Epigenetics of Memory Processes

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INTRODUCTION

Sequal studies continue to demonstrate that the histone proteins and DNA that comprise chromatin are targets of neuronal signaling pathways involved in CNS plasticity and memory formation. As it applies to cognition, we define epigenetics as the covalent modification of chromatin that influences activity-dependent changes in gene expression. These changes can be transient, underlying the dynamic regulation of gene activity states, or they can be long-term and responsible for lasting alterations in gene activity states. The combination of dynamic and stable components renders chromatin an ideal substrate for signal integration and storage of cellular information in the CNS. Indeed, studies are being published at a rapid pace demonstrating that epigenetic mechanisms mediate experience-driven changes in the CNS. Concerning the field of learning and memory, there are two basic molecular epigenetic mechanisms that are currently studied - post-translational modifications of histone proteins and direct covalent methylation of cytosines. In the following sections, we will briefly introduce the reader to these mechanisms, and then summarize the current body of literature pertaining to their role in learning and memory.

EPIGENETIC MODIFICATION OF HISTONES UNDERLYING MEMORY

Histones are proteins that organize DNA in the nucleus. There are eight histone proteins (histones 2A, 2B, 3, and 4, with two copies of each molecule) at the heart of the chromatin core DNA either coils or uncoils around this core, a process that is mediated in part by the post-translational modifications of the N-terminal tail of the histone proteins. Modifications of the tails include acetylation, phosphorylation, methylation, ubiquitination, and deubiquitination [1,2]. These modifications, along with DNA methylation, are the principal epigenetic mechanisms that help govern the activity of genes in the CNS.

These modifications have different effects on gene activity. For example, histone acetylation can lead to gene activation, while sumoylation is coupled to gene repression. Histone acetylation on the other hand is a more complex process, and can be associated with either activation or repression dependent upon the particular amino acid residue modified. Histone acetylation has been the most extensively studied epigenetic modification to date in the field of learning and memory, we will briefly review the enzymes catalyzing this reaction and how this reaction promotes gene transcription.
Enzymes known as histone acetyltransferases (HATs) catalyze the direct transfer of an acetyl group from acetyl-CoA to the ε-NH⁺ group of the lysine residues within a histone [3]. The addition of an acetyl group decreases the affinity between the protein tail and DNA, thus relaxing the chromatin structure and providing access for transcriptional machinery. Acetylated histone tails at the same time also provide a substrate for the binding of additional co-activators with domains that recognize acetylated lysines. Thus, histone acetylation is generally associated with transcriptional activation and is widely regarded as one of the epigenetic marks associated with active chromatin, often referred to as euchromatin.

Histone acetylation is also reversible, and the enzymes that catalyze the reversal of histone acetylation are known as histone deacetylases (HDACs). There are a total of eleven classic HDAC isoforms, most of which are expressed in the CNS. HDACs remove the acetyl groups from lysine residues, a reaction that promotes DNA condensation around the histone core. Trichostatin A, sodium butyrate, valproic acid, and suberoylanilide hydroxamic acid (SAHA) are the most widely used HDAC inhibitors, each having varying degrees of selectivity for the classical HDAC isoforms. The use of these inhibitors has been instrumental in helping define a role for histone modifications in adult memory formation. Furthermore, these inhibitors have potential therapeutic value in alleviating cognitive deficits.

