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Epigenetics: A Possible Mechanism of Memory

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EPIGENETICS: A POSSIBLE MECHANISM OF MEMORY

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INTRODUCTION

The mind-body connection has fascinated philosophers and scientists for centuries. How is it possible that consciousness arises from a lump of matter known as the brain? How does neurons' firing affect choice and beliefs? How do the electrochemical properties of the brain allow for the memory of events long after they've occurred? One of the most studied of these areas is that of memory. Researchers seek to understand the biological basis behind memory and how that biology is affected in individuals suffering from memory disorders.

Why is memory so difficult to comprehend from a biological standpoint? There are several facets of memory that must be satisfied by its biological mechanism: acquisition, consolidation, and extinction. To acquire a memory, the brain must first be stimulated by the environment. The outside world must be able to influence the biology behind memory. Once acquired, the memory must have the ability to become permanent, or consolidated, to accommodate long-term memory. The biological mechanism must be switched on by the environment, and remain on. Last, once memories are present, there must be a mechanism that allows them to be forgotten (otherwise known as extinction). These three requirements must be found in any biological mechanism that seeks to explain memory.

Recently, researchers have begun exploring the field of epigenetics in relation to memory. Epigenetics (literally "over genetics") is a term coined by Conrad H. Waddington to describe regulation of genetic expression by modifications of the genome that are independent of DNA sequence (Reichenberg et al. 2009; Sananbenesi and Fischer 2009). Originally, epigenetics was only used to describe stable, heritable modifications. Today, though, this distinction has blurred with the realization that epigenetic factors can be both transient and dynamic, occurring fleetingly in response to environmental factors (Day and Sweatt 2011). This is especially true in the field of cognitive epigenetics, as it is this flexibility of epigenetics that enables it to act in cognition.

Epigenetics is well suited as a candidate mechanism to explain memory as it satisfies the three requirements of the biological mechanism of memory. The epigenome is dynamic and can change in response to environment, allowing the encoding of a memory in response to a stimulus. Once activated, it may be relatively stable, thereby storing the memory across time (Day and Sweatt 2011). Despite its relative stability, its dynamic nature does allow for change, thus ensuring the possibility of extinction of the memory. Epigenetics, therefore, may be able to encode, store, and allow for extinction of memory.

EPIGENETICS

Epigenetics encompasses several processes, which may be present separately or may interact to express complex phenotypes. The two factors that have garnered the most research in the field of memory are DNA methylation and histone modifications. Both factors modify the genome, influencing transcription.

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Chromatin

In order to adequately discuss DNA methylation and histone modifications, it is necessary to first examine the organization of the genetic code. The genome consists of three levels of organization: the actual DNA sequence, the histones around which DNA is wrapped, and the chromatin, the highest level. Changes at both the DNA level and the histone level may influence the transcription of the chromatin. Chromatin is divided into two categories, according to its degree of transcription: euchromatin and heterochromatin. Euchromatin is more open and transcriptionally active while heterochromatin is less accessible to transcriptional machinery and is, therefore, transcriptionally silenced (Nelson and Monteggia 2011; Sananbenesi and Fischer 2009). Epigenetic changes at either the DNA level or histone level can alter heterochromatin to become transcriptionally active, or euchromatin to become transcriptionally silenced.

DNA Methylation

The two major pre-transcriptional epigenetic modifications are those that occur at the DNA level, such as DNA methylation, and those that occur at the histone level, such as histone modification. DNA methylation consists of adding a methyl group to the 5' carbon of cytosine within CpG sequences (sequences of cytosine-guanine nucleotides). These CpG sequences often repeat within the genome, especially within the promoter regions of genes, and are then known as CpG islands (Lubin 2011).

Methylation and Demethylation: Mechanisms

DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which transfer a methyl group from S-adenosylmethionine to the 5' carbon of cytosine (Lubin 2011). There are three distinct DNMTs in humans: DNMT1, which is primarily involved in ensuring that the methylation pattern of DNA is conserved during DNA replication, and DNMT3a and DNMT3b, which are able to methylate DNA *de novo*, at a previously unmethylated position (see Figure 1) (Nelson and Monteggia 2011). DNMTs are selective in their methylation, only methylating specific cytosines. One possible selectivity mechanism is that DNMT1s are targeted towards hemimethylated CpG islands, or islands that have methyl groups attached on one side. A second targeting technique is that of histone modification marks, which

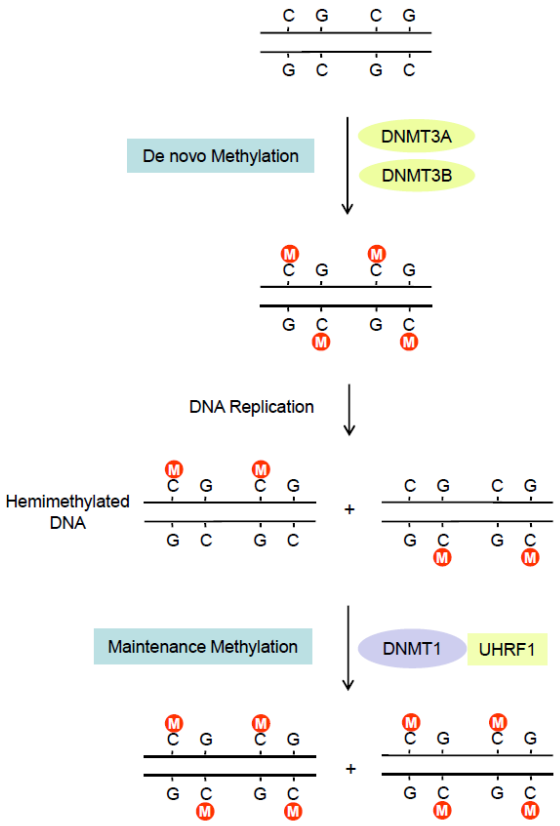

Figure 1: De novo methylation occurs via DNMT3a and DNMT3b, while maintenance replication occurs via DNMT1, which targets hemimethylated DNA. Source: Yu et al. 2011

