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Histone deacetylases govern cellular mechanisms underlying behavioral and synaptic plasticity in the developing and adult brain

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Abstract

Histone deacetylases (HDACs) are a family of enzymes that alter gene expression patterns by modifying chromatin architecture. There are 11 mammalian HDACs that are classified by homology into four subfamilies, all with distinct expression patterns in brain. Through the use of pharmacological HDAC inhibitors, and more recently HDAC knockout mice, the role of these enzymes in the central nervous system are starting to be elucidated. We will discuss the latest findings on the specific or redundant roles of individual HDACs in brain as well as the impact of HDAC function on complex behavior, with a focus on learning, memory formation, and affective behavior. Potential HDAC-mediated cellular mechanisms underlying those behaviors are discussed.

Keywords

HDAC; chromatin; learning and memory; epigenetic; synaptic plasticity

Introduction

Epigenetic studies are now providing a more complete mechanistic understanding of how signals extrinsic and intrinsic to an organism can impact long-term gene expression patterns, which undoubtedly play an important role in the emergence of complex behavioral phenotypes. Early usage of the epigenetics concept was a rebuttal to preformationism (i.e., the idea that an organism's developmental manifold is determined at conception and requires only maturation) and instinct (i.e., heritable behavioral repertoires that require no learning and are fully formed the first time they are performed) as mechanistic explanations for the complex behavior of animals, including humans. For example, the epigenetic theory forwarded by the developmental biologist Gilbert Gottlieb proposed 4 levels of analysis that must be accounted for in order to grasp the ontogeny of complex behavioral characteristics in animals - genetic activity, neural activity, behavior, and environment (including physical, social, and cultural influences). Any one of these factors is continuously influenced by the other three, and every one of them is capable of influencing the others. Development is thus

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conceptualized as a complex lattice of inputs to, and interactions between, each of these levels that leads to phenotype (Gottlieb, 2007). In the mid to late 20th century, Gottlieb and collaborators' studies convincingly refuted the popular appeal to genetic "programming" as a satisfactory account of the phenomenon of imprinting - the rapid species identification exhibited by newborn social animals, often referring to identification of a parent (not to be confused with genomic imprinting). By devocalizing mallard embryos during specific stages of embryonic development, Gottlieb was able to show that experience with their own vocalizations while still in the egg was necessary and sufficient for the postnatal recognition of maternal calls (Gottlieb, 1997). Exposure to chicken calls while still in the egg resulted in the mallards imprinting on chickens!

Although genes still appear rule to the roost when it comes to explaining the development of complex behavioral characteristics, gene regulation is now recognized to be even more complicated than previously imagined. The current formulation of epigenetics is the set of heritable and relatively enduring modifications to DNA and chromatin that impact gene expression in the absence of changes to the DNA sequence itself (Levenson and Sweatt, 2006). Utilizing the tools of cellular and molecular biology as well as behavioral models, epigenetic approaches have made the study of the cross-talk between the levels conceived by Gottlieb more accessible to mechanistic analyses and will ultimately provide a more holistic conceptualization of the developmental emergence of diverse behavioral phenotypes.

The current review will discuss the putative roles of the epigenetic regulators the histone deacetylases (HDACs) in complex behavior, with a particular focus on learning and memory, affective behavior, and addiction. We have chosen to highlight these behaviors due to the accumulation of experimental results within these fields suggesting that alterations in histone acetylation status may act as a molecular stamp of specific behavioral phenotypes. We also review data suggesting that specific HDACs play different functional roles in the developing versus the adult central nervous system. Finally, potential downstream molecular and genetic targets of the HDACs are considered, particularly in the context of the behavioral data discussed.

Epigenetic mechanisms in the adult brain respond to environmental stimuli

Neurons are post-mitotic: hence, a definition of epigenetics that stresses the *heritable* transfer of gene expression patterns does not apply (with the exception of brain regions that undergo adult neurogenesis, e.g., the dentate gyrus of the hippocampus). This review will proceed with the definition of epigenetics, proposed by Allis, Jenuwein, and Reinberg, as the sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression and silencing from the same genome (Allis, 2007).

The nucleosome is the fundamental unit of chromatin structure and consists of 146 bp of DNA wrapped around an octamer of histones. N-terminal regions of histones protrude from the nucleosome, facilitating interactions with other proteins. Epigenetic modifications can occur on either the DNA or the histones. Two of the most frequently studied epigenetic processes are DNA methylation, catalyzed by DNA methyltransferase (DNMT) enzymes, and the acetylation and deacetylation of lysine residues within histone tails. The addition of methyl groups directly to the C5 position of cytosine in CpG dinucleotides of DNA is a primary mechanism for gene silencing. The importance of gene silencing in somatic cells following cell division cannot be overstated. Maintenance of cell phenotype following cell division is reliant upon the silencing of certain genes and the sustenance of these patterns after the events that accompany inheritance at the replication fork. Imagine the physiological havoc if, following cell division, the progeny of a liver cell acquired a neuronal phenotype!