Some of the earliest evidence for the role of histone modifications in adult cognition came from studies that investigated the role of a particular HAT, cAMP Response Element Binding Protein (CBP), in long-term memory formation. Long-term memory formation is the stabilization of recently learned information, a process that evokes gene expression and structural synaptic changes in restricted regions of the brain. Mice with a truncated form of CBP were found to have significant deficits in long-term memory following several tasks, including step-through passive avoidance (a paradigm in which animals will learn to avoid the dark box of the apparatus, although they have a natural preference for dark), novel object recognition (a test based on the premise that rodents will explore a novel object more than a familiar one, but only if they remember the familiar one) and cued-fear conditioning (an associative learning paradigm in which an association is made between a neutral stimulus such as an odor and an aversive stimulus such as foot-shock) [4,5]. However, since CREB and CBP govern developmental processes, these animals also had developmental abnormalities. Thus, straightforward interpretation of data pertaining to memory is difficult. Three laboratories later developed CBP-deficient mice that were void of the effects of CBP on development [6–8]. Similar to results from previous studies, acquisition of new information (learning) and short-term memory were spared in these mice, but these mice exhibited significant impairments in novel object recognition, spatial and fear memory, and also had significant deficits in hippocampal long-term potentiation (LTP) [6–8]. In a recent topic, Marcelo Wood’s group has shown that the HDAC inhibitor sodium butyrate can establish and generate more persistent forms of long-term novel object recognition memory in both CBP mutant and wildtype mice [9].

Evidence continues to mount in support of the hypothesis that specific histone modifications are involved in long-term memory formation. In our initial studies, we investigated whether there were hippocampal histone modifications following contextual-fear conditioning in mice. Commonly used to assess hippocampal-dependent learning and memory function, it is a behavioral paradigm in which animals learn to associate a novel context (conditioned stimulus) with a mildly aversive unconditioned stimulus (foot-shock) that naturally elicits a freezing response (unconditioned response). After a few presentations of the two stimuli, animals readily learn this association and freeze in response to presentation of the context in the absence of the foot-shock (conditioned response). Their memory of this association can be assessed by returning the animal to the same context (typically 24 hours after training) and measuring their freezing behavior in the absence of the foot-shock. Freezing behavior in then used as an index to illustrate whether the animals have successfully...
and maintained memory of the association. We observed significant increases in both acetylation and phosphorylation of histone 3 (H3), but not histone 4 (H4), in mice that had learned fear, and found that these chromatin remodeling events were regulated by the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway [10, 11]. Furthermore, HDAC inhibitors (sodium butyrate and trichostatin A) were shown to enhance both LTP and the fear memory itself [11]. These observations were the first to indicate that epigenetic marking of the genome occurs in long-term memory formation, and that manipulation of epigenetic processes is a viable way to alter memory capacity.

In a landmark study in 2007, Li-Huei Tsai’s group showed that the beneficial effects of environmental enrichment on restoring learning and memory in neurodegenerative mice involves increased hippocampal and cortical H3 acetylation [12]. Also in that study, they demonstrated that the use of the HDAC inhibitor sodium butyrate is sufficient to restore learning and memory in these impaired mice. This beneficial effect of various HDAC inhibitors in improving learning and memory in non-diseased rodents and in other models of neurodegeneration and brain injury has continued to be replicated [13–16]. Tsai’s group has now been working to delineate the functions of particular HDACs in learning and memory. In a recent report, they presented data that indicate HDAC2 negatively regulates memory by suppressing the activity of genes that are necessary for synaptic plasticity, such as the brain-derived neurotrophic factor gene (BDNF) and glutamate receptor 1 gene (GLUR1) [17].

Several studies have also implicated the involvement of histone modifications at specific gene loci during fear memory formation. The gene that has undoubtedly received the most attention is BDNF, as it has been established to have an essential role in regulating neuronal structure and neural function, and is critical for the synaptic plasticity underlying long-term memory formation. This gene has also been the focus in several DNA methylation studies. In a recent report, we showed that acetylation of H3 increases at promoter IV of BDNF, and that these modifications parallel changes in expression of BDNF mRNA during the consolidation period (Fig. 23.1) [18]. Other reports also indicate that neural plasticity and long-term memory requires specific histone modifications at BDNF loci. For example, there are increased H3 acetylation levels within several regions of promoter I of BDNF following NMDA treatment in cultures of hippocampal neurons [19]. The extinction of conditioned fear in mice induces H4 acetylation around exon IV in the prefrontal cortex [20]. Additionally, chronic social defeat stress in adult mice produces a lasting down-regulation of hippocampal BDNF transcripts III and IV that are associated with increased histone methylation at the particular promoters [21]. Investigators continue to show that stressors and memories of stressful events evoke similar changes in hippocampal H3 phosphorylation and acetylation [22–25].

