Published in final edited form as:

Handb Clin Neurol. 2018 ; 148: 747–765. doi:10.1016/B978-0-444-64076-5.00048-X.

Neuroepigenetics and addiction

DEENA M. WALKER and ERIC J. NESTLER*

Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Abstract

Drug addiction involves long-term behavioral abnormalities that arise in response to repeated exposure to drugs of abuse in vulnerable individuals. It is a multifactorial syndrome involving a complex interplay between genes and the environment. Evidence suggests that the underlying mechanisms regulating these persistent behavioral abnormalities involve changes in gene expression throughout the brain's reward circuitry, in particular, in the mesolimbic dopamine system. In the past decade, investigations have begun to reveal potential genes involved in the risk for addiction through genomewide association studies. Additionally, a crucial role for epigenetic mechanisms, which mediate the enduring effects of drugs of abuse on the brain in animal models of addiction, has been established. This chapter focuses on recent evidence that genetic and epigenetic regulatory events underlie the changes throughout the reward circuitry in humans, as well as animal models of addiction. While further investigations are necessary, a picture of genetic and epigenetic mechanisms involved in addiction is beginning to emerge and the insight gained from these studies will be key to the identification of novel targets for improved diagnosis and treatment of addiction syndromes in humans.

INTRODUCTION

Drug addiction, or substance use disorder (SUD), is defined as the compulsive administration of drugs of abuse in spite of negative consequences to the individual and a loss of control over the intake of such drugs (American Psychiatric Association, 2013). From a neurobiologic perspective, addiction represents maladaptive neural plasticity in response to drugs of abuse, which involves long-term molecular alterations in key brain regions that control reward (Fig. 48.1), leading to potentially lifelong behavioral abnormalities (reviewed in Nestler (2014)). It is estimated that genetic factors contribute ~50% of the susceptibility to addiction for all abused drugs (reviewed in Wang et al. (2012)), with exposure to a range of “risk factors” contributing the other half. These risk factors include adverse early life experiences and other environmental stimuli that “prime” an individual to be more vulnerable to addiction (Pena et al., 2014). Indeed, without exposure to drugs (an environmental factor itself), the addicted phenotype would not be detected, thus highlighting the complex interplay between genes and environmental factors involved in the initiation and maintenance of the addicted phenotype (Fig. 48.2).

*Correspondence to: Eric J. Nestler, MD, PhD, Fishberg Department of Neuroscience, Mt. Sinai School of Medicine, Icahn Medical Institute Room 10-23, 1425 Madison Ave, New York NY 10029, United States. Tel: +1-212-659-5656, eric.nestler@mssm.edu.

The multifactorial nature of addiction has led to the investigation of both genetic and epigenetic mechanisms underlying the phenotype. Selective breeding studies of strains of rats displaying high and low susceptibility to morphine addiction were the first to confirm that there was a heritable component of addiction (Nichols and Hsiao, 1967). While subsequent studies utilizing selective breeding in animal models have revealed a heritable component for preference to methamphetamine (Wheeler et al., 2009) and ethanol (Gilpin et al., 2008), much of the evidence for a robust genetic contribution to addiction has come from family, twin, and adoption studies in humans. Advances in molecular biology have enabled linkage studies, those comparing genetic markers in related individuals, and association studies, those comparing markers in unrelated individuals, which initially identified candidate genes and genomic regions weakly involved in addiction. More recent investigations have expanded to genomewide comparisons in humans, which are uncovering more bona fide genetic factors. Therefore, much of the focus on the genetic component of addiction in this chapter will focus on human studies.

On the other hand, studies focusing on epigenetic mechanisms of addiction have relied primarily on animal models. In its broadest definition, epigenetics describes changes in gene expression that do not arise from changes to the DNA sequence; this generally involves alterations in histone modifications, DNA methylation, and noncoding RNAs (microRNAs (miRNAs) and long noncoding RNAs (lncRNAs); see Chapter 5). Each of these mechanisms can be altered in response to internal and external signals and provides a mechanism by which environmental stimuli can interact with an individual's genome to influence cellular responses and function, including neuroplasticity. The differing temporal effects of each mechanism are, in part, the result of their varying levels of stability – e.g., most histone modifications are exceptionally dynamic, whereas DNA methylation is generally less so (Jaenisch and Bird, 2003). Later in this chapter recent work in the field of epigenetics and addiction will be reviewed, with a major focus on histone modifications, as much of the work examining epigenetic mechanisms involved in the addicted phenotype has focused on such modifications. The much smaller number of studies focusing on the role of DNA methylation and noncoding RNAs are covered briefly as well.

