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The epigenetics of memory storage in the brain

R. Ryley Parrish,

Swati Gupta,

Farah D. Lubin

Department of Neurobiology, Evelyn F. McKnight Brain institute, The University of Alabama at Birmingham, Birmingham AL 35294-2182, USA.

Farah D. Lubin: flubin@nrc.uab.edu

Abstract

Epigenetics has been proposed as a molecular mechanism involved in encoding long-term memories. Specifically DNA methylation, an epigenetic mechanism thought to be static following cell differentiation, has been implicated as a dynamic transcription regulatory mechanism underlying the process of longterm memory storage. Now recent findings published in *Nature Neuroscience* explore the possibility that stable DNA methylation changes within the cortex contributes to memory maintenance.

The importance of gene transcription in the process of memory formation has been definitively established. Indeed, numerous studies show that coordinated activation and repression of memory-related genes in several brain regions are necessary for the proper storage of long-term memories. Additionally, protein synthesis has been conclusively implicated in the consolidation and storage of memory. Thus, investigation into the molecular and cellular processes responsible for regulating gene transcription changes necessary for long-term storage of memories has been the subject of great interest to cognitive neuroscientists.

In recent years, epigenetics has been implicated as a pivotal molecular mechanism orchestrating various transcription events at gene promoter sites in response to learning (reviewed in Jiang *et al.*, 2008). Such research has become a white-hot topic in neuroscience. Traditionally, epigenetics has been studied with respect to its role in development and was thought to be static in non-dividing cells and not subject to control by environmental influences. However, a large-body of work has established that epigenetic mechanisms mediate dynamic molecular changes within the central nervous system (CNS) in response to environmental stimuli (Alarcon *et al.*, 2004; Korzus *et al.*, 2004; Chwang *et al.*, 2006; Wood *et al.*, 2006; Bredy *et al.*, 2007; Fischer *et al.*, 2007; Lubin *et al.*, 2008). In the nervous system the two most characterized epigenetic mechanisms affecting chromatin remodeling of genes are posttranslational modification of histone proteins and the physical marking of DNA with methyl groups, of which the latter will be discussed in greater detail in the paragraphs below as it relates to activity-dependent gene regulation in the CNS.

DNA methyltransferases (DNMT) mediate the addition of methyl groups directly on to cytosine residue which is followed immediately by a guanosine residue. This nucleotide

pair is known as a 'CpG' site, which interestingly has a much higher occurrence at CpG island sites found within gene promoter regions than expected by chance (Bird, 2007). The presence of methyl groups on the cytosine can physically block transcription factors from binding and prevent the assembly of the transcription machinery (reviewed in Jiang *et al.*, 2008). Additionally, DNA methylation can also serve as a docking site for methyl binding proteins, which traditionally has been associated with transcriptional silencing of genes (reviewed in Jiang *et al.*, 2008). Conversely, recent work on activity-dependent regulation of genes in the brain demonstrate that DNA methylation can also serve to mediate activation of genes depending on the proximity of the cytosine methylated site to a transcription factor binding consensus sequence, such as the cAMP response element (CRE) binding site for the cAMP response element-binding (CREB) protein (Chahrour *et al.*, 2008; Gupta *et al.*, 2010). Thus these latest findings suggest that the occurrence of DNA methylation alone does not mediate active or repressed gene transcription, but that DNA methylation in context with the chromatin microenvironment determines regulation of gene expression.

A number of investigations in the recent decade have implicated DNA methylation as a molecular mechanism involved in synaptic plasticity and necessary for proper storage of long-term memories (Martinowich *et al.*, 2003; Colvis *et al.*, 2005; Levenson *et al.*, 2006; Jiang *et al.*, 2008; Lubin *et al.*, 2008; Nelson *et al.*, 2008; Gupta *et al.*, 2010). However, these studies have primarily focused on hippocampus-mediated synaptic plasticity and memory formation. Intriguingly, one model for long-term or remote memory storage and maintenance posits that hippocampal-dependent memories become independent of the hippocampus over time and are later stored in the cortex (Frankland *et al.*, 2004). Thus, the identification of DNA methylation events responsible for remote memory storage beyond the hippocampus has become of interest to the neurocognitive field.

