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Publication Date
2016-06-01

DOI
10.1016/j.neulet.2016.01.023

Peer reviewed

HHS Public Access

Author manuscript

Neurosci Lett. Author manuscript; available in PMC 2017 June 20.

Published in final edited form as:

Neurosci Lett. 2016 June 20; 625: 40–46. doi:10.1016/j.neulet.2016.01.023.

The role of active DNA demethylation and Tet enzyme function in memory formation and cocaine action

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Abstract

Active DNA modification is a major epigenetic mechanism that regulates gene expression in an experience-dependent manner, which is thought to establish stable changes in neuronal function and behavior. Recent discoveries regarding the Ten eleven translocation (Tet1-3) family of DNA hydroxylases have provided a new avenue for the study of active DNA demethylation, and may thus help to advance our understanding of how dynamic DNA modifications lead to long-lasting changes in brain regions underlying learning and memory, as well as drug-seeking and propensity for relapse following abstinence. Drug addiction is a complex, relapsing disorder in which compulsive drug-seeking behavior can persist despite aversive consequences. Therefore, understanding the molecular mechanisms that underlie the onset and persistence of drug addiction, as well as the pronounced propensity for relapse observed in addicts, is necessary for the development of selective treatments and therapies. In this mini-review, we provide an overview of the involvement of active DNA demethylation with an emphasis on the Tet family of enzymes and 5-hydroxymethylcytosine (5-hmC) in learning and memory, as well as in drug-seeking behavior. Memory and addiction share overlapping molecular, cellular, and circuit functions allowing research in one area to inform the other. Current discrepancies and directions for future studies focusing on the dynamic interplay between DNA methylation and demethylation, and how they orchestrate gene expression required for neuronal plasticity underlying memory formation, are discussed.

Keywords

Epigenetics; Memory; Cocaine; Demethylation; Tet

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Introduction

It is now generally accepted that gene expression is required for long-lasting forms of neuronal plasticity, cognition and memory. Furthermore, as in many other fields, the role of epigenetics in regulating gene expression is redefining how we think about normal dynamic transcriptional events and, more importantly, how the epigenome serves as a signal transduction platform that encodes past experience, integrates current experience, and can establish forms of molecular and cellular memory that poise cell function for future action. Here we focus on the modification of deoxyribonucleic acid (DNA), with an emphasis on the role of the ten-eleven translocation (Tet) family of dioxygenases involved in active DNA demethylation. For many years, as major advances have been made in the understanding of the nature of DNA methylation in neuronal function, a major question has troubled the field of epigenetics: if DNA methylation is dynamic, where are the DNA demethylases? The discovery of the Tet family enzymes and their role in active DNA demethylation is a critical step in addressing this issue, providing new insight into the complexity and power of the epigenome in action. In this mini-review, we discuss the convergent and contrasting findings surrounding the role of DNA modification and Tet-dependent mechanisms in learning and memory, as well as what little is currently known with respect to these epigenetic mechanisms in cocaine action in the brain, drug-seeking behavior, and relapse following extended periods of abstinence. The mini-review is focused on memory and addiction because the molecular and cellular mechanisms thought to underlie the acquisition and ultimately the persistent compulsive aspects of drug use are hypothesized to also serve long-term associative memory function and reward-related learning processes (e.g. [1–4]).

Dynamic DNA modification, active DNA demethylation and the Tet family of DNA dioxygenases

DNA methylation is a covalent modification that governs gene expression through a number of mechanisms, including the binding of transcription factors [5], the recruitment of methylated DNA-binding proteins and chromatin-modifying proteins leading to changes in chromatin states [6], as well as the regulation of alternative splicing, nucleosome repositioning, and retrotransposon activity [7]. Although DNA methylation was once thought to be an inherently stable mark that is not capable of rapid changes, recent findings show that DNA undergoes rapid methylation and demethylation in the adult brain. For example, DNA methylation via the accumulation of 5-methylcytosine (5-mC) is dynamically regulated in an activity-dependent manner by the same intracellular signaling cascades that are necessary for memory formation (*e.g.* [8,9]). More recently, there has been significant interest in understanding the interplay between DNA methylation/demethylation mechanisms involving 5-mC and 5-hydroxymethylcytosine (5-hmC).