Summary, there are now varied but extensive data indicating that an adult animal’s ability to form and consolidate memories depends in part on specific histone modifications in the CNS. Data also highlight the therapeutic value of HDAC inhibitors in restoring memory capacity. Despite this emerging role of histone modifications in learning and memory, several questions remain to be addressed. For example, though the current body of literature suggests that histone acetylation and phosphorylation are the two modifications supporting memory, this may be attributable to the fact that these are the two modifications that have received the most attention. Whether there is a role for histone methylation and sumoylation in memory has been largely unexplored. A further caveat of the data is that the currently available HDAC inhibitors can also alter non-histone substrates [26]. Thus, it is possible that some of the beneficial effects of therapy on memory capacity is through non-histone effects. Thus, to gain a better understanding of the role of histone modifications in memory and how they can mediate memory capacity, it will be crucial to resolve whether histone-modifying enzymes have any direct, gene-mediated functions.


**FIGURE 23.1**

Contextual-fear memory formation elicits hippocampal BDNF transcription that is associated with histone modifications at exon-specific BDNF promoters. (A) Quantitative real-time PCR data indicate that 2 hr following contextual-fear conditioning, there are significant increases in BDNF exons IV and IX mRNA in area CA1 of Context + Foot-shock animals (*p*-value significant versus Naive, Shock, and Context controls). Context exposure alone also elicits an increase in BDNF exon IVA mRNA (*p*-value significant versus Naive controls). (B) At promoter IV of the BDNF gene, there is a significant increase in acetylation of histone 3 (H3) in Context + Foot-shock animals. This increase is blocked if DNA methylation is inhibited by pre-training treatment with zebularine. BDNF = brain-derived neurotrophic factor gene.

**COVALENT MODIFICATION OF DNA UNDERLYING MEMORY**

In addition to histone modifications, DNA methylation appears to have some active role in regulating synaptic plasticity and memory. DNA methylation is a direct chemical modification that adds a -CH$_3$ group through a covalent bond. This modification occurs at cytosine-guanine dinucleotide (CpG) sequences that occur in clusters in and around gene regulatory regions as well as within intragenic regions. DNA methylation is catalyzed by a class of enzymes known as DNA methyltransferases (DNMTs) [27,28]. DNMT1, DNMT3a, and DNMT3b, the de novo DNMTs, methylate previously unmethylated CpG sites in DNA regions which have no methyl-cytosine on either DNA strand. The maintenance DNA
isoform, DNMT1, perpetuates methylation marks after cell division, regenerating the methylcytosine marks on the newly synthesized complementary DNA strand that arises from DNA replication.

DNA methylation is a process that is generally associated with suppression of gene transcription. In essence, methylation of cytosines at CpG dinucleotides recruits methyl-DNA binding proteins and HDACs to specific sites in the genome. This triggers localized regulation of the three-dimensional structure of DNA and its associated histone proteins, resulting in a higher-affinity interaction between DNA and the histone core [27,28]. In all, this ultimately suppresses transcription. However, while DNA methylation is usually associated with transcriptional suppression, it is important to point out that recent studies provide evidence that DNA methylation status alone does not always reflect that a gene is repressed, as MeCP2 can also be associated with active genes [29–31].

The idea of whether there is active DNA demethylation in post-mitotic cells, such as neurons, remains a controversial topic in the field. DNA demethylation has been historically viewed as a passive and largely irreversible process in differentiated cells, whereby multiple rounds of cell division without DNMT-mediated remethylation is necessary to erase epigenetic marks. However, recent evidence argues that there is indeed active methylation and demethylation in mature cells, mediated by either DNMT3a and 3b or Gadd45b through a glycosylase repair mechanism, though it should be noted that Gadd45-mediated demethylation remains controversial [32–37]. Furthermore, an active demethylase that can remove methyl groups has not been identified.

