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Turning over DNA methylation in the mind

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Cytosine DNA methylation is a stable epigenetic modification with established roles in regulating transcription, imprinting, female X-chromosome inactivation, and silencing of transposons. Dynamic gain or loss of DNA methylation reshapes the genomic landscape of cells during early differentiation, and in post-mitotic mammalian brain cells these changes continue to accumulate throughout the phases of cortical maturation in childhood and adolescence. There is also evidence for dynamic changes in the methylation status of specific genomic loci during the encoding of new memories, and these epigenome dynamics could play a causal role in memory formation. However, the mechanisms that may dynamically regulate DNA methylation in neurons during memory formation and expression, and the function of such epigenomic changes in this context, are unclear. Here we discuss the possible roles of DNA methylation in encoding and retrieval of memory.

Keywords: DNA methylation, epigenome, brain, memory, learning, demethylation

Introduction

A fundamental aim of neuroscience is to understand the molecular, cellular and network mechanisms for encoding, storage and expression, or recall, of memory. Inspired by the prominence of synaptic connections between neurons in the physical architecture of brain circuits, theorists of brain function have long considered synapses to be the locus of information storage and processing. As suggested by Donald Hebb, neural activity could induce changes to the strength of synapses and thereby alter future network activity in an information-preserving manner (Hebb, 1949). Since then, computational neuroscience research on learning and memory has concentrated on the ways in which networks of neurons connected by plastic synapses can give rise to the processes of memory. Meanwhile, molecular and cellular neurobiology continues to elucidate the mechanisms of neural activity-triggered strengthening and weakening of synapses, known as long term potentiation (LTP) and depression (LTD), respectively (Kessels and Malinow, 2009). This synapse-centered framework for memory research is increasingly successful, and it has enabled in vivo fluorescence microscopy to visualize the synaptic changes that accompany encoding of new memories (Lamprecht and LeDoux, 2004).

However, despite its successful record, the synaptic theory of learning and memory cannot account for all of the empirical observations. Both transcription of genes and translation of proteins, including translation occurring in ribosomes located in neuronal dendrites near the activated synapses, are necessary for LTP and LTD (McClung and Nestler, 2008). The gene regulatory contribution to activity-dependent plasticity is mediated by multiple pathways, including CREB/MAPK (Cortés-Mendoza et al., 2013). These findings raise the question of...
whether gene regulation plays a merely permissive role for memory storage, for example, by synthesizing the ion channels, receptors, trafficking proteins and other cellular components necessary for altering synapse strength? Or, alternatively, does gene regulation play an instructive role in synaptic plasticity and memory formation, enabling sophisticated and information-rich responses to specific activity patterns, which can be stably maintained or dynamically modulated? If so, mechanisms of gene regulation could influence cellular and synaptic physiology in a complex way that meaningfully contributes to the brain’s computational function. Indeed, every mammalian cell possesses a sophisticated and highly specialized network of epigenetic mechanisms that control gene expression over a broad range of timescales. An intriguing possibility is that cells, and in particular post-mitotic neurons, take advantage of such epigenetic information processing to support cognitive processes (Crick, 1984; Day and Sweatt, 2011). A key implication would be that genomic and epigenomic regulation should be considered as central elements, and not merely implementational details, in computational models of biological cognition.

Recently, this more expansive hypothesis for the role of epigenetic gene regulation in memory formation has been bolstered by evidence that covalent modifications of DNA and chromatin participate in neuronal adaptation to experience. In this review, we explore the hypothesis that DNA methylation, one of the best characterized epigenetic regulatory mechanisms, could play an instructive role in memory encoding and storage. We describe the landscape of DNA methylation in brain cells, including unique features of the neuronal methylome that suggest neurons may use distinct modes of epigenetic regulation that are not present in other cell types. In particular, we discuss evidence that DNA methylation is dynamically regulated in brain cells, with enzymatically controlled deposition and removal of methylation marks in response to neural activity. We conclude with a perspective on the potential implications of dynamical DNA methylation for the processes of memory, and future directions that will be crucial for further exploration of this possibility.

Unique Features of the Brain Methylome

DNA methylation patterns are highly dynamic through mammalian development, with numerous cell-type-specific methylation patterns detected between distinct differentiated cell types (Maegawa et al., 2010; Maunakea et al., 2010). DNA methylation patterns are established by the de novo DNA methyltransferases DNMT3A and DNMT3B, while DNMT1 maintains DNA methylation patterns following genome replication (Yoder et al., 1997; Bestor, 2000; Goll and Bestor, 2005). Furthermore, the catalytically inactive DNMT3L protein interacts with its paralogs DNMT3A and DNMT3B, acting as an adaptor protein that can stimulate the DNA methyltransferase activity (Chédin et al., 2002; Gowher et al., 2005; Wienholz et al., 2010). DNMT3L plays important roles in establishing DNA methylation patterns in gametogenesis and in embryonic stem cells (Neri et al., 2013; Vlachogiannis et al., 2015). Both DNMT1 and DNMT3a have been shown to maintain DNA methylation and regulate synaptic function in adult forebrain neurons (Feng et al., 2010). DNA methylation in the genome of most vertebrate tissues is almost exclusively located at CG dinucleotides (also called CpG sites), and has most commonly been studied in this context. The advent of high-throughput DNA sequencers has enabled deep sequencing of sodium bisulfite-converted genomic DNA, allowing identification of the exact sites, sequence context, and levels of DNA methylation throughout almost entire eukaryotic genomes, termed the DNA “methylome.” This approach has shown that the methylome of brain cells has several unique features compared with other mammalian cell types. First, DNA methylation in the CH context (mCH, where H = A, C, or T) has been identified in the brain in both neurons and glial cells (Ramsahoye et al., 2000; Xie et al., 2012; Lister et al., 2013; Guo et al., 2014). This atypical feature of the brain methylome is also present in embryonic stem cells, but is much less abundant in other differentiated tissues (Lister et al., 2009; Schultz et al., 2015). Second, there is a substantial enrichment of 5-hydroxymethylcytosine (5hmC) in brain cells (Kriaucionis and Heintz, 2009). Below we discuss in detail these different forms of DNA methylation identified in the mammalian brain.

