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How the epigenome integrates information and reshapes the synapse

Rianne R. Campbell, **Marcelo A. Wood***

Department of Neurobiology and Behavior, Center for the Neurobiology of Learning and Memory, Center for Addiction Neuroscience, Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, CA, USA.

Abstract

In the past few decades, the field of neuroepigenetics has investigated how the brain encodes information to form long-lasting memories that lead to stable changes in behaviour. Activity-dependent molecular mechanisms, including, but not limited to, histone modification, DNA methylation and nucleosome remodelling, dynamically regulate the gene expression required for memory formation. Recently, the field has begun to examine how a learning experience is integrated at the level of both chromatin structure and synaptic physiology. Here, we provide an overview of key established epigenetic mechanisms that are important for memory formation. We explore how epigenetic mechanisms give rise to stable alterations in neuronal function by modifying synaptic structure and function, and highlight studies that demonstrate how manipulating epigenetic mechanisms may push the boundaries of memory.

Learning is the acquisition of new information, and memory is the ability to retain information in the long term for later reconstruction. In the brain, cells engaged in learning and memory processes undergo persistent changes to encode new information[1,2]. To consolidate new information into memory, neurons activated during learning require distinct profiles of gene expression[3,4]. Although the mechanisms that underlie the regulation of learning-induced gene expression are not fully characterized, researchers have turned to the epigenome as a signal-integration platform through which neurons might integrate new information at the molecular level in the service of stable changes in cell function.

* mwood@uci.edu.

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Epigenome

The collective combination of chemical modifications and proteins that interact with the human genome. The epigenome is dynamically regulated, serves as a signal-integration platform and is unique to each individual.

Histone modification

A post-translational modification — such as acetylation, methylation or phosphorylation — of a histone, a protein that interacts with nuclear DNA and helps to condense genomic DNA into chromatin.

Epigenetic mechanisms are broadly defined as processes that regulate gene expression through the alteration of chromatin structure without changing nucleotide base sequences[5,6]. Five major epigenetic mechanisms that cells utilize are histone modification, histone variant exchange, nucleotide modification, non-coding RNA-mediated regulation and chromatin remodelling[7]. With the exception of non-coding RNAs, these mechanisms alter chromatin structure and function, adding a very complex layer of regulation to gene expression. These mechanisms are best known for their actions during cell differentiation and cell division[8], including processes involved in the transgenerational passage of gene-regulatory information and the integration of environmental signals for the coordination of transcriptional responses in fully differentiated cells[6].

In the last few decades, several epigenetic mechanisms have been shown to regulate learning-induced gene expression in postmitotic neurons and to establish persistent behavioural responses[9–12]. Exactly how these mechanisms persistently alter neuronal function to encode information into long-term memory remains unclear. Discrete cell populations within particular brain regions, such as the hippocampus[13,14], have been suggested to form neuronal ensembles (known as engrams) to induce long-lasting connections that are responsible for the formation of memories. Epigenetic mechanisms are hypothesized to have a role in the acquisition and maintenance of the engram, for example by modulating the encoding process through epigenetic priming and the persistence of cell function.

The signalling mechanisms involved in the coordinated firing of neural circuits and synaptic plasticity are fairly well characterized, and the molecular events that occur dynamically at the synapse are highly complex. Various signalling cascades underlie the potentiation of synaptic responses and the structural changes of activated neurons following learning[15–18]. Changes in synaptic strength arise owing to the redistribution of glutamatergic receptors and changes in the activity of adhesion proteins[18–22]. In addition, postsynaptic dendritic spines are structurally modified through the introduction and stabilization of new actin cytoskeletal elements[23,24]. The learning and memory field is beginning to understand how these stable, structural changes at the synapse are regulated in response to experience and are necessary for the long-term encoding of newly learned information.

The epigenetic mechanisms involved in memory, addiction and brain disorders have previously been comprehensively reviewed[25–29]. Nevertheless, a common and perhaps unifying aspect among these topics is the synapse. Thus, this Review highlights pioneering studies that demonstrate how epigenetic mechanisms may be employed to encode information and regulate persistent changes at the synapse. We provide an overview of classic epigenetic mechanisms in the context of the synapse. We also discuss how epigenetic mechanisms become engaged by synaptic activity and how they may in turn lead to changes in synaptic structure and function. Last, we speculate on the conceptual avenues that could be taken to better understand how epigenetic mechanisms integrate experience into stable changes of synaptic function and ultimately cause long-lasting behavioural changes.

Histone variant exchange

Exchange of variants of the canonical histone proteins (namely, H2A, H2B, H3 and H4). Histone variants include H2AZ and H3.3 and can generate specialized chromatin domains and alter the DNA accessibility and thus gene expression.

Nucleotide modification

Epigenetic modification (mark) of nucleotide bases. For example, DNA methylation involves the attachment of a methyl group to the C5 position of cytosine (5mC). 5-Hydroxymethylcytosine (5hmC) seems to be more abundant in the brain.

Epigenetics in memory

Histone modifications, DNA methylation and nucleosome remodelling (FIG. 1) are three of the best-studied mechanisms that directly modulate chromatin structure to regulate the expression of genes related to learning and memory. The studies discussed below represent highlights of the initial work examining these epigenetic mechanisms in memory formation. Evidence implicating histone variant exchange[30–32] and higher-order chromatin looping[33,34] in learning and memory is only just emerging; these mechanisms are discussed in BOX 1. RNA modifications[35,36] and non-coding RNAs[37–43] regulate gene expression without altering DNA sequences (and therefore fall under the broadest definition of epigenetic phenomena) but do not directly affect chromatin structure, and are therefore not discussed below. However, they may help to establish persistent changes in neuronal function that are necessary for memory and other long-lasting changes in behaviour.

Chromatin remodeling

In general, the rearrangement and regulation of chromatin (DNA and associated proteins) by various mechanisms, including modification (for example, histone modification) and nucleosome remodelling.

Epigenetic priming

Stable epigenetic changes (DNA modifications and exchange of transcriptional cofactors and histone variants) produced by exposure to salient stimuli that induce neuronal stimulation; these changes permit efficient transcription of memory-related genes upon re-exposure and reactivation.

Histone modifications.

One of the main entry points into understanding epigenetic mechanisms that regulate memory processes came from investigation into the role of the transcription factor cAMP-responsive element-binding protein 1 (CREB1). Transcription has long been known to be required for the formation of long-term memories[44], and pioneering work in *Aplysia*, *Drosophila* and mice demonstrated that CREB1 is crucial for memory formation (reviewed in REFS[45,46]). In addition, the finding that phosphorylation of CREB1 at serine 133 induces recruitment of the CREB-binding protein (CBP), a histone acetyltransferase (HAT)[47], prompted the hypothesis that HAT activity may be important for regulating the gene expression required for formation of long-term memory.

Indeed, early studies revealed that $Cbp^{+/-}$ mice — a model of rubinstein–Taybi syndrome — exhibit deficits in long-term memory that correlate with reductions in histone acetylation[48–54]. Later studies that dissociated the role of CBP in development from its role in the adult brain using conditional CBP-mutant mice and pharmacological inhibitors demonstrated that CBP and histone acetylation are directly involved in memory[50–57]

Histone acetyltransferase

(HAT). An enzymes that catalyses the transfer of an acetyl group from acetyl-CoA to the ε-amino group of a histone lysine residue on a histone protein.

Histone deacetylases (HDACs) have the opposite molecular effects to those of HATs, and both types of enzyme interact dynamically with one another. One of the first studies to examine the bidirectional regulation of histone acetylation and its effects on gene expression was of long-term facilitation (LTF) in *Aplysia*[11]. Application of serotonin led to phosphorylation of CREB1 on the *CEBP* promoter and increased CBP recruitment for histone acetylation and LTF. By contrast, application of the inhibitory transmitter FMRFamide led to recruitment of HDAC5, which reduced histone acetylation and displaced CREB1 at the *CEBP* promoter, blocking LTF. However, HDAC5 is not is considered to be independently responsible for the deacetylase activity of histones; both HDAC5 and HDAC4 are believed to form complexes with other HDACs to regulate histone acetylation. In addition, their ability to regulate transcription has been shown to occur through their interactions with co-repressing transcription factors[58].

Rubinstein–Taybi syndrome

A condition characterized by moderate to severe intellectual disability, short stature, distinctive facial features and broad thumbs and first toes. it is often caused by *CREBBP* (also known as *CBP*) mutations.