GENETIC COMPONENTS OF DRUG ADDICTION

Family, twin, and adoption studies

Family-based studies examine the risk of substance abuse in first-degree relatives (parents, siblings, offspring) with an SUD and suggest that addiction indeed “runs in the family.” The familial aggregation of alcoholism has been well established (reviewed in McGue (1994)). For example, siblings of ethanol-dependent probands were more likely to be dependent on ethanol when compared to controls (Bierut et al., 1998) and individuals with an alcoholic parent were five times more likely to be alcoholics (Midanik, 1983). While fewer studies have focused on other drugs of abuse, evidence suggests that first-degree relatives of substance abusers have higher rates of addiction when compared to controls (Bierut et al., 1998; Merikangas et al., 1998). Specifically, adult first-degree relatives of probands with an addiction to various drugs, including opiates, cocaine, cannabis, and/or ethanol, displayed an eightfold increased risk of a SUD (Merikangas et al., 1998). Additionally, offspring of

substance abusers were twice as likely to develop substance abuse and three times more likely to develop addiction to ethanol or marijuana when compared to children of unaffected parents (Merikangas and Avenevoli, 2000).

One drawback of family-based studies is the difficulty in distinguishing the contribution of genetic variance in individuals vs. environmental factors. Indeed, there is intriguing evidence in animal models for the complexity of this interplay between genes and environment. A recent study revealed that male rats with a history of cocaine self-administration sire offspring that show reduced predilection to self-administer cocaine (Vassoler et al., 2013), while those with a parental history of cannabis exposure show increased predilection to self-administer heroin (Szutorisz et al., 2014). It remains unknown whether such transgenerational transmission of addiction-like vulnerability is mediated behaviorally, through epigenetic modifications in sperm, or through chemical changes in seminal fluid, among other possibilities. In any event, these data raise the possibility that an individual's risk for addiction is mediated not just by genes and environment but also by additional factors that are transmitted across generations.

Adoption and twin studies have been utilized to parse the relative contribution of genetic and environmental factors to the addicted phenotype. Adoption studies compare traits in the adopted offspring with those of the adoptive and biologic parents. While there are difficulties associated with adoption studies (reviewed in Hall et al. (2013)), they have been instrumental in recognizing genetic contributions to the development of addiction by identifying a link between substance abuse in the biologic parent and adopted offspring. Adoption studies have also highlighted environmental factors important for the development of addiction by linking abuse in the adopted offspring to characteristics in the adopted parents. In particular, parental divorce or parental psychiatric disorders were identified as risk factors for the development of substance abuse in offspring (Cadoret et al., 1986, 1995, 1996).

Twin studies, on the other hand, utilize data from monozygotic (MZ) and dizygotic (DZ) twins, who presumably share much of the same environmental influences, to quantify the contribution of heritable factors. If genetics contributes to the development of substance abuse, then outcomes will be more similar between MZ than DZ twins. If environmental factors contribute to the disorder, then outcomes in MZ and DZ will be similar. Evidence from twin studies consistently estimates that the genetic contribution to substance abuse disorders is ~50% for alcoholism (Heath et al., 1993; Prescott and Kendler, 1999; True et al., 1999) and ranges from 30% to 80% for other drugs of abuse (Tsuang et al., 1996; Kendler et al., 2000, 2006; Agrawal et al., 2005; Pergadia et al., 2006). Interestingly, evidence suggests that genetic factors play a greater role in the severity of substance abuse (i.e., the development of addiction) than in earlier stages of drug use, which are seemingly influenced to a greater extent by environmental factors (Tsuang et al., 1996; Kendler and Prescott, 1998; Rhee et al., 2003; Fowler et al., 2007), highlighting the multi-factorial nature of the addiction phenotype.

