Role of DNA methylation and the DNA methyltransferases in learning and memory

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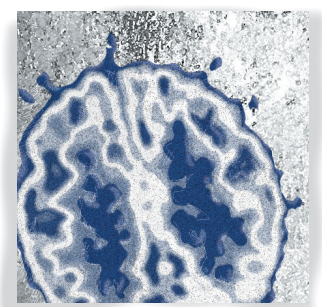

Dynamic regulation of chromatin structure in postmitotic neurons plays an important role in learning and memory. Methylation of cytosine nucleotides has historically been considered the strongest and least modifiable of epigenetic marks. Accumulating recent data suggest that rapid and dynamic methylation and demethylation of specific genes in the brain may play a fundamental role in learning, memory formation, and behavioral plasticity. The current review focuses on the emergence of data that support the role of DNA methylation and demethylation, and its molecular mediators in memory formation.

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Introduction

The term "epigenetics" was coined some 70 years ago by Sir Conrad Waddington, who theorized the existence of a necessary layer of molecular complexity beyond the genome that must be responsible for producing distinct and variable cellular phenotypes from a singular genome.[1] Waddington's theories have proven to be fundamental in abolishing "instincts" and genetic "programs" as useful conceptualizations of the ontogeny of behavior and, importantly, have initiated an appreciation of the multitude of complex influences on phenotypic expression throughout development. Epigenetics in its current formulation is more narrowly defined as the perpetuation of genetic information from a cell to its descendants without any necessary change to the genetic code itself, and has been posited as a molecular "bridge" between the information contained in the genotype and what emerges as a complex and ever-modifiable phenotype.[2]

The revolution in molecular biology that began in the 1950s, following shortly after Waddington's theoretical formulation of epigenetics, has provided scientists with tools capable of characterizing and understanding the mechanisms that comprise this layer of complexity beyond the genome (ie, the epigenome). DNA exists in a continuum of variably compacted states controlled by the structural state of chromatin, ie, the DNA and the histone proteins around which it is wrapped. Alterations to the structural state of the chromatin can have pro-

found and persistent effects on gene expression. Simply put, by establishing and maintaining the structural state of the chromatin, epigenetic processes regulate the ease in which transcription factors and other proteins can access their DNA substrates. For example, the amino acid "tails" of histone proteins are subjected to various post-translational modifications (eg, acetylation, methylation, phosphorylation) that render the chromatin relatively compact and transcriptionally inactive (ie, heterochromatin) or less compact and transcriptionally active (ie, euchromatin). Methylation at the 5-position of cytosine nucleotides within CpG dinucelotides is the only direct epigenetic modification of DNA and is associated with transcriptional silencing.[3]

Epigenetic processes have long been recognized as indispensable for appropriate embryonic and early postnatal development.[4-7] More recently it has come to light that these same mechanisms that drive critical processes in development and in mitotic cells throughout the lifespan remain dynamic in neurons that, once differentiated, are incapable of mitosis. Hence, "neuroepigenetics" describes processes that utilize the same mechanisms classically defined as epigenetic, but are clearly for functionally distinct purposes. Moreover, there is now recognition that DNA methylation itself, once thought to be the most stable of epigenetic marks, may switch between methylated and unmethylated states that render stretches of the chromatin a dynamic canvas on which epigenetic and other mechanisms can work to promote forms of plasticity necessary for long-term information storage. The hypothesis that the processes underlying stable transmission of chromatin states in dividing cells remain active in neurons for the purpose of long-term information storage is gaining significant traction and interest within neuroscience, and a better understanding of these processes is necessary for a more complete conceptualization of neural plasticity and memory.[8,9] Alterations in the structural state of chromatin appear to be highly conserved mechanisms underlying information storage in invertebrate (eg, crab, honeybee) and vertebrate (eg, rat, mouse, human) central nervous systems.[8-14]

The primary focus of the current review is to highlight the accumulating data suggesting that dynamic DNA methylation and demethylation, and the enzymes responsible for methylating and demethylating DNA, are critically involved in memory formation and behavioral plasticity. While the recognition that the structures of chromatin and DNA are rapidly modifiable in the brain adds a significant amount of complexity to our understanding of behavioral and neuronal plasticity, it also suggests a heretofore largely untapped therapeutic potential for alleviating a wide range of neurological, and other, disorders.