Nevertheless, HDAC inhibition ameliorated impairments in long-term potentiation (LTP) and deficits in long-term memory in *Cbp*$^{+/-}$ mice[50] and in mice expressing a HAT-inactive form of CBP[52], and many subsequent studies have confirmed the function of individual HDACs in memory formation[59–65]. For example, expression of a deacetylase-inactive HDAC3 mutant in the dorsal hippocampus or the basal nucleus of the amygdala enhanced conditioned context fear, indicating that the deacetylase function of HDAC3 in these regions is crucial for negatively regulating the formation of context-fear memory[59]. In addition, several studies have identified upstream mechanisms that regulate HDAC activity to influence memory processes; for example, phosphorylation of HDAC4 or HDAC5 regulates their nuclear–cytoplasmic trafficking[58,66,67], and *S*-nitrosylation of HDAC2 leads to its dissociation from chromatin[68]. Notably, HATs and HDACs have many non-histone substrates; for example, they are sometimes referred to as KATs and KDACs on the basis of their more generic lysine acetylase activity[50]. Their substrates also include proteins that regulate transcription and assist in chromatin remodelling, such as nuclear factor-κB (NF-κB) and oestrogen receptor-α (ERα) — some of which are known to influence memory processes[69–72]. Proteins that bind to acetylated histone lysine tails, such as members of the bromodomain and extra-terminal domain (BET) protein family, recruit additional protein complexes that are necessary for transcription and thus also have a role in memory formation[73,74]. Although we do not discuss them in detail here, bromodomain-containing protein 4 (BRD4) regulates activity-dependent expression of immediate-early genes, and chronic or acute treatment with a BRD4 inhibitor impairs novel object recognition in mice[74]. Thus, histone acetylation creates a permissive transcriptional state for genes required for consolidation by: increasing the accessibility of plasticity-related genes; regulating the activity of critical transcription factors through acetylation and interaction with acetylation-regulating enzymes; and recruiting histone-acetylation-recognizing proteins and, in turn, additional transcription co-activators.

Methylation of histones, primarily of their lysine and arginine residues, is another epigenetic mechanism implicated in memory formation[75–78]. Histone methylation can activate or repress transcription, depending on the residue that is methylated and the degree of methylation[7,79,80]. Over the past decade, several histone methyltransferases have been shown to be crucial for memory. For example, G9a is a histone methyltransferase that forms a complex with G9a-like protein (GLP) to catalyse the dimethylation of H3K9 (H3K9me2). Its deletion from forebrain glutamatergic neurons using *CamkII-G9a*^{flox} mice resulted in abnormal locomotor activity as well as impairments in long-term contextual and cue-conditioned fear memory[76]. In addition, these animals showed an overall enhancement of gene expression, confirming the role of G9a as a transcriptional repressor. The cognitive defects seen in the *G9a*-knockout mice are suggested to be attributable to the aberrant effects on transcriptional homeostasis. For example, neuron-specific deletion of *G9a* led to the

expression of non-neuronal genes in neurons in widespread brain regions[76]. Thus, reduced G9a expression or activity could lead to memory impairments through defects in the regulation of multiple genes, including cell-type-specific genes[81–83].

Histone deacetylases

(HDACs). enzymes that remove acetyl groups from lysine residues on DNA. Acetyl groups help to neutralize the positive charge of histones and/or serve as binding sites for bromodomain-containing proteins.

Long-term facilitation

(LTF). A form of long-term synaptic plasticity observed in *Aplysia californica*.

Ten-eleven translocation enzymes

(TETs). enzymes that convert 5-methylcytosine (5mC) DNA marks to 5-hydroxymethylcytosine (5hmC), which is enriched within gene bodies, promoters and transcription-factor-binding regions and may influence gene expression.

Similar to histone acetylation, histone methylation can influence the recruitment of transcription factors and the activity of other epigenetic enzymes to regulate gene expression. Contextual fear training leads to increased H3K4 trimethylation (H3K4me3), a transcriptionally permissive mark, at the *Zif268* promoter in the hippocampus, and this increase is accompanied by increases in local DNA methylation and reductions in local methyl CpG-binding protein 2 (MeCP2)–DNA binding[84]. In addition, histone methylation can be influenced by mechanisms of histone acetylation: systemic infusion of an HDAC inhibitor reduces fear-conditioning-induced increases in H3K9me2 (which represses transcription) in the hippocampus[84]. The above examples demonstrate the high complexity of the effects of changes in histone methylation and methyltransferase function. Several other histone methyltransferase enzymes have been implicated in memory formation (for example, histone-lysine *N*-methyltransferase 2A (KMT2A)–KMT2D)[85–89], as have other histone modifications (including phosphorylation, ubiquitylation, sumoylation, ribosylation and citrullination), discussed elsewhere[85,90].

DNA modification.

DNA methylation is a type of DNA modification[91–98] and mainly occurs symmetrically on CpG dinucleotides. Promoter methylation was originally considered to be a static mechanism to silence gene expression by recruiting methyl-CpG-binding proteins such as MeCP2 and other associated transcription-repressing protein complexes. However, DNA methylation is now considered to have more complex effects on gene expression, not only at the promoter but also in the gene body. Moreover, although DNA methylation was initially considered only to negatively regulate memory processes, neuronal activity can induce expression of several enzymes that control DNA methylation. These include DNA methyltransferases (DNMTs) and DNA-demethylating enzymes, such as growth arrest and

DNA damage-inducible protein-β (GADD45B) and ten-eleven translocation enzymes (TETs)[99–103].

During memory consolidation, de novo DNA methylation and demethylation occur within the CA1 region of the hippocampus and are enriched at both inter-genic and intronic regions[104]. These activity-induced changes in DNA methylation can correspond to the differential expression of genes that, according to gene ontology analysis, are functionally categorized under 'ion channels' and 'transcription regulation'[104,105]. From findings such as these, DNA methylation is suggested to regulate synaptic transmission and gene transcription critical for memory formation. In support of this, pharmacological inhibition of DNMT activity blocks the induction of LTP[91], and DNMT expression is upregulated following contextual fear conditioning[106]. Following a learning event, several memory-forming genes are transiently demethylated, and memory-suppressing genes are transiently methylated to promote synaptic plasticity[94,106]. Intriguingly, DNA methylation seems to mediate stable changes in the expression of memory-related genes[94]. For example, 30 days after fear conditioning training in rats, cortical expression of calcineurin, which negatively regulates memory formation, was reduced, and the gene encoding calcineurin was hypermethylated. Thus, epigenetic modifications can potentially induce stable changes in neuronal function that give rise to long-lasting behavioural changes. Examining epigenome modifications along time frames extending beyond the typical 1–24 hours studied by most laboratories will be very important to understand how epigenetic mechanisms may maintain long-term memories.

The site of DNA methylation within a gene is also important for memory-related gene expression. After reward learning, the expression of the immediate-early genes *Egr1* and *Fos* is increased in the ventral tegmental area and correlates with increases in DNA methylation specifically in the 3′ ends of their gene bodies, but not in the gene promoter[107]. Infusion of a DNMT inhibitor into the ventral tegmental area during training was sufficient to impair the acquisition of reward-related memories. This effect may be due to the regulation of intragenic DNA methylation, as in vitro application of a DNMT inhibitor reduced KCl-induced hypermethylation of intragenic regions of the *Egr1* and *Fos* genes and enhanced *Egr1 and Fos* expression. Although these results suggest that learning requires locus-specific regulation of DNA methylation, exactly how DNA methylation influences gene expression during memory formation is still being defined.

As mentioned above, mechanisms of DNA and histone modifications work in concert to regulate gene expression following learning; however, the mechanisms are not fully characterized. For example, histone methylation (H3K4me3) and DNA hydroxymethylation (5-hydroxymethylcytosine (5hmC)) were found to co-occur in intron regions of the immediate-early gene *Npas4* in CA1 of the hippocampus following the retrieval of a recent fear memory[108]. Knockdown of the histone methyltransferase MLL1 in CA1 abolished retrieval-induced increases in DNA 5hmC levels at the *Npas4* gene and prevented both fear memory and retrieval-induced increases in CA1 *Npas4* mRNA[108]. The field of DNA methylation is still in its nascent stages, and future research will focus on understanding how gene-specific and locus-specific DNA methylation affects memory processes[104,105,109,110].

Nucleosome remodelling.