will be discussed shortly. Yet a third possibility involves small non-coding RNAs, although this has not been described yet in mammals (Yu et al. 2011). This selectivity of DNMTs facilitates the creation of a methylation pattern that can be tissue-specific and event-dependent.

Methylation via DNMTs is a clear and well-known process; however, scientists are still investigating whether that process is reversible, allowing demethylation to occur (see Figure 2). While passive demethylation (i.e. demethylation due to a lack of DNMT activity) is widely accepted, active demethylation remains a puzzle. Methylation creates a covalent bond; as such, it is considered the most stable epigenetic mark (Yu et al. 2011). Over the past decade, though, there have been several reports of active demethylation.

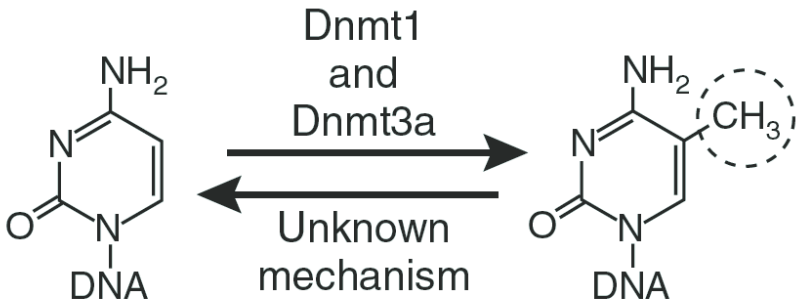

Figure 2: Cytosine group is methylated by DNMT1 and DNMT3a, as well as DNMT3b (not shown in figure) and demethylated by an unknown mechanism, although there are some possible explanations for this mechanism. Source: Korzus 2010

Two candidate mechanisms for active demethylation exist. One is an excision repair-based mechanism involving growth arrest and DNA damage-inducible (Gadd45α and Gadd45β) proteins. A recent study found that overexpression of Gadd45 proteins was correlated with global DNA demethylation while Gadd45 knockdown was correlated with hypermethylation. Presumably, Gadd45 proteins recruit DNA damage repair machinery, which replaces the methylated cytosine with an unmethylated cytosine (Barreto et al. 2007). A second possibility is that ten-eleven translocation 1 protein causes demethylation by converting 5-methylcytosine to 5-hydroxymethylcytosine, which leads to demethylation (Yu et al. 2011). Although it appears that active demethylation occurs, further research is required to elucidate its exact mechanism.

Regulation of Methylation

Methylation is regulated by DNMTs; however, how are DNMTs regulated? The exact mechanisms that regulate the expression of DNMTs are currently unclear (Nelson and Monteggia 2011). It is known, however, that DNMTs are actively regulated across different regions of the body and across a lifetime. Both DNMT1 and DNMT3a are expressed differentially in cortical neurons, especially in interneurons, in the brain of human adults. Fascinatingly, the expression of DNMT3a is greater during embryogenesis and then declines into adulthood while the expression of DNMT3b increases into adulthood (Lubin 2011). This active regulation of DNMTs indicates the dynamic nature of DNA methylation.

Impact of Methylation

DNA methylation generally causes transcriptional repression through two possible mechanisms (see Figure 3). First, it can act as a docking site for proteins that contain a methyl-binding domain, such as the methyl-CpG binding protein2 (MeCP2). These proteins, especially MeCP2, can recruit histone-modifying enzymes that aid in the formation of heterochromatin (Nelson and Monteggia 2011). Second, the methylated cytosine residues hinder transcription by repelling transcriptional activators (Yu et al. 2011). The usual result of DNA methylation is, therefore, transcriptional repression.

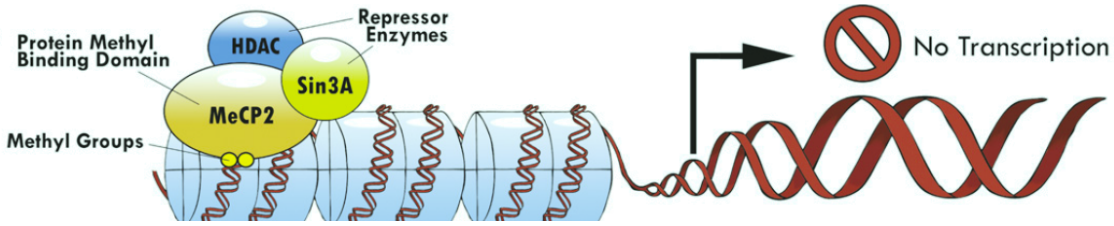

Figure 3: DNA methylation causes MeCP2 to bind to DNA, which recruits repressor enzymes, suppressing transcription. Source: Lubin 2011

Histone Modifications

The second major pre-transcriptional epigenetic regulation occurs at the histones, through histone modification. Before examining histone modifications, further explanation regarding the nature of a histone is necessary. A histone is a small, highly basic protein consisting of a globular domain and a flexible N-terminus. The histone binds DNA, which is wrapped around it. Eight histones, linked as an octamer, form a nucleosome, which consists of two molecules of each core histone: H2A, H2B, H3, and H4. The N-termini of the histones protrude from the nucleosome and are referred to as "histone-tails" (Sananbenesi and Fischer 2009). It is these histone tails that can be modified by the addition of one of several molecular groups: acetyl, methyl, phosphate, SUMO, or ubiquitin. Each of these molecular groups influences transcription differently (Mikaelsson and Miller 2011).