DNA methylation is indispensable for normal organismal development

DNA methylation plays an essential role in several developmental processes (eg, genomic imprinting, X-chromosome inactivation), in the maintenance of genome stability by silencing repetitive elements, and in maintaining tissue-specific and appropriate patterns of gene expression through cell division.[3-5,15-19] During embryonic and early postnatal development coordinated waves of methylation and demethylation ensure temporally specific patterns of gene expression that act to establish and perpetuate tissue appropriate cellular identities.[4,15,20,21] Once established in somatic cells, methylation patterns have traditionally been considered immutable.[3,22] A seminal study in 2004 by Meaney and colleagues showed that variable early postnatal levels of maternal care (eg, nursing, grooming) could alter DNA methylation patterns in neurons and that these alterations persisted into adulthood and influenced behavioral and neural responses to stress.[23]

Methylation of a cytosine nucleotide (5mC) is a thermodynamically very stable modification that is endowed with robust power to influence gene expression.[3,15,24] For example, methylation of a single site in a brain-derived neurotrophic factor (BDNF) exon promoter can silence the gene.[25,26] Transcriptional silencing is thought to occur via one of two nonmutually exclusive mechanisms; 5mC can physically restrict transcription factor and RNA polymerase II binding, or 5mC can recruit transcriptional repressor protein complexes.[3,27,28] Until recently, it was believed that 5mC occurred only in the context of CG dinucleotides; however, new findings have demonstrated the existence of substantial levels of mCH methylation where "H" represents an A,T, or C. Like 5mC, mCH is depleted in expressed genes and inversely proportional to the level of expressed transcript.[29-31]

The enzymes responsible for catalyzing the transfer of a methyl group to cytosine nucleotides, using dietary sources of s-adenosyl-l-methoinine as the methyl donor, are the DNA methyltransferases (DNMTs) and

are broadly subdivided into two categories: the de novo DNMTs, DNMT3a and DNMT3b, establish initial methylation patterns on unmethlyated DNA; and the maintenance DNMT, DNMT1, recreates already established methylation patterns on hemimethylated replicating DNA. DNMTs are essential to normal development as evidenced by the embryonic or early postnatal lethality of constitutive knockout of DNMT1 or DNMT3a.[32,33] DNMT1, DNMT3a, and DNMT3b are all expressed in the postnatal developing rat brain. DNMT1 and DNMT3a are expressed in adult neurons and oligodendrocytes, and DNMT3b expression is detectable as well, although not to the extent of DNMT3a.[30,34,35] DNMT mRNA generally reaches its highest level at around 1 week postnatal and subsequently decreases in brain.

Conditional brain-specific DNMT knockouts have yielded insights into the roles of the DNMTs in the central nervous system. A conditional DNMT1 knockout induced during embryonic development using the Cre-lox system upregulated apoptotic genes and led to degeneration of the cortex and hippocampus, abnormal morphology of dendrites, and alterations in the resting electrophysiological properties of neurons.[36,37] The conditional forebrain knockout mice survived to adulthood but, not surprisingly, evidenced severe learning and memory impairments, as they failed to show any learning curve in a spatial memory test following 13 days of training.[36,37] Another study that assayed neurological phenotypes in conditional DNMT1 knockout mice in which the knockout occurred at embryonic day 12 (E12) reported that DNMT1 deficiency led to hypomethylation in differentiated neurons and apoptosis of neurons prior to postnatal day 21 in mosaic animals.[36] DNMT3a null mice are essentially normal at birth, but quickly deteriorate and die in early postnatal development. These mice exhibit impaired postnatal neurogenesis accompanied by dramatic alterations in gene expression profiles in neural stem cells with 1253 genes upregulated and 1022 downregulated, effects likely mediated by impaired Polycomb repression of neurogenic genes.[33,38]

DNA methylation and DNMT expression are responsive to environmental stressors and cellular insults

Gene-specific DNA methylation as well as neuronal expression of DNMT enzymes, fluctuates as a result of experiences ranging from intake of drugs of abuse, associative and nonassociative learning experiences, cellular insults, and prenatal stressors.[23,25,39-66] Furthermore, in several brain pathologies the expression of the DNMTs, as well as methylation of specific gene promoters, appears aberrant.[16,49,67-81]

Endres et al reported that an increase in DNA methylation was associated with more robust brain lesions following induction of stroke using the middle cerebral artery occlusion (MCAO) model.[44] Treatment with the nonspecific DNMT inhibitor 5-aza-2'-deoxycytodine (5AZA) or mice heterozygous for DNMT evidenced a reduction in neuronal damage following MCAO. A more recent study in gerbils determined that 5 minutes of ischemia induced by bilateral common carotid artery occlusion (2VO) significantly upregulated DNMT1 expression specifically in hippocampal CA1 GABAergic neurons as well as in astrocytes 4 days after the occlusion.[82] Ninety days of chronic brain hypoperfusion induced by 2VO promoted a decrease in global DNA methylation accompanied by a decrease in expression of DNMT3a in parietal lobe cortex with no change in DNMT1 expression.[66] These findings suggest that acute vs chronic ischemic insults may differentially affect DNA methylation and expression of de novo and maintenance DNMTs in adult brain, and that targeted DNMT inhibition may offer some therapeutic potential in restricting or preventing brain damage invoked by a cerebrovascular accident.