Linkage and association studies

While twin and adoption studies establish a heritable component, presumably through variations in the DNA sequences of affected individuals, in the acquisition and maintenance of addiction, they do not identify the specific genes/alleles involved. Traditionally, linkage analysis has been used to identify the association between DNA markers and addiction status within individual families. Such studies focus on highly variable regions of the DNA (polymorphic regions) to increase the likelihood of identifying loci – e.g., containing a single-nucleotide polymorphism (SNP) – that are associated with an SUD. Association studies, on the other hand, identify allelic markers in unrelated individuals generally utilizing a case-control experimental design. Unfortunately, few linkage or association studies have identified consistent, replicable markers for SUDs to any of several drugs of abuse (Guerrini et al., 2005; Zhong and Zhang, 2005; Kuo et al., 2006; Clarimon et al., 2007; Gelernter et al., 2007; Hopfer et al., 2007; Li et al., 2007; Loh et al., 2007; Pomerleau et al., 2007; Saccone et al., 2007), thus highlighting the highly polygenic nature of the syndrome. Examples are addiction-associated polymorphisms in the vicinity of dopamine receptors (Drd2, 3, and 4) (reviewed in Gorwood et al. (2012)), GABAergic systems (Noble et al., 1998; Edenberg et al., 2004; Agrawal et al., 2006), and opioid receptors (reviewed in Hall et al. (2013)) in drug dependence, but the effect sizes are very small and only ~50% of all studies report a positive association. It should be noted that many linkage studies have focused on genes that were chosen based on *a priori* hypotheses stemming from neurobiologic mechanisms of addiction, while the genetic risk for addiction likely involves numerous genes and biochemical pathways, as well as nongenic regions of the genome, that have not yet been implicated in addiction. Going forward, it is crucial that candidate genomic regions be identified first via unbiased genomewide studies coupled with follow-up studies in animal models where the genomic loci can be manipulated and their function characterized.

Genomewide association studies

The lack of replication of linkage and association studies of candidate genes has led to the use of genomewide association studies (GWAS) as a superior method to identify common SNPs in SUD individuals. The first GWAS, which focused on multidrug addiction, was conducted in 2001 (Uhl et al., 2001), but resolution was not sufficient to implicate specific genes until 2005 (Liu et al., 2005). Those original investigations focused on addiction to nicotine (Uhl et al., 2007; Drgon et al., 2009), methamphetamine (Uhl et al., 2008b), ethanol (Uhl et al., 2008a), and multiple drugs (Liu et al., 2006). However, many SNPs initially associated with addiction by GWAS were not replicated in subsequent studies due to the small sample sizes involved (Bierut, 2011). Therefore, recent studies have used aggregate analyses of several thousands of subjects and are beginning to yield, for the first time, robust, bona fide genomic loci that are associated with nicotine, ethanol, or opiate addiction (Saccone et al., 2010; Kapoor et al., 2012; Gelernter et al., 2014a, b), including genes associated with ethanol metabolism (Gelernter et al., 2014a), cholinergic nicotinic receptors (Saccone et al., 2010; Kapoor et al., 2012), and calcium and potassium signaling (Gelernter et al., 2014b). Importantly, very few candidate genes initially hypothesized to be involved in drug addiction have been consistently identified using GWAS, emphasizing the need for

more unbiased genomewide studies involving many tens, if not hundreds, of thousands of individuals in the field of addiction.