Many post-translational modifications to histones occur, including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation. Histone acetylation is mediated by the interplay between two enzyme families: the histone acetyltransferases (HATs), which add acetyl groups to histone tails, and the histone deacetylases (HDACs) which remove acetyl groups. Acetylation of lysine residues within histone tails neutralizes their positive charge thereby “relaxing” chromatin structure due to relatively greater electrostatic repulsion from negatively charged DNA, a modification associated with an increase in gene transcription (Feng et al., 2007; Haberland et al., 2009). By contrast, HDAC action is generally associated with a decrease in gene transcription, although the identities of the specific genes impacted by HDACs are not thoroughly characterized. By maintaining acetylation patterns of core histones, HDACs and HATs can manipulate the functional state of chromatin and play an important role in governing the access of transcriptional machinery to target gene promoters (Feng et al., 2007). In a provocative recent study, Wang et al. (2009) found that both HATs and HDACs are found at transcribed regions of active genes, and they suggested that HDACs could function to “reset” the conformational state of chromatin by removing acetylation at active genes.

Adult neurons undergo epigenetic changes as a result of an organism’s interaction with the environment. Studies by Meaney, Szyf, and colleagues have demonstrated that DNA methylation patterns around the promoter region of the glucocorticoid receptor gene in infant rats change in response to the level of maternal care provided, e.g., licking, nursing (Weaver et al., 2004, 2005). Importantly, these methylation patterns persist into adulthood and affect physiological parameters underlying the stress response, as well as behavioral responses in putatively stressful situations (e.g., an elevated plus maze). Furthermore, the acetylation state of histones is responsive to synaptic activity, and reliable changes in the pattern of histone acetylation in neurons occur as a result of experience in learning tasks, acute or chronic experience with drugs of abuse, or exposure to environmental stressors (Levenson et al., 2004; Bredy et al., 2007; Barrett and Wood, 2008; Bredy and Barad, 2008; Malvaez et al., 2009).

The pattern of an animal’s genomic activity is thought to be significantly influenced by the sum of the epigenetic modifications to core histones (i.e., “histone codes”) (Strahl and Allis, 2000). The histone code hypothesis proposes that mechanisms exist within the nucleus that are capable of deciphering complex patterns of chromatin modifications and then imposing onto the underlying DNA a functional state determined by each specific code. The idea of a complex histone code together with the existence of “code readers” is an attractive hypothesis, although rigorous experimental tests of the hypothesis are largely lacking. However, a recent study introduced lysine (K) to arginine (R) mutations in yeast (*Saccharomyces cerevisiae*) histone 4 (H4) tail residues and found that, among the 4 K residues, only K16 modifications were strongly associated with distinct transcriptional outcomes. Additional modifications to K5, K8, and K12 monotonically altered regulation events, suggesting relatively simple H4-mediated effects on transcription (Dion et al., 2005).

Other variants of histone proteins also carry specific regulatory switches. For example, hyperacetylated chromatin that is also methylated on K4 within H3 is associated with a transcriptionally active locus (“euchromatin”). By contrast, if H3 is methylated on K9, that corresponding region of DNA is likely to be inactive (i.e., “heterochromatin”). Additionally, lysine residues on histone tails are not the only target of post-translational regulatory modifications. There is growing evidence that the globular domains of histones, the facets which interact with the DNA helix, can also be modified extensively and function as regulators of nucleosome mobility (Ng et al., 2002). Because acetylation (along with other lysine-directed chemical modifications) alters the charge balance between positive residues and the negatively charged DNA backbone, it is critical in the process of regulated DNA

exposure to transcription machinery. The number of possible combinations of epigenetic marks on residues of both histone tails and globular domains offers the potential of a complex molecular system capable of fine-tuning global gene-expression patterns.

Importantly, codes change as a result of environmental input, suggesting that histones essentially act as rheostats capable of shifting gene expression patterns based on the underlying codes imparted upon them by chromatin-modifying enzymes. We are now beginning experimentally to crack these codes, so to speak. For example chronic administration of drugs of abuse in animals induces increased H3 acetylation in discrete brain regions (e.g., striatum), which is associated with distinct behavioral patterns (see below). Adding to the potential functional range of the system, there is a dynamic interplay between the various epigenetic marks (e.g., histone methylation, acetylation, DNA methylation) that serve to establish relatively persistent gene expression patterns.

HDACs exhibit cell- and tissue-specific expression patterns

HDAC enzymes are present in animals, plants, and bacteria (Hildmann et al., 2007). HDACs themselves lack intrinsic DNA-binding activity and are recruited to target genes via their direct association with transcriptional activators and repressors and are typically incorporated into large multiprotein transcriptional complexes. With regard to mammalian HDACs, structural and functional differences as well as expression patterns form the basis for their grouping into at least 3 different classes (the class II HDACs are sometimes split into class IIa and IIb). Class I HDACs (HDAC 1, 2, 3, and 8), are constitutively nuclear proteins and are widely expressed (Broide et al., 2007; Haberland et al., 2009). The class II HDACs (HDAC 4, 5, 6, 7, 9, and 10) are expressed in a more cell-specific manner and shuttle between the nucleus and cytoplasm (Chawla et al., 2003; Broide et al., 2007; Haberland et al., 2009). Rounding out the list are class IV HDACs, currently consisting of one member (HDAC11), with little known of its function. The sirtuins, sometimes referred to as the class III HDACs, also possess deacetylase activity; however they are mechanistically distinct from HDACs and are reliant upon NAD^+ , as opposed to zinc, as a cofactor for enzymatic activity. [For reviews of sirtuin biology and function, see: Albani et al., 2009; Finkel et al., 2009; Haigis and Sinclair, 2010].