Modification: Mechanism

The modification of the histone tail by each group is catalyzed by a different group of enzymes. The only class of enzymes that is explored in much detail in terms of memory is that of the acetyl group. The acetyl group consists of histone acetyltransferases (HATs), which add the acetyl group, and histone deacetyltransferases (HDACs), which remove an acetyl group (see Figure 4) (Nelson and Monteggia 2011). HDACs form a significant part

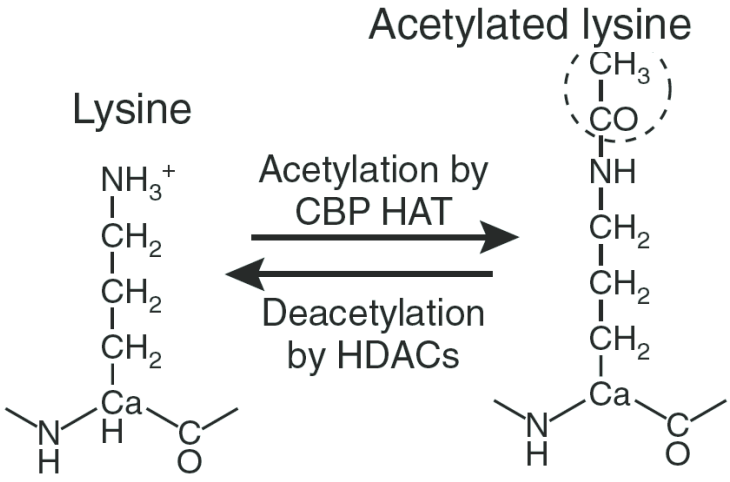

Figure 4: Acetylation of histone tails occurs via HATs, and deacetylation occurs through HDACs. Source: Korzus 2010

of cognitive epigenetics. Two classes of HDACs are Class I HDACs (HDACs 1, 2, 3, 8) and Class II HDACs, which can be further divided into Class IIa (4, 5, 7, 9) and Class IIb (6, 10). Class I HDACs are generally localized to the nucleus, although HDAC3 can be found in both the cytoplasm and the nucleus. These HDACs are highly expressed in the mature neuron, with HDAC3 having the highest expression levels in the hippocampus, cortex, and cerebellum (Haggarty and Tsai 2011; McQuown and Wood 2011). Class II HDACs shuttle between the nucleus and cytoplasm in response to phosphorylation signals and are more specifically expressed (Nelson and Monteggia 2011). Much interaction occurs between HDACs. HDAC3, for instance, can interact with HDACs 4, 5, 7, and 10 (Haggarty and Tsai 2011).

HDACs can be inhibited using small molecule probes called HDAC inhibitors, or HDACis. These probes inhibit histone deacetylase activity, thus increasing histone acetylation and generally increasing gene upregulation. Three classes of probes that have been explored in terms of memory are carboxylic acids (e.g. butyrate and valproate), hydroxamic acids (e.g. trichostatin A and suberoylanilide hydroxamic acid), and ortho-aminoanilines (e.g. MS-275) (Haggarty and Tsai 2011). Potentially, HDACis can be administered therapeutically to change the histone acetylation level.

Impact of Modification

Each group has a different effect on transcription. Acetyl and phosphate groups, which are negatively charged, reduce the affinity of the histone tail for the negatively charged DNA backbone, so the DNA has a more "open" conformation and is more easily transcribed (Lubin 2011). Additionally, acetyl groups facilitate recruitment of bromo-domain coactivators, such as chromatin-associated proteins and histone acetyltransferases, which bind the acetyl-lysine motifs and promote transcription (McQuown and Wood 2011). SUMOylation and ubiquitination have the opposite effect on transcription (Lubin 2011). Methylation, on the other hand, can activate or repress transcription depending on the methylation site and the number of methyl groups that are added. It influences transcription by serving as a docking site for activator/repressor proteins to restructure chromatin, not through its electrical charge (Lubin 2011). The varied effects of the groups on transcription allow for a large degree of precision in epigenetic regulation.

Cross-talk Between DNA Methylation and Histone Modification

DNA methylations and histone modifications often interact, producing interesting results. First, DNA methylation may change the pattern of histone modifications. Methylated CpG islands recruit proteins that interact with HDACs to mediate repression of target groups. Second, histone marks can target DNMTs to specific DNA sequences. This effect is demonstrated at the unmethylated histone H3K4, which becomes a docking site for DNMTs, resulting in *de novo* DNA methylation and transcriptional repression (Lubin 2011). Third, histone modifications are often interdependent. A hypoacetylated H3 tail is targeted in phosphorylation of H3S10 residues, and the phosphorylation of H3S28, methylation of H4K20, and dimethylation of H3K4 are interdependent on HDAC3 (McQuown and Wood 2011).

