

Epigenetic regulation in psychiatric disorders

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Abstract | Many neurological and most psychiatric disorders are not due to mutations in a single gene; rather, they involve molecular disturbances entailing multiple genes and signals that control their expression. Recent research has demonstrated that complex 'epigenetic' mechanisms, which regulate gene activity without altering the DNA code, have long-lasting effects within mature neurons. This review summarizes recent evidence for the existence of sustained epigenetic mechanisms of gene regulation in neurons that have been implicated in the regulation of complex behaviour, including abnormalities in several psychiatric disorders such as depression, drug addiction and schizophrenia.

Pathophysiological changes in the brain that are associated with psychiatric disorders or animal models of these conditions include gross differences in the sizes of specific brain regions, alterations in the morphology of subpopulations of neurons, neurochemical changes at the synaptic cleft, alterations in intracellular signalling and changes in the regulation of gene expression. Most psychiatric disorders share important features, including a substantial genetic predisposition¹ and a contribution from environmental factors. Another common attribute of psychiatric conditions is long-lasting behavioural abnormalities. In most individuals, these illnesses develop gradually and show a chronic, remitting course, often over a lifetime. Likewise, the reversal of symptoms in response to treatment occurs over weeks or months. Psychiatric medications are virtually unique in their requirement for chronic administration to achieve their full clinical effect. Accordingly, an important challenge in psychiatric research has been to identify the molecular basis of stable changes in behaviour that account for both the symptoms of mental illness and their reversal during treatment.

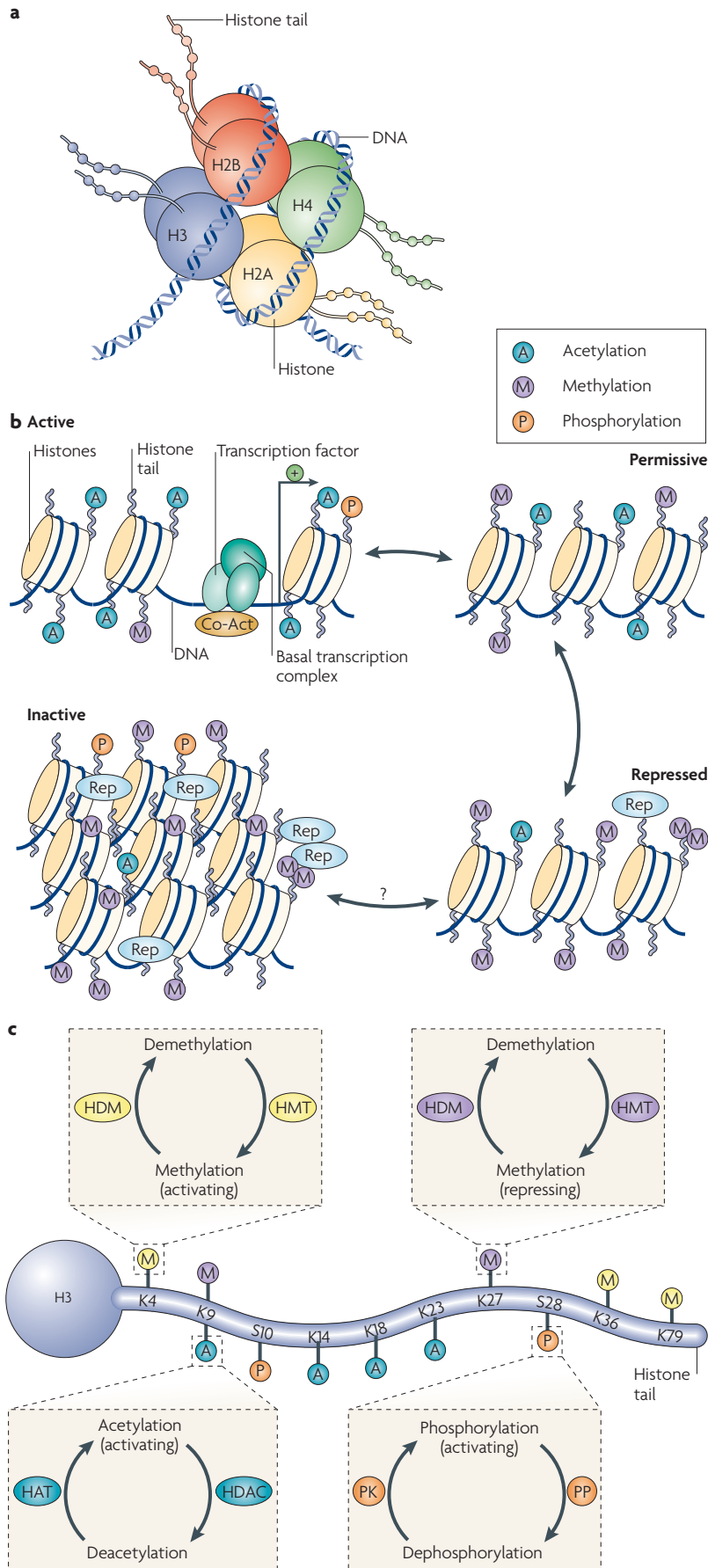
The regulation of gene expression has been proposed as one molecular mechanism that could mediate stable adaptations and maladaptations in the brain². Changes in mRNA levels have been documented in specific brain regions both in animal models of psychiatric illness and in human brains, and have been related to altered behaviour. However, it has been difficult to identify the molecular mechanisms that underlie such stable changes in gene expression; virtually all reported changes in transcription factors and other nuclear regulatory proteins in animal models revert to normal within hours or days of chronic perturbation. One exception is the induction of Δ FOSB, a FOS family transcription

factor, which accumulates in specific regions of the brain in response to many chronic stimuli (drugs of abuse, stress, antipsychotic drugs and so on), and persists for several weeks after the end of the stimulus^{3,4}. But even the induction of Δ FOSB does not persist as long as the behavioural changes. Thus, the search continues for the molecular basis of particularly stable adaptations and maladaptations in the brain.

Recent research has raised the notion that epigenetic mechanisms, which exert lasting control over gene expression without altering the genetic code, could mediate stable changes in brain function. Historically, the field of epigenetics has focused on how cellular traits can be inherited without a change in DNA sequence. Studies of epigenetic mechanisms that underlie heritable transmission have flourished in the fields of developmental and cancer biology, where the continuity of unique patterns of gene expression between parent and daughter cells is crucial. These studies have converged on a set of common enzymatic modifications to chromatin structure that can up- or downregulate gene expression in a manner that is transmissible to daughter cells. These mechanisms also regulate gene expression in neurons, but, as most neurons do not divide, chromatin modifications are instead sustained within individual cells.

This review outlines recent discoveries involving the epigenetic regulation of neurobiological adaptations that are associated with long-lasting behaviours in animal models of psychiatric conditions and in the brains of humans with these disorders. After a brief overview of epigenetic mechanisms, we focus on a small number of conditions, including depression, addiction, schizophrenia and developmental disorders, for which the supporting evidence is best established.

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Overview of epigenetic mechanisms

Chromatin is the complex of DNA, histones and non-histone proteins in the cell nucleus. Remodelling of chromatin is a dynamic process that modulates gene expression. The fundamental unit of chromatin is the nucleosome, which consists of ~147 base pairs of DNA wrapped around a core histone octamer (~1.65 turns). Each octamer contains two copies each of the histones H2A, H2B, H3 and H4 (FIG. 1a). The nucleosomal structure of chromatin allows DNA to be tightly packaged into the nucleus by organized folding⁵. Intricate chromatin remodelling mechanisms ensure that DNA remains accessible to the transcriptional machinery. These epigenetic mechanisms alter gene activity by modulating DNA–protein interactions without changing the genetic code.

