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Drugs and addiction: an introduction to epigenetics

Chloe C. Y. Wong[1], Jonathan Mill[1] & Cathy Fernandes[2]

Institute of Psychiatry, SGDP Research Centre[1] and Department of Psychosis Studies,[2] King's College London, De Crespigny Park, Denmark Hill, London, UK

ABSTRACT

Addiction is a debilitating psychiatric disorder, with a complex aetiology involving the interaction of inherited predispositions and environmental factors. Emerging evidence suggests that epigenetic alterations to the genome, including DNA methylation and histone modifications, are important mechanisms underlying addiction and the neurobiological response to addictive substances. In this review, we introduce the reader to epigenetic mechanisms and describe a potential role for dynamic epigenetic changes in mediating addictive behaviours via long-lasting changes in gene expression. We summarize recent findings from both molecular and behavioural experiments elucidating the role of epigenetic changes in mediating the addictive potential of various drugs of abuse, including cocaine, amphetamine and alcohol. The implications of these findings for molecular studies of addiction and the future development of novel therapeutic interventions are also discussed.

Keywords Addiction, alcohol, DNA methylation, drugs, epigenetics, genetics.

Correspondence to: Jonathan Mill, Institute of Psychiatry, SGDP Research Centre, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, UK. E-mail: jonathan.mill@kcl.ac.uk

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INTRODUCTION

Drug and alcohol dependence are debilitating psychiatric disorders that are common and associated with high morbidity and mortality rates [1]. Like other common complex diseases, addiction is a multi-factorial and polygenic disorder that does not conform to a simple Mendelian transmission pattern [2,3]. Family, twin and adoption studies suggest that the heritability of substance use disorders is moderate to high (ranging from 0.30 to 0.70), with shared and non-shared environmental factors also important [4]. Considerable research effort has been expended on elucidating the underlying neurobiological causes of addiction. Most aetiological studies have focused on identifying genetic variants that predispose an individual to the spectrum of addictive disorders. There has been some success in the search for underlying genetic variation predisposing to addiction, and polymorphisms in a number of candidate neurotransmitter system genes have been associated with susceptibility [5]. However, given the high heritability of substance use disorders, it is notable that the currently identified risk loci for addiction account only for a modest proportion (less than 5%) of the total genetic variance [6]. Moreover, findings from molecular genetic studies are generally characterized by non-replication, small effect sizes and significant heterogeneity. It is clear that neither genetic nor environmental factors act in isolation to increase susceptibility to addiction. For example, an approximately fivefold increase in genetic influence on alcohol consumption is observed in urban environments where there are a greater number of youths and higher alcohol sales compared to rural settings [7].

At a more basic level, the onset of addiction clearly results from the interplay between inherited predisposition (e.g. via genetic variants mediating the personality traits associated with drug-seeking behaviour and dependence) and the environment (e.g. actual exposure to drugs of abuse). Because addiction manifests only following repeated exposure to an addictive substance, identification of both the predisposing factors (inherited and environmental) and the dynamic neurobiological changes that take place in response to chronic drug exposure is key to elucidating the aetiology of addiction. Drug-taking behaviour is characterized by a three-stage cycle, including preoccupation–anticipation, binge–intoxication and withdrawal–negative affect [8]. Impulsive drug use is often associated with positive reinforcement where drug-seeking behaviour is mediated by the pleasurable effects of the drug. Following repeated

drug exposure, however, drug use becomes compulsive and negatively reinforcing, where drug-seeking behaviour is mediated by the ability of the drug to eliminate aversive emotional states associated with prolonged intoxication. Understanding the mechanism(s) that predispose individuals to the environmental factors associated with drug-taking behaviour and the systems that translate the response to environmental stimuli (e.g. drug exposure) into long-lasting cellular memories in the brain are thus fundamental to unlocking the neurobiological changes that are implicated causally in addiction.

