

REVIEWS

Transcriptional and epigenetic mechanisms of addiction

Alfred J. Robison and Eric J. Nestler

Abstract | Investigations of long-term changes in brain structure and function that accompany chronic exposure to drugs of abuse suggest that alterations in gene regulation contribute substantially to the addictive phenotype. Here, we review multiple mechanisms by which drugs alter the transcriptional potential of genes. These mechanisms range from the mobilization or repression of the transcriptional machinery—including the transcription factors ΔFOSB, cyclic AMP-responsive element binding protein (CREB) and nuclear factor-κB (NF-κB)—to epigenetics—including alterations in the accessibility of genes within their native chromatin structure induced by histone tail modifications and DNA methylation, and the regulation of gene expression by non-coding RNAs. Increasing evidence implicates these various mechanisms of gene regulation in the lasting changes that drugs of abuse induce in the brain, and offers novel inroads for addiction therapy.

Drug addiction exacts an enormous medical, financial and emotional toll on society in the form of overdose and health complications, family disintegration, loss of employment and crime. The National Institute on Drug Abuse (NIDA), part of the US National Institutes of Health, estimates that the total cost of drug abuse in the United States exceeds US\$600 billion annually, and it is particularly alarming to note a sharp increase in the abuse of prescription drugs and in drug abuse by teenagers (see the [NIDA](#) web site). These data substantiate the need for more research into the neuronal effects of drugs of abuse and the mechanisms of addiction, in the expectation of uncovering novel targets for treating and preventing addictive disorders.

Although most individuals are exposed to drugs of abuse, only a subset experience the loss of control over drug use and compulsion for drug seeking and taking that defines the addicted state. Entrance into this state is strongly influenced by both an individual's genetic constitution and the psychological and social context in which drug exposure occurs^{1–3}. Although the genetic contribution to risk for addiction is roughly 50%, the specific genes that are involved remain almost completely unknown. The addictive phenotype can persist for the length of an individual's life, with drug craving and relapse occurring even after decades of abstinence. This persistence suggests that drugs induce long-lasting changes in the brain that underlie addiction behaviours.

The many cells of an individual organism, although they contain essentially identical complements of DNA,

differentiate to form distinct tissues and organs through regulated changes in the transcriptional potential of each gene, based on environmental cues, cell-to-cell signals and other, probably random factors⁴. It is becoming clear that many of the same processes of gene regulation that are involved in the normal differentiation of cells and tissues during development are also engaged in the adult organism to mediate cellular adaptation to environmental stimuli^{5,6}. The processes that are involved in the regulation of transcriptional potential are varied and highly complex, and include activation and inhibition of transcription factors, modification of chromatin and DNA structure, and induction of non-coding RNAs. Increasing evidence supports the hypothesis that each of these mechanisms of epigenetic regulation is directly affected by drugs of abuse, and that such adaptations are one of the main processes by which drugs induce highly stable changes in the brain that mediate the addicted phenotype. This Review summarizes the findings that support this hypothesis, and highlights areas in which future research will extend this fundamental knowledge of addiction and exploit it for new therapeutics.

Drug action and gene transcription

A seemingly similar syndrome of addiction can result from exposure to a wide variety of chemical substances or even rewarding activities, from cocaine to gambling to sex. One common mechanism in these various forms of addiction is thought to be activation of the brain's reward circuitry, which centres on dopaminergic neurons in

Fishberg Department of Neuroscience and Friedman Brain Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, BOX 1065, New York, New York 10029, USA.
Correspondence to E.J.N.
e-mail: eric.nestler@mssm.edu
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Limbic system

A collection of cortical and subcortical structures that are important for processing memory and emotional information. Prominent structures include the hippocampus and amygdala.

Sensitization

Enhanced drug responsiveness — on the behavioural, cellular, and/or molecular levels — with repeated exposure to a constant dose.

the ventral tegmental area (VTA) of the midbrain and their projections to the limbic system — in particular, the nucleus accumbens (NAc; also known as the ventral striatum), dorsal striatum, amygdala, hippocampus and regions of prefrontal cortex^{7–9} (FIG. 1). This reward circuitry is activated by stimuli or pursuits that promote evolutionary fitness of the organism, such as nutrient-rich foods, sex and social stimulation. As drugs of abuse activate this circuitry far more strongly and persistently than natural rewards, and without being associated with productive behavioural outcomes, chronic exposure to drugs modulates brain reward regions partly through a homeostatic desensitization that renders the individual unable to attain sufficient feelings of reward in the absence of drug. An alternative, but not mutually exclusive, hypothesis of addiction focuses on incentive sensitization, whereby drugs alter the reward circuitry to cause increased assignment of incentive salience to drug cues, effectively making drug-associated environmental stimuli more difficult to ignore and leading to intense drug craving and relapse¹⁰. Pathological drug-induced changes in the reward circuitry further impair behavioural control over drug taking.

Virtually all rewarding drugs or activities increase dopaminergic transmission from the VTA to the NAc and other target limbic regions, although they each employ partly distinct mechanisms and in some cases involve other neurotransmitter systems as well^{7–9}. The actions of drugs on the NAc are further complicated by the cellular heterogeneity of this brain region (BOX 1). Although drugs differ in their acute mechanisms of action, the common syndrome of addiction suggests that chronic activation of these distinct, acute mechanisms induces some shared molecular adaptations in brain reward regions that mediate the lasting nature of the addictive phenotype.

We, and others, have long proposed that changes in the transcriptional potential of genes — through the actions of transcription factors, chromatin modifications and non-coding RNAs — contribute substantially to many of the neuroadaptations that result from chronic exposure to drugs of abuse¹¹ (FIG. 2). We know that many mRNAs display altered expression in brain reward regions after chronic drug exposure, which suggests that transcription of individual genes is differentially regulated under these conditions. Over the past ~5 years, studies at the chromatin level have confirmed the involvement of such transcriptional mechanisms *in vivo*. Moreover, beyond stable changes in steady-state mRNA levels, this work has shown that the ‘inducibility’ of a gene — its ability to be induced or repressed in response to the next drug exposure or some other environmental stimulus — is also altered by chronic drug exposure, and that such gene ‘priming’ or ‘desensitization’ is mediated by stable drug-induced changes in the chromatin state around individual genes (FIG. 3).

This transcriptional and epigenetic model of chronic drug action provides a plausible mechanism for how environmental influences during development can increase (or decrease) the risk for addiction later in life. For example, there is mounting evidence that stress during adolescence increases the risk of addiction, and that exposure to drugs *in utero* increases the risk in adolescence and adulthood^{12,13}. Long-lasting changes in gene transcription or in the potential for transcription that results from early-life stress or drug exposure — mediated at the chromatin level in the absence of genetic differences in the primary DNA sequence — might render an adult brain more vulnerable to the addictive process. As alterations in transcriptional potential can last for many years, this model also explains how relapse can occur despite decades of abstinence.

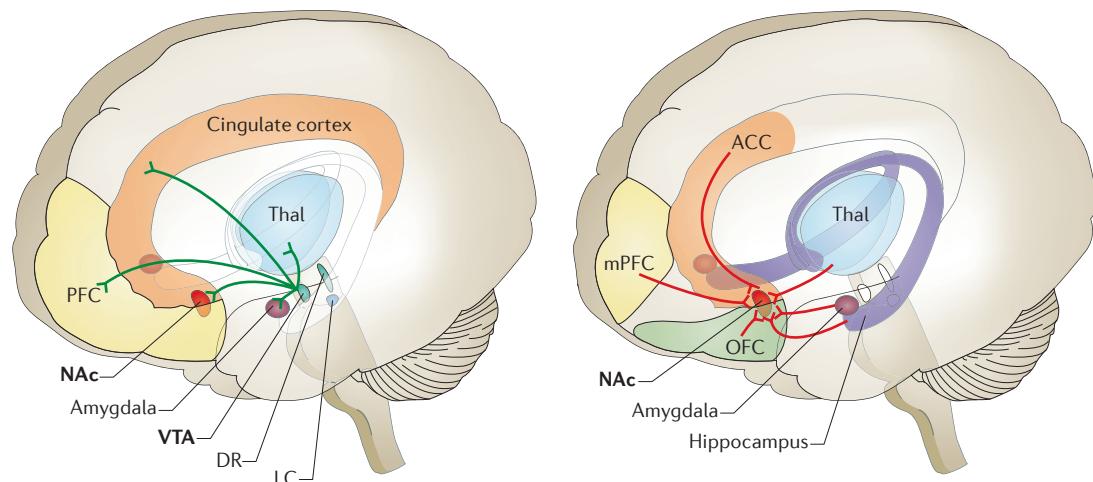
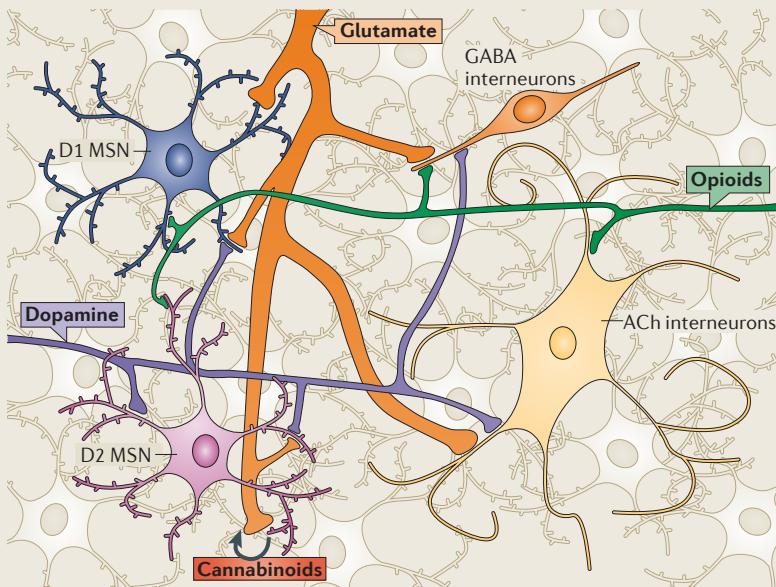


Figure 1 | Brain reward circuitry. The brain on the left depicts dopaminergic afferents that originate in the ventral tegmental area (VTA) and release dopamine in the nucleus accumbens (NAc) and many other limbic targets. Also shown are other monoaminergic nuclei — the noradrenergic locus coeruleus (LC) and serotonergic dorsal raphe (DR) — which modulate drug reward and other actions. The brain on the right highlights glutamatergic regions that are important for reward: medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), thalamus (Thal), hippocampus and amygdala, all of which send excitatory projections to the NAc. Drugs of abuse alter this reward circuitry in complex ways, and this leads to addiction.

Recent studies of rodent models of addiction have provided considerable support for this hypothesis and have contributed substantially to our understanding of *in vivo* transcriptional and epigenetic regulation in the

brain. Here, we highlight key examples of transcriptional and epigenetic mechanisms of drug action, and identify some of the novel potential targets for therapeutic intervention during the addiction process.

Box 1 | Cellular organization of the nucleus accumbens



The nucleus accumbens (NAc) is composed of multiple neuronal cell types (see the figure), with each cell type seeming to exhibit different transcriptional responses to drugs of abuse and to mediate distinct aspects of drug reward and addiction. Glutamatergic afferents from the hippocampus, prefrontal cortex and amygdala, among other regions, excite all subtypes of NAc neurons³⁶, with such excitation differentially regulating drug reward and motivation, as shown by recent optogenetic experiments^{130,131}. These excitatory inputs are modulated by dopamine afferents from the ventral tegmental area (VTA), and psychostimulant drugs such as cocaine and amphetamine act by directly prolonging the effects of these dopamine signals. Excitatory inputs to the NAc are also modulated by endogenous opioid peptides that are both expressed locally and released by input neurons. Opiate drugs thus act directly on NAc neurons that express opioid receptors, and they also promote dopamine release in the NAc indirectly by inhibiting VTA GABAergic interneurons. Cannabinoids also have a role in regulating NAc neurons — they act primarily by locally repressing the function of glutamatergic synapses.

Much work is needed to further understand the cellular specificity of drug action in the NAc. 95% of NAc neurons are GABAergic medium spiny neurons (MSNs), which can be further differentiated into those that express the D1 dopamine receptor (D1-type MSNs) along with dynorphin and substance P, and those that express the D2 dopamine receptor (D2-type MSNs) along with encephalin¹³². Drug induction of ΔFOSB^{14,133,134}, and the effects of ΔFOSB and G9a on cell morphology and behaviour, differ between D1-type and D2-type MSNs¹³⁵, and neuronal activity of these two cell types causes opposing effects on the rewarding properties of cocaine¹³⁰. In addition, acute cocaine causes extracellular signal-regulated kinase (ERK)-dependent phosphorylation of mitogen- and stress-activated kinase 1 (MSK1; also known as ribosomal protein S6 kinase α5) and of histone 3 specifically in D1-type MSNs⁷⁵, although the functional consequences of this histone modification are not yet known. By contrast, the effects of cannabinoids seem to predominate at glutamatergic synapses on D2-type MSNs¹³⁶. About 1–2% of NAc neurons are spiny large cholinergic interneurons, which have been shown to play an important part in cocaine reward¹³¹, and a similar number are GABAergic interneurons, the function of which are less well understood.