There are three DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) that have enzymatic activity in mammals, with DNMT1 and DNMT3a being the most active in neurons [10–12]. The addition of a methyl group from SAM (S-adenosyl-L-methionine) to cytosine is catalyzed by these DNMTs, resulting in 5-mC ([10]; Figure 1). 5-mC was originally thought to act as a stable transcriptional silencer [13], but it was recently

discovered that 5-mC levels are rapidly and reversibly changed at memory and synaptic plasticity-associated genes, suggesting active DNA demethylation as a result of neuronal activity [14–17]. While DNA methylation at 5-cytosine residues as well as the DNMT enzymes that are responsible for this process have been reasonably well characterized [12,18], the opposing mechanism of active DNA demethylation in differentiated neurons is only beginning to be understood. One key mechanism in DNA demethylation involves the Tet-family of methylcytosine dioxygenases. This family consists of TET1, TET2, and TET3, which participate in the conversion of 5-mC to 5-hmC [15,19]. 5-hmC is enriched within gene bodies, promoters, and transcription factor binding regions, where it may influence gene expression [20,21]. Recent findings suggest that 5-hmC not only serves as a DNA demethylation intermediate, but also functions as a stable epigenetic mark in its own right [20,21]. Its abundance and dynamic nature have therefore led to significant excitement regarding Tet enzymes and 5-hmC as this mechanism may lead to new insight into neuronal plasticity and long-term changes in behaviors related to memory and even drugs of abuse.

The Tet enzymes also mediate the conversion of 5-hmC to 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC; [15,22]; Figure 1). 5-fC and 5-caC base modifications serve as DNA demethylation intermediates subject to deamination, glycosylase-dependent excision and repair, resulting in a reversion to unmodified cytosine [23,24]. Recently, it was discovered that 5-fC can be a stable epigenetic mark [25], and can have functionally relevant effects on gene expression independent of demethylation through direct effects on DNA structure [26]. Although little is known about the functional roles of 5-fC and 5-caC [27,28] in learning and memory, what has been learned about 5-mC and 5-hmC has ignited the field by providing new insight into the mechanisms that regulate expression of genes required for neuronal plasticity and long-term changes in behavior (reviewed below), suggesting that it is critically important to also investigate the role of these base modifications as well as others, including 5-fC and 5-caC.

As one might predict, Tet enzyme activity can affect global levels of both 5-mC and 5-hmC. For example, Tet1 manipulation by viral expression of wild-type TET1 significantly decreases 5-mC levels, with a concurrent increase in 5-hmC levels in area CA1 [29] and the dentate gyrus of the hippocampus [14]. Conversely, expression of a catalytically inactive mutant TET1 in these regions produces the opposite effect [14,29]. Thus, these studies demonstrate the role of TET1 activity in actively regulating global levels of 5-mC/5-hmC in the adult hippocampus. With regard to other Tet family members, TET3-deficient zygotes from conditional knockout mice exhibit a failure to convert 5-mC into 5-hmC and levels of 5-mC remain relatively constant [30], whereas TET2 mutations lead to decreased 5-hmC levels in myeloid leukemia [31,32]. These examples of studies examining the role of Tet enzymes highlight the importance of their function in regulating the dynamic interplay between 5-mC and 5-hmC.

The Tet enzymes are highly expressed in the brain, with TET3 being the most abundantly expressed in the cerebellum, cortex, and hippocampus as compared to TET1 and TET2 [33]. Importantly, these are key regions involved in the acquisition and extinction of memory and, as described below, the functional relevance of their activity in the adult brain has recently been characterized with respect to learning and memory.