In a recent brief communication in *Nature Neuroscience*, Sweatt and colleagues undertook a series of experiments to investigate the potential role of cortical DNA methylation in remote memory storage (Miller *et al.*, 2010). The authors focused their study in the dorsal medial prefrontal cortex (dmPFC), a cortical region of the brain important in remote memory recall that has also been implicated as a possible site of memory storage. The authors correlated contextual fear conditioning with DNA methylation changes at the promoter regions of three memory-related genes: *Zif268, Reelin*, and *Calcineurin* (CaN). In this learning paradigm, animals are trained to associate exposure to a new environment with a mild footshock. The training of adult male rats resulted in two different DNA methylation events in the dmPFC. First, increases in *Reelin* DNA methylation were observed in the dmPFC at 1 hour, 1 day, and 7 days in response to contextual fear conditioning compared to control groups (context-exposure alone and footshock alone relative to naive). Similarly, increases in *CaN* DNA methylation were also observed in the dmPFC at 1 day and 7 days post-training. Secondly, *Zif268* DNA methylation levels were significantly decreased in all control groups (context-exposure alone and footshock alone relative to naive). It is important to note that the latter event is surprising considering that *Zif268* activity is associated with neuronal plasticity (Hall *et al.*, 2001; Bozon *et al.*, 2003; Lee *et al.*, 2004). Thus, the *Zif268* DNA methylation changes observed in control groups relative to naïve animals would be expected in response to fear conditioning or learning about a novel context but not with the immediate footshock treatment. These results thus suggest the possibility of a different role for *Zif268*

activity in the dmPFC compared to that in the hippocampus. Nevertheless, these findings demonstrate learning-induced cortical DNA methylation changes that further implicate a role for DNA methylation events in system memory consolidation, or possibly memory storage.

Interestingly, prior investigations have demonstrated that inactivation of the Anterior Cingulate Cortex (ACC), a subregion of the dmPFC, at 1 and 3 days posttraining does not interfere with recent fear memory; however, inactivation at 18 and 36 days disrupts remote fear memory (Suzuki *et al.*, 2004; Frankland *et al.*, 2006). In support of these findings, Sweatt and colleagues argue that the study preformed by Frankland *et al.* indeed demonstrates that memory system consolidation occurs between 3 and 18 days of training. Sweatt and colleagues further conclude that the observed changes in cortical DNA methylation in their study are also correctly timed for memory consolidation. Intriguingly, alterations in DNA methylation were present as early as 1 hour (*Zif268* and *Reelin*) and 1 day (*Reelin* and *CaN*) following fear conditioning, suggesting that either these early DNA methylation events are not memory markers, or that the dmPFC/ACC is involved in both recent memory and memory retrieval. Indeed, there is evidence that the ACC might be involved in recent memory recall and consolidation as early as 1 day post-training (Zhao *et al.*, 2005; Blum *et al.*, 2006; Leon *et al.*, 2010). Regardless, the findings described by Sweatt and colleagues demonstrate that learning triggers DNA methylation changes in the dmPFC/ACC and supports DNA methylation events in the dmPFC/ACC as an epigenetic marker for system memory consolidation and possibly memory storage.

Although numerous studies suggest that memory storage is diffusely stored throughout the brain's cortical networks, the cortical regions involved in memory storage have not been entirely elucidated to date. Thus, it is possible that DNA methylation changes could promote memory storage in a transient fashion which then leads to long-lasting structural and molecular changes at the synaptic level. Alternatively, it is also possible that in order for DNA methylation to promote memory storage, it would need to be persistent in cortical tissue.

