., 2013). The quality of maternal care has also been shown to affect the epigenetic regulation of a variety of regions in the offspring's brains, including the GAD_{67} gene involved in synthesis of GABA, the major inhibitory neurotransmitter, in prefrontal cortex, the gene for arginine

vasopressin in hypothalamus, and the gene for BDNF in prefrontal cortex and hippocampus, among other genes and regions. Translational studies in postmortem human brain have shown that a history of child abuse affects the extent of DNA methylation of the exon 1F promoter region of the gene for the GR in the hippocampus of suicide victims; thus, suicide victims and controls without histories of abuse or severe neglect in childhood had lower levels of DNA methylation of this promoter region, than suicide victims with childhood histories of abuse and neglect.

HPA axis dysregulation, increased glucocorticoid sensitivity, and hypocortisolemia can occur in patients with PTSD, which could reflect epigenetic modifications of the cytosine methylation status in promoter regions of the genes for both the GR and the FK506 binding protein (FKBP5), the latter protein regulates the efficiency of intracellular signaling mediated by the GR (Yehuda et al., 2013). There are also emerging data that the expression of these and, perhaps, other glucocorticoid related genes can be environmentally regulated throughout life. In fact, preliminary data suggest that Prolonged Exposure (PE) therapy, an evidence-based cognitive-behavioral intervention for PTSD, can regulate the epigenetic state of FKBP5, which is associated with a positive therapeutic response to treatment (Yehuda et al., 2013). As discussed, in a rat model, differences in maternal grooming of pups regulated the cytosine methylation status in hippocampus of the exon 1_7 promoter of the GR gene. Translational studies in the human confirmed that childhood adversity is associated with increased methylation of the human ortholog of the rat exon 1_7 promoter of the GR gene (i.e., the exon 1F promoter), resulting in lower expression of GR in hippocampus and a dysregulated HPA axis response to stress. These data are consistent with a literature on premorbid risk factors for PTSD. Altered levels of expression of FKBP5 appear to act as an intracellular feedback loop regulating the signaling consequences of GR activation; FKBP5 is a co-chaperone of the GR that decreases the binding of ligands to the cytosolic GR and impairs translocation of the ligand-bound GR to the nucleus from the cytoplasm. Thus, increased expression of FKBP5 would dampen activation of the GR. Ultimately, the methylation state of *FKBP51*, the gene coding FKBP5, reflects an interaction between genetic variations in the sequence of its nucleotides (i.e., polymorphisms) and childhood adversity, and the interaction of genetic variants of *FKBP51* and childhood adversity contribute to risk for major depression, suicide attempts and PTSD (Yehuda et al., 2013). Importantly, prior sensitization to stress (e.g., due to childhood adversity) can increase the risk

for PTSD after exposures to trauma as an adult. A compelling hypothesis is that epigenetic modifications of glucocorticoid related genes are likely to be responsible for both risk and perpetuation of PTSD, which is associated with low glucocorticoid levels and increased GR sensitivity.

In a small preliminary study, the effects of PE therapy on the methylation state of the promoters of the genes for GR and FKBP5 in peripheral blood mononuclear cells was studied in 16 combat veterans, eight of whom responded and no longer met diagnostic criteria for PTSD after 12 weeks of treatment (Yehuda et al., 2013). Importantly, the methylation status of the exon 1F promoter of the human gene for the GR, the homolog of the exon 1_7 promoter region of the rat gene, is influenced by a childhood history of trauma. Responders to PE therapy showed a greater number of methylated CpG sites in the GR exon 1F promoter at baseline prior to treatment; however, the extent of methylation did not change after treatment or three-month follow-up (Yehuda et al., 2013). Thus, increased methylation of the exon 1F promoter may be a predictor of a good response to PE therapy. With respect to the promoter region of the FKBP5 gene, the data suggest that the number of methylated sites decreases in treatment-responders. Thus, in patients with PTSD, who have increased sensitivity to glucocorticoids, decreased DNA methylation and up-regulated expression of FKBP5 may be a desired therapeutic goal. The upregulated expression of FKBP5 would lead to diminished glucocorticoid activation as a result of diminished GR sensitivity.

In summary, the data suggest that enduring positive effects of “psychotherapeutic” interventions may be mediated by epigenetic modifications of genes implicated in stress responsivity, such as changes in the methylation status of the promoter region of the gene for FKBP5. Conceivably, beneficial effects of nonpharmacological, psychotherapeutic interventions for PTSD and other psychiatric disorders may also be associated with changes in expression of genes implicated in neuroplasticity, such as the genes coding for reelin and BDNF. The pattern of DNA methylation of the promoter region of the GR gene may inform a history of altered stress sensitivity to traumatic exposures, especially exposures in childhood, and also be predictive of a positive response to certain therapeutic interventions. Recent work also supports the potential benefits of strategically timed pharmacological interventions to alter stress-induced changes in the epigenetic landscape (Deutsch et al., 2008a,b). For example,

treating mice with sodium butyrate, a HDAC inhibitor, around the time of their exposure to a profound stressor (i.e., forced swimming in cold water) attenuated the severity of the stressed-induced reduction of dizocilpine's antiseizure efficacy tested 24 hours after the stressful exposure; dizocilpine is a noncompetitive NMDA receptor antagonist. The dose of sodium butyrate was known to increase the acetylation status of histones in the hippocampus. These data support exploration of epigenetic interventions to address stress-induced alterations of glutamatergic neurotransmission (Deutsch et al., 2008a).

Conclusions

The data are converging to show that environmental influences, especially stressful ones, can affect expression of genes in specific brain regions; epigenetic modifications, which are covalent modifications of histone tails and DNA itself near transcription start sites, can result in enduring, even lifelong, modifications of genes mediating the stress response and neuroplasticity. The characterization of these epigenetic modifications will surely contribute to our understanding of the environmental contributions to the etiology of psychiatric disorders, especially PTSD, as well as present new avenues of possible therapeutic interventions. Epigenetic therapeutic interventions are already being explored in cancer, and there may be some translational opportunities to test them in psychiatric disorders, including PTSD.

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