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Authors
White, André O
Wood, Marcelo A

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Does stress remove the HDAC brakes for the formation and persistence of long-term memory?

André O. White[1,2] and **Marcelo A. Wood**[1,2,*]

[1] University of California, Irvine; Department of Neurobiology & Behavior; Irvine, CA

[2] Center for the Neurobiology of Learning & Memory; Irvine, CA

Abstract

It has been known for numerous decades that gene expression is required for long-lasting forms of memory. In the past decade, the study of epigenetic mechanisms in memory processes has revealed yet another layer of complexity in the regulation of gene expression. Epigenetic mechanisms do not only provide complexity in the protein regulatory complexes that control coordinate transcription for specific cell function, but the epigenome encodes critical information that integrates experience and cellular history for specific cell functions as well. Thus, epigenetic mechanisms provide a unique mechanism of gene expression regulation for memory processes. This may be why critical negative regulators of gene expression, such as histone deacetylases (HDACs), have powerful effects on the formation and persistence of memory. For example, HDAC inhibition has been shown to transform a subthreshold learning event into robust long-term memory and also generate a form of long-term memory that persists beyond the point at which normal long-term memory fails. A key question that is explored in this review, from a learning and memory perspective, is whether stress-dependent signaling drives the formation and persistence of long-term memory via HDAC-dependent mechanisms.

Keywords

epigenetics; stress; long-term memory; histone deacetylases (HDACs); histone acetylation; chromatin; gene expression

Introduction

In the search to elucidate the mechanisms involved in memory formation, researchers have been continuously faced with the following question: what mechanisms allow or initiate the formation of persistent long-term memories? It is clear that not all long-term memories are created equally as some memories last a lifetime and are particularly resistant to

* Correspondence to: Marcelo A. Wood, PhD University of California, Irvine Department of Neurobiology & Behavior Center for the Neurobiology of Learning & Memory 301 Qureshey Research Labs Irvine, CA 92697-3800 mwood@uci.edu Phone: (949) 824-2259.

interference, while others last days or weeks and are fragile. Stress in particular has been known to initiate molecular and hormonal responses in the brain that prompt the formation of persistent and resilient forms of long-term memory. Stress plays a pivotal role in the interactions between animals and their environment. The memories of stressful experiences are often linked with increased survival rates as those memories serve to predict the occurrence of similarly stressful events in the future. In this review, we will discuss epigenetic mechanisms that act as a possible gateway to long-term memory formation and how stress might create persistent long-term memories by manipulating these epigenetic mechanisms.

Since Agranoff et al. (1967) first showed that transcription was required for long-term memory formation in goldfish, our understanding of the various mechanisms that regulate gene expression necessary for long-term memory has dramatically improved (Barrett and Wood, 2008; Alberini 2009). In general, interest in epigenetics has substantially expanded over the past 2 decades. This review will promote the hypothesis that epigenetic mechanisms regulate transcription necessary for the formation and the persistence of long-term memories. There are several epigenetic mechanisms that modify and manipulate chromatin structure in the service of regulating transcription. Among these mechanisms are chromatin modification (*e.g.* histone acetylation), DNA methylation and chromatin remodeling (*e.g.* nucleosome remodeling). Chromatin modification refers to post-translation modifications of core histone proteins through methylation, acetylation and phosphorylation (Kouzarides 2007). While DNA methylation modifies DNA through the addition or removal of methyl groups, but has become significantly more complicated as discussed in Li et al. (2013). Finally, chromatin remodeling involves large multi-subunit complexes that can alter nucleosome positioning and the wrapping of DNA (reviewed in Hargreaves et al., 2011; also see Vogel-Ciernia et al., 2013, with respect to memory). These epigenetic mechanisms can repress or enhance transcription by compressing or relaxing chromatin structure, respectively. These same epigenetic mechanisms have been shown to be involved in stable changes in gene expression, especially as related to cell fate decision (cellular memory; Turner et al. 2002). Although epigenetic mechanisms have a clear role at the cellular level with regard to establishing and maintaining coordinate gene expression profiles leading to specific cell function and fate in development, whether this type of regulation extends to neurons and their ability to encode persistent long-term memories remains unclear. In the next section of this review, we will first discuss the ways in which these epigenetic mechanisms are involved in the formation of long-term memory, and then approach the question of persistence.