Temporal lobe epilepsy is associated with aberrant DNA methylation of specific genes as well as increased expression patterns of DNMT isoforms in postmortem tissue from human epileptics and in animal models of the disorder. Studies using postmortem tissue from patients with intractable epilepsy demonstrate that DNMT1 and DNMT3a protein expression are robustly increased in hippocampal tissue from epileptics,[81] and hypermethylation of the reelin gene promoter, presumably mediated by a DNMT, has been reported.[49] Using a rat model, Parrish et al recently found that kainic acid–induced epileptic activity in the hippocampus led to increased global DNA methylation in the CA1 and CA3 and decreased methylation in the dentate gyrus 6 weeks after kainate treatment.[60] Interestingly, distinct patterns of DNMT1 and DNMT3a expression were observed in hippocampal subregions immediately after (1 hour) and 6 weeks after induction of epilepsy. Decreased expression of DNMT3a persisted 6 weeks after induced seizure, whereas immediate decreases in DNMT1 ex-

pression normalized by 6 weeks. Importantly, intrahippocampal treatment with the nonspecific DNMT inhibitor zebularine decreased the latency to seizure onset and prevented the changes in global methylation and promoter methylation of the *Grin2b/Nr2b*, a gene known to play a role in epilepsy. Collectively, the results from ischemic and epileptic models suggest that wide-ranging insults can influence DNA methylation in brain. In the case of epilepsy it is not yet clear if the changes in expression are involved in the etiology of epilepsy or result from the pathology. These findings nevertheless demonstrate that genomic methylation is indeed plastic and likely plays a key role in neurological disorders and neurological responses to insult.

Prenatal stressors as well as stress paradigms administered in adulthood have repeatedly been shown to influence neuronal DNA methylation. Indeed, differential methylation of the corticotropin-releasing factor gene promoter follows maternal deprivation stress, prenatal stress, and chronic mild stress.[83-85] Variation in the diet of mice during gestation or later in development can alter the methylation status of DNA in a persistent fashion.[64,86] An increase in dietary L-methionine or treatment with the nonspecific histone deacetylase inhibitor trichostatin A (TSA) reverses the effects of poor maternal care behavior on DNA methylation and hypothalamic-pituitary-adrenal axis, and behavioral responses to stress, providing further evidence that DNA methylation marks in neurons are modifiable.[65,87] Exposure to a cat increases BDNF gene methylation in the dorsal hippocampus in rats, while simultaneously favoring a decrease in methylation of the BDNF gene in the ventral hippocampus with no change in BDNF methylation in the basolateral amygdala or the prefrontal cortex.[59] The functional significance of bidirectional methylation of the same gene in different brain regions, or how this is mediated, is not yet clear. Prenatal exposure to the environmental toxin methyl mercury hypermethylates the BDNF promoter region in the hippocampal dentate gyrus, with an accompanying hypoacetylation of histone H3 and a decrease in BDNF mRNA expression. These epigenetic alterations are associated with increased depressive-like behavior in adulthood, as assessed by the forced swim test.[88] Interestingly, BDNF expression in the hippocampal dentate gyrus is necessary for antidepressant efficacy in the forced swim test,[89] thus suggesting a mechanism whereby various stressors experienced in the intrauterine environment may relate to deleterious behavioral phenotypes in adult animals.

Exposure to drugs of abuse can have dramatic effects on DNA methylation in neurons. Tian et al reported that conditioned place preference to cocaine led to global methylation in the prefrontal cortex, and that methionine supplementation prevented the establishment of conditioned place preference for cocaine, but not morphine or food reward.[63] Bodetto et al have confirmed a role for DNA methylation in mediating the rewarding effects of cocaine. In their study, cocaine increased DNA methylation at the protein phosphatase 1 (*PP1*) gene promoter, a memory suppressor gene, and increased expression of DNMT3a.[58] DNMT3a overexpression specifically in the nucleus accumbens, a brain region often implicated in behavioral responses to drugs of abuse, has been shown to attenuate cocaine reward and increase dendritic spine density of thin spines in nucleus accumbens neurons.[50] By contrast, nucleus accumbens-specific knockout of DNMT3a potentiated conditioned place preference for cocaine. Acute vs chronic cocaine use has opposite effects on DNMT3a expression in nucleus accumbens as acute treatment increases, whereas chronic treatment decreases, DNMT3a expression in the accumbens. Administration of the DNMT inhibitor RG108 blocks cocaine's effect on spine density in the nucleus accumbens and enhances conditioned place preference for cocaine. Ethanol exposure during all 3 trimesters of embryonic development similarly has been shown to upregulate the expression of DNMT3a as well as DNMT1 and the methyl-binding protein methyl-CpG-binding protein 2 (MeCP2) in the hippocampus.[90]