Non-CG Methylation

While DNA methylation is present in the conventional CG context in neurons and glia from before birth, mCH is almost undetectable in fetal and early-infant brain cells. Starting around 1 week of age in mice and within the first 2 years in humans (Figure 1), mCH accumulates rapidly and in parallel with synaptogenesis and synaptic pruning as the brain matures (Lister et al., 2013). By adulthood, the abundance of mCH has grown to a level equivalent to mCG in the neuronal genome, and in humans mCH accounts for more than half of all neuronal methylcytosines. This mCH appears to require expression of Dnmt3a, as shown by a recent conditional knockout in mouse neurons that eliminated mCH in the cerebellum (Gabel et al., 2015). Both mCH and mCG are strongly anti-correlated with gene expression in both neurons and glia, suggesting that mCH might play a previously unrecognized role in the repression of gene expression in neurons. Indeed, mCH was reported to repress transcription in reporter assays in mouse neurons, while a conditional neuronal triple knockout of all three DNMT3 led to reduced neuronal mCH but had little effect upon mCG (Guo et al., 2014). In support of this, glial genomes, which have only 10–20% as much methylation in the CH context compared to neurons, show highly localized mCH hypermethylation within gene bodies of repressed genes that are specifically active within neurons. These genes are specifically depleted of mCH (hypomethylated) in neurons, showing a cell type-specific role for mCH.

In addition to its broad genomic distribution, mCH is widely distributed across brain regions, mammalian species, and in multiple neuronal cell types. Besides the human and mouse frontal cortex (Xie et al., 2012; Lister et al., 2013), abundant mCH has been observed in the mouse dentate gyrus (Guo et al., 2014), in chimpanzee prefrontal cortex (Zeng et al., 2012), and mouse cerebellum (Gabel et al., 2015). Importantly, mCH in
neurons and glia is most abundant at CAC positions, which is distinct from the preferred sequence context in embryonic stem cells and pluripotent cell lines (Ziller et al., 2011; Varley et al., 2013). A key question concerns whether mCH is differentially distributed across brain cell types. Cortical function relies on a balance of activity among diverse neuron types, including excitatory pyramidal cells and a wide variety of inhibitory interneurons. These neural populations arise from distinct progenitor pools located in separate brain regions, and they follow different developmental trajectories. Neurons signal via a variety of neurotransmitters and they differ in terms of multiple morphological and physiological characteristics that affect their role in supporting healthy brain network dynamics. Epigenetic profiling of specific brain cell types in adults remains technically challenging (Maze et al., 2014), but recent advances will allow profiling mCG and mCH with greater cell type specificity (Smallwood et al., 2014; Farlik et al., 2015; Mo et al., 2015). mCH, alongside mCG and other epigenetic mechanisms, thus represents a potentially information-rich substrate for shaping the cell type-specific epigenetic landscape of neurons.

**Hydroxymethylcytosine**

A second unique feature of the brain methylome is the presence of 5-hydroxymethylcytosine (5hmC). Together, mC and 5hmC can be considered as the fifth and sixth bases that, alongside A, G, T, and unmodified C, make up an epigenetically enhanced DNA code. 5hmC is highly enriched in brain tissue and is particularly concentrated in specific neuron types, such as cerebellar Purkinje cells where it is estimated to be around 40% as abundant as mCG (Penn et al., 1972; Kriaucionis and Heintz, 2009; Mellén et al., 2012). 5hmC accumulates in multiple brain areas during development (Szulwach et al., 2011), not unlike the developmental accumulation of mCH. Interest in 5hmC was stimulated by the identification of specific pathways for converting mC to 5hmC via the TET family enzymes (Tahiliani et al., 2009). Further oxidation coupled with mechanisms such as the base-excision repair pathway can lead to demethylation of sites marked by 5hmC (He et al., 2011; Wu et al., 2014). These findings have raised the possibility that the abundant 5hmC found in mammalian neurons is a transient, intermediate state at locations undergoing active demethylation. According to this view, some genomic locations may undergo a cyclical dynamic of methylation and demethylation, leading to a steady-state distribution of 5hmC in a subset of cells at any point in time. Alternatively, 5hmC in neurons could represent a stable marker that is not a precursor to further modification or demethylation (Hahn et al., 2013). Instead, developmental accumulation of 5hmC in the brain may be associated with the loss of chromatin marks associated with polycomb-mediated repression, such as H3K27me3 (Hahn et al., 2013). Further experiments to distinguish these possibilities might manipulate specific elements of the proposed methylation/demethylation pathways, preferably with both temporal and cell type specificity (Wu et al., 2014).

5hmC is enriched at specific functional genomic compartments, including actively transcribed gene bodies (Mellén et al., 2012; Hahn et al., 2013; Lister et al., 2013). It can be recognized by the transcription factor MeCP2, suggesting that it could play a role in cognitive function (Mellén et al., 2012). Intriguingly, mCH may also be capable of binding MeCP2 and inducing transcriptional repression (Chen et al., 2015).