Chromatin modification and long-term memory

There have been major inroads made into understanding the role of epigenetic mechanisms in long-term memory processes. Chromatin modification mechanisms are currently the best-studied of the epigenetic mechanisms briefly mentioned above, and within the realm of chromatin modification, histone acetylation has been worked on the most. Histone acetyltransferases (HATs) neutralize the positive charge on lysine residues, which interacts with the negative charge on the DNA phosphate backbone, through the addition of acetyl groups (for review, see Kouzarides, 2007; Day and Sweatt, 2011). This has two main effects

(Barrett and Wood, 2008). First, it physically relaxes chromatin structure, which is conducive to gene expression. Second, it provides interaction sites for bromo-domain containing proteins to build large multi-protein complexes required for transcription initiation and elongation. Conversely, histone deacetylases (HDACs) remove acetyl groups from histone tails, which returns chromatin structure into a state that in general silences gene expression (Kouzarides 2007). Although we refer to HATs and HDACs as enzymes that affect *histone* acetylation, these same enzymes have non-histone substrates, including proteins that are known to regulate gene expression.

The role of HDACs as repressors of gene expression leads to the prediction that inhibition of HDACs during the transcription-dependent consolidation phase of memory formation would result in enhancement of long-term memory. Indeed, several early studies used pharmacological approaches to inhibit HDAC activity to understand their potential role in various types of long-term memory processes. The HDAC inhibitors valproic acid (VPA), trichostatin A (TSA) and sodium butyrate (NaB) have been shown to enhance LTP, memory formation, and the extinction of memory (Guan et al., 2002; Alarcón et al., 2004; Bredy et al., 2007; Bredy et al., 2008; Fischer et al., 2007; Lattal et al. 2007; Levenson et al. 2004; Vecsey et al. 2007; Stafford et al., 2012; Yeh et al., 2004). More recent studies have examined genetic manipulation of individual HDACs, as well as the next generation of HDAC inhibitors with increased selectivity for individual HDACs, and have arrived at similar conclusions regarding the role of HDACs in memory formation (Guan et al., 2009; Hawk et al., 2011; Kim et al., 2012 McQuown et al., 2011; Malvaez et al., 2013; Rogge et al., 2013; Morris et al., 2013). From these studies (recently reviewed in Peixoto and Abel 2013) it becomes increasingly clear that HDACs play an integral role in long-term memory processes as well as long-lasting forms of synaptic plasticity.

The molecular brake pad hypothesis

The idea that HDACs and associated corepressors serve to suppress gene expression necessary for long-term memory formation was addressed in McQuown and Wood (2011) in the proposal of the “molecular brake pad” hypothesis. Briefly, this hypothesis posits that HDACs and associated co-repressor complexes (the molecular brake pads) act to inhibit transcription of genes required for long-term memory formation and maintenance. However, an activity-dependent signal of sufficient strength is able to transiently remove the brake pads to permit gene expression required for stable forms of memory. Direct predictions of this hypothesis were that HDAC inhibition would lower the threshold for long-term memory formation and also contribute to the persistence of long-term memory by manipulation of the gene expression dynamics during memory consolidation. Described below are some studies that examine the effect of non-selective HDAC inhibition and specific HDAC knockouts on threshold effects for long-term memory formation as well as effects on the persistence of long-term memory.

The molecular brake pad hypothesis raises the question of how exactly HDAC inhibition regulates gene expression that enhances long-term memory processes. Vecsey et al. (2007) addressed one aspect of this question. They showed that the effect of TSA was dependent on the interaction between the transcription factor cAMP response element-binding protein

(CREB) and the HAT CREB-binding protein (CBP). Both CREB and CBP have well-established roles in long-term memory formation. Interestingly, out of 12 cyclic-AMP response element (CRE; DNA sequence that CREB binds) containing genes examined (see Vecsey et al., 2007 for list), TSA administration only led to an increase in the expression of 2 (*Nr4a1* and *Nr4a2*) in the hippocampus after fear conditioning. These enhancements in gene expression were only observed when TSA was paired with fear conditioning and not when TSA was administered absent training. Perhaps the most surprising finding of the Vecsey et al. (2007) study was that TSA transformed a transcription-independent form of LTP (induced by a single tetanus) into a transcription-dependent form of LTP (as demonstrated by blocking with actinomycin-D). Earlier studies had shown that HDAC inhibition could enhance LTP (Levenson et al., 2004; Alarcón et al., 2004), but the transcription-dependent aspect of the enhancement had not been examined. Additionally, Vecsey et al. (2007) demonstrated that the enhancement of LTP by TSA required the interaction between CREB and CBP. Together, these results indicate that HDACs can regulate the temporal dynamics of gene expression during memory consolidation (Vecsey et al., 2007; McQuown et al., 2011) and also even be permissive (perhaps not instructive) in releasing gene expression for stabilizing potentiation (as in the LTP experiments; Vecsey et al., 2007). Also, it appears that HDAC inhibition can result in increased expression of a select subset of genes related to long-term memory formation.