Despite their known role in regulating gene expression and interactions with chromatin modifiers, the function of nucleosome-remodelling complexes (NRCs) in memory processing is somewhat understudied (FIG. 1). There are four families of NRCs (BRG1-associated factor (BAF), INO80, ISWI (imitation switch) and CHD (chromodomain helicase DNA-binding)) that regulate chromatin compaction through active sliding, ejecting or restructuring of nucleosomes[111]. NRCs are typically large protein complexes involving many protein subunits that probably dictate the specificity of their function for cell types and loci[111]. Studies in cultured *Baf53b*$^{-/-}$ hippocampal neurons revealed that the ATP-dependent BAF subunits are crucial for activity-induced dendritic outgrowth[112]: these neurons showed impairments in activity-dependent dendritic outgrowth and reduced expression of neurite outgrowth-related genes. Although RNAi-mediated reduction of other neuronal BAF (nBAF) subunits similarly affected activity-dependent dendritic outgrowth, BAF53B is the only subunit that has been studied within memory formation in vivo[113–115]. The field must further investigate how the different NRC families interact and contribute to activity-dependent gene expression and memory formation. Several mutations of NRC-encoding genes are linked to human intellectual disability disorders, including Coffin–Siris syndrome and autism spectrum disorder[26], demonstrating the role of NRCs in cognitive processes.

Nucleosome-remodelling complexes

(NrCs). Large protein complexes that, through the activity of ATP-dependent enzymes, alter histone–DNA interactions, disassemble or assemble nucleosomes, exchange histone variants or slide or reposition nucleosomes.

Histone code hypothesis

A hypothesis that posits that specific patterns of epigenetic modifications regulate specific gene expression networks for defined cell functions.

Early-phase LTP

(E-LTP). in this context, a form of potentiation that is dependent on covalent protein modifications, yet independent of gene expression. it is transient and short-lived (generally on the order of tens of minutes in slices).

Late-phase LTP

(L-LTP). in this context, a form of potentiation that is dependent on transcription and translation. it is long-lasting (generally on the order of hours in slices and hours to days in vivo).

Outstanding questions.

In summary, there is little doubt that histone modifications, DNA methylation and NRCs have a role in learning and memory. The field has yet to answer many questions about the nature of their functions. First, are any epigenetic mechanisms cell type-specific, and if so, what are their consequences? This may be especially relevant to NRCs, which exhibit cell-type-specific subunit expression. Second, do enzymes that may have histone-modifying functions in the nucleus have subcellular roles (for example, non-histone-modifying functions in the cytoplasm), and is there any crosstalk between these enzymes in different cellular compartments? Third, what are the patterns established by histone and DNA modifications, and how do they coordinate gene expression for specific cell functions (that is, in line with the histone code hypothesis[116])? Fourth, do histone modifications and other epigenomic modifications represent a molecular substrate of memory in a way that is relevant to the concept of the engram[25]?

Extinction

Weakening of a conditioned response owing to long or repeated trials of memory retrieval in which the conditioned stimulus is removed. extinction is hypothesized to result from the formation of new memories.

Reconsolidation

The re-encoding and re-stabilization of a memory after reactivation, during which time the memory is hypothesized to be labile and vulnerable to manipulation.

Remote memories

Here, memories that were encoded a long time previously and that have since become independent of the hippocampus and dependent on cortical regions of the brain (through a process sometimes termed systems consolidation).

Pushing the boundaries of memory

Epigenetic mechanisms have an important role in forming long-term memories, altering the chromatin landscape in a way that leads to a more permissive transcriptional environment for the expression of memory-promoting genes. In parallel to the identification of various epigenetic enzymes as regulators of memory processes[55,95,106,117–119], researchers have asked whether these enzymes could be exploited to expand the type or amount of information normally acquired and/or stored after a learning event or to extend the time for which it is stored.

Targeting histone modifications.

Several early studies demonstrated that histone acetylation could be observed during memory consolidation, a process during which gene expression is necessary for long-term

memory formation[75,120,121]. One study demonstrated that blocking histone deacetylation using the nonspecific HDAC inhibitor trichostatin A (TSA) changed the response to a single tetanus stimulation from a transcription-independent form of LTP known as early-phase LTP (E-LTP) to transcription-dependent, late-phase LTP (L-LTP), similar to that typically observed following multiple high-frequency tetani[122]. This finding raised the intriguing question of whether a similar phenomenon could be observed at the behavioural level when examining memory.

In an object recognition memory task, mice given the HDAC inhibitor sodium butyrate (NaB; intraperitoneally) after a single subthreshold training session that was normally insufficient to induce lasting memory exhibited robust long-term memory 24 hours later[123]. These effects seem to be persistent, as mice exhibited long-term memory after 7 days, a time point at which even mice trained over a longer session will fail to show long-term memory[123]. These results were corroborated with additional techniques using genetic focal deletions and pharmacological inhibition of HDAC3 (REFS[117,124–126]). Manipulations of HDAC3-dependent acetylation can affect the formation of not just contextual memory but also auditory memory. Rats treated with the HDAC3-selective inhibitor RGFP966 not only exhibited more robust memory for the learned association between a sound and a water reward[127] but also encoded additional, highly specific features of sounds associated with reward into memory[128]. These studies demonstrate that histone-modification mechanisms may ultimately alter the kind of information encoded during memory consolidation. These mechanisms could potentially be manipulated to increase the amount and alter the types of information being consolidated, as well as to increase information persistence in memory.

Other studies have investigated the influence of targeting histone modifications on other memory processes, including the extinction or reconsolidation of recent memories, as well as other memory types, such as remote memories. Pharmacological agents that target histone-modifying enzymes to transform these memory processes might thus have therapeutic potential for individuals suffering from traumatic long-term memories (that is, in post-traumatic stress disorder)[27]. During normal fear extinction training, histone acetylation increases on H3 and H4 histones (for example, H3K14, H3K9 and H4K8) in the hippocampus[129,130], lateral amygdala[59,61] and infralimbic prefrontal cortex[131]. Studies involving systemic or region-specific infusions of HDAC inhibitors revealed that histone acetylation can enhance the extinction of recent long-term memories, including fear-associated or drug-associated memories[129–134]. Similar studies have examined the role of histone acetylation in reconsolidation; however, the findings are conflicting, suggesting that the effects of manipulating reconsolidation by altering histone acetylation may be specific to the type of memory or learning process[109]. For example, the retrieval or reactivation of contextual memory is reported to induce acetylation of H3 and H4 in the hippocampus and lateral amygdala. Moreover, HDAC inhibitors (such as TSA) promote, and HAT inhibitors impair[57,61,135], the reconsolidation of fear conditioning, whereas HDAC inhibitors have no effect on inhibitory avoidance[136]. This difference in effect could be explained by the fact that these tasks depend on different neural systems; inhibitory avoidance tasks require an instrumental response whereas fear conditioning is a Pavlovian association. However, a more selective approach may have yielded a different result, as virus-mediated reduction of HDAC3 activity in the dorsal striatum was sufficient to accelerate the formation of habit

behaviour in instrumental learning tasks[137]. Thus, there is still much to discover regarding the role of HDACs in different brain regions and in the acquisition of different types of memory.

Epigenetic hypothesis of age-related cognitive impairments

A hypothesis proposing that the repression of chromatin and alterations in the expression of synapse-related genes lead to cognitive impairment in ageing brains.

Remote memories are thought to be more resistant than recent memories to manipulations (such as extinction paradigms) during reconsolidation after reactivation. However, administration of an HDAC2-selective inhibitor during the reconsolidation phase after spaced or massed extinction of a conditioned fear memory attenuated remote memories[68], by enhancing histone acetylation (specifically H3K9 or H3K14) and promoting the expression of neuroplasticity-related genes and LTP. Moreover, in non-treated animals, the recall of remote memory led to less histone acetylation than that observed in animals that recalled recently encoded memories. Thus, changes in histone acetylation may alter mechanisms of synaptic plasticity to make remote memories more labile and ‘recent-like’. In line with this idea is the epigenetic hypothesis of age-related cognitive impairments and preclinical evidence demonstrating that manipulating histone acetylation enzymes can rescue disease-related and age-related memory impairments[138]. However, the exact epigenetic mechanisms underlying age-related memory impairments are unknown[121,139,140]; which target genes are affected and how acetylation of their histones enhances and induces the persistence of memory processes beyond the capacity of normal memory are yet to be understood. The current progress and limitations of HDAC inhibitors are discussed in BOX 2.