In the adult rat, expression of all 11 HDACs is observed in the brain to some extent (Broide et al., 2007). The highest overall expression for any HDAC in the central nervous system is the class IV HDAC, HDAC11, which has yet to be studied in much detail. Class I HDACs are relatively highly expressed, with the exception of HDAC8 which is virtually undetectable in most areas of the brain. Two members of the class II HDACs, HDAC4 and 5, are highly expressed in brain (6, 7, 9, and 10 are expressed at low levels), with highest expression in basal ganglia, hippocampus, and cerebellum. Based on variable tissue- and cell compartment-specific expression patterns for specific HDACs in brain and other tissues, it is highly probable that there are functional differences between HDACs. Primarily through the use of pan HDAC inhibitors, HDACs have been implicated in diverse biological processes, including apoptosis, synaptogenesis, cognition, affective behavior, cancer, and neurodegenerative disorders (Bolger and Yao, 2005; Minucci and Pelicci, 2006; Hildmann et al., 2007; Barrett and Wood, 2008; Akhtar et al., 2009; Brunmeir et al., 2009; Chuang et al., 2009; Haberland et al., 2009).

Biological functions of individual HDACs have been difficult to ascertain, owing to a lack of specific pharmacological inhibitor compounds for particular HDACs. Moreover, constitutive knockout (KO) of many of the individual HDACs are lethal, underscoring the vital role played by these enzymes in normal development. HDAC6 KO mice are an exception - they are viable and have no discernible phenotype beyond hyperacetylated

tubulin (Hubbert et al., 2002; Zhang et al., 2003). HDAC5 KO mice are also viable but these mice present with cardiovascular abnormalities (Haberland et al., 2009). The cause of death in mice with constitutive KO of specific HDACs may hint at their function. For instance, HDAC1 null mice exhibit widespread growth defects; HDAC2 KO mice die shortly after birth due to severe cardiovascular defects; HDAC4 KO mice exhibit defective skeletal development; HDAC7 KO mice die early due to defective endothelial cell development (Haberland et al., 2009). Conditional KO strategies are now being employed, with the obvious benefit that the animals are viable and in many cases the KO can be made in an anatomical region-specific fashion using an appropriate promoter.

Are HDACs 1 and 2 a developmental switch underlying neuronal fate determination and excitatory synapse maturation?

The expression of class I HDACs in mouse brain is developmental-stage specific. HDAC1 and 2 are both present in neural progenitor cells; however following differentiation HDAC1 expression is primarily limited to glial cells, whereas HDAC2 is highly expressed in neurons and essentially absent from glia (MacDonald and Roskams, 2008). In other words, at some point in central nervous system development it is likely that the functional properties of HDAC1 and 2 undergo a switch, after which the adult roles of these HDACs may be established.

Accumulating data indicate a prominent role for both HDAC1 and 2 in central nervous system development. Montgomery et al. (2009) have used the Cre-lox system under the control of the GFAP promoter to delete HDAC1, HDAC2 or both HDAC1 and 2 concurrently (HDAC1/2) in the brain. HDAC1/2 KO severely disrupted cortical, hippocampal, and cerebellar organization, and the mice did not survive beyond postnatal week 1. Neuronal precursors in HDAC1/2 mice failed to differentiate into neurons, and instead underwent apoptosis. By contrast, mice with single deletions of HDAC1 or 2 were viable and exhibited no detectable developmental phenotype, prompting the authors to conclude that HDAC1 and 2 have redundant functions during neural development. Previous work has also suggested redundant roles for HDAC1 and 2 in cardiac development (Montgomery et al., 2007). However, dissociable roles for HDAC1 and 2 on cell proliferation have been documented. For example, HDAC1 is essential for unrestricted cell proliferation, by repressing the expression of cell cycle inhibitors, and although HDAC2 and 3 are upregulated in embryonic stem cells with HDAC1 KO, these class I HDACs are unable to compensate for the loss of HDAC1 function (Lagger et al., 2002). Data so far suggest that the net balance of the changes in gene expression following HDAC inhibition in adult cells favors cellular survival protection and health, although based on the work of Montgomery et al. this is not likely the case for HDAC inhibition in early development (Minucci and Pelicci, 2006; Montgomery et al., 2007, 2009). This is a clinically relevant issue, as broad-acting HDAC inhibitors are being proposed as potentially beneficial for a variety of diverse pathologies, including cancer and neurodegenerative disorders (Duvic and Vu, 2007; Kristensen et al., 2009).

Our laboratory recently reported that HDAC1 and 2 may function as a developmental “switch” that governs excitatory synapse maturation early in development (Akhtar et al., 2009). HDAC1/2 knockdown profoundly impacted excitatory synaptic transmission and the formation of synaptic contacts in cultured hippocampal neurons, without affecting inhibitory neurotransmission. Interestingly, the effects of HDAC1/2 knockdown were dissociable depending upon the maturational state of neurons, as mature neurons exhibited a profile that was distinct from immature neurons. Knockdown of HDAC1/2 during early synaptic development (5 DIV) led to an increased frequency of miniature excitatory post-synaptic currents (mEPSCs), and an increase in synaptic vesicle mobilization. The increase in

mEPSCs was mimicked by treatment with the HDAC inhibitor Trichostatin A (TSA). Single-cell recordings made from HDAC1 or 2 deficient neurons at 16 DIV, a time point at which synapses in cultured hippocampal neurons share all of the functional and structural characteristics of mature synapses formed *in vivo*, showed that knockdown of HDAC2 reduced mEPSC frequency, while HDAC1 knockdown was without effect. Furthermore, over-expression of HDAC2 produced the opposite phenotype - an increase in mEPSC frequency in mature neurons. Our data suggest that HDAC 1/2 promote the stability of synaptic networks during early time points in central nervous system development by repressing excitatory synapse maturation, and that HDAC1 and 2 do not have functionally redundant effects on synaptic transmission in immature neurons although they become functionally divergent as neurons mature.