If HDAC inhibition could transform a transcription-independent form of LTP into a transcription-dependent form, could a similar effect be observed at the behavioral level? This is the question addressed by Stefanko et al., (2009). Using an object recognition task, Stefanko et al., (2009) showed that HDAC inhibition via NaB transformed a subthreshold learning event (that does not normally result in long-term memory) into an event that generates long-term memory. This subthreshold training normally generates short-term memory, which is transcription independent. But, through HDAC inhibition, generated long-term memory, which is transcription dependent. Recall, this is a direct prediction of the molecular brake pad hypothesis (McQuown et al., 2011). Interestingly, a subthreshold learning event could not only be transformed into LTM, but also a form of LTM that was persistent, lasting beyond the time normal long-term memory for object recognition fails. Similar findings have been observed in genetically modified HDAC3-FLOX mice with a focal deletion of HDAC3 in the dorsal hippocampus (McQuown et al., 2011). Thus, both at the cellular level (with regard to LTP) and the behavioral level, HDACs appear to control the entry into stabilized potentiation and long-term memory, respectively.

Which HDACs may be involved? Several results have pointed to class I HDACs (HDAC1, 2, 3 & 8) as the critical HDACs involved in regulating gene expression required for long-term memory processes, although little is known about HDAC8. One finding that is important to understanding the role of HDACs was the demonstration that class IIa HDACs (HDAC4, 5, 7 & 9) have little to no catalytic activity due to the tyrosine instead of a histidine in a key residue of the catalytic domain (Lahm et al., 2007). This finding was confirmed for HDAC4 with regard to memory in an elegant study by Sando et al., (2012). Another significant finding was that HDAC inhibitors like SAHA, VPA, and NaB were found to confer their primary effect through the inhibition of class I HDACs with little

inhibition of other classes of HDACs (although HDAC6 is also targeted by SAHA; Kilgore et al., 2010). These findings highlighted the need to elucidate the role of specific HDACs on memory with more targeted approaches, as it appears that different classes of HDACs may serve different roles in memory formation.

Several recent studies further support a role for class I HDACs in regulating memory process. Neuronal overexpression of HDAC2, but not HDAC1, has been found to impair Morris water maze, context and cued fear conditioning (Guan et al., 2009). In addition, animals overexpressing HDAC2, but not HDAC1, had decreased dendritic spine density compared to wildtype in dentate gyrus (DG) granule cells and CA1 pyramidal neurons (Guan et al. 2009). Conversely, HDAC2 homozygous knockout mice showed enhanced context and cued fear conditioning 24 hours after training (Guan et al., 2009). The effect of HDAC2 knockouts is also reflected at the synapse as well as HDAC2 homozygous knockout mice have increased dendritic spine density in hippocampal CA1 pyramidal neurons and dentate gyrus (DG) granule cells. Interestingly, although HDAC1 and HDAC2 can be found in complexes together, they appear to have very distinct functions.

Although, in general HDACs repress memory formation by inhibiting transcription, the effects of HDACs on specific types of memory are mainly due to the complexes they form and the genes that are repressed. Bahari-Javan et al. 2012 showed that overexpression of HDAC1 enhances extinction while HDAC1 inhibition had the opposite effect. At first glance this study appears contrary to the previously explored studies that showed that inhibition of class1 HDACs facilitates extinction of fear memories. However, it appears that HDAC1, as a part of a repressor complex, facilitates extinction by down-regulating the expression of c-Fos (Bahari-Javan et al. 2012). c-Fos is up-regulated during fear conditioning but decreases during extinction, presumably through HDAC1 activity (Bahari-Javan et al. 2012). This study highlights the complex actions of different HDACs. The differential effects of HDACs, even within the same class, on persistent memory formation is likely due to the specific repressor complexes that each HDAC forms.