Histone methylation has been implicated in the initial consolidation of memories[77,85,86,89,90,141] and seems to be induced by and to regulate other memory processes (such as extinction) and memory reactivation[90,108,141,142]. The importance of histone methylation in age-related and disease-related cognitive impairments has also been explored. For example, H3K9me3 is increased in the hippocampus of aged animals compared with young-adult mice, and systemic administration of an inhibitor of the histone methyltransferase SUV39H1 improved object location and fear conditioning memory performance in aged, but not young-adult mice[78]. SUV39H1 inhibition in aged animals also restored levels of hippocampal H3K9me3, baseline levels of the synaptic glutamate receptor subunit GluR1 and the density of thin and stubby spines in the hippocampus to young adult levels. These initial findings support the hypothesis that dysregulated epigenetic mechanisms contribute to age-related cognitive dysfunction. In addition, these results demonstrate the potential therapeutic value of targeting histone methylation for rescuing age-related cognitive impairments.

Targeting DNA methylation.

DNA methylation regulates memory processes, including consolidation, extinction[103,143,144] and reconsolidation[61], as well as synaptic processes such as synaptic scaling (BOX 3), and

could be targeted to enhance memory formation. For example, in mice, overexpression of DNMT3A2 before training, similar to HDAC inhibition, resulted in the long-term memory of normally subthreshold training. In addition, inducible overexpression of DNMT3A2 in the hippocampus specifically before extinction training facilitated extinction[145]. Moreover, reducing endogenous DNMT3A2 activity in the mouse dorsal hippocampus using short hairpin RNAs abrogated extinction memories[145]. Endogenous DNMT3A2 also declines with age, and restoring DNMT3A2 levels in aged mice reduces age-related memory impairments[98]. Thus, it will be crucial to identify the loci targeted by DNMT3A2 that are relevant for hippocampus-dependent memory to better understand its mechanism in the context of memory.

The above examples demonstrate the ability of several epigenetic enzymes to push the boundaries of memory processes. Although several target genes that are necessary for these memory-enhancing effects have been identified[117,146], the precise mechanisms underlying their effects are unknown. Manipulations targeting epigenetic processes may potentially enhance memory by inducing persistent changes in the structure and function of synapses.

Epigenetics and the synapse

Over the past decade, there has been an emphasis on identifying epigenetic modifiers and remodellers involved in memory processes and synaptic plasticity. Given their important effects, epigenetic mechanisms have more recently begun to be integrated into the framework of cellular theories of memory. To better incorporate epigenetics into this framework, researchers have begun to examine the role of epigenetics in molecular processes that regulate synaptic structure and function. Here, we review theories of synaptic tagging and mechanisms of synapse-to-nucleus signalling in relation to memory formation and postulate the potential role that epigenetic mechanisms may have in these cellular processes. We highlight reports that begin to address how altering the chromatin landscape affects the expression and activity of synapse-related proteins to affect synaptic plasticity and memory formation. Other epigenetic mechanisms that do not alter chromatin structure but that have been implicated in memory processes are discussed in BOX 4. Understanding the functional relationship between the epigenome and synaptic structure and function will be necessary to better understand fundamental aspects of memory and disorders associated with memory dysfunction.

Activity-dependent changes that promote long-lasting forms of synaptic strength — for example, LTP — are crucial for encoding and maintaining information[17,24]. LTP is initiated by the activity of postsynaptic NMDA receptors (NMDARs) and calcium/calmodulin-dependent protein kinase II (CAMKII). LTP is stabilized through the activation of cell surface receptors on a potentiated subpopulation of dendritic spines, which induces changes in actin polymerization[23,147–151]. These proteins include several cell adhesion proteins, such as integrins[22,152], cadherins[21,153–155] and neurexins[18,156], which mediate the cell–cell and cell–extracellular matrix interactions that are crucial for synaptic plasticity and memory formation (FIG. 2). In addition, cadherin adhesion molecules form trans-synaptic interactions and, following neuronal activity, become increasingly localized at the synaptic membrane and promote AMPA receptor (AMPAR) stabilization[157]. Signalling pathways

downstream from these surface receptors, together with the activity of ionotropic glutamate receptors such as AMPARs, promote the local disassembly of the cytoskeleton and trafficking of additional 4 network and enable synaptic proteins, such as CAMKII, to alter dendritic spine activity.

The cytoskeleton of the dendritic spine head is rebuilt and expanded through the polymerization of filopodia-actin (F-actin). Signalling cascades initiated by calcium-dependent GTPases, including the RHOA and PAK pathways, prevent depolymerization and drive the organization of actin filaments[163]. Cell adhesion molecules facilitate activity-dependent reorganization of actin filaments to prevent potentiated spines from returning to their pre-potentiated state. For example, integrins help to link actin filament bundles to the plasma membrane and the extracellular matrix[164]. Newly synthesized proteins are required to further reconstruct the cytoskeleton and thus to consolidate and maintain the potentiation of synapses[23,147,159,165–171].

A key open question is whether epigenetic mechanisms, which can induce stable changes in cell function, initiate and maintain the learning-induced potentiation of synapses through the molecular processes outlined above. Below, we probe the relationship between epigenetics and mechanisms affecting synaptic structure and function.

Epigenetic mechanisms in synaptic tagging.

Long-lasting forms of synaptic plasticity are thought to require gene expression, protein synthesis and the formation of new synaptic connections[172,173]. However, exactly how changes in gene expression and protein synthesis give rise to synapse-specific alterations is less clear. Frey and Morris hypothesized that synapses are ‘tagged’ following stimulation and then capture newly synthesized gene products that are functionally relevant for plasticity[174]. This capture of nearby plasticity-related proteins (PRPs) facilitates molecular interactions between neighbouring dendritic spines to transform short-term plasticity into long-term plasticity, which are mediated by E-LTP and L-LTP, respectively[175–179].

Extra-coding RNAs

A form of non-coding, sense-strand rNA that is non-polyadenylated, encoded by a portion of DNA that overlaps the boundaries of another gene.

Recent evidence suggests that epigenetic mechanisms may promote long-term plasticity through synaptic tagging. In a recent study in aged animals, which show hippocampal L-LTP deficits, the HDAC3 inhibitor RGFP966 re-established synaptic tagging and capture and restored L-LTP[180]. This study investigated whether HDAC3 affects synaptic tagging by using a two-pathway ‘weak-before-strong’ experiment. In this experiment, two stimulating electrodes in the stratum radiatum of the hippocampus induce E-LTP at one synaptic input 1 (S1) of a neural pathway using a weak stimulation, and L-LTP at another synaptic input (S2) of the same neural pathway using a stronger stimulation, while a recording electrode in between the inputs records from distal apical dendrites. Unlike the stimulation at S1, the stronger stimulation at S2 synapses is hypothesized to tag synapses and to promote the expression of PRPs to stabilize potentiation. The application of RGFP966 to these

hippocampal slices transformed E-LTP at the weakly stimulated S1 input into L-LTP[180]. This finding suggests that HDAC3 inhibition may promote the expression of PRPs that are typically expressed following the stronger stimulation as at S2. However, whether HDACs in aged or young neurons deacetylate transcription factors and/or co-regulators, and/or histones, is currently unclear. Nevertheless, this work raises the possibility that epigenetic enzymes have a role in synaptic tagging and capture processes, potentially by regulating nuclear gene expression.

Enhancer RNAs

A form of non-coding rNA transcribed from active enhancers. They can control mrNA transcription, challenging the idea that enhancers are merely sites of transcription factor assembly.

Notably, local translation in dendrites can be affected by RNA modifications, which represent another type of epigenetic mechanism[181–183]. Epigenetic mechanisms are therefore hypothesized to regulate local translation in dendrites to assist synaptic tagging and potentiation and to facilitate the transcription of PRP-encoding mRNAs in the nucleus. Further experiments — in particular, looking at the effects of manipulating the localization of epigenetic enzymes in the cell — should shed light on these potential mechanisms.

From the synapse to the epigenome.

With the induction of potentiation, synapses are hypothesized to induce signalling cascades to alter gene expression to enable synapse-specific plasticity. One proposed mechanism for synapse-to-nucleus signalling is the activity-dependent nuclear translocation of synaptic proteins, which then alter transcription[160,169,184–189]. Consistent with this proposal, synaptic stimulation induces the translocation of the CREB-regulated transcriptional co-activator 1 (CRTC1) from dendrites to the nucleus, where it assists in the regulation of a set of CREB target genes[190]. However, few studies have examined how synaptic proteins that shuttle to the nucleus may induce long-lasting changes in chromatin structure[186,190–192].