In simplified terms, chromatin exists in an inactivated, condensed state, heterochromatin, which does not allow transcription of genes, and in an activated, open state, euchromatin, which allows individual genes to be transcribed (FIG. 1b). The opening of chromatin is associated with acetylation of nearby histones, although it remains unclear whether acetylation mediates or reflects chromatin decondensation. In reality, chromatin can exist in many states in between

Figure 1 | General scheme of chromatin remodelling. **a** | Picture of a nucleosome showing a DNA strand wrapped around a histone octamer composed of two copies each of the histones H2A, H2B, H3 and H4. The amino (N) termini of the histones face outward from the nucleosome complex. **b** | Chromatin can be conceptualized as existing in two primary structural states: as active, or open, euchromatin (top left) in which histone acetylation (A) is associated with opening the nucleosome to allow binding of the basal transcriptional complex and other activators of transcription; or as inactive, or condensed, heterochromatin where all gene activity is permanently silenced (bottom left). In reality, chromatin exists in a continuum of several functional states (active; permissive (top right); repressed (bottom right); and inactive). Enrichment of histone modifications such as acetylation and methylation (M) at histone N-terminal tails and related binding of transcription factors and co-activators (Co-Act) or repressors (Rep) to chromatin modulates the transcriptional state of the nucleosome. Recent evidence suggests that inactivated chromatin may in some cases be subject to reactivation in adult nerve cells, although this remains uncertain. **c** | Summary of common covalent modifications of H3, which include acetylation, methylation and phosphorylation (P) at several amino acid residues. H3 phosphoacetylation commonly involves phosphorylation of S10 and acetylation of K14. Acetylation is catalysed by histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs); lysine methylation (which can be either activating or repressing) is catalysed by histone methyltransferases (HMTs) and reversed by histone demethylases (HDMs); and phosphorylation is catalysed by protein kinases (PK) and reversed by protein phosphatases (PP), which have not yet been identified with certainty. K, lysine residue; S, serine residue. Panels **a,c** modified, with permission, from *Nature Rev. Neurosci.* REF. 62 © (2005) Macmillan Publishers Ltd.

Histones

Highly basic proteins that comprise the major protein constituents of the nucleus. Octameric complexes of histones, around which DNA is wrapped, form the nucleosome, the basic building block of chromatin.

Nucleosome

The basic building block of chromatin in which 147 base pairs of DNA are wrapped (~1.65 turns) around a core histone octamer.

Heterochromatin

The inactivated state of chromatin, in which DNA is not accessible for transcription due to covalent modifications of histones, methylation of the DNA and the binding of numerous repressor proteins.

Euchromatin

The activated state of chromatin, in which sections of DNA are accessible to the transcriptional machinery.

Ubiquitylation

Covalent addition of a small protein, called ubiquitin, to many types of proteins. Addition of multiple ubiquitin groups, polyubiquitylation, targets proteins for degradation in the proteasome. By contrast, monoubiquitylation of histones and other regulatory proteins alters their functional properties.

SUMOylation

Covalent addition of SUMO, which are small ubiquitin-related modifier proteins, to histones and many other types of regulatory proteins, which alters those proteins' function.

Nucleosome sliding

The movement of the core histone octamer relative to the DNA, which allows that DNA to be progressively transcribed into RNA.

SWI/SNF

Protein complex that partly mediates nucleosome sliding in an ATP-dependent manner. The name comes from genetic screens of yeast which identified proteins implicated in mating switching and sucrose non-fermentation. The proteins were later found to regulate nucleosome sliding.

Box 1 | Epigenetic mechanisms in neural development

Fundamental neurodevelopmental processes, such as cell fate specification and neurogenesis, are highly regulated at the level of chromatin remodelling. One of the best-established examples concerns the transcription factor neuron-restrictive silencing factor (NRSF; also known as repressor element 1 silencing transcription factor (REST)). NRSF represses neuronal differentiation by binding to conserved NRS elements (NRSEs) in gene promoters in non-neuronal cells, where it associates with one of several large repressor complexes, including the transcriptional co-repressor mSIN3a/b, nuclear receptor co-repressor 1 (N-CoR1), and coREST/histone deacetylase 2 (HDAC2)^{93,94}. In this way, NRSF keeps neural-specific genes turned off in non-neuronal cells. More recently, NRSF has been shown to modulate the expression of NRSE-containing genes in mature neurons: inhibition of NRSF leads to neuronal activation and the promotion of neurogenesis⁹⁵.

Histone acetylation, SWI/SNF-mediated remodelling and DNA methylation have also been implicated in brain development. HDAC inhibitors induce neural differentiation in embryonic cortical cells. Additionally, histone deacetylation is crucial for the timing of oligodendrocyte differentiation and myelination in the developing corpus callosum: administration of valproate (a non-selective HDAC inhibitor; see **Supplementary information S1** (table)) leads to hypomyelination, delayed expression of differentiation markers and prolonged expression of progenitor markers in these cells⁹⁶. Abnormal DNA methylation as a result of deficiency in the DNA methyltransferase DNMT1, or the methyl-CpG binding protein MBD1, causes abnormal neuronal function and postnatal death⁹⁷ or decreased neurogenesis⁹⁸, respectively. Chromatin remodelling may also be involved in the regulation of adult neurogenesis, which occurs in highly restricted brain regions: the subgranular zone of the hippocampus dentate gyrus and the subventricular zone adjacent to the striatum. HDAC inhibitors induce neuronal differentiation, suppress glial differentiation and decrease proliferation of adult hippocampal neural progenitor cells⁹⁹.

these two extremes (FIG. 1b). Portions of chromatin are highly repressed, owing to DNA and histone methylation and the binding of repressor proteins, and might never be accessible for transcription. Other portions of chromatin are in repressed or permissive states; their basal activity is low owing to histone methylation and perhaps other modifications, but the genes are available for derepression and activation in response to transcription factors and transcriptional co-activators. Chromatin remodelling modulates gene expression with high temporal and spatial resolution by permitting small groups of nucleosomes to become more or less open, which consequently enhances or inhibits access of the transcriptional machinery to specific promoter regions.

Experiments in yeast have yielded detailed information about the molecular mechanisms that control chromatin architecture to alter gene expression. Several general mechanisms have emerged and it is generally believed that their complex interactions determine the appropriate expression of specific genes in eukaryotic cells^{5,6}.

By far the best characterized chromatin remodelling mechanism in the brain is the post-translational, covalent modification of histones at distinct amino acid residues on their amino (N)-terminal tails. Such modifications include acetylation, ubiquitylation or SUMOylation at lysine (K) residues, methylation at lysine or arginine (R) residues, phosphorylation at serine (S) or threonine (T) residues, and ADP-ribosylation at glutamate (E) residues (FIG. 1c). Hyperacetylation is generally thought to promote decondensation of chromatin and an increase in gene activity, whereas hypoacetylation marks condensation and decreased activity. It has also been proposed that increased gene activity is best associated not with the level of acetylation, but with the dynamic cycling of acetylation and deacetylation. In contrast to acetylation, histone methylation can correlate with either gene activation or repression, depending on the residue undergoing

methylation⁷. Phosphorylation of histones is also associated with chromatin inhibition or activation⁶. The roles of histone ubiquitylation, SUMOylation and ADP ribosylation are less well understood^{8,9}. The diversity of histone modifications supports the 'histone code hypothesis', which posits that the sum of modifications at a particular promoter region defines a specific epigenetic state of gene activation or silencing¹⁰.

The enzymes that mediate these covalent modifications are becoming increasingly understood. Many histone acetyltransferases (HATs), which catalyse acetylation, have been identified. Several transcriptional activators contain intrinsic HAT activity^{10,11}. Histone deacetylases (HDACs) catalyse deacetylation; they also associate with several transcriptional repressors to further repress chromatin activity¹¹ (BOX 1). The balance between the opposing activities of HATs and HDACs maintains acetylation on core histones and is thought to be an important determinant of transcription. Methylation at lysine or arginine residues is mediated by histone methyltransferases (HMTs). In general, histone lysine methylation is regarded as a more stable modification than other histone modifications, which seem to be more readily reversible^{6,7}, although the recent discovery of histone demethylases (HDMs) indicates that even methylation can be reversed¹².

Several other general mechanisms of chromatin remodelling have been described, although they remain less well characterized in the nervous system. Nucleosome sliding involves the movement of the histone octamer along DNA and thereby allows the transcriptional machinery to transcribe a gene⁵. This process is facilitated by the SWI/SNF family of chromatin remodelling complexes, which use ATP-derived energy to disrupt nucleosome structure non-covalently¹¹. Histone substitution, where canonical histones within a nucleosome are switched with naturally occurring histone variants, is a further example of chromatin remodelling, although its physiological function in the brain is poorly understood⁶.