The above studies established that, in addition to classical methylation at CG positions, mammalian neurons accumulate two forms of DNA methylation that are unusual outside of the brain and pluripotent cells: mCH and 5hmC. What is the relationship between these two neuronally-enriched epigenetic marks? Bisulfite sequencing cannot, by itself, distinguish mC and 5hmC. Using TET-assisted bisulfite sequencing (TAB-Seq) 5hmC was found to be restricted to the CG context in mouse and human embryonic stem cells (Yu et al., 2012) and in mouse fetal and adult frontal cortex (Lister et al., 2013). mCH and 5hmC thus act as independent epigenetic regulatory marks, affecting distinct genomic sites.

**The Multi-Scale Brain Methylome**

To understand the potential role of different DNA modifications in cognition, it is helpful to classify them according to their temporal, spatial and genomic scale (Table 1; Figure 2). The most widespread, stable methylation patterns, such as extensive CG methylation outside of CG islands and distal regulatory elements, are shared across cell types and brain regions, persist through cellular differentiation and brain development, and are not generally altered as a function of experience. Such marks may be necessary for cellular function, as evidenced by the lethality of disruption of the maintenance methyltransferase Dnmt1 (Liao et al., 2015). However, constitutive methylation is not suited to a role in information processing, which requires a flexible, high-entropy substrate for encoding and storing the traces of specific experiences.

More dynamic aspects of the neuron methylome include the accumulation of abundant mCH and 5hmC in neurons during brain development (Kinde et al., 2015). These processes...
TABLE 1 | The multi-scale DNA methylome.

| Process type | Genomic scale | Cellular scale | Spatial scale | Time scale | Examples | Potential permissive/instructive for learning and memory | References |
|--------------|---------------|----------------|--------------|-----------|----------|----------------------------------------------------------|------------|
| Constitutive | Genome-wide   | All adult cells | Brain-wide   | Weeks/Years (lifespan) | Repressive role of promoter mCG | Permissive | Xie et al., 2012; Lister et al., 2013 |
|              | Genome-wide   | Neurons        | Brain-wide   | Weeks/Years (lifespan) | Accumulation of mCH | Permissive? Or no role? | Kriaucionis and Heintz, 2009; Szulwach et al., 2011; Mellén et al., 2012 |
|              | Genome-wide   | Dynamic (hours?) | Reduction of global mC following neuronal depolarization or fear conditioning | Likely permissive | Ma et al., 2009; Guo et al., 2011a |
|              | Chromosomal   | All adult cells | Brain-wide   | Weeks/Years (lifespan) | X-inactivation | Permissive | Lee and Bartolomei, 2013 |
|              | Megabase      | All adult cells | Brain-wide   | Weeks/Years (lifespan) | Differentially methylated valleys (DMVs); mCH deserts | Permissive | Xie et al., 2013 |
|              | 500 bp        | Specific neuron or glial cell types | Brain-wide   | Weeks/Years (lifespan) | Cell type DMRs | Permissive + Instructive? | Lister et al., 2013; Ziller et al., 2013 |
|              | 500 bp        | Specific neuron or glial cell types | Local brain region | Weeks/Years (lifespan) | Cell type and regional DMRs | Permissive + Instructive? | Lubin et al., 2008; Miller et al., 2010; Guo et al., 2011a,b; Mizuno et al., 2012; Baker-Andresen et al., 2013; Day et al., 2013 |
|              | 500 bp        | Individual cells | Dynamic (hours?) | Activity-dependent DMRs | Potentially Instructive? | | Miller et al., 2010 |
|              | 500 bp?       | Individual cells | Dynamic (hours?) | Activity-dependent 5hmC (DhMRs) | | | Hahn et al., 2013; Li et al., 2014 |
| Local        | 10 bp         | Specific neuron or glial cell types | Weeks/Years (lifespan) | Demethylation at (activity-dependent or independent) transcription factor binding sites | | Potentially instructive? | Guo et al., 2011b |

Examples of DNA methylation features that exist at different spatio-temporal scales.

unfolds over weeks (in mice) or years (human). These forms of DNA methylation affect the entire genome and, in the case of mCH, multiple neuron and glial cell types. These global patterns are thus more likely to play a neuron-specific role and may contribute to synaptic plasticity (see below). Yet, their widespread distribution makes them unlikely candidates for an information processing function.

Finally, discrete genomic regions, including gene bodies, promoters, and distal enhancers, show cell type- and brain region-specific mCG and mCH patterns (Lister et al., 2013; Ziller et al., 2013). In principle, some of these locations could be modulated in specific assemblies or circuits of cells; for example, a group of cells that contribute to a particular remembered place representation in the CA1 region of the hippocampus could, in response to a shared pattern of synaptic input, experience a coordinated modification of their DNA methylation state at particular genes or regulatory elements. Current DNA methylome profiling techniques lack the sensitivity or resolution
to identify such cell-specific methylation patterns (Maze et al., 2014), which may require bisulfite sequencing of material from small samples or even individual neurons (Smallwood et al., 2014; Farlik et al., 2015). If such patterns do exist and are at least partially regulated rather than stochastically modulated, they would be candidates for an instructive role in memory storage and information processing in the brain. They could potentially affect cellular behavior in specific and adaptive ways. However, linking these forms of DNA methylation to cognition requires understanding (1) how perturbing specific elements of the methylome may affect memory; and (2) how experience and neuronal activity can influence, and in turn be influenced by, discrete changes to individual cells’ methylome. Next, we turn to emerging evidence linking memory formation and storage with dynamic methylation patterns in brain cells.