Activity-induced synaptic proteins may influence epigenetic mechanisms. For example, expression of brain-specific fibroblast growth factor 1B (FGF1B), which is required for CA3–CA1 LTP and hippocampus-dependent learning, depends on CRTC1 in the nucleus. Whereas weak memory training induces only transient expression of FGF1B (mRNA and protein), strong training leads to sustained FGF1B expression. Following (weak or strong) training, the HDAC3–nuclear receptor co-repressor (NCOR) complex is removed from the *Fgf1b* promoter, and phosphorylated CREB and CBP are recruited in its place to induce transient expression of *Fgf1b* (0.5–1 hour following training). Unlike weak training, however, strong training leads to subsequent CRTC1-mediated exchange of CBP for the HAT KAT5 on the *Fgf1b* promoter, which in turn induces persistent expression of *Fgf1b* (2 hours following training)[193]. This KAT5 substitution was required for hippocampal synaptic plasticity and memory enhancement.

E3 ubiquitin ligase

A protein that facilitates the interaction of a target (or substrate) protein with an ubiquitin-conjugating e2 enzyme to enable the transfer of ubiquitin to the target protein.

CRTC1 is not the only synaptic protein with a potential role in regulating epigenetics. Following neuronal stimulation, the synaptic protein afadin shuttles to the nucleus to promote the phosphorylation of H3S10, a histone modification that transforms condensed heterochromatin to provide a more transcriptionally permissive, euchromatin state[194,195], and this epigenetic mechanism is required for dendritic spine remodelling[196].

Synaptophysin

A synaptic vesicle membrane protein that is ubiquitously expressed throughout the brain and has a role in synapse formation.

These are some of the first findings to demonstrate that nuclear translocation of synaptic proteins may underlie epigenetic processes required for memory. Further research examining how information is transported from the synapse to the nucleus will be needed to understand how synaptic signals induce learning-related gene expression.

INTACT

(isolation of nuclei tagged in specific cell types). A method to isolate nuclei tagged in specific cell types for further examination for specific proteins or rNAs or high-throughput sequencing.

Epigenetic regulation of the synapse: transmembrane proteins.

Integrins are transmembrane adhesion receptors that modulate dendritic morphology by mediating signals from the extracellular matrix and interacting with diffusible factors such as oestrogen and brain-derived neurotrophic factor (BDNF)[152,197,198]. Integrin activation induces postsynaptic RHO GTPase signalling, thus activating LIM domain kinase (LIMK), which in turn deactivates the actin-filament-severing cofilin, to regulate cytoskeleton dynamics[22,152,199–202]. The induction of this cytoskeleton-regulating pathway promotes LTP via the insertion of glutamatergic receptors and the expansion of the postsynaptic spine densities[160,203–206]. Although actin-related proteins and their upstream effectors have been heavily implicated in synaptic plasticity, there is less information regarding the epigenetic regulation of the expression and activity of actin-regulating proteins.

TRAP

(Translating ribosome affinity purification). A ribosome-tagging method in which a fusion protein binds ribosomal proteins and immunoprecipitation purification processes isolate biologically relevant mrNA transcripts.

Assay for transposase-accessible chromatin using sequencing

(ATAC-seq). A method for mapping genome-wide chromatin accessibility. A transposase inserts sequencing adaptors into accessible regions of chromatin, before adaptor-ligated DNA fragments are sequenced.

The nBAF subunit of the BAF53B NRC may have a role in the activation of actin-regulating pathways and the stabilization of potentiated synapses following the induction of learning[115]. In the hippocampus of wild-type mice, theta-burst stimulation (TBS) induces LTP and alters synaptic morphology by affecting actin regulation: TBS promotes increases in spines containing phosphorylated p21-activated kinase (PAK) and downstream cofilin[204]. However, compared with their wild-type counterparts, *Baf53b*$^{+/-}$ mice do not express TBS-induced LTP, and TBS-induced inactivation (that is, phosphorylation) of the actin-severing protein cofilin in the postsynaptic density is reduced. *Baf53b*$^{+/-}$ mice also fail to show activity-dependent increases in hippocampal expression of genes involved in the postsynaptic cell membrane and cytoskeleton[115]. Thus, the structural and functional LTP deficits in *Baf53b*$^{+/-}$ mice may result from altered activity-dependent-expression of synapse-related proteins. In a follow-up study, deficits in hippocampus-dependent memory and hippocampal LTP in mice lacking the BAF53B subdomain 2 were rescued by overexpression of a phosphomimetic of cofilin in the hippocampus[114]. Consistent with this work, overexpression of BAF53B within the lateral amygdala led to enhanced memory formation and thin-spine density, whereas *Baf53b* knockdown within the lateral amygdala impaired fear memory formation[113]. Together, these studies demonstrate that NRCs have a key role in synaptic plasticity and memory, perhaps by regulating actin dynamics.

Zinc-finger proteins

(ZFPs). A large family of transcription factors with finger-like DNA-sequence-specific domains. Fusion of a DNA-binding domain specific for an 18–20 bp genomic locus to a chromatin-modifying enzyme enables targeted epigenetic regulation.

From the epigenome to the synapse: GluRs.

The reorganization of the cytoskeleton expands dendritic spine heads and is associated with the trafficking of additional AMPARs to the synapse. The trafficking of AMPARs and NMDAR-dependent increases in Ca^{2+} lead to long-lasting forms of LTP[19,20,207–211]. Epigenetic regulation of GluR subunit expression occurs during critical periods of synaptic remodelling, including development[212], stress[213,214] and following drug exposure[215]. Understanding how extracellular signals influence the epigenetic regulation of synaptic GluR expression in these contexts is necessary.

After exposure to stress, AMPARs are ubiquitylated for degradation, and recent evidence suggests that this ubiquitylation results from epigenetic changes[214]. In rats, repeated stress led to glucocorticoid-receptor-dependent increases in expression of *Hdac2* and increased occupancy of HDAC2 on the *G9a* promoter, reducing G9a expression. Normally, G9a methylates the promoter of the gene encoding an e3 ubiquitin ligase, reducing its expression;

thus, in stressed animals, upregulated HDAC2 reduces G9a expression and indirectly promotes the expression of the E3 ubiquitin ligase. In stressed rats, knockdown of *Hdac2* prevented the increases in E3 and the degradation of AMPARs in the prefrontal cortex and ameliorated the stress-induced disruption of AMPAR-mediated plasticity[216,217]. These and other studies suggest that stress hormone signalling affects epigenetic regulators (including neural-restrictive silencer factor (NRSF) as well as HDAC2) that then persistently alter synaptic receptor expression, either directly or indirectly[213,218,219]. The precise molecular mechanisms giving rise to the persistence of these effects on receptor expression, and whether these effects are established and/or maintained by long-lasting epigenomic changes, remain to be understood.

From the epigenome to the synapse: presynaptic proteins.

Neurexins are presynaptic cell adhesion molecules that interact with various postsynaptic ligands to govern the connectivity of synaptic circuits (FIG. 2). Each of the thousands of expressed neurexin isoforms may show different specificities for various postsynaptic ligands (for example, different neuroligins) and thus have different effects[156]. For example, the inclusion of alternative splicing sequence 4 (SS4) in neurexin 1 interferes with postsynaptic AMPAR trafficking and represses long-term synaptic plasticity[220]. Histone modifications can regulate exon splicing by affecting either the recruitment of splice machinery or mechanisms of transcriptional elongation[221,222], although, to date, little evidence demonstrates a definitively causal role for epigenetic mechanisms in regulating mRNA splicing and isoform expression in memory.

Nevertheless, shedding new light on this topic, a recent study in the mouse dentate gyrus reported that neuronal activity drives the recruitment of the HDAC2–p66a complex onto exon 22 of *Nrxn1* to promote the inclusion of SS4 in neurexin 1 (REF.[223]). Subsequently, the histone methyltransferase SUV39H1 preserves SS4 inclusion by trimethylating H3K9 on exon 22 of *Nrxn1*. NRXN1 that contained SS4 showed reduced binding affinity for postsynaptic neuroligin 1B (NLGN1B) and synaptophysin clustered on NLGN1B-expressing cells. However, these reductions in trans-synaptic interactions were not seen in neurons from *Suv39h1*$^{+/-}$ mice, supporting the hypothesis that mechanisms downstream from histone methylation regulate synapse formation. Moreover, in mice that underwent context-specific fear conditioning followed by distraction training (that is, exposure to a neutral context following the initial recall test), knockdown of *Suv39h1* in the dentate gyrus reduced freezing in the shock-associated context compared with wild-type controls. Thus, SUV39H1 activity is crucial for memory preservation, possibly by preventing synaptic rewiring that could interfere with mechanisms of memory preservation. These results provide an example of how epigenetic mechanisms can regulate gene expression, through splicing, to preserve memories through the stabilization of synaptic structure. This study also outlines a possible mechanism by which a histone modification at a specific locus is necessary for memory persistence. Future studies — for example, using CRISPR-targeted approaches (BOX 5) — will be needed to establish a causal relationship between the histone modification and splicing, and to determine whether splicing regulation is unique to memory persistence or occurs in other memory processes.