Although these studies are important, so far they have barely scratched the surface of what promises to be an important new focus in addiction research: to overlay the alterations in transcriptional potential of genes induced by chronic exposure to drugs onto the map of cellular subtypes in the NAc. ACh, acetylcholine.

Transcription factors in addiction

The classic mechanism for the regulation of gene expression is through the actions of transcription factors: proteins that, in response to cell signalling pathways, bind to specific sequences of DNA — generally in the promoter or enhancer regions of target genes — and increase or repress the expression of these genes by respectively promoting or blocking the recruitment of the RNA polymerase II transcriptional complex. Transcription factors operate as part of large protein complexes, with their mechanisms of action eventually involving alterations in chromatin structure (see below). Although neurons contain hundreds of transcription factors, studies of adaptations induced by drugs of abuse have focused primarily on a small subset.

ΔFOSB. ΔFOSB¹⁴ is encoded by the *FosB* gene and shares homology with other FOS family transcription factors. It heterodimerizes with JUN family proteins to form activator protein 1 (AP1; also known as transcription factor AP1) complexes that bind to AP1 sites in responsive genes to regulate transcription. There is some evidence from *in vitro* studies that ΔFOSB may also homodimerize¹⁵. Although all FOS family proteins are induced transiently by acute drug exposure, chronic administration of virtually any drug of abuse induces the long-lasting expression specifically of ΔFOSB^{14,16,17}, a process that is most robust in the NAc and dorsal striatum, but is also seen in several other reward-related brain regions, including prefrontal cortex¹⁷. ΔFOSB induction in the NAc and dorsal striatum by drugs of abuse, regardless of whether the drug is investigator-administered or self-administered, occurs only in the subtype of medium spiny neuron (MSN) that expresses D1 dopamine receptors (D1-type MSNs)¹⁴ (M. K. Lobo, S. Zaman and E. J. N., unpublished observations). ΔFOSB is a carboxy-terminal truncation of full-length FOSB that is generated by alternative splicing; it lacks the two degron domains that are present in the full-length protein and that are conserved among all other FOS family proteins. This absence results in a fourfold increase in protein stability¹⁸. In addition, ΔFOSB is phosphorylated *in vivo* at serine 27 (as well as at several other sites) and this phosphorylation further stabilizes the protein by roughly tenfold, both *in vitro* and *in vivo*^{19,20}. This intrinsic and regulated protein stability is a particularly interesting feature of the molecule, as it provides a molecular mechanism by which drug-induced changes in gene expression can persist for weeks after drug intake stops.

ΔFOSB has been linked directly to several addiction-related behaviours. In adult bi-transgenic mice, in which removal of doxycycline induces ΔFOSB overexpression specifically in D1-type MSNs of the NAc and dorsal striatum, such induction causes increased locomotor sensitivity to cocaine²¹, increased conditioned place-preference to cocaine and morphine^{21,22}, and increased

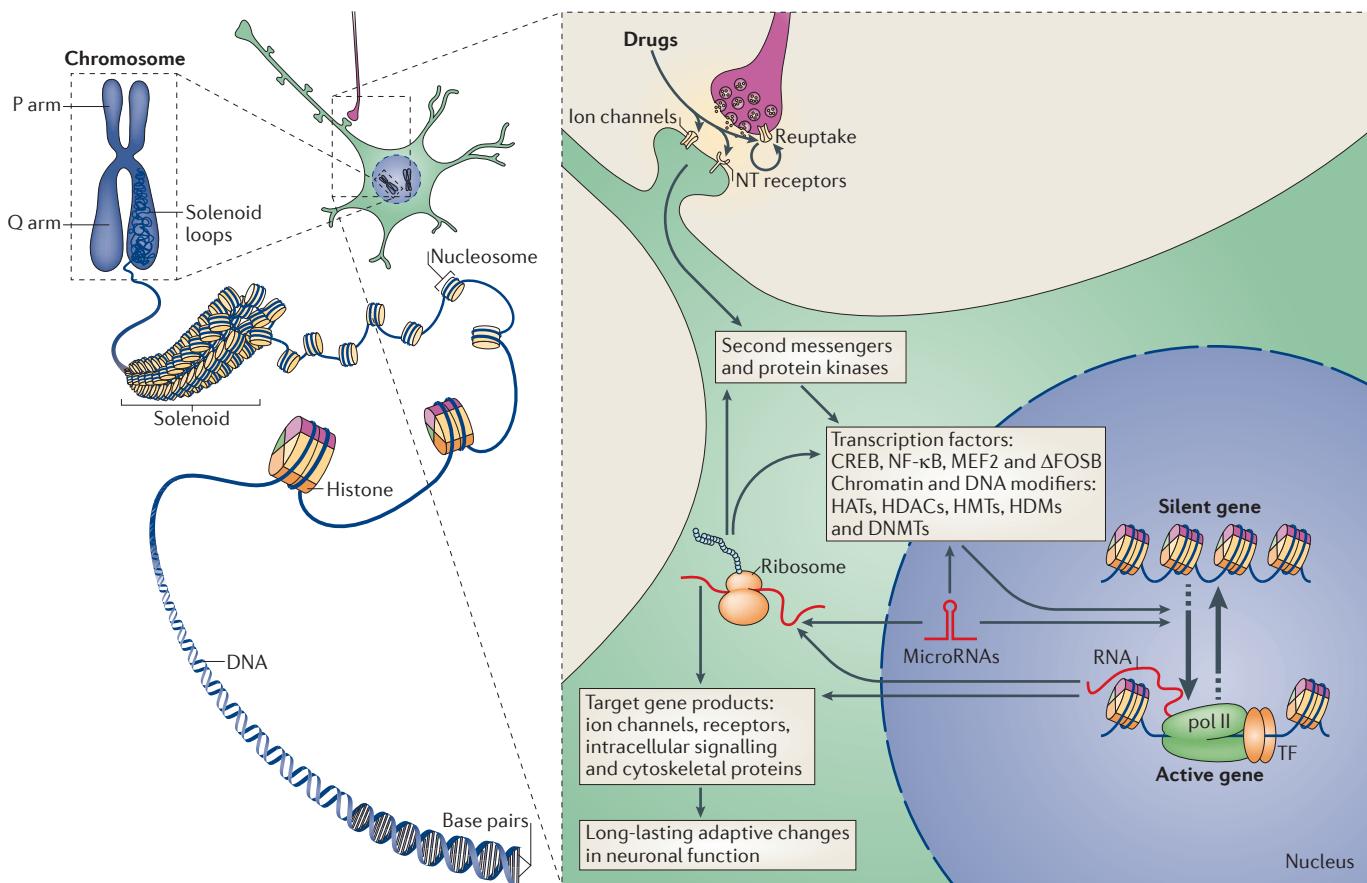


Figure 2 | Mechanisms of transcriptional and epigenetic regulation by drugs of abuse. In eukaryotic cells, DNA is organized by wrapping around histone octomers to form nucleosomes, which are then further organized and condensed to form chromosomes (left part). Only by temporarily unravelling compacted chromatin can the DNA of a specific gene be made accessible to the transcriptional machinery. Drugs of abuse act through synaptic targets such as reuptake mechanisms, ion channels and neurotransmitter (NT) receptors to alter intracellular signalling cascades (right part). This leads to the activation or inhibition of transcription factors (TFs) and of many other nuclear targets, including chromatin-regulatory proteins (shown by thick arrows); the detailed mechanisms involved in the synaptic regulation of chromatin-regulatory proteins remain poorly understood. These processes ultimately result in the induction or repression of particular genes, including those for non-coding RNAs such as microRNAs; altered expression of some of these genes can in turn further regulate gene transcription. It is proposed that some of these drug-induced changes at the chromatin level are extremely stable and thereby underlie the long-lasting behaviours that define addiction. CREB, cyclic AMP-responsive element binding protein; DNMTs, DNA methyltransferases; HATs, histone acetyltransferases; HDACs, histone deacetylases; HDMs, histone demethylases; HMTs, histone methyltransferases; MEF2, myocyte-specific enhancer factor 2; NF-κB, nuclear factor-κB; pol II, RNA polymerase II.

Self-administration

A form of operant conditioning using a drug as a reward, generally by administration through an intravenous line that is controlled directly by the animal's actions.

Medium spiny neurons (MSNs)

The main cell population of the ventral and dorsal striatum; these GABAergic projection neurons form the two main outputs of these structures, called the direct pathway (D1-type MSNs) and indirect pathway (D2-type MSNs).

Degron domains

A specific amino acid sequence that targets a protein for degradation through proteasomal or other proteolytic processes.

Conditioned place-preference

A behavioural test in which animals learn to prefer an environment that is associated with rewarding drug administration. It provides an indirect measure of drug reward.

cocaine self-administration²³. In addition, virus-mediated overexpression studies show that cocaine-mediated induction of ΔFOSB in orbitofrontal cortex, a subregion of prefrontal cortex, mediates the ability of chronic cocaine to induce tolerance to the cognition-disrupting effects of acute drug exposure²⁴. Such overexpression also enhances impulsivity during drug withdrawal, and both of these effects further promote drug self-administration^{24,25}. Importantly, genetic or viral overexpression of ΔJUND — a dominant negative mutant of JUND that antagonizes ΔFOSB and other AP1-mediated transcriptional activity — in the NAc or orbitofrontal cortex blocks these key effects of drug exposure^{14,22,24}. This indicates that ΔFOSB is both necessary and sufficient for many of the changes that are wrought in the brain by chronic drug exposure. ΔFOSB is also induced in

D1-type NAc MSNs by chronic consumption of several natural rewards, including sucrose, high fat food, sex and wheel running, and this promotes the consumption of such rewards^{14,26–30}. This implicates ΔFOSB in the regulation of natural rewards under normal conditions and, perhaps, during pathological addictive-like states.

Progress has been made in identifying the broad range of transcriptional targets (some activated and some repressed) through which ΔFOSB produces these various behavioural phenotypes in response to drug exposure^{31,32}. By regulating numerous genes that are related to dendritic spine architecture, including synaptotagmin, microtubule associated proteins, activity-regulated cytoskeleton-associated protein (ARC), actin-related proteins, cyclin-dependent kinase 5 (CDK5) and kinesin^{31–33}, ΔFOSB mediates the structural

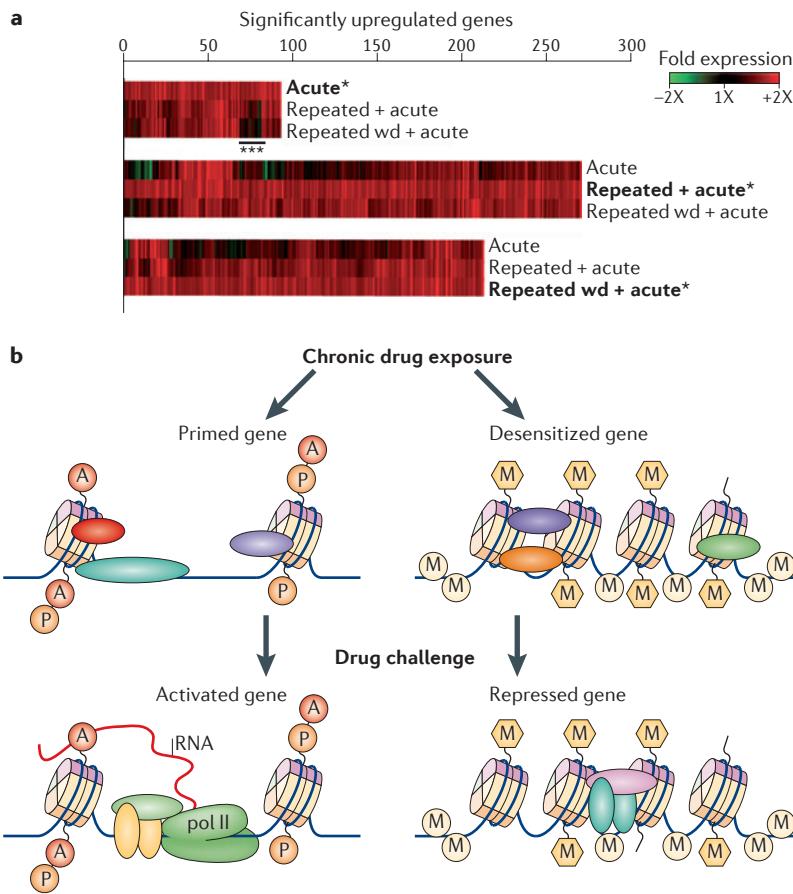


Figure 3 | Gene priming and desensitization. In addition to regulating the steady-state expression levels of certain genes, cocaine induces latent effects at many other genes, which alter their inducibility in response to a subsequent stimulus.

a | Analysis of mRNA expression after acute or chronic cocaine. Heat maps that are marked with * show all genes that are upregulated in the nucleus accumbens 1 hour after a cocaine challenge in naive animals (acute), in animals treated repeatedly with cocaine (repeated + acute) or in animals after 1 week of withdrawal (wd) from repeated cocaine (repeated wd + acute). Associated heat maps show how the same genes were affected under the other two conditions. Examples of desensitized transcriptional responses after repeated cocaine are indicated by ***. **b** | Early evidence suggests that epigenetic mechanisms are important in mediating such gene priming and desensitization, and that many of these changes are latent, meaning that they are not reflected by stable changes in steady-state mRNA levels. Instead, such changes alter chromatin structure, so that a later drug challenge induces a given gene to a greater (primed) or lesser (desensitized) extent based on the epigenetic modifications that are induced by previous chronic drug exposure. A major goal of current research is to identify the chromatin signatures that underlie gene priming and desensitization. A, acetylation; M, methylation; P, phosphorylation; pol II, RNA polymerase II. Part **a** is reproduced, with permission, from REF. 37 © (2010) American Association for the Advancement of Science.