**DNA Methylation Is Needed for Neuronal Plasticity and Memory**

Central to the processes of learning and memory is neuronal plasticity, the ability of neuronal activity to trigger lasting changes in the number and strength of synaptic connections between neurons (De Roo et al., 2008). In addition to such Hebbian plasticity, modulation of cellular properties such as intrinsic excitability or synaptic scaling can also contribute to neural plasticity (Guzman-Karlsson et al., 2014). To enable healthy neural plasticity, post-mitotic neurons of the adult brain must establish and maintain specific states of gene expression following neuronal activity in order to sustain long-term synaptic responses. Epigenetic regulatory pathways could play a key role by imparting stable states of transcriptional activity. It is now clear that epigenetic processes play critical roles in activity-dependent regulation of gene expression, and are required for adult neurogenesis, synaptic plasticity, and memory formation, consolidation and extinction (Lim et al., 2006; Miller and Sweatt, 2007; Schor et al., 2009; Feng et al., 2010; Gräff et al., 2012; Cortés-Mendoza et al., 2013; Day et al., 2013). Dnmt1 and Dnmt3a mRNA, protein, and activity are reduced by neuronal membrane depolarization (Sharma et al., 2008), and contextual fear conditioning increases Dnmt3a and Dnmt3b expression in the hippocampus (Miller and Sweatt, 2007). These data suggest that neural activity may modulate the abundance and activity of the cellular DNA methylation machinery.

**Genetic Manipulation of DNA Methyltransferases**

Disruption of the cellular pathways that establish, modify and maintain DNA methylation patterns in rodents has revealed that DNA methylation is required for learning and memory (Miller and Sweatt, 2007; Feng et al., 2010; Day et al., 2013). Conditional ablation of Dnmt1 impaired the postnatal viability, maturation and function of CNS neurons, causing abnormal
excitability and dendritic arborization, impaired synaptic long-term potentiation, and learning and memory deficits (Golshani et al., 2005; Hutnick et al., 2009). Conditional knockout of Dnmt3a in mouse forebrain neurons has implicated the de novo methyltransferase in specific complex cognitive and synaptic processes. While no differences in basal synaptic transmission were observed, Dnmt3a cKO mice displayed impaired memory formation and abnormal fear extinction, as well as spatial object memory, and induction and maintenance of hippocampal LTP (Feng et al., 2010; Morris et al., 2014). Thus, the DNA methyltransferases expressed in the adult brain appear to be essential for complex neuronal functionality, cognition, and adult behavior.

Consistent with this proposed role, the expression of Dnmt3a declines in the mouse hippocampus and cortex with increasing age in parallel with decreases in cognitive performance, hippocampus dependent memory, and euchromatic DNA methylation levels (Kang et al., 2001; Oliveira et al., 2012). A specific isoform of this methyltransferase, Dnmt3a2, is transcribed in response to neuronal activity, similar to other immediate early genes, and learning-induced Dnmt3a2 induction was reduced in the brains of aged mice (Oliveira et al., 2012). Strikingly, adenoviral delivery and expression of Dnmt3a2 in the hippocampus caused an increase in global DNA methylation levels in infected neurons and improved memory performance in fear conditioning and spatial object recognition tests. Conversely, shRNA-mediated knockdown of Dnmt3a2 in mature hippocampal neurons impaired long-term memory formation (Oliveira et al., 2012). Together, these findings suggest that activity-induced modulation of DNA methylation machinery and patterns in mature neurons is functionally important in memory formation, and that progressive reduction in Dnmt3a is involved in age-related cognitive decline in the mammalian brain.

Genetic manipulations also suggest a cognitive role for the demethylation machinery. Several recent studies have reported that impairment of the Tet dioxygenase enzymes results in impaired learning and memory. Tet1 knockout mice displayed impaired fear memory extinction and aberrantly stronger hippocampal long-term depression, together with reduced neural 5hmC and decreases in expression of neuronal activity-regulated genes that were associated with increased promoter DNA methylation (Rudenko et al., 2013). Conversely, viral-mediated overexpression of Tet1 led to a global increase in 5hmC and a decrease in mC, and impaired formation of long-term memory in a contextual fear conditioning paradigm (Kaas et al., 2013).

Pharmacological Inhibition of DNA Methyltransferases

In addition to genetic disruption of DNA methylation pathways, pharmacological inhibition of DNA methyltransferase activity has been widely used to investigate the role of this modification in neurological function. Incorporation of nucleoside analogs such as 5-aza-2′-deoxycytidine (5-azacytidine) and zebularine into genomic DNA results in hypomethylation due to their formation of a covalent bond with DNMTs, while RG108 acts as a non-nucleoside direct inhibitor of DNMTs through binding to the active site of the enzymes. Administration of these inhibitors has been used to study the involvement of DNA methylation in memory formation. For example, LTP can be blocked by infusion of DNMT inhibitors into hippocampal tissue slices, resulting in rapid demethylation of the promoters of reelin and Bdnf, which encode factors involved in synaptic plasticity (Levenson et al., 2006). Furthermore, contextual fear conditioning in rodents was reported to increase Dnmt expression in the hippocampus, and methylation and silencing of protein phosphatase 1, which encodes a chromatin remodeling regulator involved in memory suppression. Dnmt inhibition in this paradigm impeded memory formation (Genoux et al., 2002; Miller and Sweatt, 2007; Koshibu et al., 2009) and LTP (Miller et al., 2008). Moreover, infusion of DNMT inhibitors into the prefrontal cortex reduced the induction of promoter methylation at the memory suppressor gene calcineurin (CaN). This resulted in reduction of remote fear memory when administered and tested 1 month after training, but had no effect only 1 day after training, consistent with a role for DNA methylation in mediating long-term memory (Miller et al., 2010). Notably, 5-azacytidine, zebularine and RG108 were all demonstrated to disrupt remote memory. In a separate study, through infusion of 5-azacytidine into different brain regions in a cocaine-induced learning and memory model in mice, it was found that DNA methylation is required in the hippocampus for learning, and in the prelimbic cortex for memory retrieval (Han et al., 2010). Moreover, Day et al. reported that reward-related memory formation was associated with changes in DNA methylation in immediate early genes in the ventral tegmental area (VTA); the learning could be blocked through RG108 administration in the VTA (Day et al., 2013). The effect of DNA methylation on reward learning was remarkably specific to a localized brain region: disruption of methylation in brain regions adjacent to the VTA had no effect on learning (Day et al., 2013).