DNA modifications and the putative role of active DNA demethylation and Tet in learning and memory

One of the earliest studies to link dynamic DNA methylation changes to memory processes was by Boris Vanyushin at Moscow State University, who demonstrated that active variations in DNA methylation are associated with long-term memory formation [34,35]. In a more recent groundbreaking study using gene-specific approaches, Sweatt and colleagues found that contextual fear learning increases methylation within the promoter of the memory suppressor gene protein phosphatase 1 (*PP1*), whereas the promoter of *reelin*, a gene involved in synaptic plasticity, was concurrently demethylated [16]. Through the use of nonspecific DNMT inhibitors such as 5-azadeoxycytidine (5-AZA) or zebularine (zeb), the authors also found impaired fear memory consolidation. This finding seems at first counterintuitive, given that learning leads to increased 5-mC levels, which are in general associated with transcriptional repression; blocking DNA methylation by inhibiting DNMT would therefore be predicted to facilitate, not impair, learning. However, Miller and Sweatt [16] demonstrated that inhibition of DNMTs prevented methylation within the *PP1* promoter but enhanced demethylation within the promoter of *reelin* when examined 1 hr after training, supporting an old idea that memory formation requires the activation of some genes and the silencing of others. Importantly, their findings were dependent on associative fear conditioning triggering alterations in gene expression, as blocking the activity of DNMTs within the hippocampus in home cage animals did not result in changes in *reelin* or *PP1* mRNA between DNMT inhibitor-infused and vehicle-infused animals. Thus, DNMT inhibition alone is not enough to regulate reelin and PP1 gene expression. It is important to note that changes in methylation or demethylation of single genes such as PP1 or reelin give a potential snapshot of the underlying effect of methylation on gene expression required for a specific behavior. However, direct manipulation of methylation mechanisms at just PPI or reelin using a targeted molecular approach (as in [36]) can provide evidence for a more causal relationship. It is important to note that blocking DNMT in the hippocampus immediately after fear training blocked consolidation when the animals were tested 24 hr later, but the animals were still able to learn the fear conditioning task 24 hr after DNMT inhibition when they were retrained [16]. Together, the results from the Miller and Sweatt [16] study provided strong evidence showing that DNA methylation and demethylation are dynamic and reversible mechanisms that regulate the transcriptional activity involved in synaptic plasticity and learning and memory. This idea was initially quite controversial considering the lack of knowledge about the mechanisms of active DNA demethylation in the brain; however, the Tet family of enzymes and their role in iterative oxidation of methylated DNA now provides at least one powerful key epigenetic regulator involved in this dynamic process.

Thus far, TET1 is the best-characterized Tet family member with respect to learning and memory. TET1 manipulations have been shown to directly regulate several neuronal activity-dependent genes. For example, *Tet1* deletion leads to impaired hippocampal neurogenesis as well as spatial memory deficits in mice [37]. In adult neural progenitor cells lacking TET1, a group of genes involved in progenitor proliferation are hypermethylated and downregulated [37]. Thus, loss of TET1 is believed to lead to transcriptional repression of