The most highly expressed class 1 HDAC in the brain is HDAC3 (Broide et al., 2007) and both conditional and traditional knockouts of this HDAC support a role for this class I HDAC in suppressing the formation and maintenance of long-term memory. Systemic administration of the new selective HDAC3 inhibitor RGFP966 1 hour before or after a subthreshold training generated persistent long-term object location memory (Malvaez et al., 2013). In other words, RGFP966 not only transformed a subthreshold learning event into long-term memory, but also generated a form of long-term memory that lasted beyond the time normal long-term memory (in well-trained control mice) for object location fails. Similarly, HDAC3$^{flox/flox}$ mice with a focal deletion of HDAC3 in the CA1 region of the hippocampus displayed persistent long-term memory after a subthreshold learning event (McQuown et al., 2011). It is important to note however that HDACs are capable of working in concert with other corepressors and HDACs to repress transcription. While HDAC3$^{flox/flox}$ mice infused with AAV-Cre showed a predicted deletion of HDAC3 in the hippocampus, this deletion was coupled with a reduction in HDAC4, but not HDAC2, in the nucleus (matched by accumulation in the cytoplasm). This is likely due to HDAC3 interacting with HDAC4 and HDAC5 in complexes that are distinct from HDAC2, which

usually interacts with HDAC1. As HDAC4 has no catalytic activity and the effects of HDA3 are correlated with increased acetylation it appears that the effects on memory are due to deletion of HDAC3. However, loss of HDAC4 in the nucleus may alter additional mechanisms that are necessary for long-term memory formation, as suggested by Sando et al., (2012). In keeping with the molecular brake pad hypothesis, HDAC3 appears to operate as a brake mechanism for the acquisition and consolidation of persistent long-term memory formation for object location memory (and other forms of memory described in the extinction section below).

HDAC inhibition and persistent extinction memory

The early findings that HDAC inhibitors could enhance memory lead researchers to examine whether HDAC inhibition truly affected memory formation mechanisms or performance. One way to address this question was to examine the same performance measure being used to assess memory, but with regard to a fundamentally different memory process: extinction. This was initially easiest to perform with regard to fear conditioning, in which an animal learns to associate a context or cue with a shock. The performance measure is freezing. In general, a higher freezing rate reflects a stronger long-term memory. Several studies showed that HDAC inhibition could enhance freezing, which was interpreted as HDAC inhibition enhanced memory for fear (Levenson et al., 2004; Vecsey et al., 2007; Guan et al., 2009; Yeh et al., 2004). To examine extinction, the same context or cue can be presented in the absence of the unconditioned stimulus (shock) and a new association is formed. Extinction does not erase the old association, as uncovering phenomena such as renewal, reinstatement, and spontaneous recovery can reveal the original association (Bouton 1993; Pearce 1987). Extinction memory formation requires a transcription-dependent consolidation phase, thus the consolidation of extinction memories provided an excellent way in which to test the question above. That is, if HDAC inhibition occurred only during extinction consolidation, it would be predicted that HDAC inhibition would reduce freezing during extinction of contextual or cued fear. Indeed, that is what Lattal et al., (2007) found with regard to the extinction of contextual fear and by Bredy et al., (2007) with regard to the extinction of cued fear. Thus, HDAC inhibition enhances freezing during original consolidation of the association between a context or cue and a shock when HDAC inhibition occurs during consolidation, but HDAC inhibition decreases freezing during extinction consolidation. These results helped solidify the idea that HDACs function in the consolidation of memory formation, whether it is the formation of an original memory or an extinction memory, and most importantly, not the misinterpretation of potential effects on performance.

But were the effects on extinction memory similar to those observed for original memory formation with regard to subthreshold effects and persistence? Lattal et al., (2007) demonstrated that a subthreshold extinction training period that did not result in extinction could be transformed into a robust extinction memory for contextual fear by either systemic NaB or intrahippocampal delivery of TSA. There is additional evidence in the addiction literature. In the first study to show that HDAC inhibition could enhance extinction of cocaine-context associated memory, Malvaez et al., (2010) showed that NaB delivered after the first extinction trial following establishment of cocaine-induced conditioned place preference (CPP) significantly enhanced the rate of extinction. The effects on extinction by