Importantly, cellular insults, prenatal stressors, or exposure to aversive stimuli are not the only experience-driven changes in DNA methylation or DNMT expression. Single running wheel exercise sessions or week-long access to a running wheel, known to be a rewarding activity in rodents,[91] demethylate the BDNF exon IV promoter, increase BDNF mRNA and protein in the hippocampus of Sprague-Dawley rats, and can elevate levels of phosphorylated MeCP2, and subsequently silence the associated gene.[47,92] Phosphorylation of MeCP2 can lead to its dissociation from chromatin, which may favor transcriptional activation of BDNF.[93] Single exercise sessions decreased DNMT3b and DNMT1 in hippocampus in young, but not old, rats. Interestingly, long-term exercise is associated with improved learning in rodents and humans, and in en-

hanced hippocampal plasticity in rodent models, an effect that may be related to the increases in BDNF.[43]

In summary, highly variable and biologically relevant environmental experiences appear to alter the methylation state of specific regions of the genome and promote increases or decreases in the associated mRNA and protein. Long thought to be a paragon of biological stability, gene methylation, at least in the brain, may be a rather dynamic process that is altered as a result of environmental input.

DNA methylation, DNMTs, and memory

Broadly speaking, epigenetic processes have been implicated in behavioral adaptations that rely on associative and nonassociative learning processes as well as in the subsequent storage of putative memory traces in the central nervous system.[94,95] The notion that long-term memories are encoded in the methylation state of the DNA was initially proposed by Griffith and Mahler in a theoretical paper published in *Nature* ("The DNA ticketing theory of memory"), in 1969.[96] They proposed that "the physical basis of memory could lie in the enzymatic modification of the DNA of nerve cells." Similar hypotheses were further elaborated by Crick and Holliday.[97,98]

The events in the nervous system that are necessary for the formation of long-term memories are complex and not completely understood. Memory formation requires the orchestration of precise and temporally coordinated changes in gene expression, transcription factor activation and inactivation, and bidirectional changes in the expression and activity of chromatin and DNA modifying enzymes. These molecular events coalesce to produce de novo changes in the synaptic strength and connectivity within specific circuitry underlying the formation and long-term storage of new information. Moreover, the specific neural pattern responsible for the storage of the memory is retrievable in the presence or absence of the stimuli that promoted its formation. Importantly, the memory trace must be self-perpetuating, must persist in spite of the continual turnover of molecules involved in its genesis,[95,97] and can potentially last the lifetime of an organism.

The idea that dynamic changes in DNA methylation are necessary for long-term memory formation was first given empirical support by Sweatt and colleagues. Initially the Sweatt laboratory reported that treatment with nonspecific DNMT inhibitors impaired the formation of contextual fear associations.[99] A follow-up study found that experience in associative fear learning to context, a paradigm in which an animal is exposed to contiguous presentations of a novel and initially innocuous environmental context paired with an aversive footshock, could rapidly (ie, within 30 minutes) increase the methylation of the memory suppressor gene protein phosphatase 1 (*PP1*), while concurrently demethylating the promoter region of the plasticity-related gene *reelin*.[53] Experience in fear learning upregulated the expression of DNMT3a and DNMT3b in areas of the brain necessary for learning (eg, hippocampus), with no effect on DNMT1. Moreover, nonspecific inhibition of DNMTs impaired memory and prevented the methylation of *PP1*, while enhancing the demethylation of *reelin*. Following training, the methylation of *reelin* and *PP1* returned to normal within 24 hours, leading the authors to conclude that DNA methylation, while critical for memory formation, is not likely to be a mechanism of long-term storage, at least in the hippocampus.

Further studies have expanded on these initial findings and implicated methylation of the BDNF gene in associative fear learning.[51,54] Experience in a fear learning paradigm demethylates the BDNF exon III and exon IV promoters in the hippocampus, and these effects are blocked by application of the NMDA receptor antagonist MK801, indicating that they are activity-driven.[51,54] In accordance with the aforementioned Miller and Sweatt study,[53] the effects on methylation of BDNF in the hippocampus were relatively transient and observable 30 minutes and 24 hours after training. Although methylation changes in the hippocampus are relatively transient, methylation of the memory suppressor *calcineurin* is increased in the prefrontal cortex 7 days after fear conditioning, and this hypermethylation migrates to the anterior cingulate cortex as long as 1 month following initial training.[100] Infusion of DNMT inhibitors directly into the anterior cingulate cortex blocks memory retrieval 30 days after training. These data are consistent with the known roles of these brain regions in memory formation vs storage, and suggest that transient and long-lasting methylation/demethylation in distinct brain circuits is important in establishing long-term memory of fearful stimuli.