While the use of DNMT inhibitors has indicated a wide range of potential roles for DNA methylation in neuronal plasticity and memory, it remains unknown how DNMT inhibition through administration of nucleoside analogs such as 5-azacytidine and zebularine result in demethylation in post-mitotic neurons. This effect is puzzling given that the inhibitory effect of the drugs is understood to depend on incorporation into genomic DNA during genome replication. One potential mode of action could be through inhibition of cyclical demethylation-methylation processes, for example by nucleoside analog incorporation into genomic DNA after Tet- and base excision repair-mediated demethylation of 5hmC, followed by inhibition of DNMT-mediated remethylation of the cytosine analog.

These studies provide experimental support for a potential role of DNA methylation and demethylation in memory formation and synaptic and systems memory consolidation. Together, they suggest that the DNA methylation machinery and modification states in the brain may respond to experience, and could play important roles in neuronal plasticity and memory formation. However, it should be noted that the observed changes in DNA methylation in such studies are often assessed only at candidate loci, mostly at CG islands and promoter regions, consider methylation only in the CG context, and often report only partial changes in the level of methylation in the surveyed cell population. In order to gain insights into the full extent of
the DNA methylation dynamics in the brain related to neuronal plasticity and memory, it will be important to comprehensively assess all forms of DNA methylation, including mCH and 5hmC. Such studies will ideally generate single base resolution profiles throughout the entire genome, in specific subsets of cells, or even at the single cell level. Continuing advances in single cell DNA methyleome analysis (Smallwood et al., 2014; Farlik et al., 2015) and techniques for in vivo neuronal monitoring and manipulation (Deisseroth and Schnitzer, 2013), combined with decreases in the cost of DNA sequencing, will be critical for future progress in this area.

Evidence for Dynamic Methylation in Brain Cells

The emergence of a potential role for DNA methylation in the dynamic regulation of neural circuits is surprising, given the remarkable stability and conservation of the methyleome. A survey of DNA methylation patterns in a range of tissues and cell lines found evidence for dynamic methylation at only ∼22% of CG positions (Ziller et al., 2013). Whole-genome profiling studies have found highly consistent levels of methylation at individual CG and CH positions between frontal cortex samples in different adult mice or human subjects (Pearson r > 0.8, nearly as high as could possibly be observed given the sampling statistics; Lister et al., 2013). Homologous CH positions in human and mouse frontal cortex also showed highly conserved methylation (Lister et al., 2013). A large-scale study of DNA methylation in frontal cortex of 738 aged human subjects found that levels of mCG were significantly, but very weakly, correlated with sex, age, Alzheimer’s disease status, and, intriguingly, the time of the subject’s death (Lim et al., 2014). If different adult individuals, with different life experiences, have precisely conserved patterns of mCG and mCH in their frontal cortex, then how much room is there for neuronal activity-dependent modulation? Yet, even within a largely conserved methyleome that is highly consistent between different brain cells and across individuals and species, dynamic modulation of methylation at specific sites or regions could have an important impact. Localized dynamic methylation within key gene regulatory regions would not contradict the overall conservation of methylation at a global level.

Consistent with this, a growing number of studies have shown that DNA methylation can be dynamically modified by neuronal activity or by memory-forming experiences. However, the extent of such changes remains largely unknown due to the limited capability of current methyleome profiling to provide cell type-specific, genome-wide and base-resolution information. At a global level, the amount of both mC and 5hmC can be significantly reduced by neuronal activity or by memory-forming experiences. However, the extent of such changes remains largely unknown due to the limited capability of current methyleome profiling to provide cell type-specific, genome-wide and base-resolution information. At a global level, the amount of both mC and 5hmC can be significantly reduced by neuronal activity or by memory-forming experiences. 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genomic locations in the nucleus accumbens of mice exposed to cocaine (Feng et al., 2015). Reward learning experiments showed that experience-dependent dynamic methylation is targeted to specific genes (Day et al., 2013). Manipulation of Tet1 levels in the mouse brain has also been reported to modify DNA methylation at loci that display activity-induced DNA demethylation. AAV-mediated overexpression of Tet1 was shown to result in demethylation of the promoters of Bdnf and Fgf1B, which were previously observed to undergo active demethylation in response to electroconvulsive stimulation (ECS) (Martinovich et al., 2003), while shRNA-mediated knockdown of Tet1 through AAV delivery in the mouse brain abrogated ECS-induced Bdnf and Fgf1B promoter demethylation (Ma et al., 2009; Guo et al., 2011b).

A critical experimental and conceptual challenge is integrating results about dynamic DNA methylation from genome-scale profiling with findings at the level of single genes, promoters or enhancers. How widespread are changes in DNA methylation during learning? The genetic, molecular and cellular interactions that mediate different forms of learning and memory are intricate, and likely include many redundant and complementary pathways that could affect multiple genes and distal regulatory regions. Importantly, studies that reported learning-related changes to DNA methylation in activity-regulated neuronal genes like Egr1 also found no change at housekeeping genes like Gapdh (Day et al., 2013). Yet, such selective sampling does not address the vast majority of the potential learning-related methylome dynamics. It is therefore essential to analyze genome-wide data that interrogate the dynamic methylome without prior biases.