Emerging evidence has shown that epigenetic factors are important contributors to disease susceptibility for a number of common neuropsychiatric phenotypes related to addiction, including schizophrenia [9] and depressive disorders [10]. Of particular relevance to addiction, it has been shown that epigenetic processes can change dynamically in response to external factors, providing a key mechanism by which the environment can influence gene expression and, hence, phenotype. The aim of this review is to introduce the potential role that epigenetic mechanisms play in mediating addictive behaviours by triggering long-lasting changes in gene expression in response to addictive substances. Following a brief description of epigenetic processes, we discuss the relevance of epigenetics to addiction and illustrate how enduring epigenetic changes can occur in response to repeated drug exposure, potentially underlying the onset of addiction.

EPIGENETICS AND THE REGULATION OF GENE EXPRESSION

With the exception of a few rare somatic mutation events, the sequence of nucleotides comprising an individual's genome is identical across all cells in the body and remains unchanged from the moment of conception onwards. However, DNA is structurally much more complex than a simple string of nucleotides, and at a functional level the genome is anything but static. While every cell in our bodies contains the same DNA sequence, each has its own unique phenotype characterized by a specific pattern of gene expression that is in a constant state of flux. It is not only the gene-encoding DNA sequence that is important in determining the phenotype of a cell, but also the degree to which specific genes are functionally active at any particular time in development. Sequencing the genome was therefore only the first step in our quest to understand how genes are expressed and regulated. Sitting above the DNA sequence is a second layer of information (the 'epigenome') that regulates several genomic functions, including when and where genes are turned on or off.

The British biologist, Conrad Waddington, first coined the term 'epigenetics' (literally meaning 'above genetics') in the mid-20th century, introducing the concept of an epigenetic landscape to describe the ways in which cell-fates are established during development, enabling the tissues and organs of complex organisms to develop from an initially undifferentiated mass of cells. A contemporary definition regards epigenetics as the reversible regulation of gene expression, occurring independently of DNA sequence, mediated principally through changes in DNA methylation and chromatin structure [11]. Epigenetic processes are essential for normal cellular development and differentiation, and allow the long-term regulation of gene function through non-mutagenic mechanisms [12]. For a glossary of epigenetic mechanisms and a basic description of the genomic functions they perform, see Table 1.

DNA methylation

The methylation of one of the four DNA bases, cytosine, is the best understood and the most stable epigenetic modification, regulating the transcriptional plasticity of mammalian genomes (Figs 1 and 2). DNA methylation occurs when a methyl group is added to position 5 of the cytosine pyrimidine ring in a reaction catalysed by a group of enzymes called DNA methyltransferases (DNMTs). This occurs primarily where a cytosine (C) occurs next to guanine (G) in the DNA sequence ['C—phosphate link—G—', or cytosine-guanine dinucleotides (CpG)], although cytosine methylation at non-CpG positions has also been reported. The intrinsic link between DNA methylation and the regulation of gene expression is demonstrated by the observed inverse correlation between the level of promoter DNA methylation and the degree of expression of many genes [11], even though the reality is more complex, with diverse patterns across different genomic regions. The addition of a methyl group to CpG sites, over-represented in 'CpG-islands' in the promoter regulatory regions of many genes, displaces the binding of transcription factors and attracts methyl-binding proteins that instigate chromatin compaction of DNA and gene silencing. The pivotal role of DNA methylation during development is illustrated by phenotypic analyses of mice with mutations in various DNA methyltransferase (*Dnmt*) genes [13–16].

Histone modification

Another form of epigenetic mechanism acting to modulate gene expression is the post-translational modification, or changes occurring after the biosynthesis, of histones. Histones are the basic proteins around which DNA is wrapped to form nucleosomes [17]. There are various covalent histone modifications, including

Table 1 Glossary of epigenetic terms used in this paper.