Regardless of the exact mechanism involved in the mature CNS, results continue to highlight the capacity of DNA methylation to regulate synaptic plasticity and memory [18,38–42]. Initial studies in 2003 indicated that DNA methylation might play a key role in activity-dependent neural plasticity, as the methylation status of BDNF was shown to undergo dynamic changes in response to stimulation [43,44]. For example, Martinowich and colleagues demonstrated in neurons that the transcription of BDNF exon IV is suppressed by methyl-CpG binding protein 2 (MeCP2), and upon depolarization, MeCP2 is released along with HDAC1 [44]. Since these studies, 5-azadeoxycytidine (5-aza-C), which disrupts DNA methylation, and the HDAC inhibitor trichostatin A, have been shown to up-regulate specific BDNF transcripts, including exons I and IV [18,45].

Work from our own laboratory provided the first demonstration that the acute application of 5-aza-C or zebularine (a drug that also disrupts DNA methylation) to mouse hippocampal slices not only influences the methylation status of BDNF and RELN DNA, but also blocks long-term potentiation (LTP) [39,40]. The effects of their ability to disrupt synaptic plasticity have since been confirmed and extended by Lisa Monteggia’s group [42]. Using in vivo approaches, these begun to more definitively link DNA methylation with fear memory capacity. Our studies showed that DNMT3a and DNMT3b were up-regulated in the hippocampus following contextual-fear conditioning in adult rats [41]. In those same rats, we observed a decrease in methylation (demethylation) and transcriptional activation of RELN, and an increase in methylation and transcriptional silencing of the memory suppressor gene protein phosphatase 1 (PP1) [41]. Our recent efforts have focused on epigenetic regulation of the BDNF gene during fear memory formation, and have shown that contextual-fear conditioning also evokes BDNF DNA demethylation (Fig. 23.2A) [18]. These modifications are associated with localized histone modifications at specific BDNF promoters and transcription of BDNF transcription (Fig. 23.1) [18]. Furthermore, we have shown that silencing DNA methylation with various agents blocks these epigenetic changes, as well as fear memory (Fig. 23.2B) [18]. As a final point, a recent report has shown that disrupting function of MeCP2 is sufficient to impair the ability to form a fear memory (amygdala-cued-fear conditioning) [38]. All together, the available data indicate that both DNA methylation and demethylation are key components in adult memory formation.
As DNA methylation presents itself as a new mechanism underlying memory, questions remain to be answered to overcome limitations of the current data. For example, the bulk of what we know regarding the role of DNA methylation in synaptic physiology and memory is from pharmacological studies using drugs whose mechanisms we do not fully understand. Because both 5-aza and zebularine are nucleoside analogs that need to be incorporated into DNA to trap DNMT and block DNA methylation, the mechanism by which these drugs are able to alter methylation in post-mitotic neurons, or glia for that matter, is not clear. They may do so by actively demethylating DNA in non-dividing cells through a replication-independent event, such as a DNA repair process. There is only one behavioral study to date that has used a non-nucleoside compound (RG108) to directly inhibit enzyme activity. Results indicated that RG108 had similar effects on memory as that of zebularine and 5-aza-C [18]. We also do not know whether these methylation changes...
Epigenetic changes occurring in the adult hippocampus in response to contextual-fear conditioning

\[\text{Context + Foot-shock} \rightarrow \text{Histone modifications} \rightarrow \text{DNA methylation changes} \rightarrow \text{Activity-dependent gene transcription} \rightarrow \text{HDAC inhibitors} \rightarrow \text{Syntaptic plasticity} \rightarrow \text{DNA methylation inhibitors} \rightarrow \text{Increased freezing behavior (fear)}\]

**FIGURE 23.3**

Overview of the epigenetic changes that we have shown to date are responsible for supporting adult hippocampal plasticity of fear memory formation. Contiguous presentations of Context + Foot-shock elicit several histone modifications and DNA methylation changes at several gene loci. This evokes activity-dependent changes in gene transcription necessary for synaptic plasticity and behavior modifications underlying fear memory. HDAC inhibitors (e.g. trichostatin A, sodium butyrate) enhance contextual fear memory capacity, while DNA methylation inhibitors (e.g. 5-aza-2-deoxycytidine (5-aza-C), zebularine, RG108) reduce contextual fear memory capacity. RELN = Reelin gene; PP1 = protein phosphatase 1 gene; BDNF = brain-derived neurotrophic factor gene.