Conclusions for genetic contributions to drug addiction

It is clear that there is a strong (~50%) heritable component to addiction, which has been supported in family-based, adoption, and twin studies. However, investigations seeking to identify specific genetic markers for addiction in humans, until very recently, have produced inconsistent results. More recent genome-wide studies, involving the unbiased analysis of thousands of individuals, are beginning to identify genes that are significantly associated with addiction. However, these identified genes each contribute only a very small amount (typically <1%) to the overall risk, which highlights the tremendous complexity of the phenotype. In this regard, the genetic basis of addiction is similar to that for most other common, complex syndromes (type 2 diabetes, congestive heart failure, psychotic disorders), with perhaps several hundred genes comprising the genetic risk for such a syndrome, like addiction, in any given individual. Identifying those myriad genes and understanding how they combine to increase addiction risk is a major challenge of current research. One approach is to study the interactions of gene networks coupled with transcriptomic analyses in postmortem human brain tissue and animal models (Parikshak et al., 2015). Ongoing efforts are identifying networks that might be instrumental in addiction risk. Therefore, moving forward, more genomewide studies in combination with advanced bioinformatics techniques are necessary to identify the complex network of genes that determine an individual's vulnerability for addiction.

EPIGENETIC MECHANISMS OF ADDICTION

Because of the strong influence of external risk factors on the development of addiction, it has been proposed that epigenetic mechanisms regulate the long-term changes associated with phenotypic priming and the subsequent addicted phenotype. To date, a majority of the work on the epigenetic mechanisms involved in addiction has focused on the effects of drugs of abuse in animal models of addiction, with a concentration on the effects of stimulants in the nucleus accumbens (NAc), a key brain region involved in reward. Recently, work has expanded to other brain regions in the reward circuitry (Fig. 48.1), including the ventral tegmental area (VTA), prefrontal cortex (PFC), basolateral amygdala (BLA), and hippocampus (HC), as well as to other drugs of abuse. Therefore, the focus of this section will be on stimulant effects in the NAc of animal models, but will include information from other brain regions and drugs of abuse where information is available. It will be important in the future to expand such analyses to human postmortem brain tissue. A related endeavor is to perform epigenetic analyses on human blood or other peripheral tissues of living patients. However, given that drug-induced epigenetic changes in animals show considerable variations among brain regions, it will be crucial to establish that epigenetic signatures in the periphery are predictive of central mechanisms.

Histone modifications

DNA is condensed into the nucleus of the cell in a highly organized and compact manner, referred to as chromatin. The functional unit of chromatin, the nucleosome, is composed of

~147 basepairs wrapped around core histone octamers consisting of two copies of each of the following proteins: H2A, H2B, H3, and H4 (Fig. 48.3 and Table 48.1). Each histone protein can undergo numerous posttranslational modifications in which different functional groups are covalently added to amino acid residues of their N-terminal tails, e.g., acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, citullination, and ADP-ribosylation, among others. These modifications not only alter the structure of the nucleosome but also change the interaction of DNA with the associated histones, thus increasing or decreasing the likelihood of transcription at a given locus. Histone modifications are diverse, and we are only beginning to understand how the various combinations of histone posttranslational modifications influence or indicate specific transcriptional states (Maze et al., 2014; see Chapter 5). Finally, histone modifications are added or removed by a large family of enzymes, referred to as “writers” and “erasers,” respectively, making them reversible and dynamic epigenetic modifications. While a myriad of histone modifications and their interactions are likely involved in the regulation of the acquisition and maintenance of the addicted phenotype, to date, histone acetylation and methylation are the most extensively studied posttranslational modifications in the addiction field.

Histone acetylation—Histone acetylation is generally associated with a permissive transcriptional state (Fig. 48.3). By negating the positive charge associated with the lysine residues on histone tails, acetylation promotes an “open” chromatin state. While the effects of drugs of abuse are widespread, a picture of how histone acetylation is changing throughout the brain after exposure to drugs of abuse, including cocaine (Rogge and Wood, 2013), morphine (Mashayekhi et al., 2012; Wang et al., 2015), nicotine (Levine et al., 2011; Huang et al., 2013, 2014), and others, is beginning to emerge (Table 48.1). In general, acute or repeated exposure to drugs of abuse, including cocaine, ethanol, morphine, and methamphetamine, increases global (i.e., total cellular levels of) acetylation at H3 and/or H4 throughout the reward circuitry, including the whole striatum (Kumar et al., 2005), NAc (Shen et al., 2008; Renthal et al., 2009; Wang et al., 2010; Levine et al., 2011; Botia et al., 2012; Martin et al., 2012; Kennedy et al., 2013), dentate gyrus of the HC (Huang et al., 2014), and BLA (Wang et al., 2015) (Table 48.1). However, these effects may be brain region-specific as ethanol reduces total H3K9ac levels in VTA 14 hours after the last drug exposure (Arora et al., 2013) (for specifics, see Table 48.1).