Term	*Definition*
Chromatin	The complex of DNA, histones and other proteins that make up chromosomes. Chemical modifications to both DNA and histone proteins are important in regulating the structure of chromatin, which in turn regulates gene expression
DNA methylation	The addition of a methyl group at position 5 of the cytosine pyrimidine ring in CpG dinucleotides, catalysed by DNA methyltransferase (Fig. 2). Cytosine DNA methylation occurring at CpG sites, over-represented in CpG islands in the promoter regulatory regions of many genes, often displaces the binding of transcription factors and attracts methyl-binding proteins that instigate chromatin compaction and gene silencing (Fig. 1)
Epiallele	An allele that can stably exist in more than one epigenetic state and gives a distribution of phenotypes from genetically identical cells
Epigenetics	The reversible regulation of gene expression mediated principally through changes in DNA methylation, chromatin structure and small interfering RNA (siRNA)
Euchromatin	Chromatin existing in an activated open state that permits the access of the cells' transcriptional machinery to DNA, promoting gene expression
Heterochromatin	Condensed chromatin represented by the tight packaging of the DNA and histone proteins that is associated with repressed transcription. Heterochromatin obstructs the access of transcription factors and other instigators of gene expression to DNA
Histone modifications	Histones are the basic proteins around which DNA is wrapped to form nucleosome, the basic repeat unit of chromatin (Fig. 1). Covalent histone modifications, including acetylation, phosphorylation, methylation, sumoylation and ubiquitylation, modulate gene expression via alterations in chromatin structure. Histone modifications are established key components in the overall regulation of gene expression in the genome and are mediated via histone-modifying enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation of the lysine residues at the N terminus of histones proteins removes positive charges, which reduces the affinity between histones and DNA. This allows an easier access of RNA polymerase and transcription factors to the promoter region. Histone acetylation catalysed by histone acetyltransferases (HATs) often enhances transcription while histone deacetylation catalysed by histone deacetylases (HDAC) is associated with transcriptional repression
Metastable epialleles	Epialleles that can be epigenetically modified in a variable and reversible manner in response to external or stochastic factors. The epigenetic state of the epialleles is transgenerationally stable once established
siRNA (small interfering RNA)	A class of short (21–26 nucleotides in length), double-stranded RNA which is involved in the RNA interference (RNAi) pathway and suppresses the expression of specific genes. RNAi is known to cause epigenetic changes in gene transcription, mediated by both DNA methylation and histone modifications

CpG: cytosine-guanine dinucleotide.

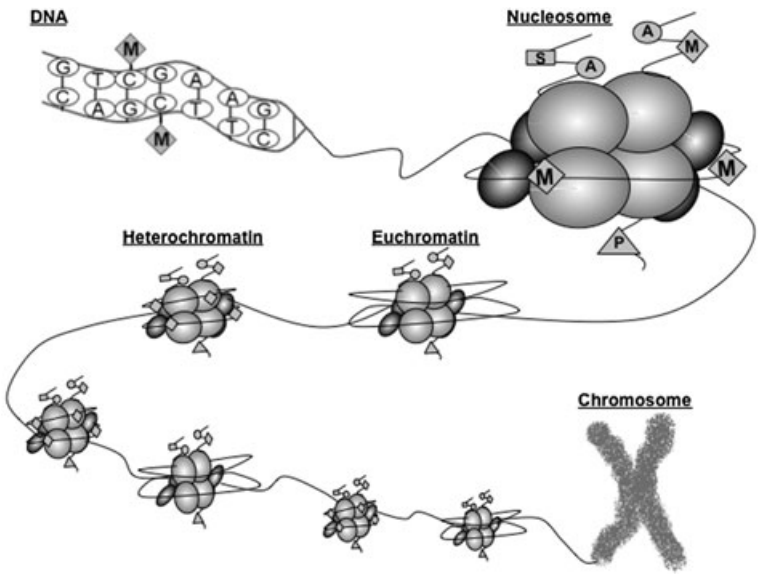

Figure 1 Epigenetic modifications to DNA and histones are associated with transcription. DNA is wrapped around a cluster of histone proteins to form nucleosomes. Changes in chromatin structure via DNA methylation (M) and histone modification including phosphorylation (P), acetylation (A) and sumoylation (S) can affect the access of transcription factors, hence gene expression (adapted from [49])