Day et al have demonstrated that the importance of DNA methylation/demethylation in memory formation

is not restricted to aversive events (eg, fear conditioning, Morris water maze).[101] Using a cued-sucrose delivery associative reward learning paradigm, the authors found that experience in the learning task increased the expression of the immediate early genes *Erg1* and *c-fos*, which were demethylated following learning. In a neuronal culture preparation, KCl depolarization did not change DNMT3a or DNMT3b expression; however, it did increase DNMT3a binding at the genomic sites (*Erg1* and *c-fos*) that underwent de novo methylation in the in vivo reward-learning experiments. DNMT inhibition before KCl treatment prevented depolarization-induced changes in DNA methylation, and pharmacological inhibition of DNMTs in the ventral tegmental area in vivo blocked reward learning without influencing motivation in general. Interestingly, treatment with DNMT inhibitors can prevent the memory enhancing effects induced by other compounds, as DNMT inhibition has been shown to prevent estrogen-induced improvements in memory.[102] Estrogen treatments increase the hippocampal expression of DNMT3a and DNMT3b, but not DNMT1. Using mice with conditional forebrain-specific double knockout of DNMT1 and DNMT3a in neurons, Feng et al reported learning and LTP deficits that were not apparent with a single knockout of either DNMT1 or DNMT3a.[103] Therefore, although experience in associative learning tasks and other stimuli appear to differentially affect de novo vs maintenance DNMT expression, it seems that each of these distinct isoforms can compensate for the lack of the other.

DNA methylation and synaptic function

The culmination of the molecular events that promote long-term memory formation leads to structural changes at synapses in brain-region specific circuits that underlie learning and memory. Therefore, it is not surprising that epigenetic modifications of chromatin have been implicated in regulating the forms of synapse plasticity believed to establish memory. Initial studies implicating epigenetic processes in synaptic plasticity focused on histone modifications such as acetylation and deacetylation.[14] Subsequently, manipulation of DNMTs in dissociated neuronal cultures and in acute brain slice experiments have implicated the process underlying addition or removal of 5mC in regulating basal neuronal function as well as plasticity within brain circuits.

Levenson et al found that treatment of a hippocampal slice preparation with the DNMT inhibitors 5AZA or zebularine impaired the magnitude of long-term potentiation (LTP) at the Schaeffer collateral-CA1 pathway.[99] LTP, widely believed to be a cellular correlate of learning, is typically induced via electrical stimulation of brain slices with robust high-frequency stimuli, which causes a demonstrable increase in synaptic responses subsequent to the high-frequency induction protocol. θ-burst stimulation, a physiologically relevant means of inducing LTP,[104] led to robust and enduring potentiation (3 hours) in vehicle-treated slices; however, treatment with either DNMT inhibitor impaired LTP magnitude and maintenance. In a follow-up study, it was shown that DNMT-inhibitor induced LTP deficits were rescued by slice application of the histone deacetylase inhibitor sodium butyrate, suggesting a crosstalk between DNA methylation and histone acetylation in the regulation of hippocampal synaptic plasticity.[105] In accordance with the observation that nonspecific DNMT inhibition impairs LTP magnitude and maintenance, Feng et al have shown that forebrain-specific conditional double knockout of both DNMT1 and DNMT3a led to similar LTP impairments.[103] Somewhat surprisingly, no effects on synaptic function were observed in DNMT1 or DNMT3a single knockouts.

Depolarization of hippocampal neuron cultures with 50 mM KCl downregulates DNMT1 and DNMT3a expression, an effect that is prevented by the sodium-channel blockers tetrodotoxin and veratridine.[61] KCl-induced increases in neuronal activity demethylate the regulatory region of the BDNF exon IV promoter, and promote the dissociation of a corepressor complex composed of MeCP2, histone deacetylases, and Sin3a from the BDNF promoter.[25,26,93] BDNF has been broadly implicated in neuronal viability, synaptic plasticity, synaptogenesis, and memory, suggesting important activity-dependent functional consequences of BDNF demethylation.[106] Although basal synapse function is normal in acute hippocampal slices treated with DNMT inhibitors,[99] DNMT inhibitors administered to dissociated hippocampal neuron cultures decrease the frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs) and demethylate the BDNF I promoter. These effects are activity-dependent, as the NMDA receptor antagonist AP5 prevented BDNF promoter demethylation.[107] Although most studies assessing the role of histone modifications and DNA methyla-

tion on LTP have examined the hippocampus, Sui et al found that high frequency stimulation-induced LTP led to demethylation of the reelin and BDNF gene promoters in the prefrontal cortex and increased acetylation of histone 3 and histone 4, marks of active transcription.[62] DNMT inhibitor treatment impaired LTP in prefrontal cortex and prevented the alterations in histone acetylation.

In future experiments it will be interesting to determine if the electrophysiological correlates of memory formation (eg, LTP) "migrate" from brain regions necessary for memory formation (eg, hippocampus) to areas more involved in memory storage (eg, anterior cingulate cortex, prefrontal cortex) and if such changes require DNA methylation status updates within this geography.

Determining an active demethylation process

Any role for rapid DNA methylation/demethylation in memory formation necessitates the existence of an active mechanism for removing 5mC from specific genes involved in plasticity and memory. In other words there must exist a "switch" on specific cytosine nucleotides that is responsive to environmental contingencies that promote associative and nonassociative forms of learning.