Tolerance
Reduced drug responsiveness with repeated exposure to a constant dose.

Dominant-negative mutant
A mutant molecule that forms heteromeric complexes with the wild-type protein's targets to yield a non-functional complex. This antagonizes the activity of the endogenous wild-type protein.

plasticity that is induced in NAc by cocaine^{34–36}: it is both necessary and sufficient for cocaine-induced increases in the dendritic spine number of NAc MSNs³⁷ (BOX 2). As discussed below, ΔFOSB controls the activity of several other transcriptional and epigenetic regulatory proteins, which then further influence NAc dendritic arborization. This suggests that ΔFOSB serves as one of the master control proteins that govern this structural plasticity. ΔFOSB also regulates proteins that are important for glutamatergic synaptic function and plasticity, including AMPA receptor subunits^{21,38} and Ca²⁺/

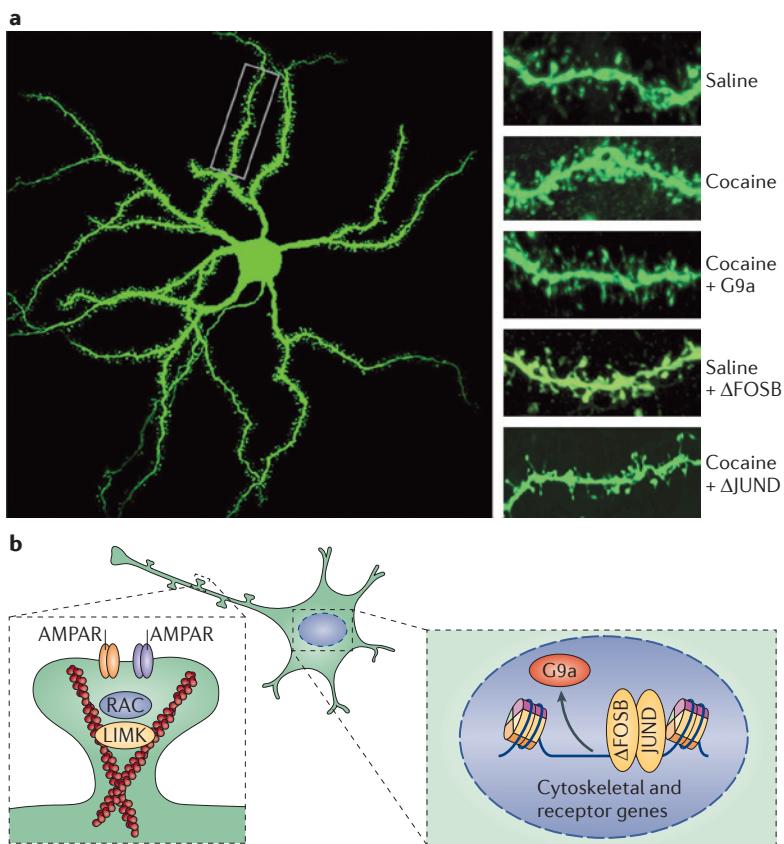
calmodulin-dependent kinase II (CaMKII)^{31,39}, which is consistent with the hypothesis that it mediates key aspects of the synaptic plasticity that is exhibited by MSNs after drug exposure^{34,40}.

ΔFOSB is far more stable than all other transcription factors that have been linked to addiction so far. Nevertheless, drug relapse can occur after decades of abstinence, a timescale dwarfing even phosphorylated ΔFOSB's prolonged turnover rate. It is possible that ΔFOSB remains stably linked to individual gene promoters for long periods of time or induces long-lasting changes to the chromatin structure of individual genes (see below) to influence relapse behaviour long after total cellular levels of the protein have returned to baseline. These possibilities remain to be investigated in future experiments.

CREB. Cyclic AMP (cAMP)-responsive element binding protein (CREB) forms homodimers that can bind to genes at cAMP-responsive elements (CREs). It primarily activates transcription after it has been phosphorylated at serine 133 (by any of several protein kinases), which allows recruitment of CREB-binding protein (CBP) that then promotes transcription (see below)^{41,42}. The mechanism by which CREB activation represses the expression of certain genes is less well understood. Psychostimulants (for example, cocaine and amphetamine) and opiates increase CREB activity, and do so acutely as well as chronically — as measured by increased phospho-CREB (pCREB) or reporter gene activity in CRE-lacZ transgenic mice — and in multiple brain regions, including the NAc and dorsal striatum^{41–43}. Experiments that involve the inducible overexpression of CREB or a dominant-negative mutant form of CREB, either in bi-transgenic mice or using viral vectors, have shown that CREB induction in the NAc, which occurs in both D1- and D2-type MSNs⁴¹, decreases the rewarding effects of cocaine and opiates^{44,45}. This promotes drug self-administration, presumably through negative reinforcement⁴⁶. CREB shows more complicated and varied responses to rewards or drugs of abuse other than cocaine and opiates. For example, chronic nicotine⁴⁷ or ethanol^{48,49} administration reduces pCREB levels in the NAc but CREB activity seems to be necessary for nicotine to establish a place preference⁵⁰. In addition, exposure to Δ9-tetrahydrocannabinol (THC, the active compound in marijuana) increases pCREB in the prefrontal cortex and hippocampus⁵¹, and stimuli that are associated with natural reward increase pCREB in the NAc⁵². Other CREB family proteins, such as inducible cAMP repressor (ICER; a product of the cAMP-responsive element modulator (CREM) gene) and activating transcription factors (ATFs), have also been implicated in the long-term actions of drugs of abuse and require further study⁵³.

CREB activity has been directly linked to the functional activity of NAc MSNs. The electrical excitability of MSNs is increased by CREB overexpression, whereas dominant-negative CREB decreases it⁵⁴. Possible differences between D1- and D2-type MSNs in this regard have not yet been explored. The observation that

Box 2 | Epigenetic regulation and dendritic spine plasticity



For changes in gene transcription and chromatin modifications to affect complex behaviours such as addiction, they must result in some functional output, such as a change in neuronal excitability (intrinsic membrane properties) or connectivity (synapse number or strength). Indeed, it is clear that nearly all drugs of abuse alter the structural connectivity of neurons in the reward circuitry, an effect that is most evident in changes in the number, shape and size of dendritic spines on medium spiny neurons (MSNs) in the nucleus accumbens (NAc)^{34–36} (see the figure, part a, which shows cocaine-induced increases in dendritic spine number that can be blocked by viral overexpression of G9a or ΔJUND, or mimicked by viral overexpression of ΔFOSB). These changes seem to be behaviourally relevant, as they correlate with behavioural sensitization³⁷. However, certain conditions that increase spine density cause opposite behavioural effects^{60,106}. Moreover, the nature of these changes varies with the abused substance, time of withdrawal and method of intake, even within a single brain region. For example, experimenter-administered cocaine increases the number of thin spines on NAc MSNs during and shortly after chronic exposure, but increases mushroom spines and dendritic complexity during withdrawal^{34,36}. Moreover, opiates and psychostimulants both induce locomotor activity acutely, and locomotor and reward sensitization chronically³⁸, whereas morphine consistently reduces NAc MSN spine density and complexity^{34,35}. Resolving this discrepancy is an important future research goal. It is also likely that structural plasticity of the NAc plays a part in volition and decision-making, as self-administered drugs generally cause larger changes in spine density than the same doses administered by experimenters^{35,36}. Although the molecular underpinnings of these structural changes remain incompletely understood, several factors that control gene transcription and chromatin regulation have been implicated (see the figure, part b). These include ΔFOSB³⁷, cyclic AMP-responsive element binding protein (CREB)⁵⁵, myocyte-specific enhancer factor 2 (MEF2)⁶⁰, G9a³⁷ and DNA methyltransferase 3A (DNMT3A)¹⁰⁶, each of which has been linked directly to cocaine regulation of NAc MSN spine density. A key goal is to now identify how these epigenetic factors control cytoskeletal and cytoskeleton-altering genes to regulate spine morphology and consequently changes in neuronal circuitry and addiction-related behaviours. LIMK, LIM domain kinase; RAC, Ras-related C3 botulinum toxin substrate. Part a, right parts are reproduced, with permission, from REF. 37 © (2010) American Association for the Advancement of Science. Part a, left part is reproduced, with permission, from REF. 34 © (2010) Cell Press.

virus-mediated overexpression of a K⁺ channel subunit in the NAc, which decreases MSN excitability, enhances locomotor responses to cocaine suggests that CREB might act as a break on behavioural sensitization to cocaine by upregulating MSN excitability⁵⁴. Numerous CREB target genes that mediate these and other effects on NAc MSNs have been identified^{31,32,41,42,44,55}. Prominent examples include the opioid peptide dynorphin, which feeds back and suppresses dopaminergic signalling to the NAc^{41,44}, as well as certain ion channels and glutamate receptor subunits that control NAc excitability^{54,55}. It is interesting to compare these effects of CREB in the NAc to similar data from the locus coeruleus, where CREB has also been found to increase neuronal excitability and thereby mediate aspects of drug tolerance and dependence (BOX 3).

NF-κB. Nuclear factor-κB (NF-κB), a transcription factor that is rapidly activated by diverse stimuli, was studied initially for its role in inflammation and immune responses, and more recently has been linked to synaptic plasticity and memory⁵⁶. NF-κB has been shown to be induced in the NAc by repeated cocaine administration, where it is required for the cocaine-induced increase in NAc MSN dendritic spine density (BOX 2) and sensitization to the rewarding effects of the drug⁵⁷. It has also been associated with nicotine dependence in humans⁵⁸. A major goal of current research is to identify the target genes through which NF-κB causes cellular and behavioural plasticity. Interestingly, cocaine-induced expression of NF-κB is mediated through ΔFOSB¹⁴, illustrating the complex transcriptional cascades that are involved in drug action. The role of NF-κB in MSN spinogenesis has recently been extended to stress and depression models⁵⁹. This finding is of particular importance considering the co-morbidity of depression and addiction, and the well-studied phenomenon of stress-induced relapse to drug abuse.

MEF2. Multiple myocyte-specific enhancer factor 2 (MEF2) proteins are expressed in the brain (including in NAc MSNs), where they form homodimers and heterodimers that can activate or repress gene transcription depending on the nature of the proteins that they recruit (for example, co-activator p300 and co-repressors known as class II histone deacetylases (HDACs) (see below)). Recent work suggests that chronic cocaine exposure suppresses striatal MEF2 activity, partly through D1 receptor-cAMP-dependent inhibition of calcineurin, a Ca²⁺-dependent protein phosphatase⁶⁰. Cocaine-mediated induction of Cdk5, which is a target gene for ΔFOSB³³, may also be involved. This reduction in MEF2 activity is required for the cocaine-induced increase in MSN dendritic spine numbers, but seems to inhibit behavioural sensitization to cocaine⁶⁰. Although these data suggest that MEF2 plays an important part in the structural and behavioural changes that result from repeated cocaine administration, they also demonstrate an apparent inconsistency between MSN spine increases and behavioural sensitization to cocaine that merits further study³⁴. Although ethanol has been shown

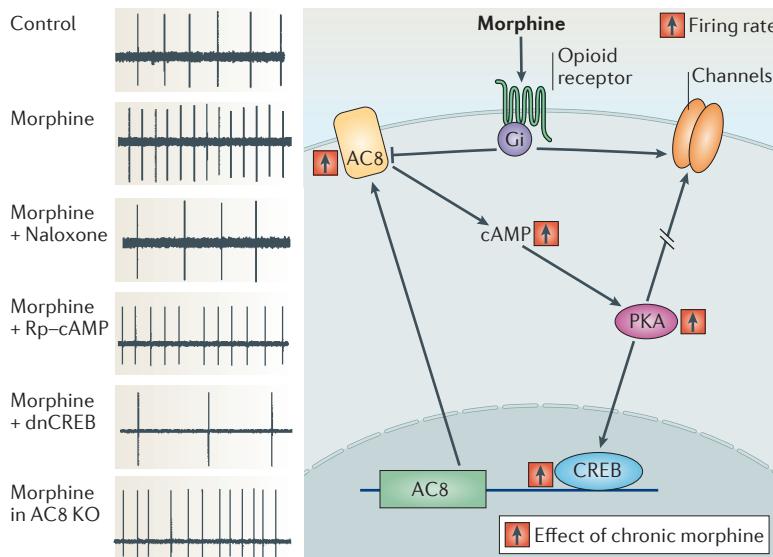
Dendritic spine
A small protrusion from a dendrite that is typically associated with synaptic input from a glutamatergic axon at its tip but may receive other inputs along its sides or neck.

to decrease MEF2 expression in rat cardiomyocytes⁶¹, little is known about the effects of other drugs of abuse on MEF2 function in the brain.