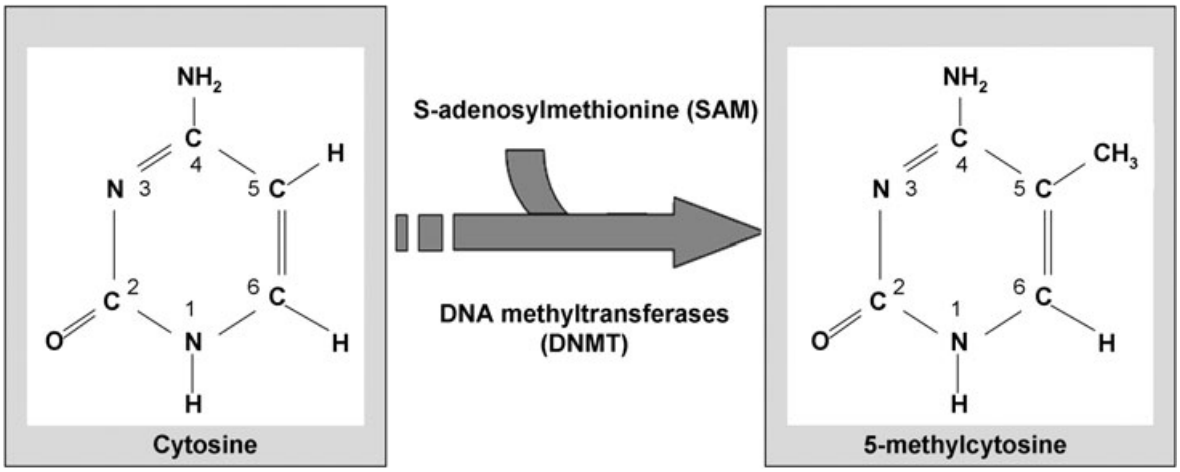

Figure 2 DNA methylation involves the addition of a methyl group at position 5 of the cytosine pyrimidine ring in cytosine-guanine dinucleotides (CpG). This reaction uses S-adenosylmethionine as a methyl donor and is catalysed by a group of enzymes called DNA methyltransferases

acetylation, phosphorylation, methylation, sumoylation and ubiquitylation, which together constitute a complex 'histone code' modulating gene expression via alterations in chromatin structure [18]. Histone modification mediated via histone-modifying enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs) are established key components in the overall regulation of gene expression in the genome [19]. Condensed chromatin (heterochromatin) is associated with repressed transcription as the tight packaging of the DNA and histone proteins obstructs the access of transcription factors and other instigators of gene expression to DNA. In contrast, when chromatin is in an activated open state (euchromatin), access of the cells' transcriptional machinery to DNA is permitted and hence gene expression can occur (Fig. 1).

While often investigated independently, epigenetic modifications to DNA and histones are not mutually exclusive, but interact in several ways. The methyl-binding protein MeCP2, for example, binds specifically to methylated cytosines, attracting histone deacetylases which hypoacetylate histones and inhibits access of transcription factors [20]. Another recent study has shown that unmethylated histone H3 residues at the lysine 4 position recruit DNA methyltransferases resulting in *de novo* DNA methylation [21].

ENVIRONMENTAL MEDIATION OF THE EPIGENOME

Mounting evidence suggests that epigenetic processes can be influenced by exposure to a range of external environmental factors, either globally or at specific loci [22]. DNA methylation, for example, has been shown to vary as a function of nutritional, chemical, physical and even psychosocial factors (e.g. stress exposure). As epigenetic changes are inherited mitotically in somatic cells, they provide a possible mechanism by which the effects of external environmental factors at specific stages in development can produce long-term changes in behaviour. The role of epigenetic mechanisms in mediating phenotypic effects of environmental stimuli is supported by evidence that the environment can induce epigenetic variation in genetically identical animals [23,24] and between identical twins [25,26]. Alleles that can exist stably in more than one epigenetic state and give a distribution of phenotypes from genetically identical cells are termed 'epialleles'. Loci that can be modified epigenetically in a variable and reversible manner in response to external or stochastic factors are defined as 'metastable epialleles' [27].

Given that the epigenetic state of metastable epialleles can be transmitted stably via cell division, the effects of external environmental factors during specific stages in development can manifest in long-term phenotypic changes via such epialleles. A classic example of the interplay between the genome, the environment and epigenetic processes is illustrated by the Agouti viable yellow allele (A^{vy}) inbred mouse strain, which demonstrates a range of coat-colour phenotypes, depending upon the epigenetic state of a large transposable element inserted upstream of the Agouti gene. The transposon contains a cryptic promoter, which expresses a phenotype characterized by yellow fur and various detrimental metabolic features, including obesity [28]. When the transposon is methylated, this phenotype is not expressed; the mice have agouti (brown) fur and are metabolically healthy. Interestingly, DNA methylation across this region, and thus phenotype, can be manipulated in the offspring by altering the diet of pregnant mothers [29,30]. Maternal dietary methyl-donor supplementation of pregnant mice

with folic acid, vitamin B_{12}, choline, betaine and genistein (the major phytostrogen in soy) were shown to increase offspring DNA methylation, leading to gene expression associated with brown fur and good metabolic health.