Conclusions and outstanding questions

As discussed above, epigenetic mechanisms are critical modulators of synaptic plasticity and memory. In turn, activity-dependent synaptic changes engage epigenetic mechanisms. Not only are enzymes that modify the epigenome necessary for various memory processes, but pharmacologically or genetically altering the function of these enzymes dramatically changes the ability of neurons to encode information. Altering epigenetic mechanisms can affect the molecular and cellular mechanisms that establish the usual limits of synaptic plasticity, memory formation and memory persistence, and may perhaps even affect how many neurons are engaged in learning and memory. In turn, the relationship between the epigenome and the synapse is probably an important factor in these processes: synaptic proteins are reported to affect epigenetic mechanisms, and vice versa. Despite correlational demonstrations of these interactions, we are still lacking a fundamental understanding of how they regulate memory.

One clear question is whether there are direct and causal mechanisms of information transfer from the synapse to the epigenome that are necessary for stable changes in neuronal function. That is, are there bona fide synaptic proteins (or other molecules, such as RNA) that are locally translated or released from the synapse upon activity-dependent stimulation that travel to the nucleus? In addition, do synaptic proteins that translocate to the nucleus directly participate in the epigenetic regulation of genes that stabilize changes in synaptic structure and function at the originally engaged synapses? To answer these questions, the coordinated cell-type-specific and locus-specific interactions between synaptic and epigenetic proteins must be examined (BOX 5).

Another key open question is whether persistent changes in the epigenome at specific synaptic protein-encoding genes establish and/or maintain stable changes in synaptic structure and function. Some of the studies discussed above begin to answer this question; however, the field has just begun to investigate how epigenetic control of synapse-related genes is regulated during memory processes. Moreover, we must examine how epigenetic regulation of established memory-associated genes affects synaptic function. Relatedly, whether the epigenome represents a form of molecular memory for past experiences, and whether learning-induced epigenomic modifications directly participate in the encoding of information that is required for the engram, are unknown[224]. Addressing questions such as these will provide insight into the dynamic relationship between the epigenome and synapse and thus will substantially advance our understanding of the molecular mechanisms underlying the formation, persistence and limits of memory.

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Box 1 |

Emerging aspects of neuronal chromatin regulation

Many aspects of the regulation of chromatin structure and modification of the epigenome are not yet fully understood. although by no means new to the field of epigenetics, new work is shedding light on how histone variant exchange and higher-order chromosomal interactions regulate neuronal gene expression.

Histones are the basic proteins around which DNa is wrapped to form nucleosomes. There are five main families of histones: the core histones H2A, H2B, H3 and H4 and the linker histone H1. There also exist non-allelic and distinct histone isoforms called histone variants. The best understood variants are those in the H2A and H3 families and include H2AZ and H3.3. An ATP-dependent nucleosome-remodelling complex replaces H2A with H2AZ, which is involved in establishing transcriptional competence and nucleosome stability and is localized around transcriptional start sites[225]. H2AZ (and two related hypervariants H2A.Z.1 and H2A.Z.2) can also be incorporated into the nucleosome by neuronal activity to affect regulation of plasticity-related genes and the formation of fear memory[30,31,226]. H3.3 is involved in nucleosome assembly and usually replaces histones at active genes (reviewed elsewhere[227]). H3.3 accumulates in the brain from embryonic development to become the predominant H3 variant in the adult brain. Histones in neuronal chromatin seem to exhibit continuous turnover, as more than 30% of the total neuronal H3.3 pool is replaced in all mouse brain regions examined within a 4-week period[32]. Furthermore, neuronal activation (through various means) induced H3.3 expression, and knocking down H3.3 expression reduced the density of hippocampal dendritic spines and impaired object recognition memory and contextual fear memory. Together, these studies demonstrate a dynamic and pivotal role for histone variants in regulating the activity-dependent gene expression required for memory processes. It will be interesting to determine whether the exchange of histone variants can affect transcription in a way that influences the effects of subsequent learning or that instils permanent memory.

On a much larger dimensional scale are higher-order chromosomal interactions, which represent another level of gene regulation with emerging importance in neuroscience. One of the main approaches to studying how chromosomal regions that are separated by massive distances (tens to hundreds of kilobases) interact is called chromosomal conformation capture[228]. In this method, chromatin is crosslinked, isolated and then digested using specific restriction enzymes; the resulting fragments are subsequently ligated and the ligated fragments are analysed using real-time PCR. The abundance of the ligated fragments correlates with the frequency of interactions between two regions. Chromosomal conformation capture has been used to demonstrate how enhancer elements loop to make contact with promoter regions and the assembly of transcriptional complexes to coordinate gene regulation[229]. it is also being used to define the 3D architecture, preferential organization and specific boundaries of the genome inside a nucleus[230] — all of which will further influence how gene expression is regulated. Determining how these long-range interactions and the 3D architecture of the genome

regulate neuronal gene expression, as recently investigated in several studies[33,231,232], will be an important area of research.

Box 2 |

Histone deacetylase inhibitors

Pharmacological histone deacetylase inhibitors (HDACis) have been used often to study the role of histone acetylation in learning and memory. there are two categories of HDACs: zinc-dependent HDACs and NAD-dependent sirtuins (SIRTs). All zinc-dependent HDACs are expressed in the brain, primarily by neurons, and are categorized on the basis of sequence similarity into class I (HDAC1–HDAC3 and HDAC8), classes IIa and IIb (HDAC4–HDAC7, HDAC9 and HDAC10) and class IV (HDAC11). Among SIRT1–SIRT7, only SIRT1–SIRT3 and SIRT5 have deacetylase activity. Several HDACis were developed for treating cancer, and several potent HDACis have shown promising preclinical effects in promoting the histone acetylation required for memory processes.

Initially, pan-HDACis demonstrated the ability of HDACs to regulate learning-induced gene expression, long-term potentiation and memory consolidation[52,122] before the roles of specific HDAC classes and isoforms were dissected. Of the class I HDACs, HDAC2 and HDAC3 strongly regulate learning and memory. Chronic treatment with SAHA (an inhibitor of class I and IIb HDACs) enhances fear conditioning memory in wild-type mice but does not further enhance fear-conditioned freezing in HDAC2-deficient mice[118], suggesting that SAHA enhances fear memory through inhibition of HDAC2. Such findings have prompted efforts to develop HDAC2-selective inhibitors for improving memory[233]. Similarly, the most abundant class I HDAC in the brain, HDAC3, also negatively regulates long-term memory[59,117,124,125,234]. Mice treated with the HDAC3-selective inhibitor RGFP966 showed enhanced long-term object location memory and object recognition memory and increased H4K8 acetylation[117,124]. Chronic administration of HDAC-isoform-selective inhibitors has, however, produced mixed results. For example, chronic homecage RGFP966 treatment did not alter dendritic spine density in the hippocampus or ameliorate memory impairments in a mouse model of alzheimer disease[235,236]. Some evidence suggests that activation of SIRT1 deacetylase activity may reduce neurodegenerative processes: in CK-p25 mice, a model of neurodegeneration, oral administration of the SIRT1 activator SRT3657 recapitulated the neuroprotective effects of caloric restriction, delaying the onset of neuronal death[237]. Although the roles of different HDAC isoforms still need to be fully characterized, such studies indicate that HDACs are pivotal regulators of neural plasticity and behaviour. For more studies using HDACis, see reFs[238,239].

The (mostly) pro-mnemonic effects of HDACis in animal models instilled high hopes for their therapeutic potential to ameliorate cognitive impairment. However, several points must be borne in mind when considering HDACis for possible therapeutic treatment. For example, although the inhibitors were developed against specific enzymes and tested for their in vitro activity on purified enzymes, they were often not tested against purified protein complexes. An inhibitor may thus disrupt protein–protein interactions among HDACs, negating much of its claimed HDAC specificity[67]. these inhibitors also show low cell-type specificity; although many inhibitors penetrate the blood–brain barrier (BBB), they do not exclusively act in particular brain regions or on specific gene sets in the brain. Nevertheless, systemic off-target effects could be reduced by increasing the

BBB permeability of these inhibitors. Soon, pharmacological agents will be able to be targeted to specific cell types.