Additional transcription factors. The transcription factors that are listed here are the ones that are most extensively studied in addiction models. However, increasing evidence links several other transcription factors to drug exposure. These include the glucocorticoid receptor,

nucleus accumbens 1 transcription factor (NAC1), early growth response factors (EGRs) and signal transducers and activators of transcription (STATs)^{11,14}. For example, glucocorticoid receptor expression is required in dopamine receptor-expressing neurons to facilitate cocaine seeking⁶² but not for molecular and behavioural responses to morphine⁶³, and polymorphisms of this gene may be associated with the initiation of alcohol abuse in teenagers⁶⁴.

Box 3 | Chronic morphine action in the locus coeruleus



The locus coeruleus is the major noradrenergic nucleus in the brain, and it has served as a useful model of opiate action^{11,139,140}. Acute morphine decreases the firing rate of locus coeruleus neurons, whereas chronic exposure to the drug allows the rate to return to baseline (a phenomenon known as tolerance) and withdrawal from morphine causes firing rates to increase dramatically over baseline (a phenomenon that is characteristic of dependence and withdrawal) (see the figure, left part)^{139,141,142}. Chronic morphine exerts these effects on firing rate partly by upregulating the cyclic AMP–cAMP-responsive element binding protein (CREB) pathway, including induction of adenyl cyclase type 8 (AC8) and CREB itself (see the figure, right part, shown by arrows in red boxes). Indeed, inhibiting or removing components of this pathway prevents the effect of chronic morphine on locus coeruleus neuron firing (see the figure, left part). As this pathway is acutely inhibited by the drug, cAMP–CREB upregulation can be seen as a classic negative feedback mechanism^{11,139}. These cellular and molecular effects of chronic morphine are independent of synaptic inputs and can be induced by direct activation of opioid receptors on locus coeruleus neurons in brain slices¹⁴². Moreover, the proposed role for CREB in locus coeruleus, which was based originally on overexpression systems, has been validated more recently by the local knockout of endogenous CREB from locus coeruleus neurons¹⁴². The nature of the ion channel (or channels) that mediate the cAMP–CREB-dependent changes in locus coeruleus excitability remains unknown, but activation of the cAMP–CREB pathway in locus coeruleus neurons is behaviourally relevant, in that it contributes to symptoms of physical opiate dependence and withdrawal, which are mediated in part by locus coeruleus activation. These studies establish the molecular details of a transcriptional mechanism of intrinsic homeostatic plasticity that is involved in the development of opiate tolerance and dependence, and have provided key insight into the chronic actions of opiates and of other drugs of abuse in several other CNS regions, including those directly related to reward, such as the nucleus accumbens and ventral tegmental area¹¹. AC8 KO, AC8 knockout mouse; dnCREB, dominant negative CREB; PKA, protein kinase A; Rp-cAMP, (Rp)-adenosine 3',5'-monophorothioate (a competitive inhibitor of cAMP-dependent processes). Left part of figure is reproduced, with permission, from REF. 142 © (2010) National Academy of Sciences.

Epigenetics of addiction

Over the past decade, research into the regulation of transcriptional potential through modification of DNA and chromatin structure has exploded. As it became clear that epigenetic change underlies adaptations in the adult organism, investigations of epigenetic mechanisms have proven fruitful in numerous fields, including drug addiction^{65,66}. Here, we describe three major mechanisms of epigenetic regulation — histone tail modification, DNA methylation and microRNAs — and summarize the major findings that have linked each of these mechanisms to addiction.

Histone tail modification. Most DNA in eukaryotic cells is densely packed into chromatin, where 147 base pairs (bp) are wrapped around a nucleosome core in ~1.7 superhelical turns⁶⁷. Nucleosomes are composed of octamers that contain four histone homodimers, one each of histones H2A, H2B, H3 and H4, with H1 binding to spans of non-nucleosomal DNA. Numerous types of post-translational modifications of the amino-terminal tails of histones alter chromatin compaction to create more ‘open’ states (euchromatin, which is transcriptionally permissive) versus ‘closed’ states (heterochromatin, which is transcriptionally repressive)⁶⁸ (FIG. 3).

Many residues in the tails of histones are covalently modified in numerous ways, resulting in a complex ‘code’ that is thought to control the accessibility of individual genes to the transcriptional machinery⁶⁹. Histone acetylation, which negates the positive charge of lysine residues in the histone tail, is associated with transcriptional activation. This process is controlled by histone acetyltransferases (HATs) and HDACs, each of which comprise multiple enzyme classes whose expression and activity are exquisitely regulated⁶⁷. Histone methylation has been associated with both transcriptional activation and repression, depending on the particular residue and the extent of methylation^{70,71}; both lysine and arginine residues can be methylated by several families of histone methyltransferases (HMTs), and this reaction can be reversed by equally diverse histone demethylases (HDMs). Histone tail modifications also include phosphorylation, ubiquitylation, sumoylation and ADP ribosylation, among many others⁶⁷. The prospect of deciphering the histone code is daunting, given the seemingly infinite number of possible patterns of histone modifications, and the possibility that a particular pattern may have various meanings, depending on the individual gene involved. Nevertheless, new tools are accelerating progress in mapping the epigenetic state of individual gene promoters and

the genome as a whole, and future research will determine the feasibility of identifying functionally meaningful chromatin codes⁷².

Multiple drugs of abuse induce changes in histone acetylation in the brain, and evidence has begun to accumulate that these modifications underlie some of the functional abnormalities found in addiction models^{66,70}. First, global (that is, total cellular) levels of H3 and H4 acetylation are increased in the NAc after acute or chronic exposure to cocaine^{65,73}, and gene promoters that show increased H3 or H4 acetylation have been mapped genome-wide³². Despite these global increases, many genes show decreased histone acetylation after chronic cocaine, raising a key question as to what governs gene-specific acetylation changes in the face of global modifications. Another key question concerns the precise intracellular signalling cascades through which cocaine induces changes in histone acetylation — there is some information that such changes may be specific to D1-type MSNs and involve regulation of growth factor-associated kinases^{74,75}. Second, alcohol withdrawal has been shown to increase HDAC activity and reduce histone acetylation in the mouse amygdala⁷⁶, and in *Drosophila melanogaster* the commonly abused inhalant benzyl alcohol regulates potassium channels that are tied to alcohol tolerance through H4 acetylation⁷⁷. Third, exposure to THC increases HDAC3 expression in trophoblast cells⁷⁸. However, this alteration was absent in a genome-wide screen of brain tissue from Δ^9 -THC-treated mice⁷⁹, demonstrating that experiments on cell lines can yield effects that are very different from those found in a complex heterogeneous tissue like the brain. These data highlight the need for further research to define the effects of drugs of abuse on histone acetylation in brain in a region- and cell type-specific manner, and to identify the specific HAT and HDAC subtypes and intracellular signalling pathways that mediate this regulation *in vivo*.

Experimental alterations in histone acetylation potently affect addiction-related behaviours. Short-term systemic or intra-NAc administration of nonspecific HDAC inhibitors potentiates place conditioning and locomotor responses to psychostimulants and to opiates^{65,73,80}. More prolonged HDAC inhibition has been reported to induce changes in the opposite direction^{81,82}, perhaps through adaptations that oppose initial enzyme inhibition. Studies of specific HDAC isoforms have yielded interesting information: overexpression of HDAC4 or HDAC5 decreases behavioural responses to cocaine^{73,80}, whereas genetic deletion of HDAC5 hyper-sensitizes mice to the chronic effects (but not to the acute effects) of the drug⁸⁰. Similarly, mutant mice with reduced expression of CBP, a major HAT in the brain, exhibit decreased sensitivity to chronic cocaine⁸³. Much additional work is needed to define the influence of specific HAT and HDAC subtypes on addiction-related phenomena.

The potential complexity involved in histone acetylation in addiction models is indicated by recent findings on sirtuins, which are considered Class III HDACs but in reality influence many non-histone proteins.

Genome-wide studies of chromatin alterations in the NAc after chronic cocaine revealed an upregulation of two sirtuins, NAD-dependent deacetylase sirtuin 1 (SIRT1) and SIRT2. Pharmacological inhibition of sirtuins decreases cocaine place preference and self-administration, whereas activation increases rewarding responses to cocaine³². SIRT1 and SIRT2 induction is associated with increased H3 acetylation and increased Δ FOSB binding at their gene promoters³², which suggests that sirtuins are downstream targets of Δ FOSB. Studies are now needed to identify the proteins that are affected by cocaine-induced regulation of these sirtuins. For example, sirtuins deacetylate several transcription factors such as forkhead box O (FOXO) proteins or NF- κ B, and serve scaffolding functions by contributing to transcriptional repressive complexes⁸⁴ — processes that now warrant study in models of cocaine addiction. These findings illustrate the ability of genome-wide efforts to identify previously unknown mechanisms that are involved in drug action.

Histone methylation is also directly regulated by drugs of abuse: global levels of histone 3 lysine 9 dimethylation (H3K9me2) are reduced in the NAc after chronic cocaine exposure³⁷, and a genome-wide screen revealed alterations in H3K9me2 binding on the promoters of numerous genes in this brain region³²; both increases and decreases were observed, indicating again that epigenetic modifications at individual genes often defy global (that is, cell-wide) changes. The global decrease in H3K9me2 in the NAc is probably mediated by cocaine-induced downregulation of two HMTs, G9a and G9a-like protein (GLP), which catalyse the demethylation of H3K9me2 (REF. 37). These adaptations mediate enhanced responsiveness to cocaine, as selective knockout or pharmacological inhibition of G9a in the NAc promotes cocaine-induced behaviours, whereas G9a overexpression has the opposite effect. Similarly, G9a downregulation mediates the ability of cocaine to increase the spine density of NAc MSNs³⁷ (BOX 2). Interestingly, there is a functional feedback loop between G9a and Δ FOSB: Δ FOSB seems to be responsible for cocaine-induced suppression of G9a, and G9a binds to and represses the *Fosb* promoter, such that G9a down-regulation may promote the accumulation of Δ FOSB that is seen after chronic cocaine³⁷. In addition, G9a and Δ FOSB share many of the same target genes.

Chronic cocaine also downregulates H3K9me3, a mark of heterochromatin, specifically in the NAc, and this change is associated with a decrease in the total amount of heterochromatin in NAc MSN nuclei and an increase in the volume of these nuclei⁸⁵. Genome-wide mapping of H3K9me3 after chronic cocaine indicates that most of the cocaine-mediated regulation of this mark occurs at non-genic regions, including at repetitive line elements, which are consequently induced by cocaine⁸⁵. Although the functional implications of this regulation are not yet known, these findings highlight the profound effects that cocaine exerts on the genome within NAc neurons.

Studies are now needed to examine the actions of other drugs of abuse on these histone endpoints, as well

Dependence

A physiological state that develops to compensate for persistent drug exposure and that gives rise to a withdrawal syndrome after cessation of drug exposure.

Histone deacetylases

Enzymes that catalyse the deacetylation of histone amino-terminal tails.

Nucleosome

The basic building block of chromatin in which 147 base pairs of DNA are wrapped (~1.7 turns) around a core histone octamer.

Histone acetyltransferases

Enzymes that catalyse the acetylation of histone amino-terminal tails.

Histone methyltransferases

Enzymes that catalyse the methylation of histone amino-terminal tails.

Histone demethylases

Enzymes that catalyse the demethylation of histone amino-terminal tails.

Sirtuins

Proteins that have been categorized as Class III histone deacetylases, but that also serve as protein deacetylases for many non-histone proteins and as part of transcription-repressive complexes, seemingly independently of catalytic activity.

as the effect of drugs on many other types of histone modifications that are known to regulate eukaryotic gene expression in other systems, in addiction models. Examples include recent, preliminary observations of chronic cocaine-mediated regulation of histone arginine methylation and poly-ADP ribosylation, of several families of chromatin remodelling proteins, and of histone variant subunits in the NAc, all of which illustrate the complexity of epigenetic changes that are associated with drug exposure^{86–89}.