Environmental mediation of the epigenome thus provides a mechanism for the gene–environment interactions currently being uncovered in psychiatry, including those interactions underlying predisposition to addiction [17]. Of particular interest to addictive behaviour, there is mounting evidence that alcohol and drugs of abuse can themselves have a direct effect on epigenetic processes, and that these alterations may contribute to the molecular basis of addiction.

EPIGENETICS AND DRUG ADDICTION

Long-lasting behavioural abnormalities are classic characteristics of psychiatric disorders, particularly where the development of mental illness is gradual, and often shows a chronic relapsing course over a life-time [31]. The reversal of symptoms in response to treatment is slow, occurring over weeks to months, and chronic administration of psychiatric medications is often required for their full clinical effect to be achieved. In the field of addiction, for example, drug relapse is one of the core features and is perhaps the most complicated clinical problem for effective treatment [32]. Drug-induced neuronal plasticity mediated via alterations in gene expression has long been viewed as a major molecular mechanism for the development of drug addiction and relapse [33–35]. Recreational drugs exert rapid effects upon behaviour by activating the reward centres, such as the mesolimbic dopamine system projecting dopaminergic neurones of the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Transient increases in members of the transcription factor Fos family, including c-fos, FosB Fra-1 and Fra-2, in the NAc and dorsal striatum are observed upon acute exposure to drugs of abuse [36]. Following repeated exposure, recreational drug use can lead to addiction, as encouraged by persistent drug reward-related memories [37]. The expression of transcription factor ΔFosB is increased severalfold in the striatum during repeated drug exposure, and often persists long after drug exposure ceases. The extraordinary stability of ΔFosB in neurones has led to the theory that it plays a significant role in the onset of addiction [33,38,39]. In addition, the expression of several other genes, including *BDNF* and activator of G-protein signalling 3 (*AGS3*), are known to be altered persistently for several weeks after the last drug administration, and manipulation of these genes in rodent models has been shown to be associated with the regulation of drug relapse behaviour [38,40–42].

Emerging evidence has demonstrated that epigenetic changes may partially mediate persistent neuroplasticity changes, as observed in drug addiction [43]. For example, the activity of DNA methyltransferases, the key enzymes in DNA methylation, has a significant role in regulating the induction of synaptic plasticity in the hippocampus [44,45]. Inhibition of DNMT activity was shown to alter the methylation states of the promoter of *reelin* and *BDNF*, two genes implicated in the induction of synaptic plasticity in the adult hippocampus [44]. DNMT inhibitors such as zebularine and 5-aza-2-deoxycytidine can also block the induction of long-term potentiation (LTP) [45]. Because DNMTs are involved in the silencing of the memory suppressor gene, *Pp1*, their inhibition during the memory consolidation period prevents the increase of *Pp1* methylation and leads to aberrant transcription of the gene and a reduction in memory formation. In addition to DNA methylation, histone acetylation has also been associated with the establishment of long-term memory. During the initial stages of consolidation of long-term association memories in a contextual fear-conditioning paradigm, a significantly enhanced level of histone H3, but not H4 acetylation, was observed in animals that underwent contextual fear conditioning, compared to controls [46]. Moreover deficits in memory formation and long-term potentiation in mice with a heterozygous mutation in CREB-binding protein (CBP) were ameliorated by treatment with histone deacetylase inhibitors [47]. These studies provide evidence that epigenetic modifications, including both DNA methylation and histone modifications such as acetylation, play an essential regulatory role in synaptic plasticity and long-term behavioural adaptation in the nervous system.