A role for HDACs in learning and memory?

It has been suggested that the central nervous system has put to use highly conserved epigenetic mechanisms necessary in development to govern the plastic synaptic events that mediate the formation of long-term memories in the adult brain (Levenson and Sweatt, 2006). The idea that epigenetic mechanisms underlie memory formation was first introduced in a theoretical paper published in *Nature* in 1969 by Griffith and Mahler who hypothesized that “the physical basis of memory could lie in the enzymatic modification of the DNA of nerve cells” (Griffith and Mahler, 1969). It is now recognized that epigenetic modifications of DNA and histones are indeed likely to play a significant role in memory formation. The conformational state of chromatin is exquisitely sensitive to experimental manipulations that foster associative learning (e.g. classical conditioning of fear responses, spatial memory tasks), and specific histone modifications, including acetylation and methylation, have been hypothesized to influence long-term memory formation by modifying promoters of transcription factors, neurotransmitter receptors, cytoskeletal proteins, and other cellular substrates (Alarcon et al., 2004; Levenson et al., 2004; Bredy et al., 2007; Lattal et al., 2007; Vecsey et al., 2007; Barrett and Wood, 2008; Nott et al., 2008; Stefanko et al., 2009).

Pharmacological and genetic manipulations of HDACs have significant effects on cognition in animal studies. The task used most frequently in experimental investigations of HDACs involvement in learning and memory is fear conditioning. Pavlovian conditioning of fear responses in rodents is used to investigate the mechanisms responsible for associative learning and the neurobiological substrates that support the acquisition and retention of emotional memory (Maren and Quirk, 2004). A handful of studies implicate chromatin-modifying enzymes in initial memory formation and in extinction of conditioned fear responses. There is an increase in acetylation of H3 in the CA1 subregion of the hippocampus following fear conditioning (Alarcon et al., 2004; Levenson et al., 2004; Keeley et al., 2006; Wood et al., 2006). Moreover, intrahippocampal or systemic treatment with HDAC inhibitors enhances both the acquisition and extinction of conditioned fear responses (Yeh et al., 2004; Lattal et al., 2007; Bredy and Barad, 2008). Extinction of conditioned fear in mice results in an increase in H4 acetylation around brain-derived neurotrophic factor (BDNF) exon IV promoter, as well as increases in BDNF exons I and IV mRNA in the prefrontal cortex (Bredy et al., 2007). BDNF has been frequently implicated in learning and memory processes, and is known to be critical for cell survival, synaptic remodeling, and the electrophysiological changes in neurons that may underlie some forms of learning (Bramham and Messaoudi, 2005). Conditional, brain-specific, HDAC2 KO mice also exhibit enhanced acquisition of associative fear learning, while overexpression of HDAC2, but not HDAC1, in the brain led to deficits in fear learning as well as poor performance in the Morris water maze task, an aversively motivated spatial memory task (Guan et al., 2009).

As far as we know, tasks that are primarily appetitive in nature (i.e., require an animal to make responses for a reward) have been largely ignored in studies of HDAC involvement in learning and memory. Therefore it is not yet clear if the effects of HDAC inhibition or KO can be considered relevant for learning and memory generally, or are instead specific for aversively-motivated learning, or learning borne under “stressful” conditions. This remains an open question, as HDAC manipulations can impact the way an animal responds to stress (see below). A recent study demonstrated that systemic treatment with glucocorticoid hormones, which are critical for the mobilization of an appropriate physiological stress response and for encoding memories associated with a salient stressor, increased histone acetylation in both hippocampus and insular cortex (Roozendaal et al., 2010). Moreover, infusions of glucocorticoid receptor antagonists directly into the insular cortex blocked sodium butyrate-induced enhancement of object recognition memory in rats, suggesting that the endocrine consequences of an engaged stress response may interact with histone acetylation status to affect memory formation in some circumstances.

A task that has been used to study cognitive performance following HDAC manipulation that does not expose the animals to aversive stimuli *per se* is novel object recognition. Following training in an open-field like chamber with two objects available to explore, normal mice spend significantly more time with a novel object in later testing sessions, after it has replaced one of the two familiar objects (Stefanko et al., 2009). Expression of truncated and inducible dominant negative forms of the HAT cyclic AMP response element binding protein (CBP) in mouse forebrain consistently and robustly leads to deficits in novel object recognition that are rescued by TSA (Alarcon et al., 2004; Korzus et al., 2004; Stefanko et al., 2009). Moreover, treatment with the HDAC inhibitor sodium butyrate in normal mice enhances performance in the novel object recognition test relative to untreated mice, and does so in a long-term fashion (Stefanko et al., 2009). Sodium butyrate-treated animals continued to prefer exploration of the novel object a week after training, a time point at which control mice exhibited no preference.