Moreover, it will be important to relate drug-induced modifications of histones, which occur at specific drug-regulated genes, with the recruitment of numerous additional proteins that ultimately constitute the transcriptional activation or repression complexes that mediate such regulation. For example, early studies have shown that cocaine-induced expression of CDK5 in the NAc involves a cascade of events, including binding of ΔFOSB to the *Cdk5* gene promoter, followed by the recruitment of CBP, increased H3 acetylation and the recruitment of specific chromatin remodelling factors, such as transcription activator BRG1 (REF. 73) (FIG. 4). Such activation also involves reduced repressive histone methylation at this promoter, which is mediated through cocaine-induced suppression of G9a. By contrast, a very different cascade mediates chronic amphetamine-induced repression of the *Fos* gene. Here, ΔFOSB binds to the *Fos* promoter and recruits HDAC1 and SIRT1, and presumably numerous other proteins⁹⁰. Also, chronic amphetamine induces increased repressive histone methylation at the *Fos* promoter, perhaps mediated through increased G9a binding⁹⁷. It is interesting that such increased G9a binding occurs despite the global decrease in G9a expression, once again highlighting gene-specific changes that occur on top of global modifications. Understanding the molecular basis of such gene-specific modifications — for example, why ΔFOSB triggers a cascade of transcriptional activation when it binds to one promoter, but a cascade of transcriptional repression when it binds to another — is a crucial goal of current research. So far, these efforts have been pursued on a protein-by-protein basis, which is experimentally painstaking. There is a major need in this field to develop tools to analyse the complete protein complexes that are recruited to individual genes in concert with drug exposure.

DNA methylation. Methylation of DNA occurs at the 5' position of cytosine nucleotides, with the resulting methyl group projecting into the major groove of the DNA double helix⁹¹. In mammals, this occurs almost exclusively in 5'-CpG-3' sequences, and methylation is common throughout the genome — ~3% of all cytosines in human DNA are methylated⁹² — with proper cytosine methylation required for normal development, genetic imprinting and X-chromosomal inactivation⁹³. CpG sequences are not evenly dispersed throughout the genome, but are concentrated in regions termed CpG islands. These are CG-rich regions that overlap with the promoters of 50–60% of human genes and are typically methylated to a much lower extent than CpG dinucleotides that are found outside of islands⁹⁴.

DNA methyltransferases
Enzymes that methylate cytosine nucleotides, in CpG sequences, in DNA.

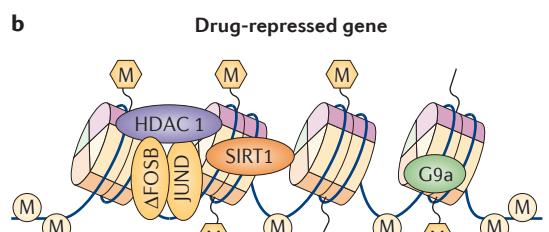
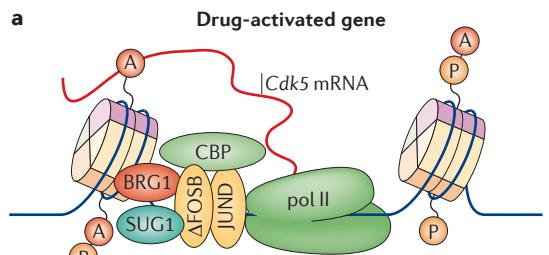


Figure 4 | Epigenetic basis of drug regulation of gene expression. The mechanisms by which chronic cocaine, through ΔFOSB, activates the cyclin-dependent kinase 5 (*Cdk5*) gene and represses the *Fos* gene. **a** | ΔFOSB binds to the *Cdk5* gene and recruits several co-activators, including cyclic AMP-responsive element (CREB)-binding protein (CBP; a type of histone acetyltransferase (HAT) leading to increased histone acetylation), transcription factor BRG1 (also known as brahma-related gene 1; a type of chromatin remodelling factor) and proteasome 26S ATPase subunit 5 (SUG1; another type of chromatin-regulatory protein). ΔFOSB also represses G9a expression, leading to reduced repressive histone methylation at the *Cdk5* gene. The net result is gene activation and increased CDK5 expression. **b** | ΔFOSB can also bind to the *Fos* gene and recruits several co-repressors, including histone deacetylase 1 (HDAC1) and sirtuin 1 (SIRT1). Additionally, the gene shows increased G9a binding and repressive histone methylation (despite global decreases in these marks). The net result is *Fos* gene repression. As transcriptional regulatory complexes contain dozens or hundreds of proteins, much further work is needed to further define the activational and repressive complexes that cocaine recruits to particular genes to mediate their transcriptional regulation and to explore the range of distinct activational and repressive complexes that are involved in cocaine action.

CpG methylation is catalysed by a family of enzymes termed DNA methyltransferases (DNMTs), some of which are responsible for the maintenance of DNA methyl states, whereas others perform *de novo* CpG methylation^{91,92}. The process of demethylation is less well understood, and may involve DNA repair mechanisms, such as growth arrest and DNA damage-inducible protein (GADD45) family members⁹² and methylcytosine dioxygenase TET1 (REFS 95–97). A variant of DNA methylation, 5-hydroxycytosine methylation, also seems to be important in gene regulation^{98,99} but has not yet been investigated in addiction models.

DNA methylation is generally considered to repress gene transcription through recruitment of co-repressor complexes (for example, HDACs and HMTs) that can

sterically hinder the transcriptional machinery or modify nucleosome structure. Such complexes involve several DNA methyl-binding domain proteins (MBDs)⁹², which are required for normal cell growth and development. Indeed, mutations in methyl CpG binding protein 2 (MeCP2), a prominent MBD, cause the majority of Rett syndrome cases and are found in a small number of patients with other autism spectrum disorders¹⁰⁰.

There are multiple known links between DNA methylation and addiction. Cocaine self-administration increases MeCP2 expression in the NAc¹⁰¹ and dorsal striatum¹⁰², and lentiviral knockdown of MeCP2 in the dorsal striatum (but not the NAc) decreases drug intake under extended but not limited access conditions¹⁰³. Hypomorphic *Mecp2* mutant mice show reduced locomotor sensitization and place conditioning after chronic amphetamine¹⁰⁴, however, the same study reported that viral knockdown of MeCP2 in the NAc increases amphetamine-induced place conditioning, whereas local overexpression decreases this behavioural response¹⁰⁴. The reasons for this discrepancy are unclear, but it seems likely that developmental abnormalities in the mutant mice, or the effects of reduced *Mecp2* expression in other brain regions, explain these differences. These findings therefore emphasize the importance of using inducible and brain region-specific tools.

Two possible mechanisms for the actions of MeCP2 in drug reward have been proposed. First, a reduction in MeCP2 prevents amphetamine-mediated increases in NAc dendritic spine density while increasing the number of GABAergic synapses¹⁰⁴. This is complemented by an increase in MeCP2 phosphorylation specifically in GABAergic interneurons in the NAc, which regulates its transcriptional activity and correlates strongly with behavioural sensitization to amphetamine¹⁰⁴. An alternative model suggests that MeCP2 represses the transcription of specific microRNAs (see below), resulting in reduced repression of brain-derived neurotrophic factor (BDNF)¹⁰³, which is also a target for CREB. BDNF has previously been described to promote cocaine self-administration¹⁰⁵, consistent with the MeCP2 data. Although these models are not mutually exclusive, further work is necessary to integrate them with our growing understanding of the multiple brain regions and cell types that are involved in reward behaviours.

A direct link between CpG methylation and addiction involves DNA (cytosine-5)-methyltransferase 3A (DNMT3A). Repeated cocaine administration dynamically regulates DNMT3A expression in the mouse NAc, with decreases seen during early phases of withdrawal and sustained increases seen at later time points^{82,106}. Experimental reduction of DNMT3A activity in the adult NAc — achieved either through virus-mediated local knockout in floxed *Dnmt3a* mice or through local infusion of a DNMT inhibitor — increases behavioural responses to cocaine, whereas DNMT3A overexpression in this region decreases these responses but also has the paradoxical effect of increasing NAc MSN spine density¹⁰⁶, similar to the effects of MEF2 manipulation in this brain region⁶⁰. Future research may identify the

specific genes whose methylation status changes in response to chronic cocaine and consequently regulates cellular and behavioural adaptations to the drug.

These observations that chronic cocaine alters DNMT3A and MBDs in the NAc and dorsal striatum raise the possibility that drug-induced changes in DNA methylation might also occur in germ cells and be passed on to subsequent generations to regulate the propensity of the offspring for addictive behaviours. The idea of such trans-generational transmission of DNA methylation changes and the resulting behavioural plasticity remains highly speculative, although recent research has shown robust effects of adult cocaine exposure in rats on cocaine responses in their progeny¹⁰⁷.

Gene priming and desensitization. Ongoing studies of chromatin regulation in addiction models support the view that epigenetic modifications at individual genes not only underlie stable changes in the steady-state levels of mRNA expression of certain genes but also alter the inducibility of many additional genes in response to some subsequent stimulus, without affecting baseline expression levels of these genes. Although such studies are still in relatively early stages of development, these types of latent epigenetic changes can be viewed as ‘molecular scars’ that dramatically alter an individual’s adaptability and contribute importantly to the addicted state.

Such priming and desensitization of genes is evident in a recently published microarray study³⁷. Numerous desensitized genes were identified: ~10% of genes whose transcription is induced acutely in the NAc by cocaine are no longer induced by a cocaine challenge after prior chronic exposure to the drug (FIG. 3a). Conversely, numerous genes are primed: genes that are not affected by acute cocaine become inducible after a chronic course of cocaine, with approximately three times more genes being induced in cocaine-experienced animals. The mechanisms that underlie such gene desensitization and priming remain incompletely understood; our hypothesis is that epigenetic mechanisms are crucial (FIG. 3b). A subset of primed genes in the NAc show reduced binding of G9a and H3K9me2 at their promoters, suggesting the involvement of this epigenetic mark³⁷. Desensitization of the *Fos* gene in the NAc, discussed above and shown in FIG. 4, involves stable increases in the binding of ΔFOSB, G9a and related co-repressors, which — although not affecting steady-state levels of *Fos* mRNA — dramatically repress its inducibility by subsequent drug exposure⁹⁰.

There is now a major need in this field to investigate the many additional chromatin mechanisms that are recruited by drug exposure to mediate gene priming and desensitization, and to understand the detailed mechanisms that target those particular genes. The goal of such studies would be to identify ‘chromatin signatures’ that underlie such long-lasting regulation. The prominence of gene priming and desensitization indicates that studies of steady-state mRNA levels per se would miss important aspects of drug regulation that

Hypomorphic

A mutation that causes a wild-type gene product to be produced at a reduced level.

are not captured at the particular time point examined. For example, the aforementioned microarray study³⁷ measured mRNA levels 1 hour after a cocaine challenge, and preliminary evidence suggests that a partly distinct set of genes show evidence of priming and desensitization at 4 hours. These observations highlight the unique utility of genome-wide assays of chromatin regulation, as such assays would reveal priming and desensitization more globally³².

MicroRNAs. Increasing attention has focused on a variety of non-coding RNAs that are important in biological regulation¹⁰⁸. These include microRNAs, which are generally around 22 bp long, are found in all mammalian cells and are post-translational regulators that bind to complementary sequences on target mRNAs to repress translation and thus silence gene expression. Like histone modifications and DNA methylation, expression of microRNAs can alter the transcriptional potential of a gene in the absence of any change to the DNA sequence, and thus can be considered an epigenetic phenomenon. Several recent studies have implicated microRNAs in addiction behaviours, and microRNAs whose expression is altered by drugs of abuse have been shown to regulate the expression of many proteins that are strongly linked to addiction¹⁰⁹.

Cocaine self-administration in rats reportedly increases expression of the microRNA miR-212 in striatum, and experimentally increasing miR-212 levels in this region decreases cocaine reward¹¹⁰. The actions of miR-212 depend on upregulation of CREB, which is known to decrease the rewarding effects of cocaine (see above), and more recent work shows that MeCP2 may interact homeostatically with miR-212 to control BDNF expression and cocaine intake¹⁰³. It has been proposed¹⁰³ that this CREB-miR-212-MeCP2-BDNF mechanism is at least partially responsible for cocaine tolerance and escalating intake. Chronic cocaine also regulates miR-124 and miR-181a in brain, where they are decreased and increased, respectively¹¹¹. miR-124 overexpression in the NAc reduces cocaine place conditioning, whereas overexpression of miR-181a has the opposite effect¹¹², suggesting that drug-induced regulation of these microRNAs may also act as a mechanism of tolerance and escalating intake. Like miR-212, miR-124 and miR-181a may operate through the CREB-BDNF pathway, as miR-124 overexpression downregulates both of these genes^{111,113}. However, these microRNAs have also been shown to affect the expression of the dopamine transporter¹¹², so their mechanisms of action are likely to be complex. Finally, argonaut 2 protein (AGO2) — which is important in microRNA-mediated gene silencing — has recently been implicated, along with several specific microRNAs, in cocaine-mediated regulation of gene expression selectively in the D2 subclass of striatal MSNs¹¹⁴.