Among the strongest candidates as molecular agents of demethylation are the ten-eleven translocation (Tet) family of proteins. Tet1, Tet2, and Tet3 are known to be bona fide mediators of 5mC demethylation in plants, and in mammalian tissue exhibit a strong preference for CpG-rich motifs.[108,109] The pathway responsible for conversion of 5mC to cytosine is thought to involve successive oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) to 5-formylcytosine (5fC) to 5-carboxylcytosine (5caC). The presence of 5hmC in the brain is significantly diminished when Tet proteins are inhibited.[110-112] All three members of the Tet protein family are capable of converting 5mC to 5hmC as well as subsequent oxidation of 5hmC to 5fC and 5caC.[108] The modified bases can then be further subjected to deamination, glycosylation, and base excision repair to result in final conversion back to a cytosine base.[108,110,113,114] Reconversion back to cytosine may require the activity of base-excision repair mechanisms.[110]

Several potential alternative pathways may mediate the conversion of 5hmC to cytosine. 5hmC can be deaminated to 5hmU by activation-induced deaminase (AID), with subsequent removal of 5hmU by thymine DNA glycosylase (TDG), methyl-binding domain protein 4 (MDB4), and single strand–specific monofunctional uracil DNA glycosylase 1 (SMUG1).[111,114-119] Thymidine glycosylase can also directly target 5fC and 5caC; however, any in vivo role of TDG in demethylation remains unclear.[119,120] Interestingly, it was recently shown that DNMTs may also act as demethylases capable of converting 5hmC to C, possibly by a direct interaction with TDG.[121] Methyl-binding domain protein 2 (MDB2) can directly demethylate DNA containing 5mC by a reaction that releases formaldehyde.[122]

Guo et al have demonstrated activity-dependent demethylation of two plasticity-related genes, fibroblast growth factor 1 (*FGF1*) and *BDNF* following electrical stimuli capable of inducing epileptiform activity.[110,111] In the hippocampus, mice with reduced levels of Tet1 were incapable of demethylating *BDNF* and *FGF* genes following seizure-inducing stimuli. Tet1 knockout mice exhibit downregulated expression of the neuronal activity-related genes *Npas4*, *c-fos*, and *Arc* and a reduction in 5hmC levels in the hippocampus and cortex with no change in 5mC.[123] Tet1 knockouts develop normally without any observable brain abnormalities, which may suggest that Tet2 or Tet3 can compensate for loss of Tet1.[124,125] Tet1 knockouts also have impaired short-term spatial memory formation and abnormal neurogenesis in neural precursor cells, but do not appear to have robust long-term memory impairments.[111,112,123,125] The knockouts surprisingly have abnormally enhanced long-term depression elicited in the hippocampus and an impaired ability to extinguish previously acquired associative memories (ie, extinction).[123] Expression of several plasticity-related genes were shown to be diminished, however, including *c-fos*, *Arc*, *Npas4*, and *Erg2* in hippocampus, likely resulting from hypermethylation of those genes. Tet1 knockout elevated promoter methylation of 478 genes and decreased methylation in 38 genes, with an overlap of 39 that were both hypermethylated and downregulated.[125] By contrast, hippocampal-targeted overexpression of Tet1 upregulated memory-associated genes including *c-fos, Arc, Erg1, Homer1*, and *Nr4a2* and impaired contextual fear learning.[112] Interestingly, overexpression of a catalytically inactive form of Tet1 also increased expression of those genes and impaired fear learning suggesting demethylase-dependent and -independent effects on learning and

gene expression.[112] Recent studies have also characterized the role of other potential demethylation factors in brain function. The growth arrest and DNA-inducible 45 (Gadd45) family of enzymes may bind to and focus the enzymatic activity of cytidine deaminases and thymidine glycosylases to specific gene promoters, thereby tagging specific genes for active demethylation.[126] Ma et al suggested that neuronal activity may focus base excision repair mechanisms to CpG promoters and this may be mediated by Gadd45 enzymes.[127] Leach et al reported that Gadd45b knockout mice are impaired in contextual fear conditioning, whereas Sultan and coworkers found improvements in contextual fear learning at 24 hours and 28 days post-training, and an enhanced late-phase LTP.[128,129] The reasons behind the contrasting results are not yet clear; however, in both cases the studies suggest that manipulations of putative demethylases can alter normal learning and memory.