Given that neuronal plasticity has a recognized role in regulating drug addiction, and epigenetic mechanisms are major mediators of long-term plasticity, epigenetic changes are probable candidates for maintaining drug addiction. Indeed, emerging evidence suggests that epigenetic mechanisms may be the molecular basis of drug-induced changes in gene expression in brain reward regions, contributing to the lasting neural and behavioural plasticity that underlies addiction [48]. Figure 3 illustrates how such epigenetic changes mediated by the interaction of inherited predispositions, environmental stimuli and exposure to drugs can trigger the long-lasting alterations in gene expression that influence susceptibility to addictive behaviours.

The role of epigenetic mechanisms in drug-related behaviours is supported by an increasing body of evidence from molecular and behavioural experiments in both animals and humans. Selected examples of epigenetic changes induced by various drugs of abuse, including alcohol, amphetamine, cocaine, nicotine and opiates, are illustrated in Table 2. Evidence from these studies not only demonstrate the imperative role of epigenetic modifications in regulating behavioural responses to drug

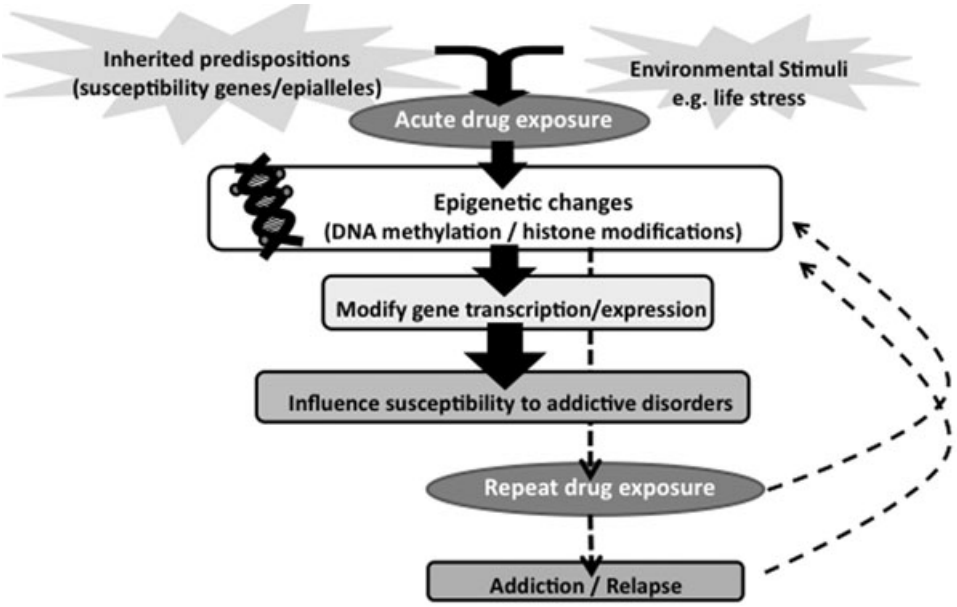

Figure 3 The proposed relationship between inherited predispositions, environmental factors, exposure to addictive substances and vulnerability to addictive disorders. When exposed to adverse environmental stimuli, individuals carrying susceptibility genes or epialleles predisposing to addictive behaviours may have an increased risk of developing addiction. Acute drug use may produce enduring alterations in gene expression via epigenetic changes that influence susceptibility to addictive disorders. Enhanced vulnerability to drugs of abuse will then feed back into increased risk of future drug use (as shown by the dashed arrow) that bring about further modifications to the epigenome and gene expression

exposure, but also shed light on the complex mechanism of drug addiction. However, it is worth noting that only a few epigenetic studies on addiction have been performed in humans, and the majority of findings presented in Table 2 are from studies in animals.

FUTURE DIRECTIONS: IMPLICATIONS FOR MOLECULAR STUDIES OF ADDICTION AND THE DEVELOPMENT OF NOVEL THERAPEUTIC INTERVENTIONS

In this paper, we have introduced the importance of epigenetic processes in regulating gene expression and described the role that dynamic epigenetic changes may play in mediating addictive behaviours via long-lasting transcriptional changes following repeated drug exposure. There is emerging evidence for the role of epigenetic mechanisms underlying addictive behaviours to various types of drugs of abuse, including cocaine, amphetamine, alcohol, nicotine and opiates. The current rapid growth in epigenetic research, combined with new high-throughput technologies for epigenomic profiling, could potentially revolutionize our understanding of the molecular changes associated with addiction and exposure to drugs of abuse.