Memory and Synaptic Plasticity

Even with an understanding of epigenetics, an understanding of memory is still required. Memory on a cellular and molecular level is a much studied but little understood phenomenon. The accepted model of memory claims that memories are encoded as connections between neurons. In this model, neuronal connections are altered as new memories are encoded, either by growing new dendrites or altering synaptic strength (Squire 2011). This model is known as synaptic plasticity.

Long-term potentiation (LTP) and long-term depression (LTD) are two examples of synaptic plasticity. LTP refers to the enhanced synaptic transmission between two neurons, resulting from a short burst of high-frequency stimulation of the presynaptic fibers. LTD can be seen as the opposite of LTP; it involves decreased synaptic transmission due to low-frequency stimulation of the presynaptic fibers (Squire 2011). Both phenomena are persistent, lasting for hours or even weeks, yet they are triggered by a transient stimulus (Squire 2011). LTP, LTD, and long-term memory (LTM) share this feature of a short stimulus affecting long-term neuronal change, as well as input specificity and an inability to be maintained in the presence of protein synthesis inhibitors (Day and Sweatt 2011). Interestingly, enhancements or impairments in spatial memory are demonstrated in animals that have increases or decreases in hippocampal LTP, respectively (Nelson and Monteggia 2011). These shared features strongly suggest that LTP is involved in memory formation.

The next question that scientists ask involves the molecular changes that occur with LTP and LTM. The rapid turnover of proteins within a cell belies the long-term effects of LTP (Day and Sweatt 2011). There must be some kind of maintenance molecule to overcome the loss of acquired changes (Yu et al. 2011). Crick postulated in 1984 that this maintenance molecule would form multimers or at least dimers, with each monomer able to exist in a modified (+) or unmodified (-) mode. If a monomer is (+), then even if molecular turnover causes a newly synthesized linked monomer to be (-), the maintenance enzyme would alter it to become (+) (Yu et al. 2011). This feature matches the activity of DNMTs in methylation of hemimethylated DNA perfectly (see above). Additionally, since both LTP and LTM cannot be maintained in the presence of protein synthesis inhibitors, it seems that changes in gene expression are a necessary component of memory formation (Day and Sweatt 2011; Nelson and Monteggia 2011). Together, these features suggest that epigenetic regulation may act to mediate LTP, and through LTP, memory formation.

Epigenetics and Memory

Evidence of Epigenetic Changes with Learning

The Global Epigenome

In the past decade, much evidence has shown that the epigenome changes in response to learning or memory events. The DNA methylation status of the genome changes drastically. According to Sananbenesi, exposing rats to fear conditioning causes a brief rise in the hippocampal levels of DNMT3a and DNMT3b, presumably changing DNA methylation levels (Sananbenesi and Fischer 2009). This was found to be true in a study performed by Feng and his colleagues that found a global 20% demethylation in DNMT1 and DNMT3a conditional double knockout mice that exhibited memory impairments. That study also demonstrated that 84 genes were upregulated more than 1.5-fold and 7 genes were downregulated more than 1.5-fold in

these double knockout mice (Feng et al. 2010). It has been shown that contextual fear conditioning triggers *de novo* DNMT gene expression in the adult hippocampus (Lubin 2011). DNA methylation appears to be affected in learning and memory.

Besides causing changes in DNA methylation, learning and memory events appear to alter histone modifications. According to Mikaelsson, novel taste learning in rats resulted in heightened acetylation of histone H2A and H4 in the insular cortex (Mikaelsson and Miller 2011). Rats displayed a transient increase in histone acetylation 1 hour after exposure to fear conditioning (Mikaelsson and Miller 2011; Sananbenesi and Fischer 2009). Correspondingly, HAT activity, which serves to acetylate histones, increased in the amygdala following cued fear conditioning, an amygdala- and hippocampal-dependent form of associative learning (Nelson and Monteggia 2011). Learning and memory events appear to cause increased histone acetylation.

Other histone modifications altered by learning include phosphorylation and methylation. Increased histone H3 phosphorylation occurs at Ser10 residue in hippocampal area CA1 during the formation of contextual fear memory (Mikaelsson and Miller 2011). Contextual fear conditioning also increases histone H3 phosphoacetylation, H3K4 trimethylation, and H3K9 dimethylation in the hippocampus (Nelson and Monteggia 2011). Thus, increases in histone phosphorylation and methylation are involved in learning and memory formation.

BEHAVIORAL PHENOTYPE OF EPIGENETIC CHANGES

Behaviorally, learning and memory appear to be dependent on DNA methylation and histone modifications changes. In the aforementioned study by Feng and his colleagues (2010), it was shown that the DNMT double knockout mice, which had lost much of their ability to dynamically regulate DNA methylation levels, performed poorly in the Morris water-maze task, a hippocampus-dependent learning and memory task. Generally, DNMT double knockout mice had impaired spatial learning and memory ability. Other studies sought to determine the effect of the blockade of DNMT activity using DNA methylation inhibitors as opposed to mutated mice. It was found that inhibition of DNA methylation by intrahippocampal injections of 5-aza or zebularine (two DNMT inhibitors) severely impaired memory consolidation (Sananbenesi and Fischer 2009). These studies seem to prove DNMTs are necessary for long-term memory (LTM) formation.