DNA methylation in disorders presenting with cognitive impairment

The brain exhibits dynamic patterns of DNA methylation and DNMT expression during aging,[73,130-132] and the transcription of key memory-related genes declines in aging.[133] Consistent with this observation is that in aged animals impairments in LTP magnitude and maintenance are observed when weak LTP induction protocols (ie, near-threshold) are used.[133] Siegmund et al have shown changing patterns of DNA methylation patterns at various gene loci in human brain, with a general trend for increasing methylation over the lifespan in the 50 loci examined.[130] Inappropriate methylation of the activity-related cytoskeleton-associated protein (Arc), a known actor in synaptic plasticity and memory, may play a role in age-related memory impairments. Old rats have higher levels of methylation of the Arc promoter than do adult rats, and the transcription of Arc is reduced in the aged hippocampus after learning events relative to younger animals.[133-135] In addition, inhibition of Arc interferes with maintenance of LTP, likely due to its role in synaptic α-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid (AMPA) receptor trafficking.[136] Oliviera et al have demonstrated an aging-associated decrease in the expression of DNMT3a2, one of two transcripts from the DNMT3a gene, in the hippocampus of aged mice.[137] DNMT3a2 is structurally identical to DNMT3a1 except it lacks 219 amino acids in the N- terminus, is associated with euchromatin, and appears to act as an immediate early gene. Learning-induced activation of DNMT3a2 was shown to be impaired in aged mice and overexpression of DNMT3a2, which increased global DNA methylation in the hippocampus, improved performance in fear learning and object location memory tasks.

Specific gene methylation alterations have been shown in postmortem brain tissue from Alzheimer's disease patients, and these patients exhibit an accelerated rate of age-related change in methylation.[16,68,75] Alzheimer's-related alterations in DNA methylation may be complex and vary by brain region, as global hypomethylation has been shown in entorhinal cortex in postmortem Alzheimer's disease tissue,[68] as well as hypermethylation in the dorsolateral prefrontal cortex.[16]

Debilitating psychiatric disorders including schizophrenia, bipolar disorder, and major depressive disorder have been linked to aberrant DNA methylation. Mill and coworkers examined genomic DNA from 125 postmortem brains of schizophrenics, bipolar, and nonpsychiatric patients and concluded that DNA methylation is significantly altered in major psychiatric disorders.[76] Schizophrenia in particular is characterized by aberrant DNA methylation.[70-72,74,80,138] The *reelin* and *GAD1* promoter regions are hypermethylated in the brains of schizophrenic patients, and DNMT1 expression is upregulated.[67,71,80] The *GAD1* gene may be of particular interest as it codes for glutamic acid decarboxylase, the enzyme responsible for synthesis of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain.[77]

Studies using animal and cell culture models have corroborated the findings from human schizophrenics. Noh et al demonstrated that an antisense-driven knockdown of DNMT1 in mouse cortical neuron cultures was accompanied by an increase in reelin expression and suggest that reduced reelin and GAD67 protein may be due to DNMT1-mediated hypermethylation of their promoters.[57] Treatment with the histone deacetylase inhibitors TSA or valproate can decrease GAD1 promoter methylation, decrease *DNMT1* expression in mouse cortex, and increase the expression of *reelin* and *GAD67*.[40,139] Increased reelin and GAD67 expression were associated with the dissociation of MeCP2-containing corepressor complexes from their promoter regions. Interestingly, treatment with the DNMT inhibitors 5AZA and zebularine had the same effects. Deficits

in the ability to inhibit a startle response to an auditory stimulus when that stimulus is preceded by a smaller magnitude auditory stimulus are observed in human schizophrenic patients as well as in animal models of schizophrenia.[140] Our laboratory has recently found a dissociable impact of conditional forebrain knockout of DNMT1 and DNMT3a on prepulse inhibition in mice (unpublished data). DNMT1 knockout mice showed an enhanced inhibition of their startle response at all prepulse stimulus magnitudes tested, effects in opposition to the impairments in prepulse inhibition observed in schizophrenia models. By contrast, the DNMT3a knockout mice were indistinguishable from controls.

Significant stressors experienced in the prenatal environment may predispose an individual to the development of a psychiatric disorder in adulthood. Restraint stress experienced by a pregnant mouse leads to increases in DNMT1 and MeCP2 binding at the reelin and GAD1 promoter regions, changes that resemble those observed in postmortem samples from schizophrenic brain.[141]

Aberrant DNA methylation may also play a role in major depressive disorder. Polymorphisms in the *DNMT3b* gene were recently found to be associated with suicide attempts in depressed patients.[78] Postmortem brain samples revealed higher levels of global methylation. McGowan et al reported increased methylation of the glucocorticoid receptor gene in postmortem brain tissue from suicide victims that had experienced child abuse, findings that are consistent with the behavioral abnormalities observed in animal models of insufficient postnatal care.[23,142] Overexpression of DNMT3a in the nucleus accumbens has been demonstrated to induce depressive-like behavior in mice, whereas inhibition of DNMTs in the nucleus accumbens has antidepressant-like effects in a chronic social defeat model as well as in the forced swim test.[50,143]

Future questions

One hurdle in the way of a better understanding of the role of DNA methylation, and chromatin modification in general, in memory is the robust and dynamic interplay between the various enzymes and proteins capable of altering the chromatin. 5mC, by poorly understood signaling pathways, is able to recruit methyl-binding proteins and subsequently large chromatin remodeling complexes that are believed to stably mark stretches of the genome. The methyl-binding protein MeCP2 recognizes single 5mC sites and is thought to further recruit transcriptional corepressor complexes. Interestingly, approximately 95% of cases of the neurodevelopmental mental retardation syndrome Rett Syndrome are caused by mutations of MeCP2, with some similar phenotypes apparent in cases of MeCP2 duplication syndrome, which involves a duplication of the Xq28 chromosomal region harboring MeCP2.[144,145] Using a mouse model of MeCP2 duplication syndrome, our laboratory has shown that a 50% increase in MeCP2 in brain promotes motor coordination deficits, anxiety, learning and memory, and LTP impairments.[146] Therefore, the molecular events proceeding from DNA methylation followed by binding of MeCP2 appear to be of critical importance for cognitive function and synaptic plasticity.