Beneficial effects of HDAC inhibition have been observed in mouse models of Alzheimer’s disease, spurring hope that HDAC inhibitors may prove useful in treating symptoms of neurodegenerative disorders. Enhanced retention of learned fear associations, and improved spatial memory following central and peripheral treatments with sodium butyrate, have been shown in a mouse model of neurodegeneration that utilized inducible expression of p25 protein in the brain or in Tg2576 mice, an established transgenic model of Alzheimer’s (Fischer et al., 2007; Ricobaraza et al., 2009). Using the HDAC inhibitors sodium valproate or SAHA, as well as sodium butyrate, Kilgore et al. (2010) found beneficial effects on fear memory retention in the APP^{swe} transgenic Alzheimer’s model. It is not yet known by what mechanisms HDAC inhibition can rescue or prevent memory deficits in these models; however, by stimulating expression of the chaperone protein clusterin (a.k.a apolipoprotein J), which is important for clearance of amyloid β peptides in the brain, HDAC inhibitors may have preventative effects on amyloid β aggregation (Nuutinen et al., 2010).

Corroborating the results from behavioral studies demonstrating improved memory with HDAC inhibition are findings showing that HDAC inhibitors enhance the induction of long-term potentiation (LTP) at hippocampal synapses and also in the amygdala, two brain regions that are essential for associative learning (Levenson et al., 2004; Yeh et al., 2004; Vecsey et al., 2007; Barrett and Wood, 2008). LTP, an activity-dependent increase in synaptic strength, is widely regarded as an electrophysiological correlate of learning and memory formation (Bliss and Collingridge, 1993; Kim and Linden, 2007). Induction of LTP triggers histone acetylation at H3 and H4 in hippocampus, and perfusion of hippocampal slices with sodium butyrate and TSA has been reported to enhance LTP at the Schaffer-CA1 synapse (Levenson et al., 2004). Using relatively weak stimulation parameters (i.e., a single

train of 100 Hz stimulation), HDAC inhibitor treatment transforms early-phase LTP, which is protein synthesis-independent, into sustained and robust late-phase LTP, which is reliant on protein synthesis (Vecsey et al., 2007).

Many important questions remain about the role of HDACs in learning and memory and several types of studies are warranted to address these questions. It is important to note that a number of learning and memory studies utilize HDAC inhibitors such as TSA or SAHA which seem to preferentially hit Class I but not Class II HDACs, suggesting some specificity to the effects of these drugs (Kilgore et al., 2010). Within learning and memory paradigms it will be important to determine if HDAC manipulations differentially affect memory encoding or storage – as discussed, both appear to be affected in the fear conditioning paradigm. What connection, if any, can be made between studies using HDAC inhibitors in adult animals and studies that KO specific HDACs early in development? It is difficult to reconcile that HDAC2 KO early in embryonic development yields a similar learning phenotype to that seen following acute treatments with relatively non-specific and broad-acting HDAC inhibitors in adult animals. What is striking is the consistent observation of beneficial effects of HDAC inhibition on cognitive function, as well as the enhancement of plastic events at synapses that likely support learning and memory. One general function of the HDACs may be to maintain a “homeostatic” level of gene activation, potentially in a developmental-stage specific fashion, thereby acting as a check on the system-wide deleterious effects of unfettered transcription at critical periods during development or in the adult brain. Unchecked transcription of the downstream gene targets of HDACs may be manifested as enhanced synaptic plasticity, as suggested by recent data generated in our laboratory, among others (Akhtar et al., 2009).

A role for HDACs in affect regulation?

Chronic environmental stressors are a known trigger for many cases of affective disorder, including major depression and anxiety disorders (Kendler et al., 1999). Interestingly, acute versus chronic stress appears to affect histone acetylation patterns differentially in brain regions that are involved in emotion and affect. Chronic social defeat stress, a paradigm in which a mouse is repeatedly exposed to a larger “aggressor” mouse to which it consistently loses confrontations, is associated with long-term acetylation of H3 in the nucleus accumbens (NAcc), a brain region that is critical for mediating reward-related behavior (Covington et al., 2009). Chronic social defeat downregulates HDAC5 mRNA in the NAcc, while chronic treatment with the antidepressant imipramine increases HDAC5 expression in animals that have been chronically subjected to social defeat (Renthall et al., 2007). By contrast, overexpression of HDAC5 antagonizes the antidepressant effects of imipramine (Tsankova et al., 2006). Direct injection of the HDAC inhibitors SAHA or MS-275 chronically into NAcc prevented the social avoidance typically observed in socially defeated mice (Covington et al., 2009). Importantly, infusion of the HDAC inhibitor had antidepressant action in the forced swim and sucrose preference tests, thereby establishing that HDAC inhibition can rescue several depressive-like symptoms (e.g., anhedonia, immobility, reduced social interaction). Using microarrays the authors also demonstrate that chronic MS-275 infusion into NAcc reverses the pattern of gene expression observed following social defeat in a fashion similar to chronic treatment with fluoxetine (Covington et al., 2009).