A second vital epigenetic change in memory and learning is that of histone modifications. HATs, which act to acetylate histones, include the cAMP response element-binding (CREB)-binding protein (CBP) and P300. Both are necessary for hippocampal synaptic plasticity and long-term memory formation in both novel objects and contextual fear conditioning paradigms (Mikaelsson and Miller 2011; Sananbenesi and Fischer 2009). In fact, mouse models that lack CBP and its HAT function demonstrate attenuated histone acetylation as well as impaired LTM (McQuown and Wood 2011). LTM is seemingly dependent on HATs.

Since HATs appear to be necessary for LTM, one would expect HDACs to be negative regulators of LTM; this has been shown to be true in mutant mouse models. Mice deficient in HDAC5 show enhanced learning in cocaine conditioned place preference, while mice that overexpressed HDAC4 or HDAC5 demonstrated a weakened expression of cocaine conditioned place preference (McQuown and Wood

2011). Additionally, HDAC2 deficient mice experienced enhanced memory formation and synaptic plasticity while forebrain overexpression of HDAC2 (but not HDAC1) caused impaired memory formation and synapse formation (Nelson and Monteggia 2011). A study undertaken by McQuown and her colleagues demonstrated that HDAC3 deficiency enhanced LTM formation to such a degree that HDAC3 deficient mice that received subthreshold training (3 minutes) in a novel object recognition test did experience LTM formation, and those mice that had completely lost HDAC3 function retained this memory for seven days, which is longer than the normal retention of object memory (2011). It seems that HDACs negatively regulate LTM.

Several studies have sought to confirm that HDACs negatively regulate LTM by examining the effect of HDAC inhibitors (HDACis) in wild-type mice. The previously mentioned study by McQuown and her colleagues also found enhanced learning in wild-type mice that were injected with an HDACi, RGFP136 (2011). These mice experienced LTM even with subthreshold training conditions. A second study, performed by Vecsey and his colleagues (2007), proved that HDAC inhibition in the hippocampus enhances memory consolidation for hippocampus-dependent learning by microinjecting mice with trichostatin A (TSA), an HDACi, directly after conditioning, and measuring their level of freezing when exposed again to the conditioned context. Mice injected with TSA showed a notable enhancement in memory as compared to control groups. This study also ruled out HDACi inhibition enhancement of memory retrieval, as opposed to consolidation, by microinjecting the mice with TSA four hours before re-exposure to the conditioned context: results were comparable for experimental and cued conditioning (Vecsey et al. 2007). Additionally, the study repeated the protocol using non-hippocampal dependent conditioning in order to prove that the memory enhancement is due to the microinjections into the hippocampus; again, no differences were found between the TSA- and control-treated mice (Vecsey et al. 2007). It appears that HDACis do enhance LTM formation in wild-type mice.

HDACis have also been found to ameliorate loss of other epigenetic functions, such as those caused by inhibition of DNMTs or loss of HATs. DNMT inhibitors impair memory, but this effect appears to be reversed by administration of TSA, an HDACi, prior to the test (Day and Sweatt 2011). Loss of CBP, as mentioned above, impairs LTM, but not short-term memory, in a number of learning and memory tests. Administration of HDACis to *Cbp* mutant mice restores their memory function, probably because some CBP HAT activity remained active (Nelson and Monteggia 2011). Oddly enough, when the same HDACis were administered to CBP conditional knockout mice, which lacked any expression of CBP in excitatory neurons of the forebrain, no restoration of memory function was observed; this was most probably due to the complete deficiency of CBP (Nelson and Monteggia 2011). A similar effect was observed in the aforementioned study by Vecsey and his colleagues (2007). Memory enhancement was observed in $CREB^{+/+}$ mice that were injected with TSA but not in the CREBαΔ (CREB-deficient) mice that were injected with it. It appears that HDACis can act in a limited capacity to reverse memory impairment caused by deficiencies in other epigenetic functions.

Effect of Epigenetic Changes on LTP and LTD

After concluding that epigenetic changes heavily influence memory formation, the question is whether they influence synaptic plasticity (e.g. LTP and LTD). DNA methylation does appear to play a role in both LTP and LTD. A number of studies in mutant mouse models demonstrate that DNA methylation is necessary for LTM formation. In a study performed by Feng and his colleagues (2010), the mice that were deficient in DNMT1 and DNMT3a, showed attenuation of LTP and enhanced induction of LTD. Importantly, DNMT KO mice show no LTP after 1-2 hours, but the base response is still there, so the DNMTs must be acting in memory acquisition, not in initial synaptic transmission (Day and Sweatt 2011). One explanation of why DNA methylation is so significant in LTP focuses on MeCP2, one of the mechanisms by which DNA methylation acts to repress transcription. MeCP2 mutant mice show impairments in hippocampal LTP, hippocampal LTD, and cortical LTP, while MeCP2 overexpressing mice show enhanced hippocampal LTP. Apparently, DNA methylation may mediate LTP through MeCP2.

Other studies have sought to confirm that DNA methylation influences synaptic plasticity by examining the results of treatment with DNMT inhibitors. Brain slices treated with DNMT inhibitors show no LTP after 1-2 hours, but the base response is still there, so the DNMTs must not be influencing the synaptic transmission itself (Day and Sweatt 2011). Specifically, the LTP in hippocampal slices that were treated with the DNMT inhibitors zebularine and 5-aza was shown to be reduced in magnitude (Nelson and Monteggia 2011). DNA methylation is significant to synaptic plasticity.