Epigenetic studies of brain-specific processes such as addiction and psychiatric disease are subject to several limitations that make them particularly hard to undertake successfully [49]. A major limitation to the optimal study of addiction-associated epigenetic changes is that researchers may require post-mortem human brain tissue. This is because epigenetic changes are often tissue- (and cell-) specific, and thus most likely to be apparent in the brain areas in which addiction is primarily manifest. Unfortunately, high-quality post-mortem brain samples that have been well-characterized for addiction and/or drug exposure are a limited resource, but it is likely that peripheral tissues can still provide other valuable information. Epigenetic studies of human neurobiological processes such as addiction are also limited by insufficient knowledge about the 'normal' epigenetic patterns that characterize different brain regions and cell types. However, it is hoped that current initiatives such as the NIH Epigenomic Roadmap [50], which aims to catalogue patterns of epigenetic variation across different cell- and tissue-types, will be useful in this regard. Additionally, little is known about how factors such as age, sex and environmental exposure influence epigenetic patterns, so samples should be matched as carefully as possible for all potential confounding variables. For these reasons, the use of rodent models, as exemplified by many of the examples discussed in this paper, will be of great value in furthering our understanding of the role of epigenetic processes in addiction.

Table 2 Selected examples of drug-induced epigenetic changes.

Type of drug	*Species*	*Reference*	*Key findings*
Alcohol	Human	[55]	• A significant increase in global DNA methylation has been reported in alcoholic patients compared to normal controls
	Rat	[56]	• Rats exposed to excessive ethanol, at levels equivalent to human binge drinking, demonstrated tissue-specific alteration in histone H3 acetylation at lysine9
	Mouse	[57]	• Exposure to chronic ethanol treatment altered DNA methylation at the NMDA receptor Nr2b gene in mice
	Rat	[58]	• Enhanced levels of histone H3 and H4 acetylation, CBP and neuropeptide Y (NPY), as well as decreased levels of HDAC activity, in rat amygdala were found to be involved in the anxiolytic effects of acute ethanol • Moreover, the development of anxiety in alcohol withdrawal was associated with decreased histone acetylation and increased HDAC activity in the amygdala • The administration of a known HDAC inhibitor, trichostatin A, prevented the development of alcohol withdrawal-related anxiety in rats by recovering the diminished levels of histone acetylation and inhibiting the augmented HDAC activity following chronic alcohol exposure
	Drosophila	[59]	• Behavioural tolerance observed in drosophila following the administration of a single dose of benzyl alcohol, for example, was shown to be mediated via histone H4 acetylation across the promoter region of Slo, a gene with an established role in the induction of tolerance to sedative drugs
Alcohol and nicotine	Human	[60]	• DNA methylation at the monoamine oxidase-A (MAOA) gene was shown to be associated significantly with alcohol and nicotine dependence in women
Amphetamine	Mouse	[61]	• Alteration in expression of *Mecp2* in the nucleus accumbens (NAc) altered both amphetamine-induced locomotion and the rewarding properties of amphetamine in conditional place preference
	Mouse	[62]	• Following chronic amphetamine administration, ΔFosB, a key transcription factor involved in the behavioural responses to cocaine, has been shown to accumulate in the striatum in mice • The up-regulation of ΔFosB desensitizes c-fos mRNA induction to a subsequent drug exposure by binding to the c-fos promoter and recruits histone deacetylase 1 (HDAC1), which deacetylases nearby histones and attenuates gene activity
	Mouse	[63]	• The administration of HDAC inhibitors butyric acid (BA) and valproic acid (VPA) was shown to potentiate amphetamine-induced behavioural sensitisation in mice • An increased level of H4 hyperacetylation in the striatum was observed upon administration of VPA and amphetamine, while their co-treatment induced an additive effect on histone H4 acetylation • The occurrence of the chronic amphetamine induced-sensitized response following amphetamine challenge was blocked by repeated administration of butyric acid (BA) and VPA • The enhanced amphetamine-induced behavioural sensitization caused by HDAC inhibitors has also been shown to be associated with increased associative learning and memory, suggesting that histone deacetylation accounts at least partially for the induction and maintenance of the behavioural responses to amphetamine