Electroconvulsive shock (ECS), an efficacious treatment in some cases of severe depression, can induce differential chromatin remodeling patterns at specific BDNF promoter regions, dependent upon whether the ECS is acute or chronic. Chronic ECS increased H3 acetylation at BDNF promoters III and IV, and was correlated with increased expression of the corresponding BDNF transcripts (Tsankova et al., 2004). Acute ECS, however, was

associated with increased H4 acetylation. Previous studies in our laboratory have demonstrated that BDNF, particularly in the dentate gyrus of the hippocampus, is necessary for antidepressant responses in the forced swim test in mice, an animal model that is effective at predicting clinically efficacious antidepressant drug treatments (Adachi et al., 2008). The HDAC inhibitors sodium butyrate and valproate improve performance in the tail suspension and forced swim tests, two similar models, and also enhance the efficacy of fluoxetine in the forced swim test (Semba et al., 1989; Schroeder et al., 2007).

A recent study reported that patients with major depressive disorder exhibit increased expression of HDAC2 and HDAC5 mRNA in leukocytes (Hobara et al., 2010). Increased mRNA was only observed in depressive states and was not apparent during remission. Bipolar patients, by contrast, had increased expression of HDAC4, but a decrease in HDAC6 and 8 mRNA relative to control subjects. Importantly, antidepressant or mood stabilizing medications had no effect on HDAC expression, indicating that the drug treatments themselves were most likely not responsible for altered HDAC expression in the patients suffering a mood disorder. Of course, these observations say little if anything about HDAC expression or function in the central nervous system in these patients. Covington et al. (2009) found increased H3 acetylation in the NAcc, and a decrease in HDAC2 in the NAcc in postmortem tissue from depressed patients. Although the analysis did not distinguish between patients who had or had not been taking antidepressant medication, it was discovered that, by itself, chronic treatment with the antidepressant fluoxetine did not change HDAC2 expression in the NAcc in mice.

Thus far there is accumulating evidence consistent with the idea that one mechanism of antidepressant action may be the targeting of chromatin-modifying enzymes, which can restore gene expression patterns that are more conducive to healthy affective states. Changes in gene expression patterns induced by histone-modifying enzymes may underlie the switch from healthy adaptations to chronic stressful stimuli to the development of a psychiatric disorder. It is possible that the reorganization of the structural architecture of chromatin, and the gene expression patterns promoted by such restructuring, may help to explain the lag time between onset of antidepressant treatment and the appearance of a beneficial clinical response.

A role for HDACs in addiction?

It has been hypothesized that the transition from acute and recreational drug use to addiction involves long-term changes in gene expression patterns in brain regions critical for reward processing (Kumar et al., 2005; Wallace et al., 2008). In support of this hypothesis is the observation that drug-induced alterations in chromatin structure occur in the NAcc and these alterations can be consistently dissociated, depending upon the temporal nature of drug administration. Similar to what is observed following exposure to stressors, rats given acute treatments with cocaine display increased acetylation of H4 in the promoter regions of the immediate early genes *fosB* and *cfos*, whereas chronic exposure preferentially induces hyperacetylation of H3 around the *fosB*, cyclin-dependent kinase 5 (*CDK5*), and *BDNF* gene promoters (Brami-Cherrier et al., 2005; Kumar et al., 2005; Levine et al., 2005). Following chronic cocaine administration, voluntary running, and exposure to stressors, the transcription factor Δ fosB (a splice variant of the *fosB* gene) accumulates in the NAcc, and has been shown to inhibit Fos expression via recruitment of HDACs to the *cfos* promoter (Werme et al., 2002; Kumar et al., 2005; Tsankova et al., 2006; Wallace et al., 2008). Furthermore, overexpression of Δ fosB in the NAcc in mice leads to increased cocaine self-administration, as well as increased intakes of naturally rewarding sucrose solutions and an increase in voluntary running in a running wheel (Werme et al., 2002; Wallace et al., 2008). These data strongly implicate Δ fosB and the Fos family of transcription factors as important

players in mediating an animal's interactions with rewarding stimuli, and suggest that epigenetic modifications support long-term alterations in gene expression that contribute to reward-related behavior.

Interestingly, overexpression of HDAC4 in mouse NAcc reverses the acetylation patterns, as well as behavioral adaptations induced by chronic cocaine administration (Kumar et al., 2005). Specifically, HDAC4 expression dramatically decreased preference for an environment that was previously associated with cocaine treatment (i.e., conditioned place preference), and reduced the level of H3 acetylation in striatum. Also, treatment with the non-specific HDAC inhibitors TSA and sodium butyrate enhanced the locomotor activating effects of cocaine, as well as preference for a cocaine-paired environment.

Another class II HDAC, HDAC5, has also been implicated in mediating neural alterations that result from chronic exposure to drugs of abuse and other stressors. High levels of HDAC5 are expressed in the NAcc, and Nestler and co-workers have shown that genetic manipulations of HDAC5 in the NAcc profoundly influence an animal's sensitivity to, and interactions with, cocaine (Renthal et al., 2007). Phosphorylated levels of HDAC5 are greater in mice with a history of cocaine administration as opposed to acute experience. Moreover, overexpression of HDAC5 in the NAcc robustly diminished conditioned place preference for a cocaine-paired environment, an effect that was blocked by TSA treatment. Alternatively, HDAC5 KO mice exhibited the opposite phenotype, an enhanced sensitivity to cocaine, provided the animals were treated chronically with cocaine. HDAC5 KO mice showed normal responses to acute cocaine treatment, suggesting that HDAC5 may ultimately be important for the behavioral transition from acute to chronic drug use.