The ACC has been implicated in remote memory recall and as a possible site of memory storage (Suzuki *et al.*, 2004; Frankland *et al.*, 2006), but its role as a memory storage site has not been established. Therefore, Sweatt and colleagues further examined the presence of DNA methylation at *Zif268, Reelin*, and *CaN* gene promoters 30 days post fear conditioning in the ACC. The authors found a decrease in *Zif268* DNA methylation in all control groups (context-exposure alone and footshock alone relative to naive). Furthermore, they found a robust increase in *CaN* DNA methylation 30 days after contextual fear conditioning that was specific to the associative learning paradigm. The increase in *CaN* DNA methylation in the ACC at 30 days corresponded with a significant decrease in *CaN* mRNA transcript at 30 days post-training and a decrease in *CaN* protein expression 2 hours after retrieval. Thus, the observed *CaN* DNA methylation changes in the ACC may indeed be a marker for memory storage. Together, these findings provide further evidence for the role of DNA methylation in long-term memory formation and also demonstrate that the ACC might be a site for long-term memory storage via an epigenetic mechanism.

To further implicate DNA methylation events in the ACC for memory storage, the authors administered DNMT inhibitors into the ACC 30 days post fear conditioning and prior to test. DNMT inhibition significantly reduced freezing behavior compared to vehicle controls. In addition, DNMT inhibition resulted in a significant decrease in *CaN* DNA methylation in the ACC after memory retrieval. Thus, these results suggest that DNMT activity and subsequent DNA methylation are necessary for remote memory recall. However, the question still remains as to the specific role of DNA methylation events during memory storage. Indeed, these findings provide supporting evidence for the role of the ACC as a necessary site for remote memory retrieval, however the effect of DNMT inhibition on freezing behavior suggest that disrupting DNA methylation events interferes with retrieval of remote memories, but does not distinguish between memory maintenance and memory retrieval mechanisms. Thus, it would be interesting to determine the effect DNMT inhibition on DNA methylation events in the ACC of animals at 30 days post-training without initiating memory retrieval. Alternatively, to further investigate the role of DNA methylation in memory maintenance versus memory retrieval one could potentially administer DNMT inhibitors at the 18–20 day time point post fear conditioning with testing at the 30 day time point. The predicted outcome would be that DNMT inhibition at this later time point (18–20 days) prior to testing might disrupt the DNA methylation changes triggered with training but should not have an affect on memory retrieval, thus allowing one to further determine the importance of DNA methylation events in long-term memory storage.

In summary, the studies performed by Sweatt and colleagues strongly suggest a possible role for DNA methylation in both remote memory retrieval and storage within the ACC. These findings solicit further investigations into the role of not only DNA methylation but also other epigenetic mechanisms as potential molecular processes involved in memory maintenance within the cortex. Understanding and elucidating the different epigenetic players is essential as these mechanisms occur in tandem and not in isolation to influence gene transcription. Future studies in the field should also attempt to identify the specific CpG sites being modified by methylation and their location within a given gene promoter region with respect to consensus sequences that serve as docking sites for transcription factors such as CREB and NF-κB, both of which have been implicated in memory formation (Meffert *et al.*, 2003; Lubin and Sweatt, 2007; Gupta *et al.*, 2010). This information is pivotal in understanding whether DNA methylation events serve as either a transcription activating or repressive mechanism to mediate memory formation. Interestingly, the authors found that *Zif268* DNA methylation levels in the ACC remain persistently low across the behavior groups with increasing time points compared to naïve controls. The immediate-early gene *Zif268* has been shown to be a memory permissive gene whose mRNA expression peaks transiently after learning within the hippocampus (Hall *et al.*, 2001; Bozon *et al.*, 2003; Lee *et al.*, 2004; Lubin and Sweatt, 2007; Gupta *et al.*, 2010). Therefore, in light of the importance of *Zif268* gene expression in the hippocampus to mediate memory formation (Hall *et al.*, 2001; Bozon *et al.*, 2003; Lee *et al.*, 2004; Lubin and Sweatt, 2007), further studies should be directed at elucidating the role of this immediate early gene in the ACC in the storage of remote memories. Other potential future investigations include examining the effect of DNMT inhibition on cellular plasticity such as long-term potentiation and long-term depression in the ACC (Sacktor, 2008). Outcomes from such

studies will provide important new information at the synaptic level as to the functionality of hypermethylation of genes such as *CaN* and demethylation of *Zif268* in the ACC induced by learning. Overall, future work in this area has the potential to enrich our knowledge of the far-reaching effects of these epigenetic mechanisms in post-mitotic cells in the nervous system subserving long-term memory formation and storage.

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