Another important consideration is whether chronic and acute treatment may differentially affect memory processes. HDACis can induce differential effects depending on the behavioural context, therefore different treatments may need to be designed for specific cognitive impairments in different disease states. We are still in the early stages of understanding how to target HDACs and their complex roles in memory. However, given their dynamic roles and powerful effects, efforts dedicated to characterizing and manipulating HDAC mechanisms promise to be of therapeutic value.

Box 3 |

DNA modification and cell-wide synaptic plasticity

Although DNA methylation enzymes have been implicated in learning and memory, the underlying cellular mechanisms remain fairly unclear. A new perspective was provided by recent studies identifying a relationship between DNa methylation and cell-wide synaptic plasticity. In one study in cultured hippocampal neurons, treatment with the sodium channel tetradotoxin (TTX), which reduces synaptic activity and triggers global synaptic upscaling, increased the expression of ten-eleven translocation enzyme 3 (TET3)[240], which oxidizes 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) to initiate DNA demethylation. Indeed, TET3 was crucial for homeostatic synaptic upscaling, a cell-wide synaptic plasticity mechanism (distinct from synapse-specific, Hebbian forms of plasticity)[241]. Short hairpin RNA-mediated knockdown of TET3 considerably increased the amplitude of miniature glutamatergic excitatory postsynaptic currents (mEPSCs) measured by whole-cell patch clamp[240]. By contrast, neurons overexpressing TET3 exhibited smaller mEPSC amplitudes, suggesting that increases and decreases in the levels of TET3 bidirectionally affect excitatory synaptic transmission. Importantly, these effects on synaptic transmission were attributable to changes in DNA oxidation, but not to changes in oxidation-independent functions that TET enzymes are known to have.

Similar results were reported in a study examining DNA methyltransferases (DNMTs)[242]. The small-molecule DNMT competitive inhibitor RG108 drove synaptic upscaling in cultures of cortical pyramidal neurons, and knockdown of *Dnmt1* and *Dnmt3a* blocked this effect. In contrast to the study in hippocampal neurons described above[240], in which TTX increased the expression of TET3, but not TET1 or TET2, this study in cortical neurons[242] showed that chronic TTX treatment increased the expression of TET1, but not TET3 (or DNMT1 or DNMT3). This discrepancy could be attributable to many factors but does suggest that cell-type specific mechanisms may be in play. Together, these two studies, and earlier studies implicating methyl CpG-binding protein 2 (MeCP2), which binds methylated DNa[243–245], reveal a more causal relationship between DNA methylation mechanisms and synaptic scaling.

What these synaptic upscaling observations imply about normal learning and memory processes in the brain is not clear. Nevertheless, these results provide new and unusual insights into the regulation of cell-wide synaptic plasticity by epigenetic mechanisms and potentially provide new insight into understanding how manipulations of histone modification or DNA methylation could drive long-term memory for otherwise subthreshold learning events; promote formation of long-term memories that persist beyond the normal lifespan of other memories; enable more neurons than normal to be engaged during learning; or gate the encoding of additional features during learning (observations discussed in the main text).

Box 4 |

Additional epigenetic mechanisms in memory processes to explore

There is still much to be discovered regarding the finer details of the epigenetic mechanisms discussed in this article, and new epigenetic mechanisms involved in memory processes are still being identified.

For example, accumulating evidence suggests that several types of RNA species, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), can regulate synapse-related gene expression and memory formation[35,143,246–249], with their ability to regulate neural gene expression at least partly attributable to their epigenetic functions. For example, the expression of extra-coding rNAs transcribed from DNa overlapping with the boundaries of the immediate-early gene *Fos* is necessary for formation of fear memory[37]. *Fos* extra-coding RNA molecules may interact with DNMTs and thus direct site-specific DNa methylation and *Fos* gene regulation. In addition, although not yet seen in the context of memory processing, some evidence from embryonic mouse fibroblasts suggests that certain enhancer rNAs interact with CREB-binding protein (CBP) to stimulate histone acetylation and transcription[250].

Moving forward, focus should be placed on understanding how gene regulators interact with one another and coordinate their influence on transcription. in one example of such efforts, deletion of *Hdac3* (encoding histone deacetylase 3) from the hippocampus ameliorated impairments in both memory and synaptic plasticity that were caused by mutations of the gene encoding the BAF53B subunit of the BRG1-associated factor (BAF) nucleosome-remodelling complex[251]. One possible explanation for this rescue is that HDAC3 deletion could have led to enhanced histone acetylation at memory-related genes that may have promoted a permissive chromatin structure despite the absence of normal nucleosome remodelling. Alternatively, BAF53B disruption might have impaired memory by preventing histone-acetyltransferase-dependent histone acetylation, which was restored by the deletion of HDAC3.

The complexity of gene regulation by epigenetic mechanisms such as these must be thoroughly investigated; these research pursuits will be critical to elucidating how cells integrate information to induce long-lasting changes in behaviour and should point to novel therapeutic avenues for human disorders associated with mutations in genes encoding neuronal BAF complex subunits, including BAF53B.

Box 5 |

Tools to study epigenetic processes

The advent of recent genetic tools will assist in the pursuit of answering the open questions outlined in this article. For example, deep-sequencing methods such as single-cell RNA sequencing will be useful to analyse how subpopulations within cell types of the brain are uniquely altered during memory formation and updating[252]. Methods that allow for isolation or manipulation of specific cell types, such as iNTACT (isolation of nuclei tagged in specific cell types) and TrAP (translating ribosome affinity purification), will also prove useful. The best implementation of these techniques would be to overlay sequencing results (for example, RNA sequencing with chromatin immunoprecipitation (ChIP) sequencing) to obtain a more accurate representation of changes in gene expression. Researchers can also turn to Hi-C and ATAC-seq (assay for transposase-accessible chromatin using sequencing) to examine the long-term changes in chromatin structure that enable a memory to be sustained and recalled. To provide more causal evidence of how epigenetic mechanisms regulate cellular and behavioural plasticity, researchers could use tools such as zinc-finger proteins (ZFPs) and CRISPR–Cas9 to induce locus-specific epigenetic modulations[139,249,253,254]. Furthermore, temporally specific manipulations such as optogenetic control of transcription factors and epigenetic enzymes will enable the control of gene expression within specific brain regions[255].

To further advance this work, the field must use techniques that control locus-specific epigenetic mechanisms in a temporally specific manner. Given the sophisticated techniques available, the role of epigenetics in cellular and behavioural functions can be examined simultaneously. For example, changes in gene expression can be induced by CRISPR–dCas9, and changes in the function of individual cells can be assessed using calcium imaging. Ultimately, understanding the epigenetic regulation of synaptic plasticity may reveal gene regulation mechanisms that, when disrupted, lead to abnormalities in cellular function that are observed in cognitive disorders.