Alcohol intake, known to have anxiolytic effects acutely, has also been shown to induce changes in histone acetylation patterns and a decrease in class I and II HDAC expression in the amygdala, a brain region involved in mediating anxiety-like behavior (Pandey et al., 2008). Withdrawal from alcohol following chronic administration was associated with decreased H3 and H4 acetylation, increased amygdalar HDAC expression, and increased anxiety-like behavior in the elevated plus maze and dark/light box in rats, all of which could be prevented by TSA administration during the alcohol withdrawal phase.

What are the downstream targets of HDACs that influence complex behavior?

Less than 10% of all genes are regulated by HDACs, and HDAC inhibition may lead to gene repression as well as activation, depending on the nature of the gene (Fass et al., 2003; Glaser et al., 2003; Kato et al., 2004; Nusinzon and Horvath, 2005; Peart et al., 2005). Indeed, the ratio of upregulated to downregulated genes appears to be approximately 1:1, which strongly suggests that HDACs should not be characterized as global transcriptional repressors (Nusinzon and Horvath, 2005; Minucci and Pelicci, 2006). However, upregulation of specific genes following HDAC inhibition does not rule out secondary effects that may result in transcriptional repression (e.g. disinhibition of a repressor).

Thus far, gene expression data have largely been gathered following non-specific HDAC inhibitor treatments, so it is not yet known to what degree antagonism of specific HDACs will correspond with results using inhibitor compounds, and any distinctions should provide clues to understanding the functional roles of individual HDACs. Given that HDACs appear to only regulate a subset of genes, it is possible that those genes are specifically involved in mediating plastic cellular events (e.g., synapse development, activity-dependent changes in synaptic strength, cardiovascular hypertrophy), both during development and adulthood. Guan et al. (2009) found that HDAC2 binds to several synaptic plasticity-related genes-

BDNF, *Erg1*, *Creb1*, *CBP*, *nrn1*, NMDA receptor subunit genes, and *fos*, as well as genes important for synapse formation. The fact that HDAC2 binds at CREB and CBP gene promoters led the authors to suggest that HDAC2 may contribute to a well-established CREB-CBP pathway to regulate activity-dependent gene expression and learning and memory. Additionally, coREST was discovered to preferentially associate with HDAC2 relative to HDAC1. The coREST complex is an important regulator of neuron-specific genes, for example genes encoding ion channels, synaptic vesicle proteins, and neurotransmitter receptors (Schoenherr and Anderson, 1995). Following treatment with the HDAC inhibitor MS-275, Covington et al. (2009) found upregulation of various genes involved in dendritic remodeling (e.g., *SLIT*, *TGF α* , *JNK*, and *Rho*), which is interesting in relation to data suggesting that alterations in dendritic morphology may be involved in depression (Krishnan and Nestler, 2008). In addition, regulation of various transcription factors was noted (e.g., CREB, REST, coREST, STAT, *nrn1*), which partially overlap the findings with HDAC2 KO described above.

The *Nr4a1* and *Nr4a2* genes, which code for the immediate early transcription factors Nurr77 and Nurr1 respectively, have previously been implicated in memory, affective behavior, and addiction (von Herten and Giese, 2005; Colon-Cesario et al., 2006; Rojas et al., 2007). Mice with knockdown of Nurr1 exhibit poor learning in the aversively motivated passive avoidance task, and an increase in depressive-like behavior in the forced swim task (von Herten and Giese, 2005; Rojas et al., 2007). Abel and co-workers have shown that increased expression of *Nr4a1* and *Nr4a2* mRNA is observed two hours after training in fear conditioning. Treatment with TSA immediately after context fear conditioning increased the acetylation of H3 and H4 at the promoters of *Nr4a1* and *Nr4a2* and enhanced the expression of *Nr4a1* and *Nr4a2* mRNA relative to wild type and CREB mutant mice, an effect not observed following TSA treatment without fear conditioning experience (Vecsey et al., 2007). The authors suggest that these genes may play a role in mediating the learning enhancements that follow HDAC inhibition. Additionally, it has been shown that TSA treatment leads to recruitment of RNA polymerase and transcription factor IIB to the *Nr4a1* promoter *in vitro* (Fass et al., 2003).

It is not yet known how histone acetylation is regulated at specific genes. HDAC3 may bind directly to transcription factors, together with HDAC4, 5, or 9, and class II HDACs can target specific genes for repression through N-terminal regulatory domains that mediate interactions between these HDACs and certain transcription factors, [e.g., myocyte-enhancing factor-2 (MEF2)] (Durst and Hiebert, 2004). Indeed, HDAC4, 5, and 7 do not appear capable of deacetylating histones on their own and require interaction with HDAC3 or transcription factors (Fischle et al., 2002), suggesting a complex interplay between these factors.