neurogenesis-related genes through promoter hypermethylation and perhaps impaired demethylation [37]. In contrast to these findings, TET1 knockout mice in the Rudenko [38] study exhibit normal long-term spatial memory. The discrepancy between these findings is not clear. Although no other learning and memory tasks were used to interrogate the functional relevance of TET1 in the Zhang et al. [37] study, additional tasks were used by Rudenko et al. [38]. For example, TET1 mutant mice exhibited normal memory acquisition in Pavlovian fear conditioning (a paradigm in which an animal is exposed to contiguous presentations of a novel and initially innocuous environmental context paired with an aversive footshock), but impaired extinction of both contextual fear memory and spatial reference memory [38]. These findings support the idea that there may be divergence in the signaling cascades and epigenetic mechanisms that are activated in order support acquisition versus extinction processes. However, the findings of Tsai and colleagues point to key differences between memory acquisition/consolidation and extinction paradigms, which can lead to distinct effects on memory in the presence of the same genetic manipulation [38]. For instance, the memory extinction paradigm they used may not have provided a sufficiently strong stimulus to overcome TET1 deficiency, but a stronger stimulation such as presentation of a conditioned stimulus (CS) and foot shock used during memory acquisition may have resulted in the activation of additional transcriptional machinery and other Tet proteins, thus compensating for the lack of Tet1 (as discussed in [38]). This interpretation is supported by the observed differences in gene expression in TET1 knockouts following acquisition versus extinction training. Furthermore, developmental compensatory effects mediated by the other Tet family members owing to the whole genome knockout of TET1 may have led to the opposing observations.

TET1 mutant mice showed downregulation of both mRNA and protein levels of Npas4 and c-Fos (a downstream target of Npas4) in the cortex and hippocampus compared to controls, as well as hypermethylation of the Npas4 promoter following extinction training [38]. The Npas4 promoter region contains multiple CpG dinuleotides, and 5-hmC and TET1 are greatly enriched at the transcription start sites and gene bodies of numerous genes with high CpG content [38,39]. Indeed, in the hippocampus, TET1 knockout also leads to a significant decrease in the expression of Arc and Egr2 [38]. In contrast, using a viral approach to overexpress a catalytically inactive form of TET1 (TET1m), Kaas et al. [29] found that a TET1 knockdown (Tet1m) resulted in increased expression of cFos, BDNF, Arc, Egr1, Homer1, and NR4a2 14 days after viral injection. As alluded to above, there are fundamental differences between gene knockout [38] and dominant negative approaches [29], which may, in part, explain this discrepancy. Moreover, to add further complexity to the issue, both the catalytically inactive dominant negative TET1m as well as overexpression of wild-type TET1, resulted in increased expression of cFos, BDNF, Arc, Egr1, Homer1 and Nr4a2 [29]. Thus, it will be essential in future studies to examine 5-mC, 5-hmC, and TET1 occupancy along the promoter regions and coding/non-coding regions of these genes to define with greater resolution how learning-dependent changes in 5-hmC regulate their expression.

In contrast to Rudenko et al. [38], Kaas et al. [29] also found that overexpression of the catalytically inactive mutant form of TET1, which was shown to globally increase 5-mC in the CA1 region of the hippocampus, resulted in impaired long-term memory for

hippocampal-dependent contextual fear conditioning. Similarly, overexpression of wild-type TET1, which globally decreased 5-mC and increased 5-hmC in CA1, also resulted in impaired long-term memory for contextual fear. Thus, the TET1 knockout mouse exhibits normal memory for fear [38], and overexpression of either a dominant negative form of TET1 or wild-type TET1 impairs memory for fear [29]. These studies highlight our need to further understand these mechanisms, particularly considering that the findings of Kaas et al. [29] intriguingly suggest that TET1 regulates the expression of genes required for memory formation in a manner that may be independent of its catalytic activity. Indeed, Tet3 can also function independent of its enzymatic activity: Tet3 is a direct binding partner with O-linked *N*-acetylglucosamine transferase and colocalizes with this transferase on chromatin at active promoters enriched for H3K4me3, which is critically involved in transcriptional activation [40,41]. Therefore, it is likely that other members of the Tet family are also capable of influencing gene expression independent of active DNA demethylation.