Global alterations in acetylation provide insight into general changes in transcription in response to drugs of abuse. To understand further the functional outcomes of such changes it is necessary to identify sites of altered histone acetylation at specific genomic regions. Chromatin-immunoprecipitation (ChIP) (Table 48.2) has been used to identify drug-induced increases in pan-acetylation of histone H3 for a number of genes in specific brain reward regions. A common set of genes with enriched histone acetylation, consisting of immediate early genes and those involved in neuro-plasticity, is beginning to emerge in the literature (reviewed in Feng and Nestler (2013)) (for specifics, see Table 48.1). While acute and repeated exposures to drugs of abuse have similar effects on global acetylation levels, histone acetylation at specific loci is dependent on duration of exposure and seemingly reflects functional differences in the role of specific genes in addiction. For example, H4ac is

increased at the *cFos* promoter, an immediate early gene, after acute, but not chronic, cocaine treatment, consistent with a desensitization of *cFos* expression only after chronic drug exposure (Kumar et al., 2005; Renthal et al., 2008). In contrast, *Bdnf* and *Cdk5* promoters, genes associated with cell signaling, display enriched H3ac only after chronic drug exposure (Kumar et al., 2005; Mashayekhi et al., 2012; Schmidt et al., 2012), consistent with induction of these genes after chronic exposure to both cocaine (Kumar et al., 2005; Schmidt et al., 2012) and morphine (Mashayekhi et al., 2012). These results suggest that, at the molecular level, the initial response to drugs of abuse is very different from chronic exposures. Understanding the molecular targets of acute and chronic exposures can provide insight into the brain changes associated with acquisition, maintenance, and perhaps extinction of the addicted phenotype and may be helpful in identifying potential therapeutics and/or biomarkers for different stages or severity of addiction.

Genomewide studies have been instrumental in identifying genes involved in drug-related behaviors and addiction susceptibility. Using ChIP-chip (Table 48.2), an earlier method for analyzing genome-wide changes in chromatin modifications, Renthal et al. (2009) carried out the first genomewide map of pan-acetylation of histone H3 and H4 in the NAc in response to chronic cocaine. The authors reported increases of H3ac or H4ac at 1696 genes in the NAc and reduced levels of acetylation at only 206 genes, consistent with increased acetylation being the predominant modification. Interestingly, there was little overlap of genes displaying alterations of H3ac and H4ac, and most genes did not follow the expected expression pattern predicted by altered histone modifications: hypoacetylation with decreased gene expression and hyperacetylation with increased gene expression (Renthal et al., 2009). These data indicate that additional gene-regulatory mechanisms work in concert with histone acetylation to control drug-induced changes in gene expression.

Drugs of abuse are thought to alter histone modifications in part by affecting the activity or expression levels of histone acetyltransferases (HATs) and histone deacetylases (HDACs), the enzymes that add or remove acetylation of lysine residues, respectively (Jaenisch and Bird, 2003). Regulation of histone acetylation through manipulation of HAT and HDAC expression has yielded valuable information regarding the role of acetylation in drug-elicited behavioral responses (Feng and Nestler, 2013; Rogge and Wood, 2013) (Table 48.3). In general, decreasing histone acetylation in the NAc either through activation/overexpression of HDACs or inhibition/knockdown of HATs, blunts the behavioral responses to drugs of abuse. On the other hand, increasing histone acetylation in the NAc through pharmacologic inhibition or knockout of HDACs or viral-mediated overexpression of HATs initially facilitates the behavioral effects of many drugs of abuse. However, these effects are sensitive to the timing of HDAC inhibition in relation to exposure to drugs of abuse (Nestler, 2014) (for specifics, see Table 48.3). For example, sustained HDAC inhibition in the NAc can blunt behavioral responses to cocaine by suppressing gene expression through the induction of repressive histone mechanisms (see below) (Kennedy et al., 2013).