Other drugs of abuse have also been linked to microRNAs. Opioid receptor activation downregulates miR-190 in cultured rat hippocampal neurons in a beta arrestin 2-dependent manner¹¹⁵, and the let-7 family of microRNA precursors is upregulated by

chronic morphine exposure in mice¹¹⁶. Interestingly, the mu opioid receptor is itself a direct target for let-7, and the resulting repression of the receptor has been suggested as a novel mechanism for opiate tolerance¹¹⁶. In zebrafish and in cultured immature rat neurons, morphine decreases miR-133b expression, and this might influence dopamine neuron differentiation¹¹⁷. In addition, both acute and chronic alcohol exposure upregulates miR-9 in cultured striatal neurons, and this may contribute to alcohol tolerance through regulation of large-conductance Ca^{2+} activated K^+ (BK) channels¹¹⁸. miR-9 seems to preferentially downregulate BK channel isoforms that are sensitive to alcohol potentiation, perhaps shifting BK channel expression towards more tolerant subtypes¹¹⁹. miR-9 also targets the D2 dopamine receptor¹¹⁹ and so probably influences alcohol reward.

In the future, next-generation sequencing of microRNAs in several brain regions after exposure to drugs of abuse will be essential to uncover how specific microRNAs (and, eventually, the genes that they control) are regulated. Indeed, this process has already begun, as such screens are revealing that numerous microRNAs are regulated in the NAc by chronic cocaine^{114,120}. For example, cocaine-mediated regulation of the miR-8 family suggests novel mechanisms for drug-induced alterations in the neuronal cytoskeletal and synaptic structure¹²⁰. Exploring this mechanism in drug-induced regulation of NAc dendritic morphology is an important line of future investigation.

Future directions

This Review has summarized the increasing array of findings that support a role for regulation of the transcriptional potential of myriad genes in the brain's maladaptations to drugs of abuse. The mechanisms of transcriptional and epigenetic regulation are themselves varied and highly complex, and future studies are needed to catalogue the vast number of regulatory events that occur as well as to understand the precise underlying mechanisms that are involved. One key question is what controls the recruitment or expulsion of individual transcriptional regulatory proteins to a particular target gene. Our hypothesis is that the underlying epigenetic state of that gene is a crucial determining factor. However, if this is the case, what controls the formation and maintenance of distinct epigenetic states at particular genes? Also, what are the intracellular signalling cascades that transduce the initial drug action at the neurotransmitter-receptor level to the neuronal nucleus to regulate the epigenetic state of specific subsets of genes?

The existing literature on transcriptional and epigenetic mechanisms of addiction is limited in several key ways. So far, most studies have employed conditioned place-preference and locomotor sensitization paradigms. Although these behavioural assays provide useful insight into an animal's sensitivity to the actions of drugs of abuse on the brain's reward circuitry, they do not provide direct measures of drug reinforcement or addiction per se. Instead, the field needs to make greater use of drug self-administration and relapse

Box 4 | Sex differences in drug addiction: epigenetic mechanisms?

Addiction research has historically neglected female subjects, particularly in animal studies, although both human and animal studies have found robust sex differences in drug responses^{143,144}. In self-administration studies with various drugs, female rats are more responsive in general and exhibit particularly enhanced responses in the transition phases of acquisition or relapse compared to the maintenance phase^{145,146}. In addition, the locomotor effects of many psychostimulants are greater in female rats^{147,148}. Although, in general, ovariectomy reduces these differences and oestrogen administration increases them, this is not true of all drugs of abuse, and some contradictory results have been reported¹⁴³. These data suggest that drugs of abuse have differential effects on the two sexes, and that the reward system may be different between men and women; clinical evidence supports these hypotheses. Women usually have a later age of onset for substance abuse, although they progress to addiction more rapidly than men¹⁴⁹. In the specific case of cocaine, women report shorter periods of abstinence, have greater drug intake and respond more strongly to cue-induced craving¹⁴³. These differences may be directly related to the brain's reward circuitry, as men have been reported to show greater striatal dopamine release than women in response to psychostimulant challenges¹⁵⁰. Interestingly, stress upregulates the expression of DNA methyltransferases (DNMTs) and DNA methyl-binding domain proteins (MBDs) in the nucleus accumbens (NAc)¹⁰⁶; these effects predominate in females and inhibition of DNMT3A in the NAc of female rats increases natural reward¹⁵¹, suggesting that the sexes may undergo differential epigenetic regulation of the reward circuitry. Furthermore, as activation of the reward circuitry by sexual behaviour induces ΔFOSB^{27,29,30} and other regulators of transcription, there is little doubt that future studies will reveal further sexual dimorphism in the regulation of transcriptional and epigenetic mechanisms by drugs of abuse — findings that may have important consequences for treatment.

assays, which are considered the best available animal models of addiction^{121–123}. Similarly, in most studies the drugs of abuse were experimenter-administered, but we know that drugs exert some distinct actions when self-administered or given within a particular environmental context. Studies that move beyond the relatively short time frames of most current experiments are also needed to examine transcriptional and epigenetic endpoints after much longer periods of drug exposure and longer periods of withdrawal from drug exposure. Such studies might lead to a molecular hypothesis that explains the phenomenon of relapse in human addicts after years or even decades of abstinence. In addition, studies should be extended from investigating cocaine action in NAc, which has been the main focus so far, to investigating several other drugs and several other reward-related brain regions. Future studies of gene regulation will better inform drug discovery efforts as they increasingly incorporate experimental paradigms that better model human addiction.

Another limitation of the existing literature is the reliance of many studies on overexpression systems — viral or transgenic — which often induce levels of expression that are far greater than those seen under normal conditions or even after drug treatment. Such overexpression of transcription factors, chromatin-regulatory proteins or their dominant-negative mutants, can lead to non-physiological changes in gene expression and subsequent alterations in cell morphology, physiology and/or behaviour. It is reassuring that many of the phenomena described above, resulting from studies that utilized overexpression systems, have been validated with other methods. For example, the genes that are regulated by overexpression

of ΔFOSB in the NAc of inducible bi-transgenic mice³¹ overlap extensively with genes that show enrichment of endogenous ΔFOSB binding after cocaine exposure³². Similar caveats exist for the use of constitutive knockout animals, in which loss of a gene in early development and in all tissues makes it difficult to interpret any changes that are observed in drug regulation involving a single brain region of an adult animal. Ultimately, a truly accurate understanding of the transcriptional and epigenetic regulation of the addiction process will require the generation of novel tools that control protein expression with greater spatial, temporal and accumulation precision.

Methodological advances in epigenetics are needed as well. Current levels of experimental proof of epigenetic mechanisms of drug action have so far involved the overexpression or deletion of a given epigenetic protein (for example, an HAT, HDAC, HMT or a DNMT) within a brain region of interest. However, such manipulations affect the epigenetic states of perhaps thousands of genes without targeting those genes that are specifically altered by drug exposure. Being able to experimentally manipulate the epigenetic state of an individual gene within a discrete brain region of an adult animal would represent a major advance for the field. Tools such as artificially designed zinc-finger proteins¹²⁴ or sequence-specific transcription activator-like effectors (TALEs)¹²⁵, which are designed to bind specific DNA sequences *in vivo*, would offer exciting possibilities for future studies. Similarly, all genome-wide studies of drug-induced epigenetic changes in the brain so far have used total extracts of brain regions, even though we know that drugs produce very different effects on distinct neuronal and non-neuronal cell types within a given region. Genome-wide epigenetic analyses in a cell type-specific manner are crucially needed in addiction research¹²⁶.

Advances in bioinformatics are also needed. Genome-wide studies of transcription factor binding and chromatin modifications generate enormous datasets, which require the development of better tools to effectively mine the resulting data. For example, it will be crucial to overlay such epigenetic analyses with genome-wide changes in RNA expression and to compare data obtained from animal models with those from human post-mortem brain tissue. On a similar note, the findings from studies on drug regulation of gene expression reviewed here must be integrated with findings obtained at several other levels of analysis. How do individual differences in genome sequences relate to individual differences in epigenetic regulation? Do drug-induced epigenetic modifications occur in peripheral tissues such as blood, and do any such changes reflect addiction-relevant phenomena? Recent studies, for example, have found altered levels of methylation of the monoamine oxidase A (MAOA) and MAOB gene promoters in the blood of smokers^{127,128}. Additionally, altered methylation of MAOA in lymphoblasts is associated with nicotine and alcohol dependence in women but not in men¹²⁹, emphasizing the need for studies of sex differences in epigenetic regulation in addiction models, which until now have focused almost exclusively on male animals (BOX 4).

As information on transcriptional and epigenetic mechanisms of addiction accumulates, it is essential to integrate it with equally important information regarding post-transcriptional (translational and post-translational) regulation to obtain a complete understanding of how chronic exposure to a drug

of abuse changes the brain to cause addiction. The ultimate goal of this research is to understand basic principles of neuronal and behavioural adaptation and, ultimately, to identify new targets for the treatment of addictive disorders and new methods for their prevention.