As mentioned above, TET1 has been found to have the lowest expression among the Tet family enzymes in the cortex and hippocampus [33]. That may not mean much as what matters most is the activity of the enzyme. In contrast to TET1, TET3 is the most highly expressed member of the Tet family in the cortex and hippocampus [33]. It is currently difficult to compare the literature with regard to the effect of neuronal activity or learning-induced expression of Tet family enzymes as most studies examine only one enzyme and each study focuses on a different sub-region of the brain. However, two studies have shown that KCl-induced depolarization of either hippocampal neurons [29] or cortical neurons [42] leads to decreases in TET1 expression. Following fear conditioning, TET1 expression is also decreased in the CA1 region, with no change in TET1 expression in the infralimbic (IL) prefrontal cortex [42], which is preferentially associated with extinction. Following fear extinction training, TET3 expression is significantly increased in the IL [42], suggesting a key role in regulating gene expression during the consolidation of extinction memories.

Li et al. [42] found a dramatic redistribution of 5-hmC in response to fear extinction training in tissue collected 2hr following training, coinciding with effects on several histone modifications and alterations in gene expression. For example, extinction led to a decrease in 5-hmC within intronic and intergenic regions, but increased levels at gene promoters, the 5′-UTR, the 3′UTR, and within coding regions [42]. These findings support the interpretation that, at least with regard to fear extinction memory, the accumulation of 5-hmC is associated with a transcriptionally permissive chromatin environment, although the pattern of distribution depends on the genomic locus. Importantly, TET3 expression was enhanced in the IL following extinction training and intra-IL TET3 knockdown was associated with impaired extinction learning. Examination of a specific gene of interest, *Gephyrin*, which is necessary for GABA receptor function and extinction, showed that *Gephyrin* expression during extinction correlates with increased 5-hmC and TET3, but not TET1, occupancy within the Gephyrin gene locus [42]. Blocking TET3 expression prevented extinction-dependent increases in 5-hmC at the Gephyrin locus. Together, these findings reveal a key role for TET3, but not TET1, in the IL during the consolidation of fear extinction memory. Similar to many findings in the Tet literature with regard to learning and memory, there is a discrepancy in findings with regard to TET1 and extinction. Li et al. [42] found that specific knockdown of TET1 had no effect in the IL during extinction, yet Rudenko et al. [38]

observed significant impairments in extinction of fear and spatial memory in a TET1 knockout. A key experiment is to determine whether ameliorating expression of TET1 in the mutant mouse rescues the extinction memory phenotype, which would strengthen the interpretation that TET1 is indeed involved in extinction memory, and weaken the interpretation that a secondary event led to the extinction phenotype in those knockout animals. In any case, these studies underscore the need to learn more about the expression, function, and cell-type-specific regulation of gene expression during memory processes carried out by the Tet family enzymes.

As the Tet family enzymes are becoming central to understanding the mechanisms of active DNA demethylation involved in learning and memory, they are also beginning to shed light on similar events required for drug-associated memory processes. Bredy and colleagues [7] recently set forth the idea that experience-dependent variations in DNA methylation represent a form of metaplasticity that functions to prime the genome to respond to later events by regulating the transcriptional capability for specific neuronal functions. This hypothesis, and epigenetic mechanisms involving 5-mC/5-hmC and potential other DNA modifications, may be the key to understanding how drugs of abuse establish long-lasting changes in neuronal plasticity, circuit level changes, and ultimately persistent changes in behavior leading to compulsive drug use or even relapse following years or decades of abstinence.

DNA methylation and demethylation and Tet enzyme involvement in cocaine-seeking behavior

Drug addiction is a complex, relapsing disorder in which compulsive drug-seeking and drug-taking behavior can persist despite aversive consequences [1,43]. Continued drug-use induces adaptive changes in the central nervous system that ultimately lead to a complex set of neurobiological consequences including tolerance, physical dependence, sensitization, craving, and relapse. These long-lasting effects may be the result of drug-induced modifications in DNA/chromatin that lead to stable changes in cellular function. A major goal of addiction research is to gain an understanding of the neuroanatomical and molecular changes that underlie the onset and persistence of drug addiction, as well as the pronounced propensity for relapse observed in addicts [44,45]. Epigenetic mechanisms have been found to be central to synaptic plasticity and memory, as well as to the behavioral responses to drugs of abuse [46]. Key epigenetic modifications may be involved in establishing more stable transcription profiles that produce stable changes in cellular and molecular processes, ultimately leading to persistent changes in behavior. The specific epigenetic changes in response to drugs of abuse and underlying drug-seeking behaviors remains an area of active investigation. Tet enzyme-mediated demethylation of 5-mC to 5-hmC is a powerful epigenetic mechanism that may help to elucidate the molecular processes leading to long-lasting changes in brain regions underlying drug seeking and relapse behavior.