Table 1 | Examples of diseases of chromatin remodelling

| Disease | Chromatin defect | Clinical features |
|--|---|--|
| Rubinstein–Taybi syndrome ^{62,70,109} | Heterozygous mutations in <i>CBP</i> | Autosomal dominant inheritance; mental retardation, abnormal facial features, blunted growth |
| Fragile X syndrome ^{62,105} | Hypermethylation of DNA at the <i>FMR1</i> and <i>FMR2</i> promoters, caused by trinucleotide repeat expansion | X-linked inheritance; most common inherited form of mental retardation, signs of autistic behaviour, macrocephaly, long and narrow face with large ears, macroorchidism, hypotonia |
| Coffin–Lowry syndrome ^{70,106,107} | Mutation in <i>RSK2</i> , which can interact with CREB and CBP and can phosphorylate H3 <i>in vitro</i> ¹⁰⁶ | X-linked inheritance; psychomotor retardation, craniofacial and skeletal abnormalities |
| Rett syndrome ^{62,70–72} | Mutations in <i>MeCP2</i> | X-linked, predominantly affecting girls; pervasive developmental disorder associated with arrested brain development, cognitive decline and autistic-like behaviour |
| Alpha-thalassemia/mental retardation syndrome, X-linked (<i>ATR-X</i>) ⁷⁰ | Mutations in the <i>ATRX</i> gene encoding XH2 — a member of SWI/SNF family of proteins; defective chromatin remodelling thought to downregulate the α -globin locus | X-linked inheritance; mental retardation, haemolytic anaemia, splenomegaly, facial, skeletal and genital anomalies |
| Immunodeficiency–centromeric instability–facial anomalies syndrome (ICF) ⁷⁰ | Mutations in <i>Dnmt3B</i> ; hypomethylation at centromeric regions of chromosomes 1, 9 and 16 | Autosomal recessive; mild mental retardation, marked immunodeficiency, facial anomalies |
| Myotonic dystrophy ¹⁰⁷ | Abnormal CTG repeat expansion at the 3' UTR of the <i>DMPK</i> gene favours chromatin condensation, affecting the expression of many neighbouring genes | Autosomal dominant; mild mental retardation, myotonia, abnormal cardiac conduction, insulin-dependent diabetes, testicular atrophy, premature balding |
| Prader–Willi syndrome ¹⁰⁸ | Rare forms caused by abnormal imprinting (DNA methylation) of paternal chromosomal region 15q11–13 | Mild mental retardation, endocrine abnormalities |
| Angelman syndrome ¹⁰⁸ | Rare forms caused by abnormal imprinting (DNA methylation) of maternal chromosomal region 15q11–13 | Cortical atrophy, cerebellar dysmyelination, cognitive abnormalities |

CBP, CREB binding protein; CREB, cyclic AMP-response element binding protein; *DMPK*, DM1 protein kinase; *Dnmt3B*, DNA methyltransferase 3B; *FMR1*, fragile X mental retardation protein 1; *MeCP2*, methyl-CpG-binding protein 2; *RSK2*, ribosomal S6 kinase 2; SWI/SNF, mating switching and sucrose non-fermenting complex; UTR, untranslated region; XH2, X-linked helicase 2.

Histone substitution

A type of chromatin remodelling where histone constituents of the nucleosome can be replaced by other naturally occurring histone variants.

X-chromosome inactivation

Chromatin remodelling on a very large scale, whereby one of two X chromosomes in all cells of a female organism are inactivated by DNA hypermethylation. Once that chromosome is silenced, it remains inactive for the life of the organism.

Genetic imprinting

A process where only the maternal or paternal allele of a gene is expressed in the offspring. The other, inactivated allele is transcriptionally silenced through DNA methylation at CpG-rich domains.

DNA methylation is another important mechanism of gene repression. It occurs by transfer of a methyl group from S-adenosyl methionine (SAM) to cytosine residues at the dinucleotide sequence CpG, and is catalysed by DNA methyltransferases (DNMTs)⁶⁷. Although CpG sequences throughout the genome are usually heavily methylated, those at the promoter regions of genes, specifically at CpG clusters or islands, are methylated to a much lesser extent, and the amount of DNA methylation at a promoter correlates with the extent of gene inactivation. The functional significance of DNA methylation is best established in X chromosome inactivation and genetic imprinting; abnormal imprinting can lead to neurodevelopmental diseases (BOX 1; TABLE 1). More recently, DNA methylation has been implicated in the regulation of gene activity in the adult brain under normal and pathological conditions (see below).

Patterns of DNA methylation are intricately linked to patterns of histone modification. Methyl binding domain proteins (MBDs), such as methyl-CpG-binding protein 2 (**MeCP2**), can be recruited to methylated DNA. MBDs are associated with large protein complexes containing HDACs and HMTs, which further repress gene transcription⁷. Thus, DNA methylation and histone methylation and deacetylation are intricately interconnected, each representing epigenetic hallmarks of the silenced promoter.

Signalling pathways in chromatin remodelling

Although chromatin remodelling is best understood for its influence on neural development (BOX 1), increasing evidence suggests a role in regulating mature, fully differentiated neurons. During synaptic transmission, neurons respond to neurotransmitters by receptor-mediated intracellular signal transduction events, which, among other actions, activate or inhibit transcription factors. The regulation of transcriptional activity by transcription factor binding to DNA depends on interactions of the transcription factors with many co-activators or co-repressors and the underlying structure of chromatin. Chromatin remodelling is thus intimately linked to activation or repression of genes by synaptic activity. Such mechanisms regulate the expression of specific sets of neuronal genes that are important for neural activity, survival, morphology and ultimately the integrated regulation of complex behaviour.

We are beginning to understand how intracellular signalling in the brain regulates chromatin remodelling. The best-established mechanism involves the transcription factor cyclic AMP (cAMP)-response element binding protein (**CREB**). The activation of several signalling pathways involving cAMP, Ca²⁺ and extracellular signal regulated kinase (ERK) leads to the phosphorylation of CREB at Ser133 (FIG. 2). CREB phosphorylation triggers