Histone acetylation, as well, appears to play a role in synaptic plasticity, as studies using mutant mice models demonstrate. HDAC2 overexpressing mice showed impaired hippocampal LTP (Nelson and Monteggia 2011) as well as decreased dendritic spine density and synapse number (Haggarty and Tsai 2011), while HDAC2 forebrain-specific KO mice showed enhanced LTP (Nelson and Monteggia 2011) and increased synapse number (Haggarty and Tsai 2011). Additionally, heterozygous *Cbp* mutant mice showed impaired hippocampal late-phase LTP with a normal stimulation protocol; however, with a stronger stimulation protocol, no impairment was observed. Histone acetylation is crucial for synaptic plasticity.

Several studies have sought to confirm the involvement of epigenetic changes in synaptic plasticity, using the effects of HDACis on wild-type mice. The study performed by Vecsey and his colleagues (see above) demonstrated that HDACis enhanced LTP in hippocampal slices from wild-type mice (Vecsey et al. 2007). Their controls included mice that were injected with TSA and actinomysin D, a substance that prevents transcription, to prove that HDACis act through transcription-dependent mechanism. Most HDACis appear to have this effect on LTP. Treatment of hippocampal slices with TSA and sodium butyrate, an HDACi, resulted in enhanced LTP induction at Schaffer-collateral synapses, while treatment of amygdala-containing slices with TSA resulted in enhancement of forskolin-induced LTP (Nelson and Monteggia 2011). Last, treatment of hippocampal slices with suberoylanilide hydroxamic acid enhanced late-phase LTP in wild-type mice but had no effect on HDAC2 KO mice, showing that HDACis are effective due to their effect on HDACs

(Nelson and Monteggia 2011). HDACis do enhance LTP in wild-type mice, confirming a role for histone acetylation in LTP.

Other studies examined the ability of HDACis to compensate for the effects of other epigenetic deficiencies. HDACis compensate for the loss of DNMT activity through DNMT inhibitors; treatment with TSA prior to testing reverses the effect of DNMT inhibitors on LTP (Day and Sweatt 2011). Additionally, HDACis can somewhat attenuate the effects of a loss of CBP function. The treatment of hippocampal slices with suberoylanilide hydroxamic acid does ameliorate the LTP deficit generally observed in $Cbp^{+/-}$ mice (Nelson and Monteggia 2011). However, TSA treatment of CREBαΔ mice and mice with a genetic disruption between CREB and CBP did not enhance LTP. Apparently, HDACis can compensate somewhat for the loss of other epigenetic changes.

MECHANISM OF EPIGENETIC CHANGES AND MEMORY

Now that it is clear that epigenetic changes are involved in memory, the next step is to determine how they influence memory. Intriguingly, as explained above, memory appears to be dependent on both histone acetylation and DNA methylation (see Figure 5). This is rather incongruous as histone acetylation increases transcription while DNA methylation decreases transcription. The apparent inconsistency can be explained by viewing these epigenetic modifications as gene-specific, so that histone acetylation upregulates some genes whereas DNA methylation downregulates other genes. There are many genes, as well as some non-histone substrates of histone-modifying enzymes, that are regulated by these epigenetic changes.

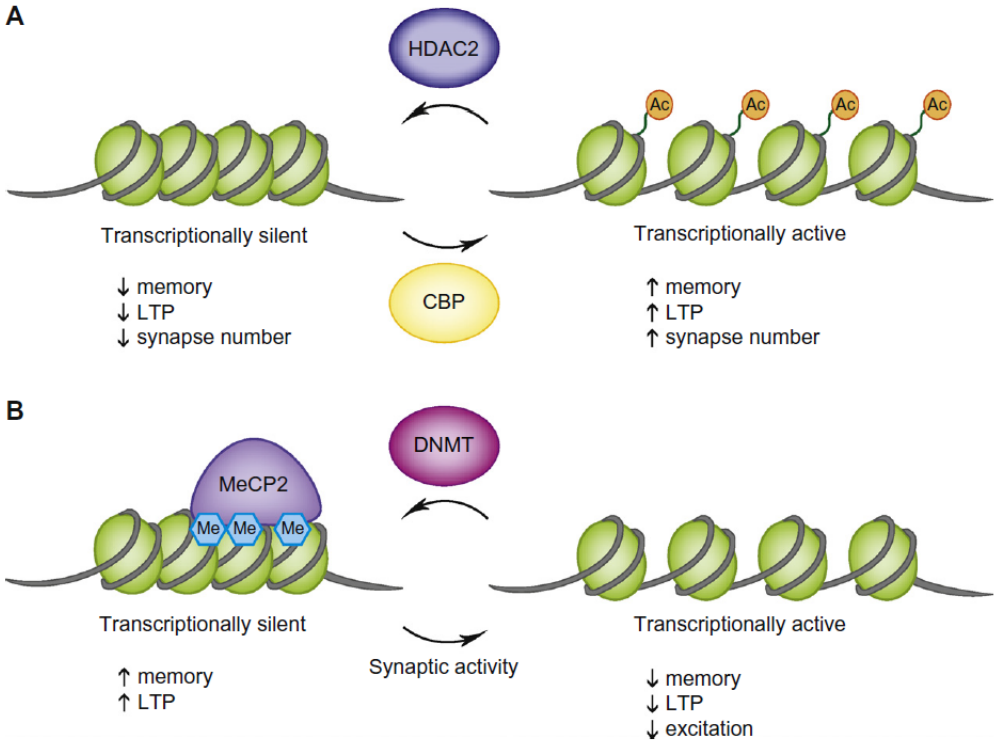

Figure 5: Histone acetylation and DNA methylation are necessary for memory formation and LTP. Histone deacetylation and DNA demethylation impair memory formation and LTP. Source: Nelson and Monteggia 2011