NaB were context-dependent and time-dependent. For example, NaB had no effect if administered outside the extinction consolidation window at 10 hours after the extinction trial. A key question was whether the enhanced extinction was persistent. One way to measure the persistence of extinction or strength of extinction is to examine cocaine-induced reinstatement. Cocaine led to the robust reinstatement of preference in vehicle control mice, but had no effect on mice treated with NaB during extinction (Malvaez et al., 2010). Recently, Malvaez et al., (2013) showed similar findings for the manipulation of HDAC3 using a selective HDAC3 inhibitor called RGFP966. RGFP966 could facilitate extinction of cocaine-induced CPP in a manner that blocks cocaine-induced reinstatement. Systemic RGFP966 resulted in specific histone acetylation changes in H4K8 and H3K14 in the infralimbic cortex (IFC) versus hippocampus and nucleus accumbens (NAc) during extinction consolidation (Malvaez et al., 2013). The increased H4K8Ac correlated with increased c-Fos expression in the IFC while chromatin immunoprecipitation showed that indeed the promoter of c-Fos had reduced HDAC3 binding and increased H4K8Ac at the promoter. These data suggest that although RGFP966 was delivered systemically, specific effects can be observed in the IFC during extinction consolidation. This is supported by earlier work by Stafford et al. (2012) who demonstrated that direct infusion of sodium butyrate into the IFC, but not the prelimbic cortex, results in persistent extinction of fear memory enhancements. Furthermore, sodium butyrate administered following weak extinction induced behavioral extinction, IFC H3K14Ac, and c-Fos expression consistent with strong extinction (Stafford et al., 2012). In summary, these findings suggest that HDAC3, and potentially other class I HDACs other than HDAC1, are key regulators of extinction memory formation in a manner that controls both the consolidation of extinction memory and the persistence or strength of extinction. These findings and others were recently reviewed in the context of the role of epigenetic mechanisms in extinction and reconsolidation by Lattal and Wood (2013).

The studies examining the role of HDACs in memory formation and the consolidation of extinction memory suggest that HDACs prevent the encoding of information into long-lasting forms of synaptic plasticity and memory. As proposed in McQuown and Wood (2011), HDACs may be key to preventing us from encoding most of what we perceive and information that we process every day. It is intriguing that HDAC inhibition can affect both the transformation of subthreshold training events into long-term memory formation as well as the generation of persistent long-term memory. This latter point is better demonstrated by the extinction studies performed to date. Although much more work is necessary to truly understand the mechanisms by which HDAC inhibition leads to persistent effects on memory processes, a different field of research may yield significant insight into physiological conditions that affect HDAC activity resulting in persistent memory—the stress field, which is discussed in the next section.

Stress and emotionally salient memories

This section will discuss the possibility that under certain conditions stress-induced cellular signaling results in the inhibition of HDACs and corepressor complexes leading to enhanced memory formation and persistent long-term memory (two aspects we have discussed above). This is not meant to be a comprehensive review of stress-dependent modulation of memory

(which is the topic of other reviews in this special issue of *Neurobiology of Learning and Memory*). We will discuss the evidence for how stress related events might alter the epigenetic landscape resulting in enhanced long-term memory formation and persistence of memory.

It is well established that stressful events have the capability to initiate a cascade of neurochemical release in the central and peripheral nervous systems (for a review, see Joëls et al., 2011; McClelland et al., 2011; Maras and Baram, 2012). These neurochemicals include neuromodulators such as corticotropin-releasing hormone (CRH), epinephrine and glucocorticoids. For example, stressful or emotionally arousing events (such as fear-conditioning) lead to release of CRH in brain areas involved in memory such as amygdala (Roozendaal et al., 2002) and hippocampus (Chen et al., 2001). Additionally, the adrenal gland releases epinephrine, which initiates the downstream release of norepinephrine in the basal lateral amygdala (BLA; Roozendaal and Quirarte, 2002; Roozendaal et al., 2006). The release of these neuromodulators and hormones in the brain are regulated through the hippocampal-hypothalamic-adrenal stress system (reviewed in Joëls and Baram 2009).

To briefly overview, acute stress events (events signaling potential or perceived threat) initiate the release of CRH in the hypothalamus that acts on CRH receptors in the pituitary gland. The pituitary then releases corticotropin, which induces the release of glucocorticoids. Glucocorticoids are capable of crossing the blood brain barrier to bind to glucocorticoid receptors (GRs) throughout the brain. GRs, being nuclear hormone receptors, have the ability to translocate to the nucleus and bind to the glucocorticoid-responsive DNA elements at promoter and enhancer regions of select genes. GRs interact with transcription factors as well as HATs and HDACs to modulate gene expression (Beato and Sánchez-Pacheco, 1996; Revollo and Cidlowski, 2009). Membrane bound GRs are also capable of signaling via the cAMP/PKA-signaling pathway, which results in the phosphorylation of CREB, which is considered a necessary step towards activating gene expression profiles necessary for long-term memory formation (Roozendaal et al., 2010; Kim et al., 2013; see Figure 1). Therefore, stress hormones and neuromodulators can work independently or in concert to produce changes in the molecular landscape inside cells and can engage gene expression required for long-term memory processes.