However, using genome-scale profiling techniques such as MethylC-seq or TAB-seq (discussed above) it may be difficult to achieve the sensitivity required for detecting DNA methylation changes of ~5-10% that are frequently reported by studies using targeted sequencing, and which reflect changes occurring in only a small sub-population of cells. This limitation arises because the expense of genome-wide profiling is often prohibitive for generating high coverage (say, >30-fold) for multiple replicates (Shin et al., 2014). Even if such data were available, a conceptual challenge remains in interpreting the many changes in mC and 5hmC that correlate with specific experiences or forms of neural activity. Some of these methylation dynamics may play a causal role for downstream modulation of transcriptional activity, for example by altering the binding of methylation-sensitive transcription factors (Schübeler, 2015), while others may be a consequence rather than a driver of changes in transcription. Such dynamic methylation could have different effects in different cell types, so cell type-specific profiling is an essential goal.

### Outlook and Open Questions

Although the presence of methylated nucleotides in DNA has long been known, the development of tools for high-throughput, genome-wide profiling (Maze et al., 2014) has established that the landscape of DNA methylation is complex and highly regulated at multiple spatial and temporal scales. In mammalian neurons, the methylome is distinguished from other cell types by the abundant presence of both mCH and 5hmC. Emerging evidence for a dynamic role for the DNA methylation and demethylation machinery in the cellular response to neural activity and in plasticity raises many intriguing questions. As we have argued, understanding the potential role of DNA methylation in cognitive processes such as learning and memory requires integrating information across scales, from the whole genome to individual gene promoters and even single modified bases, potentially ultimately requiring single cell resolution. It also requires methods for interrogating dynamic DNA methylation and determining the kinetics of such processes. To date, such studies have been limited by the requirement for relatively large quantities of genomic DNA for genome-scale methylome profiling. Furthermore, how such dynamic DNA methylation may be specifically targeted to highly localized genomic regions in an activity-dependent manner is currently unknown, and will likely require in depth studies into the targeting of the DNA methylation and demethylation machinery by specific proteins or non-coding RNAs that have the capacity to facilitate sequence-specific recruitment.

A key open question is the relative distribution of DNA methylation (mCG, mCH, and 5hmC) across specific neuronal and glial cell populations. Both excitatory and inhibitory neurons respond to neural activity by expressing common early-response transcription factors, such as Npas4, yet the downstream consequences are highly cell type-specific (Spiegel et al., 2014). We would thus expect that DNA methylation, in particular dynamic methylation patterns that may be involved in the cellular response to activity, will be distinctly regulated in different excitatory and inhibitory neuronal cell types. Supporting this, the abundance and distribution of 5hmC is cell type-specific in the cerebellum, where Purkinje neurons, granule cells and Bergmann glia harbor distinct amounts of the mark (Kriaucionis and Heintz, 2009; Mellén et al., 2012). Addressing this issue is challenging because it requires purifying nuclear material from defined cell types. Recent advances in cell sorting using marker genes (Molyneaux et al., 2015), transgenic mouse lines (Sugino et al., 2014), nuclei labeling and isolation (Deal and Henikoff, 2010), as well as single-cell analysis (Smallwood et al., 2014; Farlik et al., 2015) will help to uncover the role that DNA methylation is playing at the level of specific neuron types (Mo et al., 2015). By combining these molecular techniques with behavioral and systems neuroscience approaches, we may soon be positioned to learn whether DNA methylation is a necessary but merely permissive enabler of learning and memory, or whether DNA methylation and other epigenetic regulatory mechanisms are more deeply involved in the information processing function of the brain.

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References

Baker-Andresen, D., Ratu, V. S., and Bredy, T. W. (2013). Dynamic DNA methylation: a prime candidate for genomic plasticity and behavioral adaptation. *Trends Neurosci.* 36, 3–13. doi: 10.1016/j.tins.2012.09.003

Bestor, T. H. (2000). The DNA methyltransferases of mammals. *Hum. Mol. Genet.* 9, 2395–2405. doi: 10.1093/hmg/9.16.2395

Chédin, F., Lieber, M. R., and Hsieh, C.-L. (2002). The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16016–16021. doi: 10.1073/pnas.262443999

Chen, L., Chen, K., Lavery, I. A., Baker, S. A., Shaw, C. A., Li, W., et al. (2015). MeCP2 binds to non-CG methylated DNA as neurons mature, influencing transcription and the timing of onset for Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 112, 5509–5514. doi: 10.1073/pnas.1505909112

Cortés-Mendoza, J., Díaz de León-Guerrero, S., Pedraza-Alva, G., and Pérez-Martínez, L. (2013). Shaping synaptic plasticity: the role of activity-mediated epigenetic regulation on gene transcription. *Int. J. Dev. Neurosci.* 31, 359–369. doi: 10.1016/j.jidraeu.2013.04.003

Crick, F. (1984). Neurobiology: memory and molecular turnover. *Nature* 312, 101. doi: 10.1038/312101a0

Day, J. J., and Sweatt, J. D. (2011). Epigenetic mechanisms in cognition. *Neuron* 70, 813–829. doi: 10.1016/j.neuron.2011.05.019

Day, J. J., Childs, D., Guzman-Karlsson, M. C., Kibe, M., Moulden, J., Song, E., et al. (2013). DNA methylation regulates associative reward learning. *Nat. Neurosci.* 16, 1445–1452. doi: 10.1038/nn.3504

De Felipe, F., Marco, P., Fairén, A., and Jones, E. G. (1997). Inhibition of synaptic recognition and regulation of non-CG methylation in the adult mammalian brain. *Hum. Mol. Genet.* 6, 1386–1397. doi: 10.1093/hmg/6i.1219