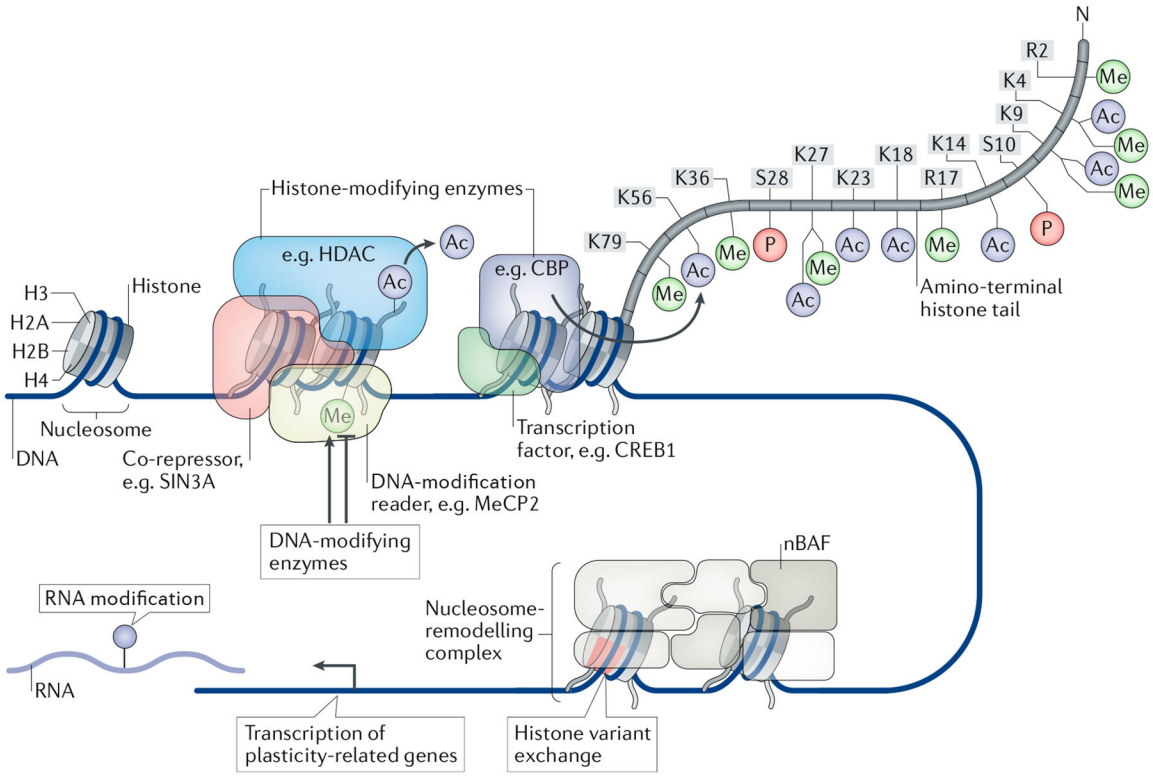

Fig. 1 |. regulation of synaptic plasticity-related gene expression through epigenetic mechanisms. Several epigenetic mechanisms have been identified as regulators of gene expression important for synaptic plasticity and memory formation. For instance, histone acetylation mediated by the activity of histone acetyltransferases, such as CREB-binding protein (CBP), can facilitate memory-related gene expression. CBP is recruited by the transcription factor cAMP-responsive element-binding protein 1 (CREB1) and promotes a permissive transcription environment by adding acetyl groups onto the lysine tails of histones. By contrast, histone deacetylases (HDACs) remove acetyl groups from histone tails and act in concert with associated co-repressor transcription factors to reduce gene expression (for example, transcriptional co-repressor SIN3A). Gene expression can be repressed by the interaction with epigenetic enzymes, such as HDAC–repressor complexes or methyl-CpG-binding protein 2 (MeCP2), which binds to methylated DNA. DNA methylation is controlled by several DNA-modifying enzymes, including DNA methyltransferase 3A (DNMT3A) or DNMT3B and ten-eleven translocation enzymes (TETs), which reportedly repress or permit gene expression depending on the region of DNA that is methylated. Nucleosome-remodelling complexes, such as the neuronal BRG1-associated factor (nBAF) complex, interact with DNA and histones to potentially regulate chromatin structure and synapse-related gene expression through insertion of histone variants, nucleosome sliding, nucleosome eviction and chromatin looping. Although RNA-modifying enzymes do not directly affect chromatin structure, they do influence the rate of mRNA translation and the localization of RNAs, including at the synapse.

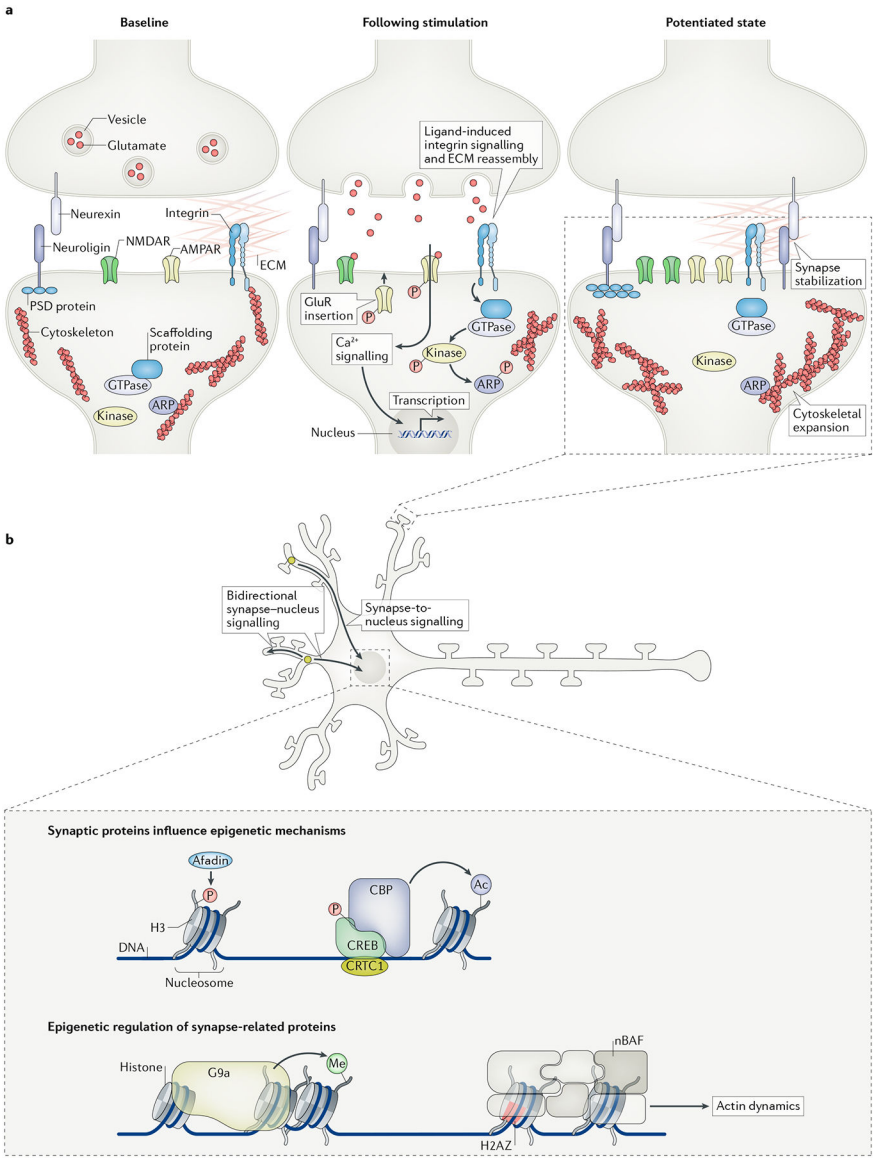

Fig. 2 |. synaptic plasticity and interactions between the epigenome and synapse.

a | Dendritic spines are filopodia-actin (F-actin)-rich protrusions that receive information from neighbouring cells via several types of surface receptor (left). The strength of synaptic transmission correlates with the size of dendritic spines. Synapses undergo changes in actin polymerization to rapidly expand the dendritic spine head and translocate synaptic proteins (middle). Multiple signalling cascades are activated to facilitate different aspects of synaptic plasticity. First, the activation of the ionotropic NMDA receptor (NMDAR) initiates several calcium-dependent signalling cascades important for regulation of synaptic protein activity and nuclear transcription. Second, the interaction of presynaptic neurexin and postsynaptic neuroligin cell adhesion molecules stabilizes transient synaptic contacts for synapse specification. Third, integrin receptors detect extracellular matrix (ECM) signals and promote the disassembly of cytoskeleton proteins. One downstream integrin mechanism is the activation of cofilin or other actin-related proteins (ARPs), which leads to the

depolymerization and reorganization of actin filaments. Fourth, AMPA receptors (AMPARs) are trafficked to the postsynaptic density (PSD). Together, these mechanisms rebuild the dendritic spine head, increase the concentration of glutamatergic receptors and regulate the translocation of synaptic proteins (right). **b** | Following neuronal stimulation, synaptic proteins can translocate to the nucleus or induce signalling cascades to promote transcription. Although the time points are not fully characterized, epigenetic regulation of transcription is proposed to regulate memory and synaptic plasticity. It is hypothesized that epigenetic mechanisms alter chromatin structure to permit transcription of genes crucial for immediate cellular responses (such as immediate-early genes) and synaptic potentiation. Evidence suggests that synaptic proteins can translocate and interact with epigenetic modifiers to potentially also induce long-lasting changes in gene regulation. Along these same lines, epigenetic mechanisms regulate the expression of synapse-related genes (for example, genes important for cytoskeleton polymerization) and thus influence synaptic structure and function. In order to fully understand how changes to the epigenome and synapse lead to long-lasting changes in behaviour, it is critical to further explore how bidirectional interactions between the synapse and nucleus occur to persistently alter neuronal function. CBP, CREB-binding protein; CREB, cAMP-responsive element-binding protein; CRTC1, CREB-regulated transcriptional co-activator 1; GluR, glutamate receptor; nBAF, neuronal BRG1-associated factor.