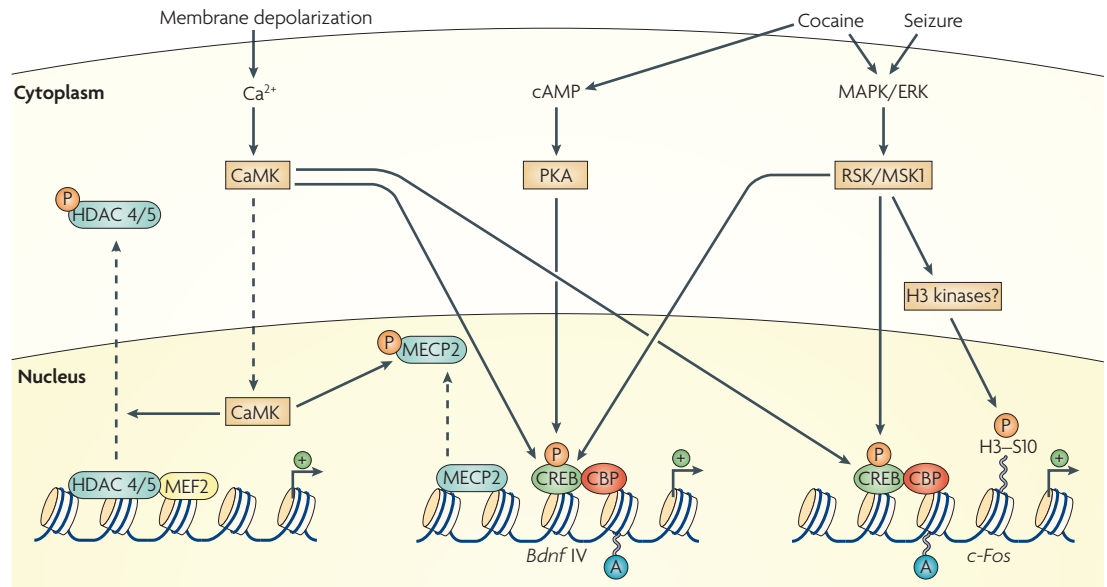


Figure 2 | Regulation of chromatin remodelling via intracellular signalling cascades. Multiple kinases such as protein kinase A (PKA), calcium/calmodulin-activated protein kinases (CaMKs), ribosomal S6 kinase (RSK) and mitogen- and stress-activated protein kinase 1 (MSK1), which are activated by intracellular signalling cascades involving cyclic AMP (cAMP), calcium- and mitogen-activated protein/extracellular signal-regulated protein kinase (MAPK/ERK), respectively, can phosphorylate the transcription factor cAMP-response element binding protein (CREB). This causes it to associate with the histone acetyltransferase CREB-binding protein (CBP). CBP, in turn, acetylates nearby histones and thereby promotes transcriptional activation. This mechanism of regulation has been described for, among other genes, *c-Fos* and brain-derived neurotrophic factor (*Bdnf*). Membrane depolarization and calcium influx activate CaMKs. CaMKII-induced phosphorylation of the repressor methyl-CpG-binding protein 2 (MeCP2) is proposed to be important for the release of this repressor from the *Bdnf* promoter P4 and the subsequent transcriptional activation of the *Bdnf* gene. CaMKs also phosphorylate class II histone deacetylases (HDACs) 4 and 5 in neurons, which results in their translocation out of the nucleus. At certain promoters, the phosphorylation of HDACs also decreases their affinity to the activator myocyte enhancing factor 2 (MEF2) and promotes further transcriptional activation. Acute cocaine administration, presumably acting through dopamine receptors, activates MSK1 in striatal neurons. This results in the downstream phosphorylation of histone H3 at the *c-Fos* promoter with concomitant induction of *c-Fos* transcription. The effect of MSK1 may be indirect.

the recruitment of CREB-binding protein (CBP), a transcriptional co-activator whose intrinsic HAT activity acetylates nearby histones, which loosens the chromatin and allows subsequent transcriptional activation. CBP is important for normal learning and memory, and mutations of CBP cause **Rubinstein-Taybi syndrome**, a form of mental retardation in humans (see below).

Several external stimuli can induce rapid changes in histone modifications in the brain, but the intracellular mediators of these signals are poorly defined. For example, cocaine and antipsychotic drugs induce acetylation of histone H4 and phosphoacetylation of histone H3 in the striatum (a brain region that is important for the behavioural effects of these drugs; see below)¹³⁻¹⁵. Among the genes that show the most marked histone changes, which can be identified by use of chromatin immunoprecipitation (ChIP) assays, are immediate-early genes, such as *c-Fos*. *c-Fos* transcription is induced rapidly in the brain by numerous stimuli, including cocaine, antipsychotic drugs and seizures. These stimuli trigger rapid and transient enrichment of H4 acetylation and H3 phosphoacetylation at the *c-Fos* promoter in neurons, in association with transcriptional activation of *c-Fos*. However, the signalling pathways through which these stimuli modify histones

are unknown. One study implicated the ERK pathway, in particular mitogen- and stress-activated kinase 1 (MSK1), in cocaine induction of H3 phosphorylation and *c-Fos* activation in the striatum¹³, although it remains unclear whether MSK1 directly phosphorylates H3 or induces its phosphorylation by other kinases. Likewise, the ERK pathway may mediate H3 phosphoacetylation in response to seizures¹⁶. Several other kinases can phosphorylate H3 in non-neuronal cells, but their actions in the brain are unexplored¹⁷. Therefore, although H3 phosphoacetylation has been directly associated with *c-Fos* induction by acute stimuli, much work is needed to understand the underlying molecular mechanisms involved.

Indeed, one of the main challenges in the field is to elaborate the precise steps through which neural activity and synaptic transmission signal to the nucleus to regulate the enzymes and other proteins that mediate chromatin remodelling. Insight into these pathways has come from studies of non-neural cells. The activation of cellular Ca²⁺ pathways in muscle leads to the activation of CaMKs (calcium/calmodulin-activated protein kinases), which phosphorylate class II HDACs. This phosphorylation triggers the shuttling of the enzyme out of the nucleus, and results in increased histone

Chromatin immunoprecipitation (ChIP). A method that enables the identification of histone modifications or transcriptional regulatory proteins at a given gene promoter. In the assay, DNA is crosslinked to nearby proteins by light fixation, the material is sheared, then immunoprecipitated with an antibody to a particular protein of interest, and genes in the final immunoprecipitate are quantified by polymerase chain reaction.

Immediate-early genes
Genes that are induced rapidly and transiently without the need for new protein synthesis. Many immediate-early genes, such as *c-Fos*, control the transcription of other genes, and thereby provide the early stages in the control of the production of specific proteins.

acetylation (FIG. 2). This pathway has now been demonstrated in hippocampal cells¹⁸ and cerebellar granule neurons¹⁹, indicating that chromatin signalling mechanisms in different tissues overlap substantially.

It is unclear how histone acetylation can be regulated only at specific genes, but this is believed to involve multi-protein complexes. For example, class II HDACs can target specific genes for repression through N-terminal regulatory domains that mediate interactions between these HDACs and certain transcription factors, such as myocyte enhancing factor 2 (MEF2) (FIG. 2). Independent of histone deacetylation, class II HDACs can recruit cyclin-dependent kinase 5 (CDK5) to phosphorylate MEF2 and repress its transcriptional activity^{19,20}. However, little is known about these regulatory mechanisms in the brain. Among neural genes, brain-derived neurotrophic factor (*Bdnf*) is one of the most studied for its regulation by chromatin remodelling (BOX 2).

The above studies underscore the importance of dynamic chromatin remodelling in the transcriptional response to acute stimuli in neuronal cells. However, much research on psychiatric disorders is focused on neuroadaptations that evolve slowly but can cause lasting changes in brain circuitry. The next sections provide evidence for such stable neuronal regulation at the level of chromatin remodelling as it relates to the pathogenesis and maintenance of complex psychiatric disorders.

Epigenetic mechanisms in depression

Depression is a common, chronic and debilitating disease. Although many patients benefit from antidepressant medication, electroconvulsive seizures (ECS) or psychotherapy, only about half of depressed patients show a complete remission, which underscores the need for more effective agents²¹. The mechanisms that precipitate depression, such as stress, are incompletely understood. One mystery of the disease is its long-lasting nature and delayed response to antidepressant treatment. This persistence is thought to be mediated by slowly developing but stable adaptations, which might include epigenetic regulation.

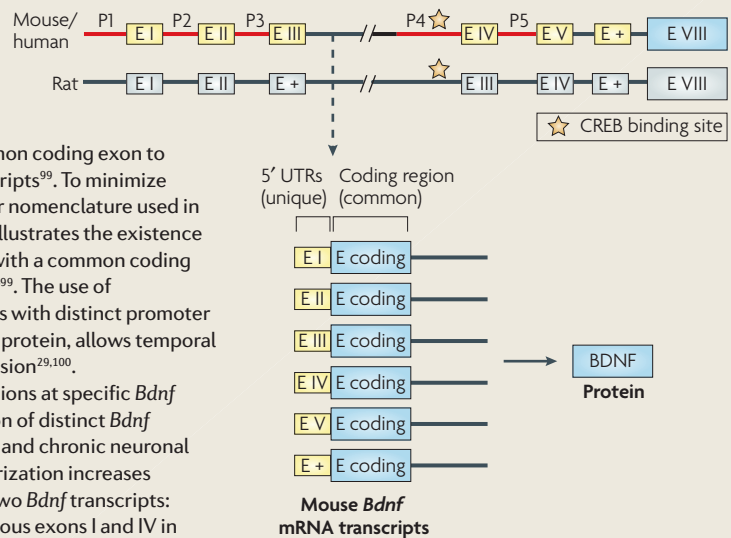
To investigate such adaptations, one study examined changes in histone modifications after chronic ECS in the rat hippocampus²², a brain region implicated in the pathophysiology and treatment of depression^{23,24}. Like antidepressant medications, ECS is effective only after repeated administration, indicating that long-term adaptations at the level of gene expression might be involved. Chronic ECS upregulates the expression of *Bdnf* and *Creb* in the hippocampus, and such upregulation has been shown to mediate antidepressant activity in animal models²⁵⁻²⁷. Chronic ECS produced chromatin remodelling changes that were very different from those seen after acute ECS, and that were detected at distinct *Bdnf* promoter regions. Chronic ECS increased H3 acetylation

Box 2 | *Bdnf*: activity-dependent regulation at the level of chromatin remodelling

The most recent analysis indicates that the brain-derived neurotrophic factor gene (*Bdnf*) contains at least nine short 5' non-coding exons in rodents and humans, each of which can be alternatively spliced to a common coding exon to generate at least nine different transcripts⁹⁹. To minimize confusion, the figure depicts the older nomenclature used in most previous studies^{14,22,29,100-104}, but illustrates the existence of additional non-coding exons (E+) with a common coding exon E VIII as described most recently⁹⁹. The use of alternatively spliced mRNA transcripts with distinct promoter regions, all of which encode the same protein, allows temporal and spatial specificity of BDNF expression^{29,100}.