- Kendler, K. S., Myers, J. & Prescott, C. A. Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. *Arch. Gen. Psychiatry* **64**, 1313–1320 (2007).
- Volkow, N., Rutter, J., Pollock, J. D., Shurtliff, D. & Balcer, R. One SNP linked to two diseases — addiction and cancer: a double whammy? Nicotine addiction and lung cancer susceptibility. *Mol. Psychiatry* **13**, 990–992 (2008).
- Goldman, D., Oroszi, G. & Ducci, F. The genetics of addictions: uncovering the genes. *Nature Rev. Genet.* **6**, 521–532 (2005).
- Henikoff, S. & Matzke, M. A. Exploring and explaining epigenetic effects. *Trends Genet.* **13**, 293–295 (1997).
- Sutherland, J. E. & Costa, M. Epigenetics and the environment. *Ann. NY Acad. Sci.* **983**, 151–160 (2003).
- Fraga, M. F. et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl Acad. Sci. USA* **102**, 10604–10609 (2005).
- Koob, G. F. & Le Moal, M. *Neurobiology of Addiction*. (Academic Press, London, 2005).
- Kalivas, P. W. & Volkow, N. D. The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry* **162**, 1403–1413 (2005).
- Hyman, S. E., Malenka, R. C. & Nestler, E. J. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.* **29**, 565–598 (2006).
- Robinson, T. E. & Berridge, K. C. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Brain Res. Rev.* **18**, 247–291 (1993).
- Nestler, E. J. Molecular basis of long-term plasticity underlying addiction. *Nature Rev. Neurosci.* **2**, 119–128 (2001).
- Andersen, S. L. & Teicher, M. H. Desperately driven and no brakes: developmental stress exposure and subsequent risk for substance abuse. *Neurosci. Biobehav. Rev.* **33**, 516–524 (2009).
- Malanga, C. J. & Kosofsky, B. E. Does drug abuse beget drug abuse? Behavioral analysis of addiction liability in animal models of prenatal drug exposure. *Brain Res. Dev. Brain Res.* **147**, 47–57 (2003).
- Nestler, E. J. Review. Transcriptional mechanisms of addiction: role of DeltaFosB. *Phil. Trans. R. Soc. Lond. B* **363**, 3245–3255 (2008).
- Jorissen, H. J. et al. Dimerization and DNA-binding properties of the transcription factor DeltaFosB. *Biochemistry* **46**, 8360–8372 (2007).
- Hiroi, N. et al. FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. *Proc. Natl Acad. Sci. USA* **94**, 10397–10402 (1997).
- Perrotti, L. I. et al. Distinct patterns of DeltaFosB induction in brain by drugs of abuse. *Synapse* **62**, 358–369 (2008).
- Carle, T. L. et al. Proteasome-dependent and -independent mechanisms for FosB destabilization: identification of FosB degron domains and implications for DeltaFosB stability. *Eur. J. Neurosci.* **25**, 3009–3019 (2007).
- Ulery, P. G., Rudenko, G. & Nestler, E. J. Regulation of DeltaFosB stability by phosphorylation. *J. Neurosci.* **26**, 5131–5142 (2006).
- Ulery-Reynolds, P. G., Castillo, M. A., Vialou, V., Russo, S. J. & Nestler, E. J. Phosphorylation of DeltaFosB mediates its stability *in vivo*. *Neuroscience* **158**, 369–372 (2009).
- Kelz, M. B. et al. Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* **401**, 272–276 (1999).
- Zachariou, V. et al. An essential role for DeltaFosB in the nucleus accumbens in morphine action. *Nature Neurosci.* **9**, 205–211 (2006).
- Colby, C. R., Whisler, K., Steffen, C., Nestler, E. J. & Self, D. W. Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. *J. Neurosci.* **23**, 2488–2493 (2003).
- Winstanley, C. A. et al. DeltaFosB induction in orbitofrontal cortex mediates tolerance to cocaine-induced cognitive dysfunction. *J. Neurosci.* **27**, 10497–10507 (2007).
- Winstanley, C. A. et al. DeltaFosB induction in orbitofrontal cortex potentiates locomotor sensitization despite attenuating the cognitive dysfunction caused by cocaine. *Pharmacol. Biochem. Behav.* **93**, 278–284 (2009).
- Wermel, M. et al. Delta FosB regulates wheel running. *J. Neurosci.* **22**, 8133–8138 (2002).
- Wallace, D. L. et al. The influence of DeltaFosB in the nucleus accumbens on natural reward-related behavior. *J. Neurosci.* **28**, 10272–10277 (2008).
- Teegarden, S. L., Nestler, E. J. & Bale, T. L. Delta FosB-mediated alterations in dopamine signaling are normalized by a palatable high-fat diet. *Biol. Psychiatry* **64**, 941–950 (2008).
- Hedges, V. L., Chakravarty, S., Nestler, E. J. & Meisel, R. L. Delta FosB overexpression in the nucleus accumbens enhances sexual reward in female Syrian hamsters. *Genes Brain Behav.* **8**, 442–449 (2009).
- Pitchers, K. K. et al. DeltaFosB in the nucleus accumbens is critical for reinforcing effects of sexual reward. *Genes Brain Behav.* **9**, 831–840 (2010).
- McClung, C. A. & Nestler, E. J. Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nature Neurosci.* **6**, 1208–1215 (2003).
- Renthal, W. et al. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron* **62**, 335–348 (2009).
- The authors used ChIP-chip analysis to identify genome-wide patterns of gene regulation induced by chronic cocaine administration, and identified numerous novel target genes of ΔFOSB and CREB, including sirtuins.**
- Bibb, J. A. et al. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* **410**, 376–380 (2001).
- Russo, S. J. et al. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* **33**, 267–276 (2010). **Drugs of abuse alter dendritic spine morphology in the NAC, and this morphological plasticity may contribute to addiction-related behaviours. This article provides a comprehensive review of this growing field, and highlight new methods for more accurate and detailed quantification of dendritic spine morphology.**
- Robinson, T. E. & Kolb, B. Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* **47**, 33–46 (2004).
- Kalivas, P. W. The glutamate homeostasis hypothesis of addiction. *Nature Rev. Neurosci.* **10**, 561–572 (2009).
- Maze, I. et al. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* **327**, 213–216 (2010). **This study provided the first link between repressive histone methylation and the actions of cocaine and ΔFOSB, and demonstrated a crucial role for this molecular pathway in mediating the effects of repeated cocaine on dendritic spine morphology of NAC MSNs as well as on reward-related behavioural plasticity.**
- Vialou, V. et al. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nature Neurosci.* **13**, 745–752 (2010).
- Robison, A. J. et al. Chronic cocaine engages a feedback loop involving ΔFosB and CamKII in the nucleus accumbens. *Soc. Neurosci. Abstr.* **39**, 909.23 (Washington DC, 12–16 Nov 2011).
- Wolf, M. E. & Ferrario, C. R. AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neurosci. Biobehav. Rev.* **35**, 185–211 (2010).
- Carlezon, W. A. Jr, Duman, R. S. & Nestler, E. J. The many faces of CREB. *Trends Neurosci.* **28**, 436–445 (2005).
- Briand, L. A. & Blendy, J. A. Molecular and genetic substrates linking stress and addiction. *Brain Res.* **1314**, 219–234 (2010).
- Edwards, S., Graham, D. L., Bachtell, R. K. & Self, D. W. Region-specific tolerance to cocaine-regulated cAMP-dependent protein phosphorylation following chronic self-administration. *Eur. J. Neurosci.* **25**, 2201–2213 (2007).
- Carlezon, W. A. Jr. et al. Regulation of cocaine reward by CREB. *Science* **282**, 2272–2275 (1998).
- Barrot, M. et al. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proc. Natl. Acad. Sci. USA* **99**, 11435–11440 (2002).
- Larson, E. B. et al. Over-expression of CREB in the nucleus accumbens shell increases cocaine reinforcement in self-administering rats. *J. Neurosci.* (in the press).
- Pluzarev, O. & Pandey, S. C. Modulation of CREB expression and phosphorylation in the rat nucleus accumbens during nicotine exposure and withdrawal. *J. Neurosci. Res.* **77**, 884–891 (2004).
- Misra, K., Roy, A. & Pandey, S. C. Effects of voluntary ethanol intake on the expression of Ca²⁺/calmodulin-dependent protein kinase IV and on CREB expression and phosphorylation in the rat nucleus accumbens. *Neuroreport* **12**, 4133–4137 (2001).
- Li, J., Li, Y. H. & Yuan, X. R. Changes of phosphorylation of cAMP response element binding protein in rat nucleus accumbens after chronic ethanol intake: naloxone reversal. *Acta Pharmacol. Sin.* **24**, 930–936 (2003).
- Brunzell, D. H., Mineur, Y. S., Neve, R. L. & Picciotto, M. R. Nucleus accumbens CREB activity is necessary for nicotine conditioned place preference. *Neuropsychopharmacology* **34**, 1993–2001 (2009).
- Rubino, T. et al. Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral Delta9-tetrahydrocannabinol in rats. *Neuropsychopharmacology* **32**, 2036–2045 (2007).
- Shiftlet, M. W., Mauna, J. C., Chipman, A. M., Peet, E. & Thielis, E. Appetitive Pavlovian conditioned stimuli increase CREB phosphorylation in the nucleus accumbens. *Neurobiol. Learn. Mem.* **92**, 451–454 (2009).
- Green, T. A. et al. Induction of activating transcription factors (ATFs) ATF2, ATF3, and ATF4 in the nucleus accumbens and their regulation of emotional behavior. *J. Neurosci.* **28**, 2025–2032 (2008).
- Dong, Y. et al. CREB modulates excitability of nucleus accumbens neurons. *Nature Neurosci.* **9**, 475–477 (2006).
- Huang, Y. H. et al. CREB modulates the functional output of nucleus accumbens neurons: a critical role of N-methyl-D-aspartate glutamate receptor (NMDAR) receptors. *J. Biol. Chem.* **283**, 2751–2760 (2008).
- Meffert, M. K., Chang, J. M., Wiltgen, B. J., Fanselow, M. S. & Baltimore, D. NF-κB functions in synaptic signalling and behavior. *Nature Neurosci.* **6**, 1072–1078 (2003).
- Russo, S. J. et al. Nuclear factor κB signaling regulates neuronal morphology and cocaine reward. *J. Neurosci.* **29**, 3529–3537 (2009).
- Sullivan, P. F. et al. Candidate genes for nicotine dependence via linkage, epistasis, and bioinformatics. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **126B**, 23–36 (2004).
- Christoffel, D. J. et al. IκappaB kinase regulates social defeat stress-induced synaptic and behavioral plasticity. *J. Neurosci.* **31**, 314–321 (2011). **This study combined confocal microscopy with electron microscopy and electrophysiology to paint a more complete picture of functional synaptic changes in NAC MSNs after chronic stress, and provides a model for future studies of synaptic plasticity in addiction paradigms.**