A better understanding of the cross talk between epigenetic pathways is critical for mechanistically determining how HDACs influence gene expression, and ultimately behavior. HDACs cannot bind directly to DNA and are instead recruited to their gene targets through their associations with transcriptional activators and repressors. For example HDAC1 and 2 are known to form part of a multiprotein co-repressor complex with methyl-CpG binding protein 2 (MeCP2), a protein that binds to methylated CpG dinucleotides (Dannenberget al., 2005; Monteggia and Kavalali, 2009). Although the functional relevance of this interaction is not yet known, interruptions to the normal arrangement of this complex are likely be clinically relevant, as the neurodevelopmental disorder Rett Syndrome is caused by mutations in *MECP2* (Chahrour and Zoghbi, 2007; Monteggia and Kavalali, 2009). The MeCP2-HDAC1/2 complex may be important for the stabilization of heterochromatin, and TSA treatment can relieve transcriptional repression by MeCP2 (Jones et al., 1998; Nan et al., 1998). HDACs clearly interact with the enzymes responsible for

adding methyl groups to DNA. DNMT1, important for maintenance methylation of DNA, recruits HDAC1 and 2 as well as the histone methyltransferase Suv39h1 (Espada et al., 2004). HDAC1 and 2 have also been shown to interact with DNMT3a and DNMT3b, *de novo* DNMTs (Fuks et al., 2000; Fuks et al., 2001). An example of a functional outcome of DNMT-HDAC interaction has been demonstrated for neurite outgrowth. DNMT3b activity is important for neurite outgrowth, and this is mediated by DNMT3b interaction with HDAC2 (Bai et al., 2005).

HDACs do more than simply chase tails – their deacetylase domains also act on non-histone proteins. The bulk of experimental effort, and interpretation of experimental results in studies of HDAC function, has focused on the effects of HDACs on chromatin structure. These interpretations may be misleading, in the sense that HDAC interactions with non-histone proteins tend to be ignored, both conceptually and empirically. The known non-histone substrates of HDACs are diverse, and it is beyond the scope of this review to discuss them in detail; moreover the list is continuously growing [for a review of non-histone substrates see (Glozak et al., 2005)]. Acetylation of non histones can increase or decrease the DNA binding affinity of target proteins, increase or decrease transcriptional activation, increase or decrease protein stability, and promote or disrupt protein-protein interactions (Glozak et al., 2005). HDAC6, the main cytoplasmic deacetylase, may be important for synapse remodeling by acetylating the cytoskeletal protein α -tubulin (Glozak et al., 2005). Activity-dependent synaptic remodeling events are widely considered to be important for memory formation.

Much remains unknown about the downstream targets of HDAC activity, and the same can be said about the upstream effectors that induce changes in HDAC activity. The movement of HDAC4 to the nucleus in cultured hippocampal neurons is dependent on synaptic activity and believed to be stimulated by Ca^{2+} influx, which indicates one possible mechanism for input-specific gene expression (Chawla et al., 2003). *In vitro* studies have indicated that phosphorylation of serine residues in the C-terminal portion of HDAC1 or 2 enhances its activity, promotes the formation of co-repressor complexes, and regulates shuttling to the nucleus (Cai et al., 2001; Pflum et al., 2001; Smillie et al., 2004). The activation of cellular Ca^{2+} pathways in muscle activates CaMKs (calcium/calmodulin activated protein kinases) which phosphorylate class II HDACs (Tsankova et al., 2007). Although this work is in its infancy, elucidating the molecular pathways by which synaptic activity triggers chromatin- and DNA-modifying enzymes will substantially advance our understanding of the contribution of epigenetic mechanisms to complex behavior.

Concluding Remarks

Gene function cannot be studied in isolation from environmental influence, and environmental input and an animal's own behavior hold great sway over gene expression patterns. Therefore it is arguable whether there is a biologically-relevant partition between these influences. An organism's interaction with its environment impacts the pattern of epigenetic marks on histones and DNA, which is of course superimposed on an established sequence-specific genotype and the accumulated neurobiological consequences of environmental and behavioral experience. Revisiting Gottlieb's four levels of analysis of behavioral development it is evident that epigenetic mechanisms add another level of complexity, but have also spurred the use of cell and molecular biological tools to address the mechanisms by which these influences on phenotype interact. An enormous diversity of phenotypes can emerge from a single genotype, and epigenetic processes are likely involved in this variability. For example early life experience has been shown to set DNA methylation patterns at specific gene promoters, which has profound effects on adult behavior (Weaver et al., 2004, 2005, 2006). It cannot be ruled out that a similar role exists for acetylation.

Acetylation patterns on specific histones are dynamically regulated by HDACs and HATs and the resulting modifications impact gene expression patterns that contribute to diverse behavioral phenotypes (e.g. learning, reward-related behavior, affective states).

Acute versus chronic environmental manipulations elicit differential effects on histone modifications and the enzymes that mediate those modifications. These observations are likely relevant to the development of psychiatric disorders, as long-term deleterious changes in affective behavior are more frequently associated with chronic rather than acute exposure to stressors. Understanding the molecular mechanisms that facilitate the transition from acutely beneficial physiological responses to a pathophysiological phenotype may go a long way toward treating a diverse array of disorders (e.g., major depression, addiction, cancer). Critical to understanding this transition is the discovery of the molecular “scorekeepers” that maintain memory for whether an animal has been exposed to, for example, a drug of abuse repeatedly as opposed to infrequently. Given that H3 and H4 acetylation are able to differentially mark chronic vs. acute exposure to cocaine, it is possible that HAT and HDAC interactions with specific transcription factors (e.g., Δ fosB) can differentially modify the histone code. An understanding of the various functional roles of the HDACs in the developing and adult central nervous system will ultimately advance our understanding of behavioral ontogeny as well aid in the development of treatments that target a disorder’s epigenetic roots.

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