Indeed, unique chromatin modifications at specific *Bdnf* promoters drive differential expression of distinct *Bdnf* splice variants in the context of acute and chronic neuronal stimulation. Acute membrane depolarization increases calcium-dependent transcription of two *Bdnf* transcripts: exons I and III in rats and the homologous exons I and IV in mice^{100,101}. Neuronal activity can trigger the phosphorylation of the repressor methyl-CpG-binding protein 2 (MeCP2), which regulates activity-dependent induction of *Bdnf* expression and provides a possible mechanism for the regulation of *BdnfIII* after neuronal stimulation^{102,103}. Phosphorylation of MeCP2 depends on calcium/calmodulin-activated protein kinase II (CaMKII) (FIG. 2). Rapid activity-dependent changes in the DNA methylation of specific *Bdnf* promoters have also been observed in neurons^{35,69}.

Chromatin remodelling at *Bdnf* promoters is also altered after *in vivo* stimulation of neuronal activity, such as after the induction of seizures in rodent models. An acute pilocarpine-induced seizure increases levels of H4 acetylation most prominently at the P2 promoter of *Bdnf* in the rat hippocampus¹⁰⁴. Acute electroconvulsive seizure similarly increases histone H4 acetylation at *Bdnf* P2, in correlation with a robust increase in *Bdnf* transcription²². In addition to the transient induction of H4 acetylation, seizure activity induces the rapid and transient phosphoacetylation of H3 in hippocampal neurons¹⁶. As discussed in the main text, more sustained changes in H3 acetylation and methylation at the *Bdnf* promoter have been seen in mouse models of depression and antidepressant treatment²⁹ (FIG. 3). CREB, cyclic AMP-response element binding protein; E I-V, exons I-V; P, promoter; UTR, untranslated region.



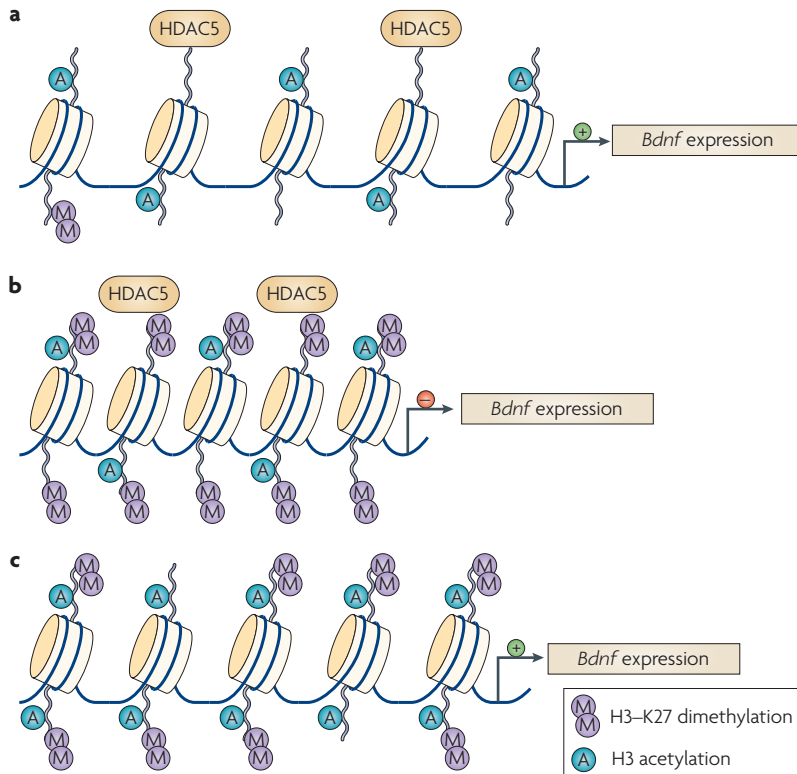


Figure 3 | Regulation of the *Bdnf* gene by social defeat. **a** | In the absence of stress, the chromatin state of brain-derived neurotrophic factor (*Bdnf*) is at a basal level, characterized by moderate levels of histone H3 acetylation and virtually no H3-K27 dimethylation. In this state, histone deacetylase 5 (HDAC5) might repress unnecessary activation of BDNF and maintain a chromatin balance. **b** | Chronic defeat stress induces the specific and prolonged dimethylation of histone H3-K27. This induces a more ‘closed’ chromatin state at *Bdnf* promoters P3 and P4, and a corresponding repression of *Bdnf* transcripts III and IV expression. H3 acetylation and HDAC5 regulation are not affected after chronic defeat stress alone, corroborating the idea that the main repressive marker after chronic stress is histone methylation. **c** | Chronic imipramine (antidepressant) treatment after defeat stress downregulates *Hdac5* expression and increases H3 acetylation, with little if any change in H3-K27 dimethylation. Imipramine-dependent H3 hyperacetylation at the *Bdnf* promoters P3 and P4 allows partial ‘reopening’ of the repressed chromatin state caused by defeat stress, and results in transcriptional reactivation of the *Bdnf* gene. K, lysine residue.

(instead of H4 acetylation as seen after acute ECS) at *Bdnf* promoters 3 and 4, in correlation with increased expression of the corresponding *Bdnf* transcripts (BOX 2).

Chronic social defeat stress, an animal model of depression²⁸ that mimics many symptoms of human depression, also alters chromatin regulation of *Bdnf*²⁹. Prolonged exposure to an aggressor induces lasting changes in mouse behaviour such as social avoidance, which are reversed by chronic (but not acute) treatment with antidepressants^{28,29}. At a molecular level, chronic defeat stress in mice induces sustained downregulation of the expression of two splice variants of *Bdnf*, *BdnfIII* and *BdnfIV*, in the hippocampus²⁹. These changes are reversed only after chronic treatment with the antidepressant imipramine. Chronic defeat stress induces robust and long-lasting increases in H3-K27 dimethylation, a repressive modification, specifically at the promoters of the downregulated *Bdnf* transcripts.

This histone modification was present four weeks after the cessation of defeat stress and was not reversed by antidepressant treatment, indicating that chronic defeat stress imposes a long-lasting marker of repression at these *Bdnf* promoters (FIG. 3). Rather, chronic imipramine seems to reverse repression of the *Bdnf* gene by inducing H3 acetylation, as well as H3-K4 methylation — an activating modification (FIG. 1) — at the same promoters. Direct support for this hypothesis comes from the observation that chronic imipramine downregulates *Hdac5* expression in the hippocampus, but only in animals previously subjected to chronic social defeat. Moreover, viral-mediated overexpression of *Hdac5* in this region prevents imipramine’s restoration of *Bdnf* levels as well as the drug’s antidepressant effects²⁹. Further support for this scheme is the observation that systemic administration of sodium butyrate, a nonspecific HDAC inhibitor (see **Supplementary information S1** (table)), acts as an antidepressant in models of depression, including social defeat^{29,30}.

The mechanism by which chronic antidepressant treatment upregulates H3-K4 methylation *in vivo* is unclear, but a recent *in vitro* study may provide some insight. Demethylation of histone H3-K4 is catalysed by BHC110/LSD1, an enzyme that has a close structural homology to monoamine oxidases. Monoamine oxidase inhibitors, which are a class of antidepressants, can increase global levels of H3-K4 methylation and cause transcriptional derepression of specific genes *in vitro*³¹. The importance of HMT and HDM inhibition for the action of antidepressants remains unknown, but this can now be tested because distinct HMTs and HDMs act on H3-K4 versus H3-K27. Together, these studies implicate chromatin remodelling in the formation of stable neuronal adaptations (molecular scars) in the hippocampus that might underlie some of the long-lasting changes in behaviour which are induced by chronic stress and reversed by chronic antidepressants. The studies also raise the possibility that specific HDAC, HMT and HDM inhibitors might be useful as antidepressant treatments (FIG. 3).