60. Pulipparacharuvil, S. *et al.* Cocaine regulates MEF2 to control synaptic and behavioral plasticity. *Neuron* **59**, 621–633 (2008). **This work implicated MEF2 as a key regulator of structural and behavioural plasticity to cocaine, and suggested that reductions in MEF2 activity in the NAc may act as a compensatory mechanism to limit behavioural responses to the drug.**
61. Chen, L. *et al.* Chronic ethanol feeding impairs AMPK and MEF2 expression and is associated with GLUT4 decrease in rat myocardium. *Exp. Mol. Med.* **42**, 205–215 (2010).
62. Ambroggi, F. *et al.* Stress and addiction: glucocorticoid receptor in dopaminergic neurons facilitates cocaine seeking. *Nature Neurosci.* **12**, 247–249 (2009).
63. Barik, J. *et al.* Glucocorticoid receptors in dopaminergic neurons, key for cocaine, are dispensable for molecular and behavioral morphine responses. *Biol. Psychiatry* **68**, 231–239 (2010).
64. Desirivres, S. *et al.* Glucocorticoid receptor (NR3C1) gene polymorphisms and onset of alcohol abuse in adolescents. *Addict. Biol.* **16**, 510–513 (2011).
65. McQuown, S. C. & Wood, M. A. Epigenetic regulation in substance use disorders. *Curr. Psychiatry Rep.* **12**, 145–153 (2010).
66. Renthal, W. & Nestler, E. J. Epigenetic mechanisms in drug addiction. *Trends Mol. Med.* **14**, 341–350 (2008).
67. Borrelli, E., Nestler, E. J., Allis, C. D. & Sassone-Corsi, P. Decoding the epigenetic language of neuronal plasticity. *Neuron* **60**, 961–974 (2008).
68. Berger, S. L. The complex language of chromatin regulation during transcription. *Nature* **447**, 407–412 (2007).
69. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
70. Maze, I. & Nestler, E. J. The epigenetic landscape of addiction. *Ann. NY Acad. Sci.* **1216**, 99–113 (2011).
71. Su, I. H. & Tarakhovsky, A. Lysine methylation and ‘signaling memory’. *Curr. Opin. Immunol.* **18**, 152–157 (2006).
72. Rumbaugh, G. & Miller, C. A. Epigenetic changes in the brain: measuring global histone modifications. *Methods Mol. Biol.* **670**, 263–274 (2011).
73. Kumar, A. *et al.* Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **48**, 303–314 (2005).
74. Schroeder, F. A. *et al.* Drug-induced activation of dopamine D₁ receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. *Neuropharmacology* **33**, 2981–2992 (2008).
75. Bertran-Gonzalez, J. *et al.* Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* **28**, 5671–5685 (2008).
76. Pandey, S. C., Ugale, R., Zhang, H., Tang, L. & Prakash, A. Brain chromatin remodeling: a novel mechanism of alcoholism. *J. Neurosci.* **28**, 3729–3737 (2008).
77. Wang, Y., Krishnan, H. R., Ghezzi, A., Yin, J. C. & Atkinson, N. S. Drug-induced epigenetic changes produce drug tolerance. *PLoS Biol.* **5**, e265 (2007).
78. Khare, M., Taylor, A. H., Konje, J. C. & Bell, S. C. Delta9-tetrahydrocannabinol inhibits cytotrophoblast cell proliferation and modulates gene transcription. *Mol. Hum. Reprod.* **12**, 321–333 (2006).
79. Parmentier-Batteur, S., Jin, K., Xie, L., Mao, X. O. & Greenberg, D. A. DNA microarray analysis of cannabinoid signaling in mouse brain *in vivo*. *Mol. Pharmacol.* **62**, 828–835 (2002).
80. Renthal, W. *et al.* Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* **56**, 517–529 (2007).
81. Romieu, P. *et al.* Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *J. Neurosci.* **28**, 9342–9348 (2008).
82. Kim, W. Y., Kim, S. & Kim, J. H. Chronic microinjection of valproic acid into the nucleus accumbens attenuates amphetamine-induced locomotor activity. *Neurosci. Lett.* **432**, 54–57 (2008).
83. Levine, A. A. *et al.* CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proc. Natl. Acad. Sci. USA* **102**, 19186–19191 (2005).
84. Finkel, T., Deng, C. X. & Mostoslavsky, R. Recent progress in the biology and physiology of sirtuins. *Nature* **460**, 587–591 (2009).
85. Maze, I. *et al.* Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. *Proc. Natl. Acad. Sci. USA* **108**, 3035–3040 (2011). **This study used ChIP-Seq to show drug-induced regulation of repetitive genomic sequences in the NAc after repeated cocaine administration. This supports the utility of such genome-wide methods in revealing new mechanisms of drug action.**
86. Sun, H. *et al.* Cocaine and stress regulates ATPase-containing chromatin remodelers. *Soc. Neurosci. Abstr.* **39**, 9.14 (Washington DC, 12–16 Nov 2011).
87. Damez-Werno, D. *et al.* Histone arginine methylation in the nucleus accumbens in response to chronic cocaine and social stress. *Soc. Neurosci. Abstr.* **39**, 9.16 (Washington DC, 12–16 Nov 2011).
88. Kennedy, P. J. *et al.* Differential histone H2A variant expression in the nucleus accumbens following repeated exposure to cocaine or morphine. *Soc. Neurosci. Abstr.* **39**, 9.15 (Washington DC, 12–16 Nov 2011).
89. Scobie, K., Damez-Werno, D., Sun, H., Kennedy, P. J. & Nestler, E. J. Role of poly(ADP-ribosylation) in drug-seeking behavior and resiliency to stress. *Soc. Neurosci. Abstr.* **39**, 9.18 (Washington DC, 12–16 Nov 2011).
90. Renthal, W. *et al.* Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. *J. Neurosci.* **28**, 7344–7349 (2008).
91. Newell-Price, J., Clark, A. J. & King, P. DNA methylation and silencing of gene expression. *Trends Endocrinol. Metab.* **11**, 142–148 (2000).
92. Kim, J. K., Samaranayake, M. & Pradhan, S. Epigenetic mechanisms in mammals. *Cell. Mol. Life Sci.* **66**, 596–612 (2009).
93. Chahrour, M. & Zoghbi, H. Y. The story of Rett syndrome: from clinic to neurobiology. *Neuron* **56**, 422–437 (2007).
94. Wang, Y. & Leung, F. C. An evaluation of new criteria for CpG islands in the human genome as gene markers. *Bioinformatics* **20**, 1170–1177 (2004).
95. Guo, J. U., Su, Y., Zhong, C., Ming, G. L. & Song, H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell* **145**, 423–434 (2011). **This study provides the first demonstration of TET1-mediated demethylation of DNA in the adult brain, laying the groundwork for future *in vivo* studies that may provide insight into the role of DNA demethylation in addiction-related phenomena.**
96. Williams, K. *et al.* TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. *Nature* **473**, 343–348 (2011).
97. Wu, H. *et al.* Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. *Nature* **473**, 389–393 (2011).
98. Pastor, W. A. *et al.* Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. *Nature* **473**, 394–397 (2011).
99. Ficz, G. *et al.* Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. *Nature* **473**, 398–402 (2011).
100. Chahrour, M. & Zoghbi, H. Y. The story of Rett syndrome: from clinic to neurobiology. *Neuron* **56**, 422–437 (2007).
101. Host, L., Dietrich, J. B., Carouge, D., Aunis, D. & Zwilley, J. Cocaine self-administration alters the expression of chromatin-remodelling proteins: modulation by histone deacetylase inhibition. *J. Psychopharmacol.* **25**, 222–229 (2011).
102. Cassel, S. *et al.* Fluoxetine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. *Mol. Pharmacol.* **70**, 487–492 (2006).
103. Im, H. I., Hollander, J. A., Bali, P. & Kenny, P. J. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nature Neurosci.* **13**, 1120–1127 (2010). **This work provided an alternative, but not mutually exclusive, mechanism for MeCP2 action in addiction models to that presented in reference 104. The authors showed that MeCP2 controls cocaine intake through microRNA-mediated regulation of BDNF, demonstrating the complex interactions among the various mechanisms of epigenetic modifications in the drug-exposed brain.**
104. Deng, J. V. *et al.* MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. *Nature Neurosci.* **13**, 1128–1136 (2010).
105. Graham, D. L. *et al.* Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nature Neurosci.* **10**, 1029–1037 (2007). **This study provided novel evidence that MeCP2 activity in NAc regulates synaptic responses to psychostimulants, and established a link between MeCP2 and behavioural sensitization (see also REF. 103).**
106. LaPlant, Q. *et al.* Dmrt5a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nature Neurosci.* **13**, 1137–1143 (2010). **This study provided crucial evidence of the importance of DNMT3A in the NAc in regulating cellular and behavioural plasticity to repeated cocaine exposure.**
107. Vassoler, F. M., White, S. L., Ortinski, P. I., Sadri-Vakili, G. & Pierce, R. C. Paternal transmission of a cocaine resistance phenotype in male offspring. *Soc. Neurosci. Abstr.* **21**, 9.2 (Washington DC, 12–16 Nov 2011).
108. Taft, R. J., Pang, K. C., Mercer, T. R., Dinger, M. & Mattick, J. S. Non-coding RNAs: regulators of disease. *J. Pathol.* **220**, 126–139 (2010).
109. Li, M. D. & van der Vaart, A. D. MicroRNAs in addiction: adaptation’s middlemen? *Mol. Psychiatry* **24** May 2011 (doi: 10.1038/mp.2011.58).
110. Hollander, J. A. *et al.* Striatal microRNA controls cocaine intake through CREB signalling. *Nature* **466**, 197–202 (2010). **This study provided a detailed mechanism by which miR-212 enhances CREB signalling in dorsal striatum after cocaine, and demonstrated a key role for this adaptation in blunting the sensitivity of animals to the motivational effects of the drug.**
111. Chandrasekar, V. & Dreyer, J. L. microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. *Mol. Cell. Neurosci.* **42**, 350–362 (2009).
112. Chandrasekar, V. & Dreyer, J. L. Regulation of MiR-124, Let-7d, and MiR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. *Neuropharmacology* **56**, 1149–1164 (2011).
113. Rajasethupathy, P. *et al.* Characterization of small RNAs in Aplysia reveals a role for miR-124 in constraining synaptic plasticity through CREB. *Neuron* **63**, 803–817 (2009).
114. Schaefer, A. *et al.* Argonaute 2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction. *J. Exp. Med.* **207**, 1843–1851 (2010).
115. Zheng, H. *et al.* mu-Opioid receptor agonists differentially regulate the expression of miR-190 and NeuroD. *Mol. Pharmacol.* **77**, 102–109 (2010).
116. He, Y., Yang, C., Kirkmire, C. M. & Wang, Z. J. Regulation of opioid tolerance by let-7 family microRNA targeting the mu opioid receptor. *J. Neurosci.* **30**, 10251–10258 (2010).
117. Sanchez-Simon, F. M., Zhang, X. X., Loh, H. H., Law, P. Y. & Rodriguez, R. E. Morphine regulates dopaminergic neuron differentiation via miR-133b. *Mol. Pharmacol.* **78**, 935–942 (2010).
118. Pietrzykowski, A. Z. *et al.* Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. *Neuron* **59**, 274–287 (2008).
119. Pietrzykowski, A. Z. The role of microRNAs in drug addiction: a big lesson from tiny molecules. *Int. Rev. Neurobiol.* **91**, 1–24 (2010).
120. Eipper-Mains, J. E. *et al.* MicroRNA-Seq reveals cocaine-regulated expression of striatal microRNAs. *RNA* **17**, 1529–1543 (2011). **As the role of microRNAs in addiction becomes clearer, the requirement for an unbiased approach to identifying microRNA-regulated genes becomes more pressing. Here, RNA-Seq was used to identify novel messages regulated by cocaine-induced microRNAs in the striatum, with a particular focus on regulation of synaptically located microRNAs.**
121. Pelloix, Y., Everett, B. J. & Dickinson, A. Compulsive drug seeking by rats under punishment: effects of drug taking history. *Psychopharmacology* **194**, 127–137 (2007).
122. Pickens, C. L. *et al.* Neurobiology of the incubation of drug craving. *Trends Neurosci.* **34**, 411–420 (2011).
123. O’Connor, E. C., Chapman, K., Butler, P. & Mead, A. N. The predictive validity of the rat self-administration model for abuse liability. *Neurosci. Biobehav. Rev.* **35**, 912–938 (2011).
124. Laganiere, J. *et al.* An engineered zinc finger protein activator of the endogenous glial cell line-derived neurotrophic factor gene provides functional neuroprotection in a rat model of Parkinson’s disease. *J. Neurosci.* **30**, 16469–16474 (2010).

125. Zhang, F. *et al.* Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. *Nature Biotech.* **29**, 149–153 (2011).
126. Cheung, I. *et al.* Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. *Proc. Natl. Acad. Sci. USA* **107**, 8824–8829 (2010). **Understanding the epigenetic regulation of addiction behaviours will require the delineation of individual genes whose chromatin structure is altered by drug exposure in specific regions and cell types within the brain. This study validated a method for cell-type specific ChIP-Seq on brain tissue that makes such studies possible.**
127. Philibert, R. A. *et al.* The effect of smoking on MAOA promoter methylation in DNA prepared from lymphoblasts and whole blood. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **153B**, 619–628, (2010).
128. Launay, J. M. *et al.* Smoking induces long-lasting effects through a monoamine-oxidase epigenetic regulation. *PLoS ONE* **4**, e7959 (2009).
129. Philibert, R. A., Gunter, T. D., Beach, S. R., Brody, G. H. & Madan, A. MAOA methylation is associated with nicotine and alcohol dependence in women. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **147B**, 565–570 (2008).
130. Lobo, M. K. *et al.* Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* **330**, 385–390 (2010). **Using cell-type specific expression strategies and optogenetic control of neuronal activity, this study showed that activation of D1-type and D2-type MSNs enhances and suppresses behavioural responses to cocaine, respectively. The authors implicated neurotrophin signalling in mediating these opposite responses (see also REF. 131).**
131. Witten, I. B. *et al.* Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* **330**, 1677–1681 (2010). **This study showed that cholinergic interneurons in the NAc are activated by cocaine, and that optogenetic suppression of this activity blocks cocaine conditioning, providing an important, complementary mechanism to those proposed by reference 130.**
132. Self, D. W. in *The Dopamine Receptors* 2nd edn (ed. Neve, K. A.) 479–524 (Humana Press, New York, 2010).
133. Nye, H. E., Hope, B. T., Kelz, M. B., Iadarola, M. & Nestler, E. J. Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. *J. Pharmacol. Exp. Ther.* **275**, 1671–1680 (1995).
134. Lee, K. W. *et al.* Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc. Natl. Acad. Sci. USA* **103**, 3399–3404 (2006).
135. Maze, I. *et al.* C9a regulates cocaine-induced behavioral and transcriptional plasticity in a cell-type specific manner. *Soc. Neurosci. Abstr.* **57.4** (California, 13–17 Nov 2010).
136. Singla, S., Kreitzer, A. C. & Malenka, R. C. Mechanisms for synapse specificity during striatal long-term depression. *J. Neurosci.* **27**, 5260–5264 (2007).
137. Li, Y., Acerbo, M. J. & Robinson, T. E. The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. *Eur. J. Neurosci.* **20**, 1647–1654 (2004).
138. Russo, S. J., Mazei-Robison, M. S., Ables, J. L. & Nestler, E. J. Neurotrophic factors and structural plasticity in addiction. *Neuropharmacology* **56**, 73–82 (2009).
139. Nestler, E. J. & Aghajanian, G. K. Molecular and cellular basis of addiction. *Science* **278**, 58–63 (1997).
140. Van Bockstaele, E. J., Reyes, B. A. & Valentino, R. J. The locus coeruleus: a key nucleus where stress and opioids intersect to mediate vulnerability to opiate abuse. *Brain Res.* **1314**, 162–174 (2010).
141. Han, M. H. *et al.* Role of cAMP response element-binding protein in the rat locus ceruleus: regulation of neuronal activity and opiate withdrawal behaviors. *J. Neurosci.* **26**, 4624–4629 (2006).
142. Cao, J. L. *et al.* Essential role of the cAMP-cAMP response-element binding protein pathway in opiate-induced homeostatic adaptations of locus coeruleus neurons. *Proc. Natl. Acad. Sci. USA* **107**, 17011–17016 (2010). **This study detailed a signalling mechanism by which long-term exposure to morphine induces homeostatic plasticity intrinsic to locus coeruleus neurons, which involves induction of CREB and its downstream targets, such as adenylyl cyclase 8. These adaptations result in enhanced excitability of the neurons and partly mediate physical opiate withdrawal.**
143. Fattore, L., Altea, S. & Fratta, W. Sex differences in drug addiction: a review of animal and human studies. *Womens Health* **4**, 51–65 (2008).
144. Carroll, M. E. & Anker, J. J. Sex differences and ovarian hormones in animal models of drug dependence. *Horm. Behav.* **58**, 44–56 (2010).
145. Lynch, W. J. & Carroll, M. E. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology* **144**, 77–82 (1999).
146. Roth, M. E. & Carroll, M. E. Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. *Pharmacol. Biochem. Behav.* **78**, 199–207 (2004).
147. Caillol, S. & Mormede, P. Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. *Brain Res.* **842**, 200–205 (1999).
148. Robinson, T. E., Becker, J. B. & Presty, S. K. Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. *Brain Res.* **253**, 231–241 (1982).
149. Hernandez-Avila, C. A., Rounsville, B. J. & Kranzler, H. R. Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. *Drug Alcohol Depend.* **74**, 265–272 (2004).
150. Munro, C. A. *et al.* Sex differences in striatal dopamine release in healthy adults. *Biol. Psychiatry* **59**, 966–974 (2006).
151. Hodes, G. E., Christoffel, D. J., Golden, S. A., Ahn, H. F. & Russo, S. J. Sex differences in epigenetic regulation of stress-related disorders. *Soc. Neurosci. Abstr.* **219.01** (Washington DC, 12–16 Nov 2011).

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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