	Rat	[64]	• Rats exposed to subchronic amphetamine treatment showed differential DNA methylation and mRNA expression of Dnmt1
Cocaine	Rat	[65]	• MeCP2, a key transcriptional repressor, was shown to control the effects of cocaine on striatal brain-derived neurotrophic factor (BDNF) levels via homeostasis interactions with microRNA-212
	Mouse	[66]	• Repeated cocaine administration reduced global levels of histone 3 lysine 9 (H3K9) demethylation in the nucleus accumbens of mice • This reduction in H3K9 level was mediated by down-regulation of histone methyltransferase G9a, one of the key histone modification enzymes • Repression of G9a after repeated cocaine administration increased the plasticity of nucleus accumbens neurones and enhanced the preference for cocaine
	Mouse	[67]	• Cocaine sensitivity was mediated via histone acetylation at the c-fos gene promoter in the striatum in mice
	Mouse	[68]	• Decreased cerebral volume and significantly lower sustained visual–spatial attention and spatial working memory were observed in the offspring of male mice exposed to cocaine • Such phenotypical changes might be caused by the observed altered expression levels of DNMT1 and DNMT3 in the germ-cell rich seminiferous tubular tissue of the male mice exposed to cocaine
	Mouse	[69]	• The levels of global DNA methylation and expression of selected genes in hippocampal neurones were altered significantly in the male offspring of cocaine-exposed mothers at 3 and 30 days postnatum, compared to the offspring of non-exposed mothers
	Rat	[70]	• Acute cocaine administration induced transient H4 hyperacetylation of various genes, e.g. cFos and FosB • Chronic cocaine injection did not have an effect on the c-fos promoter, but resulted in H3 hyperacetylation of the FosB promoter, as well as the Cdk5 and Bdnf genes • These histone modifications were shown to be long-lasting, persisting for at least 1 week after the last cocaine injection
	Rat	[52]	• Administration of HDAC inhibitors extinguished cocaine-induced conditioned place preference in mice
	Rat	[53]	• Administration of HDAC inhibitors decreased cocaine self-administration in rats
Nicotine	Human	[71]	• A DNA methylation status signature at the monoamine oxidase-A (MAOA) gene is associated with smoking status
	Human and mouse	[72]	• Significant and persistent demethylation of *MAOB* promoter was found in former smokers (abstinent for over 10 years) and current smokers when compared to non-smokers • The role of tobacco smoke in mediating DNA methylation change is supported by significantly higher level of nucleic acid demethylase activity in mice exposed to cigarette smoke compared to controls
	Mouse	[73]	• Injection of nicotine down-regulated Dnmt1 mRNA and protein expression in mice, as well as decreasing DNA methylation level of the promoter region of glutamic acid decarboxylase 67 (GAD67), one of the two enzymes that synthesize GABA in the brain
Opiate	Human	[74]	• Increased DNA methylation in the promoter of opioid receptor mu 1(OPRM1) gene was seen in methadone-maintained former heroin addicts compared to controls

GABA: gamma aminobutyric acid; HDAC: histone deacetylase; NMDA: N-methyl-D-aspartate.

Because epigenetic mechanisms are dynamic and reversible, chemical agents that alter the modification of histones or the methylation of DNA might prove to be potent candidates for therapeutic interventions. Moreover, the identification of specific epigenetic patterns associated with specific disease phenotypes might be useful biomarkers for early disease diagnosis and preventive intervention. Promising results have been seen in the cancer field, where epigenetic drugs and therapies are used as effective anti-cancer therapeutics [51]. Although epigenetic research is still largely unexplored in the field of addiction, recent findings demonstrating that administration of HDAC inhibitors extinguished cocaine-induced conditioned place preference in mice [52] and decreased cocaine self-administration in rats [53] highlight the potential therapeutic use of drugs that target epigenetic processes. Recent technological advances in epigenomic profiling have made it feasible to screen the entire DNA methylome with individual base-pair resolution [54]. We are now at the exciting stage where it is possible to assess the ways by which drugs of abuse alter specific epigenetic processes in the brain with the aim of identifying the molecular mechanisms underlying addiction.

Declarations of interest

None.

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