Epigenetic Proteins

Interestingly, several enzymes that are instrumental in epigenetics are themselves regulated by epigenetics. First, DNMT1, the enzyme that methylates DNA, is itself a target of histone methylation enzymes (Lubin 2011). Second, HDAC1, which acts to deacetylate histones, is a substrate of histone acetylation enzymes (Lubin 2011). Third, the HATs p300, CBP, and p300/CBP-associated protein, are their own targets; each can act to acetylate itself, a process known as auto-acetylation (Lubin 2011). They can also be deacetylated by HDAC3, in combination with a corepressor known as nuclear receptor co-repressor 1 (McQuown and Wood 2011). Histone-modifying enzymes modify both DNA methylation enzymes and other histone-modifying enzymes.

Memory Genes

Several genes that are known to be involved in memory are regulated via epigenetics. The promoter of *reelin*, which enhances LTP, shows a robust response when exposed to DNMT inhibitors while learning (Mikaelsson and Miller 2011). DNMT inhibitors also modify DNA methylation in the adult brain at the promoter of *bdnf*, a gene that is crucial for memory (Day and Sweatt 2011). Electroconvulsive treatment, which causes LTP, decreases the methylation level of specific regulatory regions of *bdnf* (Yu et al. 2011). Interestingly, *bdnf* promoters are differentially methylated in memory. *Bdnf* exon I promoter is demethylated by chronic network activity caused by picrotoxin treatment of cultured neurons, as well as contextual exposure to living animals; the methylation level of this promoter is correlated with object recognition memory task performance (Yu et al. 2011). *Bdnf* exon IV promoter, which is generally basally-repressed by MeCP2, becomes demethylated and, thereby, expressed following high potassium induced neuronal depolarization in rodent primary neuron culture (Yu et al. 2011). Interestingly, *bdnf* demethylation may be active, caused by Gadd45β, as discussed above, since Gadd45β-KO mice displayed no significant demethylation at regulatory region of *bdnf* exon IX in response to electroconvulsive treatment, thus downregulating *bdnf* expression (Lubin 2011). Both *reelin* and *bdnf* are regulated by DNA methylation.

Bdnf is also regulated by histone modifications. Fear conditioning causes the upregulation of *bdnf* exons I and IV, which is associated with increased histone acetylation and phosphorylation at those promoters. Additionally, extinction of fear conditioning in mice is associated with an increase in histone H4 acetylation around the promoter of *bdnf* exon IV. *Bdnf* is regulated epigenetically to influence LTM formation.

Two memory suppressor genes, *Re1n* and *Pp1* are also regulated by DNA methylation. Interestingly, although DNMT inhibitors upregulate *Re1N* and *Pp1*, DNMT1 and DNMT3a conditional double knockout mice have normal expression of both genes (Feng et al. 2010; Nelson and Monteggia 2011). PP1 may actually participate in regulating other epigenetic modifications at the promoters of *Creb* and *nuclear factor-kappa B (NF-κB)*. *Pp1* and *Re1N* may be epigenetically modified during memory formation.

Transcription Genes

Several genes that are involved in transcription experience epigenetic modification during memory formation. *Nr4a1* and *Nr4a2*, which are both immediate

early genes (IEGs), acting to transcribe other genes, are regulated by histone acetylation. After TSA-induced memory enhancement, CBP-dependent expression of *Nr4a1* and *Nr4a2* occurred (Vecsey et al. 2007). Additionally, increased *Nr4a2* expression was observed in the area of focal HDAC3 deletion in the dorsal hippocampus two hours after subthreshold training (McQuown and Wood 2011). Remarkably, the silencing of *Nr4a2* through small interfering RNA attenuates the memory enhancing effects of HDAC3 deficiency in novel object memory (Haggarty and Tsai 2011), indicating that *Nr4a2* may interact with CBP and/or HDAC3 in their epigenetic roles, besides for being regulated by them.

A second IEG is *Egr1*, which is also influenced by histone acetylation. *Egr1* is upregulated in the hippocampus by associative learning. This appears to be mediated by BDNF, which causes HDAC2 to leave chromatin. H3 and H4 in the *Egr1*promoter are thereby acetylated, causing transcription. The expression of *Egr1* is affected by histone acetylation.

A third transcription factor is NF-κB, previously discussed in the context of PP1, which may act to regulate it. The promoter of *NF-κB* experiences reduced phosphorylation during novel object recognition (Mikaelsson and Miller 2011). NF-κB itself has been implicated in the induction of synaptic plasticity and initial formation of LTM. One of its subunits, p65/Re1A, is actually activated as one of the non-histone substrates of histone-modifying enzymes. It is the target of both histone methylation and histone acetylation enzymes (Lubin 2011). HDAC2 acts to negatively regulate it, and treatment with TSA results in prolonged p65 acetylation with a resulting increase in NF-κB DNA binding activity as well as enhanced memory formation. This enhancement in memory is attenuated by inhibitors of NF-κB DNA binding activity, indicating that this may be a mechanism whereby HDACis cause enhanced memory formation (Lubin 2011). NF-κB is regulated epigenetically to influence memory formation.