Deal, R. B., and Henikoff, S. (2008). A simple method for gene expression and chromatin profiling of individual cell types within a tissue. *Dev. Cell* 18, 1030–1040. doi: 10.1016/j.devcel.2010.05.013

Deisseroth, K., and Schnitzer, M. J. (2013). Engineering approaches to illuminate brain structure and dynamics. *Nat. Neurosci.* 16, 586–577. doi: 10.1038/nn.3263

Farlik, M., Sheffield, N. C., Nuzzo, A., Datlinger, P., Schöngger, A., Klughammer, J., et al. (2015). Single-cell DNA methylation sequencing and bioinformatic inference of epigenomic cell-state dynamics. *Cell Rep.* 10, 1386–1397. doi: 10.1016/j.celrep.2015.02.001

Feng, J., and Nestler, E. J. (2013). Role of Tet1 and 5-hydroxymethylcytosine in cocaine action. *Nat. Neurosci.* 16, 5509–5514. doi: 10.1016/j.neuropharm.2014.01.001

Hahn, M. A., Qin, R., Wu, X., Li, A. X., Zhang, H., Wang, J., et al. (2013). Dynamics of 5-hydroxymethylcytosine and chromatin marks in mammalian neurogenesis. *Cell Rep.* 3, 291–300. doi: 10.1016/j.celrep.2013.01.011

Han, J., Li, Y., Wang, D., Wei, C., Yang, X., and Sui, N. (2010). Effect of 5-aza-2-deoxycytidine microinjecting into hippocampus and prelimbic cortex on acquisition and retrieval of cocaine-induced place preference in C57BL/6 mice. *Eur. J. Pharmacol.* 642, 93–98. doi: 10.1016/j.ejphar.2010.05.050

He, Y.-F., Li, R.-Z., Li, Z., Liu, P., Wang, Y., Tang, Q., et al. (2011). Tet-mediated formation of 5-carboxyhydroxyme and its excision by TDG in mammalian DNA. *Science* 333, 1303–1307. doi: 10.1126/science.1210944

Hebb, D. O. (1949). *The Organization of Behavior.* New York, NY: Wiley & Sons.

Hutnick, L. K., Golshani, P., Namihira, M., Xue, Z., Matynia, A., Yang, X. W., et al. (2009). DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. *Hum. Mol. Genet.* 18, 2875–2888. doi: 10.1038/hmg/ddp222

Huttenlocher, P. R., and Dahlshofer, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *J. Comp. Neurol.* 387, 167–178

Kaa, G. A., Zhong, C., Eason, D. E., Ross, D. L., Vachhani, R. V., Ming, G.-L., et al. (2013). TET1 controls CNS 5-methylcytosine hydroxylation, active DNA methylation, gene transcription, and memory formation. *Neuron* 79, 1086–1093. doi: 10.1016/j.neuron.2013.08.032

Kang, Y. K., Koo, D. B., Park, J. S., Choi, Y. H., Lee, K. K., and Han, Y. M. (2001). Differential inheritance modes of DNA methylation between euchromatic and heterochromatic DNA sequences in ageing fetal bovine fibroblasts. *FEBS Lett.* 498, 1–5. doi: 10.1016/S0014-5793(01)02472-3

Kessels, H. W., and Malinow, R. (2009). Synaptic AMPA receptor plasticity and behavior. *Neuron* 61, 340–350. doi: 10.1016/j.neuron.2009.01.013

Kinde, B., Gabel, H. W., Gilbert, C. S., Griffith, E. C., and Greenberg, M. E. (2015). Reading the unique DNA methylation landscape of the brain: non-CpG methylation, hydroxymethylation, and MeCP2. *Proc. Natl. Acad. Sci. U.S.A.* 112, 6800–6806. doi: 10.1073/pnas.1411269112

Koshub, K., Gräff, J., Beulens, M., Heitz, F. D., Berchtold, D., Russig, H., et al. (2009). Protein phosphatase 1 regulates the histone code for long-term memory. *J. Neurosci.* 29, 13079–13089. doi: 10.1523/jneurosci.3610-09.2009

Kriaucionis, S., and Heintz, N. (2009). Protein phosphatase 1 regulates the histone code for long-term memory. *J. Neurosci.* 29, 13079–13089. doi: 10.1523/jneurosci.3610-09.2009

Lamprecht, R., and LeDoux, J. (2004). Structural plasticity and memory. *Nat. Rev. Neurosci.* 5, 45–54. doi: 10.1038/nrn1301

Lee, J. T., and Bartolomei, M. S. (2013). X-inactivation, imprinting, and long noncoding RNAs in health and disease. *Cell* 152, 1308–1323. doi: 10.1016/j.cell.2013.02.016

Levenson, J. M., Roth, T. L., Lubin, F. D., Miller, C. A., Huang, I.-C., Desai, P., et al. (2006). Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. *J. Biol. Chem.* 281, 15763–15773. doi: 10.1074/jbc.M511762200

Liao, J., Karnik, R., Gu, H., Ziller, M. J., Clement, K., Tsankov, A. M., et al. (2015). Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7120–7125. doi: 10.1073/pnas.1318906111

Lim, A. S. P., Srivastava, G. P., Yu, L., Chibnik, L. B., Xu, J., Buchman, A. S., et al. (2014). 24-hour rhythms of DNA methylation and their relation with rhythms...
of RNA expression in the human dorsolateral prefrontal cortex. PLoS Genet. 10:e1004792. doi: 10.1371/journal.pgen.1004792