Moreover, all studies to date have investigated epigenetic mechanisms in memory using a target-gene approach to assessing changes at specific candidate gene loci. Finally, we do not know yet whether epigenetic changes contribute to the maintenance and persistence of memory. It has only been shown that they are involved in the early stages of memory formation and consolidation. The best evidence that they might sub serve memory persistence is in the studies that demonstrate that these epigenetic changes that underlie the persisting effects, or “memory”, of early-life experiences [46–51].

**SUMMARY**

It is becoming increasingly clear that epigenetic mechanisms have some necessary role in the dynamic nature of the adult CNS in response to the environment, and that epigenetic regulation of gene transcription facilitates memory formation (Fig. 23.3). Studies also indicate that manipulation of such mechanisms can restore learning and memory deficits in several adult models of neurodegeneration and brain injury. Thus, one of the most important lessons that future studies will continue to address is whether epigenetic drugs can alleviate cognitive deficits in humans, particularly those associated with age-related neurological
disorders (i.e. Alzheimer's) and neuropsychiatric disorders (such as schizophrenia). Indeed studies continue to demonstrate that epigenetic alterations occur in these patients [52–55]. The continued study of epigenetic marks in the regulation of cognition and in postmortem tissue promises a future where we will fully appreciate the role of epigenetic molecular mechanisms in CNS control, and importantly, the viability of epigenetic therapy treatment to alleviate the growing prevalence of memory dysfunction in society.

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Transgenerational Epigenetics

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INTRODUCTION

The regulation of gene expression through epigenetic modifications provides a dynamic path through which environmental experiences can lead to persistent changes in cellular phenotype. This plasticity plays an important role in mediating cellular differentiation and the potential stability of these modifications can lead to persistent and inheritable variations in gene expression. Though there are numerous types of epigenetic mechanisms, studies of environmentally-induced changes in the epigenome have focused primarily on DNA methylation and post-translational modification of histone proteins. The process of DNA methylation whereby cytosine is converted to 5-methylcytosine is mediated by methyltransferases which either promote maintenance (i.e. DNMT1) or de novo DNA methylation (i.e. DNMT3) [1-3]. The process of methylation is dependent on the presence of methyl donors (provided by nutrients such as folic acid, methionine, and choline) and the transcriptional repression associated with DNA methylation is sustained through the binding of proteins such as MeCP2 [4]. Histone proteins, which form the core of the nucleosome, also significantly alter gene expression through their interactions with DNA. Histones can undergo multiple post-translational modifications, including methylation, acetylation, and ubiquitination, which can alter the accessibility of DNA and the density of chromatin structure. In particular, histone acetylation is associated with increased transcriptional activity whereas histone deacetylation is associated with transcriptional repression [1,5].

The role of epigenetic mechanisms in mediating the long-term effects of environmental experiences is a rapidly expanding field of study, and it has become evident that experiences throughout the lifespan can induce modifications to the epigenome. Moreover, these epigenetic changes can have implications for neurobiology, physiology, and behavior of an organism and can contribute to the "epigenesis" of phenotype as described by Cattell in the 1940s, in which the term "epigenetics" has its roots [6]. Within the study of mammalian development, the quality of interactions between parents and offspring is a particularly salient aspect of the early environment and there is converging evidence in numerous experimental paradigms for parental influences on the regulation of gene expression and behavior [7-10]. Though maternal effects have been well established in literature, there is increasing evidence for paternal regulation of offspring development and behavior.