Stressful events early in life might also leave lasting epigenetic marks on the organism. In rats, some mothers naturally display high levels of nurturing behaviours, such as licking, grooming and arched-back nursing (high-LG-ABN), whereas others have low levels of such behaviours (low-LG-ABN)³². Offspring of high-LG-ABN mothers are less anxious, have attenuated corticosterone responses to stress and display increased expression of glucocorticoid receptor (*GR*) mRNA and protein in the hippocampus when compared to pups of low-LG-ABN mothers³³. The upregulation of *GR* mRNA specifically involves the alternatively spliced variant *GRI₁*. The promoter that drives expression of this variant is brain specific, and contains a binding consensus sequence for nerve growth factor inducible factor A (NGFI-A), the expression of which is upregulated in the pups of high-LG-ABN mothers. A study that compared the methylation status at individual CpG sites within the NGFI-A consensus sequence region of the *GRI₁* promoter between the offspring of high- and

DNA demethylases

Enzymes, not yet molecularly characterized, that demethylate CpG residues in DNA. Active DNA demethylation may also occur through alternative mechanisms such as DNA repair and deamination.

ChIP on chip

A method that enables a global analysis of genes associated with a particular histone modification or transcriptional regulatory protein. Immunoprecipitated chromatin is analysed on a microarray gene chip, enriched in promoter regions.

SACO

Serial analysis of chromatin occupancy, an alternative method to ChIP on chip, is used to obtain a genome-wide appreciation of the genes that bind a particular histone modification or transcriptional regulatory protein. Instead of hybridizing the immunoprecipitated DNA to a microarray, the DNA is directly sequenced.

low-LG-ABN mothers found that low-LG-ABN pups have increased methylation at these sites³⁴. This difference in methylation emerged in the first week of life and persisted into adulthood. As adults, the offspring of mothers with low levels of nurturing behaviour were at a molecular disadvantage — their methylated *GRI₇* promoter prevented binding of the transcriptional enhancer NGFI-A, effectively disrupting the normal transcriptional regulation of the *GR* gene. Although this epigenetic methylation was long-lasting, it could be reversed by infusion of the class I and II HDAC inhibitor trichostatin A (TSA) (see **Supplementary information S1** (table)), which increased levels of H3 acetylation, cytosine demethylation and the binding of NGFI-A at the *GRI₇* promoter in the low-LG-ABN-raised offspring. Interestingly, cross-fostering also reversed the differences in methylation at that site³⁴.

DNA methylation has traditionally been viewed as an irreversible epigenetic marker; however, this study indicates that patterns of DNA methylation might be reversible even in adult neurons. Enzymes that mediate DNA demethylation in the brain have yet to be identified. Another important concept illustrated by the reversal of DNA methylation by an HDAC inhibitor is the interaction between histone modifications and DNA methylation. It has been proposed that demethylation might occur through activation of second messenger signals that lead to the recruitment of HATs, which, through increased histone acetylation, would allow DNA demethylases greater access to the *GR* promoter³³. Other examples of rapidly reversible changes in DNA methylation in the adult brain have been reported in learning and memory³⁵ (see below).

Together, these studies indicate that several epigenetic modifications, including lasting changes in histone acetylation, histone methylation and DNA methylation, are important in animal models of stress, depression and antidepressant treatment. However, this work has so far focused only on the *Bdnf* and *GR* genes, and only on the hippocampus. Work is now needed to investigate other brain regions that have been implicated in depression and its treatment²⁴, and to investigate the involvement of additional genes in mediating the long-term effects of stress and antidepressant treatments on gene transcription.

Epigenetic mechanisms in addiction

Drugs of abuse control human behaviour by hijacking the brain's natural reward centres, including the mesolimbic dopamine system. This circuit includes dopaminergic neurons of the ventral tegmental area (VTA), which project directly to the nucleus accumbens (NAc) — the ventral portion of the striatum. During the past few decades, much has been learned about the brain's reward pathway and how drugs of abuse usurp its functions^{36,37}, but it is unclear why addictive behaviours persist long after drug abstinence, underlying high rates of relapse. Many studies have identified drug-induced changes in mRNA levels in the VTA, NAc and other relevant brain regions^{38–43}. Some of these changes in gene expression persist even after months of abstinence⁴⁴. The longevity of the changes has driven research into chromatin remodel-

ling as the molecular basis of sustained, even life-long, alterations in gene expression in brain reward regions⁴⁵.

An acute dose of cocaine induces the expression of *c-Fos* and *FosB* in the striatum, and this induction is associated with transient increases in H4 acetylation within 30 min of drug injection^{13,14}. CBP, with its intrinsic HAT activity, is an important mediator of cocaine-induced acetylation of histones at the *FosB* gene, and probably serves this function for other genes as well⁴⁶. Acute cocaine administration also induces H3 phosphoacetylation at the *c-Fos* gene promoter only¹⁴, and this effect requires the protein kinase MSK1, as mentioned above¹³.

Chronic cocaine treatment and self-administration activate and repress many distinct genes compared with acute treatment. *FosB*, for example, is induced by both acute and chronic cocaine exposure, but acute exposure produces H4 acetylation whereas chronic treatment causes H3 acetylation¹⁴. Genes that are selectively induced in the chronic state, such as *Cdk5* and *Bdnf*^{44,47}, also seem to be acetylated selectively on H3. Interestingly, cocaine induction of histone acetylation at the *Bdnf* promoter builds during a week of withdrawal¹⁴, and precedes the progressive increase seen in BDNF protein and mRNA levels in this region⁴⁴. A similar switch from H4 to H3 acetylation is seen in the hippocampus after acute versus chronic ECS²², indicating that H3 acetylation may represent a common, chromatin-mediated mark of persistently or repeatedly activated genes. Little is known about the catalytic selectivity of HATs and HDACs for the specific acetylated residues on H3 and H4. It would be interesting to determine whether this switch from H4 to H3 acetylation is mediated by the recruitment of distinct HATs or HDACs to regulated genes after acute versus chronic treatment.

Cocaine regulation of histone acetylation at the *Cdk5* and *Bdnf* genes highlights the importance of exploring genome-wide chromatin structure — by use of ChIP on chip^{48,49} or SACO⁵⁰ assays, for example — to identify many other genes, the dysregulation of which at the chromatin level contribute to cocaine addiction. Genome-wide epigenetic approaches have yielded exciting results in the fields of developmental and cancer biology^{48,49}, and efforts to carry out similar studies in addiction are now underway. Preliminary evidence from our laboratory has identified several hundred genes that are significantly hyper- or hypoacetylated after chronic cocaine treatment^{51–53}, and future studies will focus on the role of these chromatin changes in the pathogenesis and maintenance of addiction.

As mentioned earlier, the transcription factor ΔFOSB is implicated in the transition to an addicted state^{3,4}, and accounts for >25% of all changes in steady state mRNA levels induced by cocaine in the NAc⁴¹. ChIP assays have shown that the induction of one of these mRNAs, *Cdk5*, by cocaine represents a direct, activating effect of ΔFOSB on the *Cdk5* gene¹⁴ (FIG. 4). By contrast, *Bdnf* is not a direct target of ΔFOSB, indicating that a different mechanism is involved in the induction of this gene. Induction of *Cdk5*, in turn, partly mediates the effects of chronic cocaine treatment on dendritic remodelling in the NAc⁵⁴. These findings support a model in which the accumulation of

Circular dichroism

A form of spectroscopy, involving the differential absorption of left- and right-handed polarized light, used to study the structure of complex molecules.

Δ FOSB interacts with chromatin remodelling factors at specific promoters to regulate genes that are important for the development and maintenance of addiction (FIG. 4).