Although several studies have investigated the role of activity-dependent methylation and demethylation of DNA with regard to learning and memory (discussed above), similar studies focusing specifically on drug-seeking behaviors are limited. The first study to

investigate the role of DNA methylation with regard to general reward-related behavior used a rat model of reward learning in which an audio cue fully predicted sucrose delivery, and nosepokes during the presentation of the cue alone reflected experience-dependent associative memory formation. This study found that reward learning produced changes in DNA methylation at genes that were upregulated in dopamine neurons following learning. Inhibiting DNA methylation in the ventral tegmental area (VTA) impaired the formation of the sucrose cue-reward association [47]. The VTA is a brain region that is widely implicated in the natural reward circuitry as well as the circuitry underlying drug-associated behaviors. Drugs of abuse first act on VTA projections to the forebrain, resulting in dopamine release within key brain regions that mediate addiction such as the medial prefrontal cortex, basolateral amygdala, and the nucleus accumbens (NAc; [44]). The NAc plays a critical role in reward circuits. Interestingly, Day et al. [47] found that even though reward-learning altered gene expression in the NAc core, blocking DNA methylation in this region using RG108 (a small molecule DNMT inhibitor) prior to sucrose reward conditioning did not alter the cue-reward association, when examined 1 hour after the completion of the final conditioning session. There are several possible interpretations with regard to this finding, but perhaps this points to the VTA and NAc having different requirements for DNA methylation activity during reward-learning.

Incubation of craving was the focus of a very recent study to investigate DNA methylation underlying cocaine-seeking behavior. The prolonged period of withdrawal after extended access cocaine self-administration results in ‘incubated,” or heightened, craving [48]. Yadid and colleagues [49] found that withdrawal periods and cue-induced cocaine-seeking were associated with broad, time-dependent enhancement of DNA methylation changes that occurred in gene promoters at the whole genome level as well as selected candidate genes related to drug addiction. These changes in DNA methylation, which were partly negatively correlated with gene expression, were observed during incubation of cocaine craving and were found in 47 candidate genes that haven been shown to be involved in cocaine addiction. These findings support the notion that epigenetic changes involve altering outputs of complex networks, instead of a small number of proteins.

To examine the effect of DNMT inhibition, Massart et al. [49] used intra-NAc injections of RG108 and found attenuated cue-induced cocaine seeking after prolonged withdrawal. Specifically, animals received infusions of RG108 into the NAc during the end of withdrawal training and immediately underwent extinction session. NAc epigenetic analysis was conducted immediately after the extinction test. This was a long-lasting effect that persisted for 1 month, whereas the methyl donor SAM had the opposite effect. They hypothesized that RG108 inhibits incubated cue-induced cocaine craving by driving demethylation of specific genes. Pharmacological targeting of two proteins (estrogen receptor 1 and cyclin-dependent kinase 5) encoded by genes that were demethylated by DNMT inhibitor treatment decreased cue-induced cocaine seeking. Because single intra-NAc manipulations produced robust, long-lasting effects, it was concluded that DNA methylation patterns may result in stable and dynamic changes through the reprogramming of the epigenetic mechanisms underlying withdrawal and cocaine seeking behaviors [49,50]. This study provides strong evidence for the idea that DNA methylation and demethylation mechanisms can lead to long-term, persistent effects on behavior.