Lim, D. A., Suárez-Fariñas, M., Naef, F., Hacker, C. R., Menn, B., TAKEBASHI, T., et al. (2006). In vivo transcriptional profile analysis reveals RNA splicing and chromatin remodeling as prominent processes for adult neurogenesis. Mol. Cell. Neurosci. 31, 131–148. doi: 10.1016/j.mcn.2005.10.005

Lister, R., Mukamel, E. A., Nery, J. R., Urlich, M., Puddifoot, C. A., Johnson, N. D., et al. (2013). Global epigenomic reconfiguration during mammalian brain development. Science 341:1237905. doi: 10.1126/science.1237905

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., et al. (2009). Human DNA methylation at base resolution show widespread epigenomic differences. Nature 462, 315–322. doi: 10.1038/nature08514

Mohan, A. K., Loh, A., Zhang, B., Mahal, G., et al. (2015). Neuronal activity-induced Gadd45β promotes epigenetic DNA demethylation and adult neurogenesis. Science 323, 1074–1077. doi: 10.1126/science.1166859

Maegawa, S., Hinkal, G., Kim, H. S., Shen, L., Zhang, L., Jiang, Z., et al. (2010). Widespread and tissue specific age-related DNA methylation changes in mice. Genome Res. 20, 332–340. doi: 10.1101/gr.98626.109

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., et al. (2003). DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 302, 880–883. doi: 10.1126/science.1080482

Maunakea, A. K., Nagarajan, R. P., Bilenky, M., Ballinger, T. J., D’Souza, C., Fouse, S., et al. (2008). DNA methylation variation. Nature 452, 212–216. doi: 10.1038/nature06610

Sharma, R. P., Tun, N., and Grayson, D. R. (2008). Depolarization induces downregulation of DNMT1 and DNMT3a in primary cortical cultures. Epigenetics 3, 74–80. doi: 10.4161/epi.3.2.6103

Shen, J., Ming, G.-L., and Song, H. (2014). Decoding neural transcriptional and epigenomes via high-throughput sequencing. Nat. Neurosci. 17, 1463–1475. doi: 10.1038/nn.3814

Smallwood, S., A. S., Lee, H., Angermueller, C., Krueger, F., Saadeh, H., et al. (2014). Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. Nat. Methods 11, 817–820. doi: 10.1038/nmeth.3035

Spiegel, I., Mardinaly, A. R., Gabel, H. W., Bazinet, J. E., Couch, C. H., et al. (2014). Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs. Cell 157, 1216–1229. doi: 10.1016/j.cell.2014.03.058

Sugino, K., Hempel, C. M., Okaty, B. W., Armon, H. A., Kato, S.,Dani, V. S., et al. (2014). Cell-type-specific repression by methyl-CpG-binding protein 2 is biased toward long genes. J. Neurosci. 34, 12877–12883. doi: 10.1523/jneurosci.2674-14.2014

Szulwach, K. E., Li, X., Li, Y., Song, C.-X., Wu, H., Dai, Q., et al. (2011). 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat. Neurosci. 14, 1607–1616. doi: 10.1038/nn.2959

Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324, 930–935. doi: 10.1126/science.1170116

Varley, K. E., Gertz, J., Bowling, K. M., Parker, S. L., Reddy, T. E., Paul-Behn, F., et al. (2013). Dynamic DNA methylation across diverse human cell lines and tissues. Genome Res. 23, 555–567. doi: 10.1101/gr.147942.112

Vlachogiannis, G., Niederhuth, C. E., Tuna, S., Stathopoulou, A., Vlachogiannis, G., Niederhuth, C. E., Tuna, S., Stathopoulou, A., et al. (2010). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Cell 141, 1413–1426. doi: 10.1016/j.cell.2010.07.022

Wu, H., Wu, X., Shen, L., and Zhang, Y. (2014). Single-base resolution analysis of active DNA demethylation using methylase-assisted bisulfite sequencing. Nat. Biotechnol. 32, 1231–1240. doi: 10.1038/nbt.3073

Xie, W., Barr, C. L., Kim, A., Yue, F., Lee, A. Y., Eubanks, J., et al. (2012). Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. Cell 148, 816–831. doi: 10.1016/j.cell.2011.12.035

Yoder, J. A., Soman, N. S., and Bestor, T. H. (1997). DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. J. Mol. Biol. 270, 385–395. doi: 10.1006/jmbi.1997.1125
Yu, H., Su, Y., Shin, J., Zhong, C., Guo, J. U., Weng, Y.-L., et al. (2015). Tet3 regulates synaptic transmission and homeostatic plasticity via DNA oxidation and repair. Nat. Neurosci. 18, 836–843. doi: 10.1038/nn.4008

Yu, M., Hon, G. C., Szulwach, K. E., Song, C.-X., Zhang, L., Kim, A., et al. (2012). Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome. Cell 149, 1368–1380. doi: 10.1016/j.cell.2012.04.027

Zeng, J., Konopka, G., Hunt, B. G., Preuss, T. M., Geschwind, D., and Yi, S. V. (2012). Divergent whole-genome methylation maps of human and chimpanzee brains reveal epigenetic basis of human regulatory evolution. Am. J. Hum. Genet. 91, 455–465. doi: 10.1016/j.ajhg.2012.07.024

Ziller, M. J., Gu, H., Müller, F., Donaghey, J., Tsai, L. T.-Y., Kohlbacher, O., et al. (2013). Charting a dynamic DNA methylation landscape of the human genome. Nature 500, 477–481. doi: 10.1038/nature12433

Ziller, M. J., Müller, F., Liao, J., Zhang, Y., Gu, H., Bock, C., et al. (2011). Genomic distribution and inter-sample variation of non-CpG methylation across human cell types, PLoS Genet. 7:e1002389. doi: 10.1371/journal.pgen.1002389

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