Further studies are needed to identify the enzymes that mediate these cocaine-induced chromatin remodelling events. Histone and DNA modifying complexes are obvious targets. A recent report found that chronic cocaine treatment increases MeCP2 and MBD1 in the adult brain⁵⁵, both of which bind to methylated DNA and aid in transcriptional repression. Several proteins that have been implicated behaviourally in drug addiction are now being rediscovered as histone modifying enzymes. For example, CDK5 phosphorylates an HDAC-interacting protein, which then alters chromatin structure⁵⁶. Another example is the transcriptional regulator named nucleus accumbens 1 (NAC1), which is highly induced by cocaine in the NAc and interacts with HDAC3 and HDAC4 (REF. 57). Possible effects of CDK5 and NAC1 on chromatin structure in animal models of addiction remain unexplored, but these studies might provide mechanistic insight into how drugs of abuse can direct chromatin-modifying enzymes to specific genes. Further work is also needed to examine cocaine regulation of other chromatin-modifying enzymes, and their specific involvement in shaping the addicted state.

The manipulation of chromatin remodelling enzymes by pharmacological inhibition, viral-mediated gene transfer and genetic mutations in mice have shown that cocaine-induced changes in chromatin structure are behaviourally relevant. HDAC inhibitors increase histone acetylation and potentiate cocaine-induced locomotor activity and cocaine reward, as measured by conditioned place preference^{14,53}. Conversely, viral-mediated overexpression of *Hdac4* or *Hdac5* in the NAc had the opposite effect. Consistent with these findings, mice deficient in CBP show reduced cocaine-induced locomotor activity⁴⁶. Together, these findings indicate that the ability of cocaine to increase histone acetylation is important for the behavioural effects of the drug, and set the stage for more in-depth analysis of HDAC inhibitors and related pharmacological and genetic tools in paradigms of cocaine self-administration and relapse.

There are also a few reports of chromatin changes after chronic ethanol administration^{58–60}. An early study used circular dichroism to show that chronic ethanol induced a more open structure to chromatin, consistent with a switch from heterochromatin to euchromatin⁵⁸. Subsequently, changes in histone acetylation and DNA methylation have been reported in alcoholic patients^{59,60}.

Epigenetic mechanisms in other disorders

Learning and memory. The formation of long-term memories, by analogy to pathophysiological mechanisms of depression and addiction, is thought to entail lasting changes in gene expression, and there is growing evidence that histone modifications and DNA methylation may be involved^{35,61,62}. Mice deficient in CBP exhibit memory deficits in several hippocampus-dependent memory tests, including spatial mazes, contextual fear conditioning and novel object recognition, and administration of an HDAC inhibitor can restore

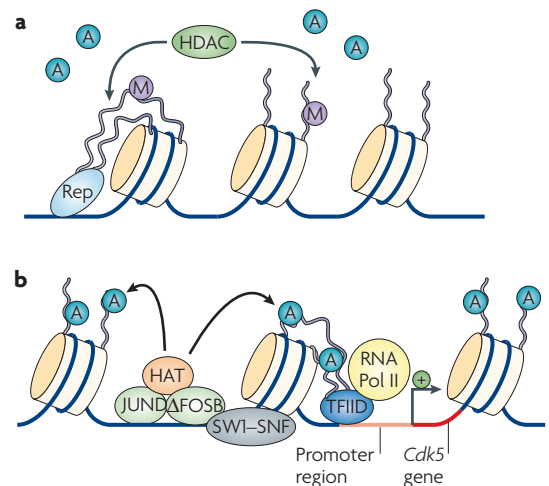


Figure 4 | Proposed chromatin remodelling events at a cocaine-activated gene. **a** | Repressed state of chromatin, where a site-specific repressor (Rep) recruits a histone deacetylase (HDAC) complex, which removes acetyl groups (A) from histone amino-terminal tails. Gene inactivation may also involve other modifications, such as methylation (M) of histone tails. **b** | Active state of chromatin around a cocaine-activated gene (for example, cyclin-dependent kinase 5 (*Cdk5*)), where a cocaine-induced transcriptional activator (for example, an activator protein 1 dimer composed of Δ FOSB–JUN) recruits a histone acetyltransferase (HAT) and a chromatin remodelling complex (mating switching and sucrose non-fermenting complex, SWI/SNF), which induce acetylation (and perhaps demethylation and other modifications) of histone tails and repositioning of nucleosomes. These actions facilitate the binding of general transcription factors and the basal transcriptional apparatus (for example, transcription factor IID (TFIID) and RNA polymerase II (PolII)) to the promoter. Modified, with permission, from REF. 45 © (2005) Society for Neuroscience.

normal long-term memory formation in the mutants^{62–64}, and even enhance it in normal animals^{62,65}. Contextual fear conditioning and/or activation of the ERK pathway, which is thought to contribute to memory formation, increase levels of H3 phosphoacetylation in the CA1 area of the hippocampus^{62,66}. H4 acetylation, another common marker of chromatin activation, was not regulated in these learning and memory models⁶⁵, substantiating the hypothesis that H3 acetylation might mark chronic and stable events, whereas H4 acetylation might signify more acute and dynamic changes. In addition, there is early evidence that the chromatin remodelling complexes polycomb and trithorax, which contain HMT activity, contribute to the epigenetic regulation of contextual fear conditioning⁶⁷. Synaptic plasticity is believed to contribute to the formation of long-term memories and also seems to have an epigenetic component: in *Aplysia*, H4 acetylation around the *C/EBP* (CAATT-enhancing binding protein) promoter is altered after long-term potentiation (LTP) and long-term depression⁶⁸, and HDAC inhibitors promote LTP in mammalian neurons⁶⁵.

Recent findings have also implicated changes in DNA methylation in learning and memory. In contrast to the

more traditional view that DNA methylation is a highly stable modification, they suggest that DNA methylation may be subject to rapid and dynamic regulation in the nervous system. Contextual fear conditioning induced DNA methyltransferase 3A (*Dnmt3A*) and *Dnmt3B* expression in CA1 of the hippocampus, and administration of the DNMT inhibitors zebularine and 5-aza-2'-deoxycytidine blocked the induction of both contextual fear conditioning⁶⁹ and hippocampal LTP³⁵. Fear conditioning also caused rapid methylation and silencing of the protein phosphatase 1 (*PPI*) gene promoter⁶⁹, a gene known to be important for LTP and memory formation. Interestingly, fear conditioning also induced demethylation of the reelin promoter, indicating that both DNA methylation and demethylation are highly regulated. Some of these effects may be mediated by protein kinase C (PKC), the activation of which *in vitro* decreased reelin promoter methylation and increased *Dnmt3A* expression³⁵. PKC also induced H3 acetylation, which seemed to depend on DNMT activity; this provides another example of the intimate interplay between histone modifications and DNA methylation in chromatin remodelling. However, one question raised by these studies is the mechanism by which zebularine and 5-aza-2'-deoxycytidine lead to demethylation of genes in adult neurons. Thus, these drugs are generally thought to act through incorporation into DNA during mitosis, thereby preventing methylation of the new DNA strand. Further work is needed to understand their actions in postmitotic, mature neurons.

Several developmental conditions, including common forms of mental retardation, can be attributed, at least in part, to disruption in the brain's epigenetic machinery^{62,70} (TABLE 1).

Rett syndrome. *Rett syndrome* is an X-linked, pervasive developmental disorder that is associated with arrested brain development and cognitive decline. It is considered an autism spectrum disorder, given the prominence of social and language abnormalities in many patients, and predominantly affects girls. Patients develop normally until 6–18 months of age, but thereafter begin to lose acquired language and motor skills and develop neurological and psychiatric symptoms, including stereotyped motor movements, seizures, autonomic instability, breathing irregularities, severe cognitive decline and autistic-like behaviour⁷¹.