When considering the fundamental role of DNA methylation in the NAc for reward-based behaviors, it is worth comparing the Day et al. [47] and Massart et al. [49] studies. Blocking DNA methylation in the NAc core before sucrose reward conditioning did not alter cue-reward association [47], whereas DNA methylation inhibition in the NAc prior to retrieval of cocaine-associated memory formation caused long-lasting attenuation of cue-induced cocaine seeking [49]. However, there are several key differences in these studies. Day et al. [47] used a simple natural reward-related model to study associative memory, whereas Massart et al. [49] used a more complex model of self-administration to study cocaine incubation of craving. Furthermore, Day et al. [47] focused their investigation on the initial acquisition phase of memory formation whereas Massart et al. [49] blocked DNA methylation in the NAc during the retrieval of cocaine-associated memory by exposing the animals to the cocaine-associated cue during an extinction test session. These key differences, in addition to the notion that DNA methylation regulation of natural reward-related associative memory may differ from that of incubation of cocaine craving, all point to reasons for the observed differences. Taken together, these studies highlight the need for the field to continue examining this epigenetic regulatory mechanism and to extend the role of DNA methylation and active demethylation to other brain regions known to subserve natural reward associations and drug-associated behaviors such that similarities and differences in these behaviors can be further elucidated.

With regard to active DNA demethylation, there is currently only one published study examining TET1 in cocaine action. TET1 has recently been shown to be regulated by cocaine and involved in cocaine-conditioned place preference [51], a behavioral model that is widely used to study the association formed between the rewarding properties of a drug and the environment in which the drug is given. Repeated cocaine administration resulted in decreased TET1 mRNA and protein in the Nac. However, the levels of TET2 and TET3 were unchanged when examined 24 hours after repeated cocaine treatment. The decrease in TET1 mRNA and lack of change in TET2 and TET3 following cocaine treatment suggests that TET1 is regulated in the NAc by cocaine, whereas TET2 and TET3 are not. Investigation of the levels of TET1 mRNA in the NAc of cocaine addicted humans revealed that TET1 mRNA levels were reduced [51]. TET1 knockdown in the NAc also facilitated the acquisition of cocaine-conditioned place preference. Conversely, overexpression of TET1 in the NAc weakened conditioned place preference, indicating that TET1 expression in the NAc is sufficient to diminish cocaine reward. These findings show that TET1 serves to negatively regulate cocaine reward and that a cocaine-dependent decrease in TET1 expression in the NAc could contribute to increased drug sensitivity [51].

To understand the effect of reduced TET1 expression in the NAc, Feng et al. [51] examined the total levels of 5-mC and 5-hmC, but found no global changes, suggesting that any changes were going to be site specific. In addition, TET2 and TET3 are more highly expressed than TET1 in the NAc, as in other brain regions, which could potentially compensate for TET1 reduction. In any case, the authors mapped 5-hmC and found accumulation of this epigenetic mark within the putative enhancers and coding regions of genes that are known to play a role in cocaine action. Induction of 5-hmC correlated with gene expression as well as alternative splicing, both of which were cocaine-dependent. However, the authors failed to detect a correlation between RNA-seq gene expression

analyses and 5-hmC at putative enhancer elements, indicating that further detailed studies are necessary to completely understand their relationship [51]. Of note, previous studies found that decreased TET1 led to the decreased conversion of 5-mC to 5-hmC and thus to promoter hypermethylation and transcriptional repression [29,37,38]. Nestler and colleagues showed that TET1 downregulation leads to increased 5-hmC correlating with gene expression [51]. One plausible explanation for this is that previous studies found effects at gene promoters, whereas Feng et al. [51] identified 5-hmC increases at gene bodies, with DNA modifications likely to have different effects at these distinct regions [7] [48]. TET1 has also been shown to lead to both transactivation and repression [39] [52], which will likely be dependent on temporal and cell-type specific effects as well as unique interactions with specific repressor and activator transcriptional complexes. Importantly, changes in 5-hmC at certain loci lasted for at least one month after cocaine exposure [51], suggesting that these modifications participate in long-lasting changes in cell function underlying long-term changes in drug-seeking and perhaps even relapse behavior. Aside from the role of DNA methylation and demethylation in the regulation of gene expression, the ability to establish persistent changes in neuronal plasticity that ultimately guides long-term changes in behavior will highlight the power of neuroepigenetics.