The interaction between DNA methylation and histone acetylation has important functional consequences. DNMT inhibition can impair memory formation as well as LTP in hippocampal slices, and these effects are reversed by treatment with nonspecific histone deacetylase inhibitors.[105] Drugs that promote the acetylation of histones may facilitate the loosening of chromatin by leading to the release of methyl-binding proteins (eg, MeCP2[147]). Our laboratory has shown that pharmacological inhibition of DNMTs in cultured hippocampal neurons decreases mEPSCs, however this effect is occluded in MeCP2 knockout neurons.[107]

Another large gap remaining in the pursuit of a more complete understanding of the role of DNA methylation in memory formation is determining the mechanisms both upstream and downstream of the epigenetic alterations as well as how individual epigenetic modifiers are activated in distinct environmental and cellular contexts. For instance how are DNMTs or Tet proteins directed in a sequence-specific manner? Although the factors that guide a DNMT to a specific methylated site are not known for certain, it is believed that interactions with transcription factors and other chromatin proteins play a critical role.[70] Heterochromatin is characterized by distinct epigenetic marks, eg, methylated lysines 9 (H3K9) and 27 (H3K27), and DNA CpG methylation, which are associated with further recruitment of methyl-binding proteins, histone deacetylases, and other proteins. These histone modifications and the enzymes that catalyze their formation are known to interact with DNMTs and methyl-binding proteins. For example, the

histone protein H1d recruits DNMT1 and DNMT3b, and the histone methyltransferases SUV39H1 and G9a recruit DNMT3a.[148] It has previously been shown that the miR-29 family of micro RNAs is capable of targeting DNMT3a, DNMT3b, and the Tet proteins, potentially establishing a balance between methylation and demethylation at specific genomic targets.[114,149] Vire et al have highlighted the importance of Polycomb group proteins as links between the methylation of histones and DNA methylation.[150] Trimethylation of histone 3 at lysine 9 (H3K9) and H4K20 appears to be a prerequisite for subsequent methylation of a gene. Following H3K9 binding, heterochromatin protein 1 (HP1) can associate with DNMT3a by direct binding to its plant homeodomain (PHD) motif.[148]

Other significant questions include the following. Why is methylation seemingly quite dynamic in postmitotic neurons? How dynamic are chromatin states and the molecular agents that confer specific states resulting from environmental experience? Are there distinct roles for different DNMT or Tet isoforms in methylation and demethylation in specific tissues or in response to distinct signals? In spite of the inherent complexity of the epigenome, great strides are being made to determine its role in the central nervous system. These advances are, and will continue to be, essential for understanding diverse processes including memory formation, responsiveness to stressors and neurological insults, and the etiology of psychiatric disorders. ❑

Papel de la metilación del ADN y de las ADN-metiltransferasas en el aprendizaje y la memoria

La regulación dinámica de la estructura de la cromatina en las neuronas postmitóticas juega un importante papel en el aprendizaje y en la memoria. Históricamente la metilación de los nucleótidos de citosina ha sido considerada como la más potente y la menos modificable de las marcas epigenéticas. La acumulación de datos recientes sugiere que la rápida y dinámica metilación y desmetilación de genes específicos en el cerebro puede jugar un papel fundamental en el aprendizaje, la formación de la memoria y la plasticidad conductual. Este artículo se enfoca en la aparición de información que apoya el papel de la metilación y desmetilación del ADN, y sus mediadores moleculares en la formación de la memoria.

Rôle de la méthylation et des méthyltransférases de l'ADN dans l'apprentissage et la mémoire

La régulation dynamique de la structure de la chromatine dans les neurones post-mitotiques joue un rôle important dans l'apprentissage et la mémoire. Historiquement, la méthylation des nucléotides de la cytosine est la marque épigénétique la plus forte et la moins modifiable. D'après une série de données récentes, une méthylation et une déméthylation rapides et dynamiques de gènes spécifiques du cerveau pourraient jouer un rôle fondamental dans l'apprentissage, la formation de la mémoire et la plasticité comportementale. La mise au point actuelle s'intéresse aux nouvelles données en faveur du rôle de la méthylation et de la déméthylation de l'ADN et de ses médiateurs moléculaires dans la formation de la mémoire.

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