Rett syndrome is an epigenetic disorder, because it is caused by mutations in the gene that encodes the transcriptional repressor MeCP2 (REF. 72). Several studies have begun to define the influence of MeCP2 in the CNS and the pathophysiology of Rett syndrome at behavioural, functional and molecular levels^{73–80}. Mice that lack *Mecp2* recapitulate many aspects of the neurological^{73,79} and behavioural^{76,80} phenotypes of Rett syndrome. Moreover, transgenic overexpression of *Mecp2* in mutant mice can rescue the Rett-like phenotype⁷³. Learning, memory and synaptic transmission are also impaired in one mouse model of the disease⁷⁷. Electrophysiology studies in MeCP2-deficient mice have shown that MeCP2 is important for synaptic function^{74,78}. MeCP2 deficiency

causes a global reduction in cortical synaptic excitation with concomitant increased inhibition (but without any changes in the intrinsic electrophysiological properties of individual neurons), resulting in an overall shift towards synaptic inhibition in cortical neurons⁷⁴. There is also a regulatory relationship between the presynaptic function of MeCP2 and its ability to silence transcription⁷⁸. First, cultured hippocampal neurons from *Mecp2*-knockout mice show a decreased frequency of spontaneous excitatory postsynaptic potentials and increased short-term synaptic depression, substantiating the previous findings that MeCP2 deficiency causes synaptic inhibition. Furthermore, treatment of wild-type hippocampal cultures with the HDAC inhibitor TSA effectively reduced transcriptional silencing, and recapitulated the synaptic changes seen in MeCP2^{-/-} cultures. Importantly, these effects of TSA could be blocked by actinomycin D, an inhibitor of DNA synthesis⁷⁸. These results suggest that a deficiency in MeCP2 leads to overactive gene transcription, which in turn causes a selective impairment in presynaptic excitatory function. An encouraging recent finding is that reversal of an MeCP2 deficit in mice, even after Rett-like symptoms appear, can reverse many of these symptoms⁸¹, which parallels the ability to reverse synaptic deficiencies caused by MeCP2 mutation in fully differentiated neurons⁷⁸. These findings suggest the possibility of effectively treating Rett syndrome in humans even after it has become symptomatic.

The molecular features of the pathogenesis of Rett syndrome are also under study. In MeCP2-deficient mice, levels of BDNF are reportedly elevated¹⁰² or reduced⁷⁵; however, deletion of *Bdnf* causes earlier onset of Rett-like symptoms and *Bdnf* overexpression can rescue some behavioural and electrophysiological defects⁷⁵. *Bdnf* is a presumed target of MeCP2 transcriptional repression and is likely to be one of many such targets that are involved in Rett syndrome^{75,102,103}.

Schizophrenia. There is mounting evidence that epigenetic mechanisms are involved in the pathogenesis of schizophrenia^{82,83}. Much of this work has focused on epigenetic alterations at the reelin promoter. Reelin is a glycoprotein that is expressed during development and in adult GABA (γ -aminobutyric acid)-containing neurons, and is important for proper neural positioning during brain development. Post-mortem studies of patients with schizophrenia reveal significant downregulation of reelin expression in several brain regions that is not associated with neuronal loss⁸⁴. The reelin promoter contains a large CpG island, indicating that DNA methylation might be important for regulating its expression⁸⁵. Repeated methionine administration causes hypermethylation of the promoter and results in downregulation of reelin transcription in the heterozygous *Reln*^{+/-} mouse model of schizophrenia⁸². Methionine treatment also induced MeCP2 binding to the reelin promoter⁸⁶. By contrast, treatment with the methylation inhibitor 5-aza-2'-deoxycytidine upregulated reelin expression *in vitro*⁸⁵. Interestingly, a role for methylation in the pathogenesis of schizophrenia was suggested decades ago by clinical studies, in which

Valproate

A commonly used anticonvulsant and antimanic medication. Among many other actions (for example, direct effects on the brain's GABA pathways), valproate is a nonspecific inhibitor of class I and class II HDACs.

treatment with the methylating agent SAM elicited psychotic episodes in some patients with schizophrenia⁸⁷.

Histone remodelling might also contribute to schizophrenia. The antipsychotic drugs haloperidol and raclopride (both dopamine receptor D₂ antagonists) rapidly induce phosphoacetylation of H3 in the mouse striatum, specifically at the *c-Fos* gene promoter¹⁵. Treatment with valproate (an anticonvulsant and mood stabilizer) that, among other actions, inhibits HDACs, increased reelin expression *in vitro* and *in vivo*^{82,85}, and this effect was accompanied by decreased methylation of the reelin promoter. Furthermore, valproate attenuated schizophrenia-like behavioural abnormalities in a methionine-induced epigenetic mouse model of the illness. Other HDAC inhibitors produced similar results⁸⁸. Although the mechanisms by which HDAC inhibitors reduce DNA methylation are unknown, it is thought that hyperacetylation can regulate the accessibility of DNMT1 to promoter regions or that it might induce DNA demethylase activity^{84,89,90}.

One hypothesis that has been proposed for how epigenetic malfunction may contribute to schizophrenia is that hypermethylation (regulated by DNMT1 and other chromatin-related complexes) downregulates multiple genes (such as reelin) in GABA-containing neurons, causing dysfunction of GABA-mediated neuronal circuitry. Higher brain function would be impaired because synchronization with other neuronal networks would be disrupted as a consequence. Agents that can reactivate gene expression, such as inhibitors of DNMT1 or HDACs, might therefore provide improved pharmacological treatment for schizophrenia⁸⁴. The administration of valproate in conjunction with antipsychotic medication has been shown to accelerate the onset of the antipsychotic effects in patients with schizophrenia⁹¹. However, valproate has many actions in addition to its HDAC inhibitor activity, and so direct evidence for this hypothesis remains lacking.

Future directions

There is much evidence that epigenetic regulation is involved in neurogenesis, neuronal plasticity and learning and memory, and in disorders such as depression, addiction, schizophrenia and cognitive dysfunction. Changes in histone modifications and DNA methylation have been found both globally and at the promoters of genes that have been implicated in these phenomena. For example, chromatin remodelling at the *Bdnf* promoter is associated with neuronal activity, seizures, chronic stress, cocaine addiction and Rett syndrome; at the reelin promoter with a mouse model of schizophrenia; and at the *Cdk5* promoter with drugs of abuse. However, chromatin remodelling is likely to affect many more

genes, and it is important to investigate this in both animal models of psychiatric conditions and post-mortem human brain tissue. Coordinated gene expression arrays and ChIP on chip arrays are likely to be helpful in elucidating the promoter gene targets for histone modifications as well as the *in vivo* binding sites of transcriptional activators or repressors in relevant brain regions. Such studies will provide a global picture of epigenetic regulation, which is currently lacking. Genome-wide epigenetic approaches have yielded exciting results in the fields of developmental and cancer biology^{48–50}, and efforts to carry out similar studies in addiction and depression are ongoing. Many genes show abnormal levels of histone acetylation or methylation after chronic cocaine administration or stress, and future studies will focus on the influence of these global changes in chromatin on the pathogenesis of addiction and depression, and on identifying the transcription factors that mediate this regulation. It will be necessary to use a system-based biological approach to interpret the data from such studies⁹².

The lack of specific inhibitors for many epigenetic regulators impedes progress in understanding the mechanisms by which chromatin remodelling contributes to psychiatric disorders. As just one example, all available HDAC inhibitors act broadly and inhibit all Class I and Class II HDACs (see **Supplementary information S1** (table)). Moreover, certain HDACs (Class III HDACs, termed sirtuins, as well as HDAC6, a Class IIb HDAC) can deacetylate proteins other than histones (for example, tubulin, MEF2, the tumour suppressor p53 and the DNA repair protein Ku70), and these non-histone actions complicate interpretation of the inhibitors' biological effects. Inhibitors that are specific for particular HDACs or other chromatin modifiers would allow us to identify the specific players in the epigenetic regulation of psychiatric phenomena. Such specific inhibitors would also have the potential of being used as new treatment agents for these disorders. In addition, it will be important to generate mouse models that lack particular chromatin-related genes in order to study their molecular and behavioural influences.

Lasting changes in chromatin modifications in animal models often occur only after chronic behavioural manipulations, which try to mimic the long-lasting behavioural changes associated with psychiatric disorders. A better understanding of the mechanisms by which such stable changes are brought about would not only advance our knowledge of the basic neurobiology of these illnesses, but might also provide new therapeutic avenues for disorders such as depression, addiction, schizophrenia and neurodevelopmental illnesses.

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Competing interests statement

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DATABASES

The following terms in this article are linked online to:

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 Δ FOSB | *Bdnf* | CBP | *c-Fos* | CREB | *FosB* | GR | MeCP2 | OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
Rett syndrome | Rubinstein-Taybi syndrome | schizophrenia

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