Conclusion

Thus far, studies investigating the role of DNA modifications underlying natural reward and drug action have focused their attention on the VTA and the NAc. Future experiments examining these mechanisms in other brain regions that are known to mediate drug-associated memories will significantly advance our basic understanding of the role of epigenetic regulatory mechanisms in drug-induced neuroadaptations and increased vulnerability to relapse following abstinence. Additional experiments using intricate behavioral paradigms including self-administration, conditioned place preference, and sensitization are also needed in order to study more complex memory processes underlying drug-seeking behaviors such as memory extinction, reconsolidation, and reinstatement. Finally, investigation into the role of active demethylation in the extinction and reconsolidation of drug-associated memories may help to elucidate key differences in these memory mechanisms, and identify novel therapeutic approaches for the treatment of substance abuse.

Acknowledgments

The authors thank Rowan Tweedale for helpful editing of this manuscript. This work was supported by grants from the National Institute of Mental Health (NIMH) and National Institute on Drug Abuse (NIDA) (MH101491, DA025922, DA036984) to M.A. Wood, and NIMH, National Health and Medical Research Council (APP1040251, APP1069570), and the ARC-SRI: Science of Learning Research Centre (SR120300015) to T.W. Bredy. ChemDoodle was used to draw the molecular structures shown in Figure 1. *ChemDoodle*: Chemical Publishing Software (Version 7.0.2). Available from http://www.chemdoodle.com.

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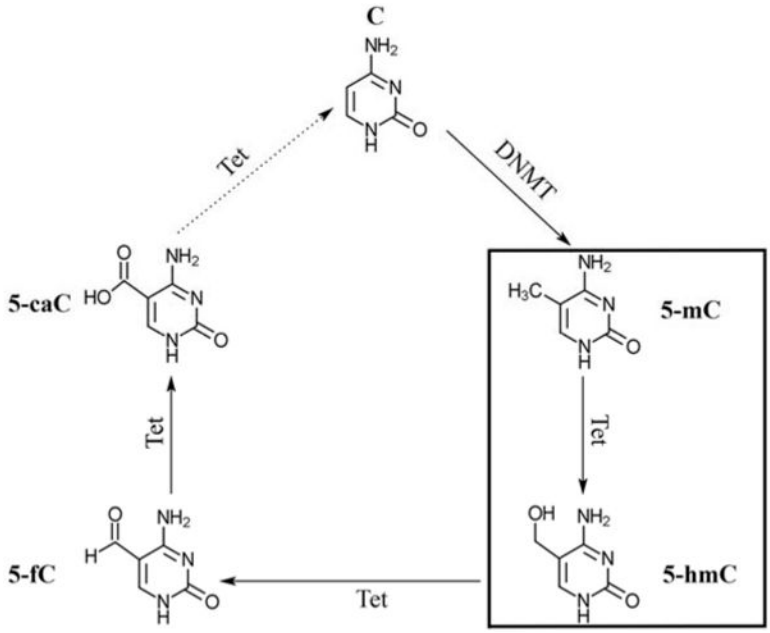

Figure 1.

Schematic representation of the DNA methylation/demethylation pathway. Boxed reaction is the demethylation step on which we focus this review. *C*, cytosine; *5-mC*, 5-methylcytosine; *5-hmC*, 5-hydroxymethylcytosine; *5-fC*, 5-formylcytosine; *5-caC*, 5-carboxylcytosine; *DNMT*, DNA methyltransferase; *Tet*, Ten-eleven translocation enzyme. (see also [53,54]).