Both CRH and GRs are involved in the consolidation of stress-related memories (for a review, see McGaugh 2004; Roozendaal et al., 2007; Joëls et al., 2009). Post-training infusions of CRH into the amygdala enhanced inhibitory avoidance (Liang & Lee 1988). Immediate post-training bilateral infusions of CRH receptor antagonist [9 – 41]-α-helical CRH into the BLA, but not the adjacent central nucleus of the amygdala (CEA), impaired retention of inhibitory avoidance training (Roozendaal et al., 2002). Corticosterone (glucocorticoid released in rodents) has previously been shown to modulate the consolidation of emotional memory (de Kloet et al., 1999; McGaugh and Roozendaal, 2002; Sandi and Pinelo-Nava, 2007; Roozendaal et al., 2008). Roozendaal et al., (2010) built on those studies by further elucidating the mechanism underlying corticosterone's effect on memory consolidation. They showed that a systemic post-training injection of corticosterone enhanced object location and recognition memory in rats. Similar to HDAC inhibition discussed above, a subthreshold training (that does not lead to long-term memory) was

converted into an event that resulted in long-term memory formation (Roozendaal et al., 2010). The corticosterone-induced enhancements in object location and recognition memories were also coupled with increased acetylation in brain areas involved in those forms of memory (hippocampus and insular cortex, respectively). Increased acetylation is often a marker for a relaxed chromatin state that is permissive to gene expression (Kouzarides 2007). Therefore, these results suggest a potential role for chromatin modification in mediating the effects of stress on memory consolidation.

The observed increased acetylation following subthreshold learning paired with corticosterone delivery led Roozendaal et al. (2010) to examine whether there was an interaction between the memory enhancing effects of both HDAC inhibition and corticosterone administration. In addressing this question, they showed that memory enhancements in object recognition memory resulting from HDAC inhibition required GR signaling and protein kinase A activity. Inhibition of either GR or PKA activity also completely blocked any memory enhancements by corticosterone (Roozendaal et al., 2010). Together, these results suggested that GR signaling via PKA would engage CREB-CBP mediated gene expression during memory consolidation.

It had previously been shown that although HDAC inhibition results in increased acetylation, the memory enhancing effect is dependent an interaction between phosphorylated CREB and the HAT CBP (Vecsey et al., 2007). Therefore, Roozendaal et al. (2010) explored whether mice with a CBP mutation would show the aforementioned memory enhancements after corticosterone administration. *Cbp* mutant mice, carrying a triple point mutation in the phospho-CREB (KIX) binding domain of CBP ($CBP^{KIX/KIX}$ mice), have impaired long-term memory formation (Wood et al., 2006). And while hippocampal slices treated with TSA from wildtype mice show enhanced LTP, slices from $CBP^{KIX/KIX}$ mice treated with TSA showed no such enhancements (Vecsey et al., 2007). Roozendaal et al. (2010) showed that an immediate post-training corticosterone injection ameliorated the long-term memory deficits exhibited by $CBP^{KIX/KIX}$ mice for object recognition (hippocampus-independent task), but not object location long-term memory (hippocampus-dependent task). This suggests that the corticosterone-dependent memory enhancement in object location memory requires CREB:CBP-dependent gene expression. Yet, corticosterone-dependent enhancement of object recognition does not require CREB:CBP. Together, these findings highlight the important differences in the molecular mechanisms underlying corticosterone-mediated regulation of memory formation in different brain regions.

A functional interaction between CREB and the HAT CBP is required for corticosterone-dependent memory enhancement in object location memory, suggesting CBP-mediated histone acetylation is pivotal for corticosterone effects on memory. Increased acetylation of histone H3K14 was associated with enhanced long-term memory in brain areas associated with object location and recognition tasks (Roozendaal et al., 2010). In addition, GRs have been shown to enhance ERK1/2 dependent kinases MSK1 and Elk-1 and ultimately, an increase in acetylation at histone H3K14 after a forced swim task (Gutiérrez-Mecinas et al., 2011). This increased acetylation at histone H3K14 was linked to increased c-Fos induction in dentate neurons (Gutierrez-Mecinas et al., 2011). Histone acetylation at H3K14 was

found to require GR activity, as pretreatment administration of GR antagonists abolished the changes in acetylation generated by the forced swim task (Bilang-Bleuel et al., 2005; Chandramohan et al., 2007). Therefore, GR signaling appears to result in increased histone acetylation, likely leading to facilitated gene expression and/or maintained gene expression that ultimately leads to formation of powerful memories for stressful events.