Other IEGs and transcription factors include *c-Fos*, transcription factor p53, and MEF2. Increased *c-Fos* expression was noted in the area of focal HDAC3 deletion in the dorsal hippocampus two hours after subthreshold training (McQuown and Wood 2011). Transcription factor p53 is a non-histone substrate of histone-methylating and histone-acetylating enzymes (Lubin 2011). MEF2, a transcription factor important for regulation of structural plasticity genes, can be deacetylated by HDAC3, thus terminating the transcription of plasticity genes.

Immune Function Genes

Interestingly, several genes that are involved in immune function may be involved in memory as well, including *MHC 1*, *Stat1*, and *calcineurin*. *MHC 1* is highly upregulated in DNMT double knockout mice (Feng et al. 2010). *Stat1*, which is important for synaptic function in CNS and learning/memory, is also highly upregulated in neuronal cells of DNMT double knockout mice (Feng et al. 2010). *Calcineurin* is regulated by a methylation change that occurs in contextual fear conditioning (Yu et al. 2011). These genes may be involved in signaling pathways that influence memory formation.

Other Non-histone Targets of Epigenetic Enzymes

There are several other non-histone substrates of chromatin-modifying enzymes. The estrogen receptor alpha is targeted by both histone methylation and histone acetylation enzymes. Both tubulin and the glucocorticoid receptor are substrates of histone-acetylating enzymes. The function of these proteins in memory is unclear.

Regulation of Epigenetic Changes in Memory

Epigenetics clearly influences memory via regulation of genetic transcription. How, though, is epigenetics itself modulated by the physical cause of memory, neuronal stimulation? Clearly, increased synaptic activity triggers DNA methylation changes and histone modification changes. N-methyl-D-aspartate receptor activation is a crucial part of the signaling pathway. It activates MAPK signaling which is instrumental in hippocampal H3 acetylation (Sananbenesi and Fischer 2009). The ERK/MAPK pathway is crucial for heightened acetylation of H2A and H4 in insular cortex due to novel taste learning in rats, histone acetylation associated with hippocampus-dependent fear memory, and histone H3 phosphorylation during formation of contextual fear memory (Mikaelsson and Miller 2011). Additionally, N-methyl-D-aspartate receptor activation is actually linked to both *bdnf* DNA methylation and changes in the levels of histone H3K4me3 at the *bdnf* promoter IV in response to contextual fear conditioning (Lubin 2011). Other pathways that may be involved in DNA methylation mediation are the protein kinase C and NF-κB pathways (Lubin 2011). Synaptic activity may act through N-methyl-D-aspartate receptor activation to initiate signaling cascades that cause epigenetic modifications to occur.

Future Directions

Although it is clear that epigenetics is heavily involved in memory formation, there are still many questions that must be clarified. These questions can be classified into three categories: those regarding memory alone, those involving epigenetic changes and memory, and those investigating therapeutic potential of epigenetics. According to Haggarty (2011), it is crucial to understand the roles of the individual genes implicated in memory in order to comprehend the ways in which epigenetic modifications affect them. Before understanding memory, it is unfeasible to understand how epigenetics affects memory.

A second direction is to examine the ways in which epigenetic modifications affect memory. One problem involved is that of cross-talk between epigenetic modifications (e.g. histone acetylation affecting DNA methylation, etc.). Researchers should undertake to study the epigenome and neuron as a whole, investigating all aspects of memory formation in order to understand how they interact (Haggarty and Tsai 2011). Another part of epigenetic modification that must be clarified is that of how a cell-wide modification affects synapse selectivity. Although there are several theories that attempt to explain this, no studies have examined it in depth (Day and Sweatt 2011). A third difficulty is that of differentiating between epigenetic modifications that are transient and activity-induced and can therefore be implicated in acquisition, and those that are more stable and likely involved in consolidation (Yu et al. 2011). Answering these puzzles is a significant step in understanding epigenetics.

The last class is that of examining the potential of HDACi as a therapeutic drug. The memory enhancement ability of HDACi seems to indicate its utility as a therapeutic drug for cognitive diseases such as Alzheimer's disease and Huntington's disease. Several questions must be answered, though, before it can be used clinically. First, the selectivity of different HDACis must be determined. Since non-histone substrates and histone substrates are involved, it is necessary to determine how different HDACis will affect each of them. Currently, most HDACis affect all HDACs, which may be too general for therapeutic utility. More research should be undertaken to find other, more specific, HDACis (Haggarty and Tsai 2011). Additionally, the absorption, distribution, metabolism, excretion, and pharmacokinetics of HDACis must be studied (Haggarty and Tsai 2011). Although HDACis have great potential, much must be answered before they can be used.

CONCLUSION

Epigenetic modifications play a large role in memory formation. They modify genetic expression of many genes and proteins that are involved in transcription and memory formation. Although it is still unclear how exactly epigenetics fulfills the requirements of a molecular mechanism of memory formation, it definitely holds much potential for future research and investigation of its role. The mind-body connection may be elucidated after all.

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