HDAC inhibition also requires the interaction between CREB and CBP in order to enhance memory for contextual fear (Vecsey et al., 2007) as well as object location (Stefanko et al., 2009; Barrett et al., 2011). The 1.0 mg/kg dose of corticosterone used in Roozendaal et al. (2010) produces plasma corticosterone levels akin to a moderate level of stress (de Quervain et al., 1998; Okuda et al., 2004). It suggests that a moderate level of stress signaling is capable of removing the HDAC brake pads to enhance memory formation in a CREB:CBP-dependent manner (as observed under conditions of HDAC inhibition alone; best demonstrated in study by Haettig et al., 2011). In summary, results from the Roozendaal et al. (2010) study provide support for the idea that GR signaling can remove the molecular brake pads to memory formation and perhaps even lead to the persistence of memory.

Which HDACs might be involved? We speculate that HDAC3 would be a primary target of stress induced signaling, especially via GR-mediated signaling. HDAC3 has been found to be in complexes with CBP and both enzymes have opposing action on shared histone substrates. Thus, HDAC3 is a likely target, but other HDACs may also be involved depending on the cell type, duration of stress, type of stress, etc. There is obviously much more work to be done in this area of behavioral epigenetics and understanding exactly how epigenetic mechanisms are involved in stress modulated memory processes. At the basic level, we hardly understand how the epigenome and chromatin architecture change with different durations and forms of stress. There are striking studies showing that early life experience can have long-lasting effects via epigenetic mechanisms on CRH expression in the hypothalamus leading to altered synaptic connectivity in CRH-expressing cells (reviewed in Karsten and Baram, 2013), as well as paternal and maternal stress (and thus prenatal stress in offspring) can result in reprogramming of offspring stress pathways (reviewed in Bale 2011; Rodgers et al., 2013). Thus, developmental periods also must be included in a complete understanding of how stress and different forms of stress modify the epigenome and lead to persistent changes in neuronal function and ultimately behavior.

Summary

In conclusion, although much more work is necessary to completely understand underlying mechanisms, it appears that HDACs are key negative regulators of memory formation and perhaps even the persistence of long-term memory. Furthermore, stress-induced signaling may remove HDACs in an abnormal manner that drives the transformation of subthreshold learning events into robust long-term memory and persistent long-term memories. As there is a constant interplay between HDACs and HATs (especially as indicated by HDAC inhibition or deletion of specific HDACs that leads to a significant increase in histone acetylation by HATs), there is obviously a key role for HATs in these mechanisms. Both corticosterone and HDAC inhibition require CREB:CBP interaction, and result in increased acetylation, presumably changing gene expression dynamics (as discussed in McQuown et

al., 2011). Are these mechanisms underlying the persistent maladaptive memories that characterize PTSD? Can these same mechanisms be used for the persistent extinction of such memories? These are important questions for future studies.

Currently, the far majority of clinical trials for PTSD involve cognitive behavioral treatment (CBT) and exposure therapy. These rely on habituation to cues or extinction of cues associated with PTSD symptoms (*e.g.* re-experiencing symptoms, avoidant symptoms, and symptoms of increased arousal). At least one clinical trial is examining the ability of D-cycloserine (DCS), an antibiotic that has been shown to facilitate memory and extinction of memory (reviewed in Davis et al., 2006), to improve outcome of CBT and exposure therapy. This would presumably be by facilitating extinction processes engaged by CBT and exposure therapy. It is worth speculating that as selective HDAC inhibitors progress in their development and our understanding of their effects, they too could ultimately be used to facilitate extinction when used in combination with CBT and exposure therapy. Perhaps this approach would even lead to persistent extinction of otherwise intrusive and maladaptive memories characterizing PTSD symptoms. Similar approaches could even be envisioned with regard to addiction and the extinction of cues eliciting drug-seeking behaviors. These are exciting times with regard to understanding how individual experience and the environment impact our epigenome, change neuronal plasticity, and ultimately have long-lasting effects on behavior. The more we understand at the basic level, the sooner we will be able to adapt this understanding to therapeutic development that has the potential to revolutionize diagnosis, prognosis, and treatment of numerous disorders.

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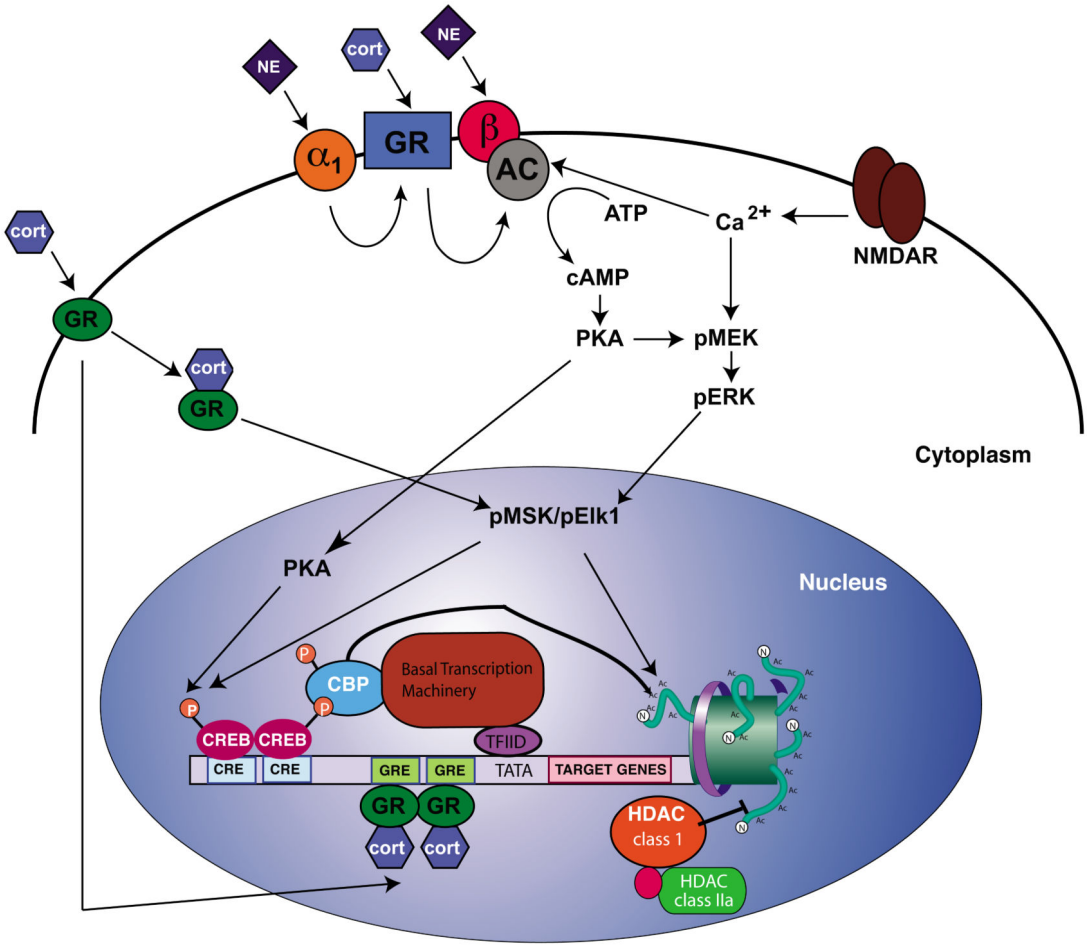

Figure 1. Modulation of transcription by the stress-dependent signaling

HDACs and associated corepressors (the molecular brake pads) act to suppress gene expression necessary for long-term memory formation and maintenance (McQuown and Wood, 2011). However, a stress-dependent signal of sufficient strength is capable of transiently removing the brake pads to permit gene expression required for persistent long-term memory. Stressful events trigger the release of cortisol (cort) and norepinephrine (NE) that bind to their respective receptors on post-synaptic neurons. Cortisol binds to membrane-bound glucocorticoid receptors (GRs) that in association with NE-bound adrenergic receptors (α1 and) lead to the activation of adenylate cyclase (AC) and the cAMP/PKA-signaling pathway. This ultimately results in the phosphorylation of CREB (Roozendaal et al., 2010). PKA is also capable of initiating the ERK/MAPK signaling cascade (pMEK/perk/pMSK/pElk1) to phosphorylate CREB. CREB binding protein (CBP; a HAT), once associated with phosphorylated CREB, interacts with the basal transcription machinery to acetylate histones thereby promoting transcription. Cortisol can also bind to nuclear hormone glucocorticoid receptors (GRs) that translocate to the nucleus where they can have several effects. GRs dimerize and bind to glucocorticoid-response elements (GREs) within regulatory and promoter regions. If GREs are in close proximity to the TATA box then GRs can promote the binding of transcription factor II D (TFIID) and the basal transcription machinery. During this process, the molecular ‘brake pads’ (HDACs and associated co-

repressors) are released from the promoter, allowing histone acetylation. It is hypothesized that GR removes the molecular ‘brake pads’ to persistent long-term memory formation similar to the effect of HDAC inhibitors (HDACi) on class I HDACs. The combined action of these various interacting signaling cascades is to regulate transcription required for the formation and maintenance of long-term memory.