Recent studies are beginning to identify more selective effects of individual HDACs and HATs in drug-induced behaviors (for specifics, see Table 48.3). For example, overexpression of HDAC4 (Wang et al., 2010) or HDAC5 (Renthal et al., 2007), or inhibition of CREB-binding protein (a HAT) (Levine et al., 2005; Malvaez et al., 2011), weakens the locomotor

response to cocaine (Levine et al., 2005; Malvaez et al., 2011) and acquisition of conditioned place preference (CPP) (Renthal et al., 2007; Wang et al., 2010). Focal deletion of HDAC1, but not HDAC2 or 3, in the NAc mimics the effect of long-term HDAC inhibition by attenuating locomotor responses to cocaine (Kennedy et al., 2013). Interestingly, focal deletion of HDAC3 in the NAc enhances cocaine CPP (Rogge et al., 2013), as does knockout of HDAC5 (Renthal et al., 2007), an effect not observed with HDAC9 (Renthal et al., 2007). The opposing effect observed with HDAC3 could be explained by the finding that HDAC3 plays an important role in drug-induced memory consolidation, as pharmacologic inhibition of HDAC3 enhances extinction of cocaine-induced memories after CPP (Malvaez et al., 2013). Finally, the class III HDACs, termed sirtuins (SIRT1s), are implicated in the actions of cocaine and morphine (Renthal et al., 2009; Ferguson et al., 2013, 2015). Viral-mediated overexpression of SIRT1 or 2 in the NAc enhances cocaine and morphine reward, whereas the local knockout of SIRT1 has the opposite effect (Ferguson et al., 2013, 2015). This action of SIRT1, which is opposite to what has been observed with class I and II HDACs, appears to be mediated in large part via the deacetylation of nonhistone proteins, such as the transcription factor, FOXO3a (Ferguson et al., 2015). While it is apparent that differences in histone acetylation state are involved in the behavioral response to acute and prolonged exposure to drugs of abuse, further studies are necessary to identify how these alterations work in concert to produce a temporally specific histone landscape that is indicative of the various stages of addiction.

Taken together, these data support the conclusion that cocaine is a potent regulator of histone acetylation in the NAc (Renthal et al., 2007, 2009). However, histone lysine acetylation is not the only modification contributing to expression changes and the “histone code” regulating these alterations is exceptionally complex (Jenuwein and Allis, 2001), including acetylation of additional residues as well as numerous other types of histone modifications. Further investigations, which focus on additional histone modifications, are necessary to develop a complete understanding of how each modification (or combination of modifications) regulates gene expression in response to drugs of abuse.

Histone lysine methylation—Histone lysine methylation, another well-characterized histone modification, is associated with either activation or repression of gene expression depending on which residues are methylated and the number of methyl groups added. Indeed, translating the role of histone methylation and its responses to drugs of abuse is much more complex. To date, most of the work on drugs of abuse has focused on the role of repressive histone methylation (Nestler, 2014). However, a recent study in NAc using ChIP-seq coupled with RNA-seq (Table 48.2) investigated how combinations of methylation marks (H3K4me1, H3K4me3, H3K36me3 – all activating marks; and H3K9me2, H3K9me3, H3K27me3 – all repressive marks) correlate with altered transcript levels after exposure to repeated cocaine. While global changes across the genome were not observed, enrichment of H3K4me1 or H3K4me3 at specific gene regions was correlated positively with increased transcription levels, whereas enrichment of H3K9me2, H3K9me3, or H3K27me3 was negatively correlated with transcription (Feng et al., 2014). Other studies have found that repeated exposure to cocaine decreases global levels of H3K9me2 and H3K9me3 in NAc (Maze et al., 2010), whereas repeated opiate exposure (Sun et al., 2012) only decreases

H3K9me2 in this brain region. By contrast, methamphetamine decreases H3K27me2 and increases H3K4me2 and H3K4me3 globally in NAc when exposure is coupled with CPP but not in the animals' home cage (Aguilar-Valles et al., 2014), again suggesting that regulation of histone methylation in response to drugs of abuse is complex and likely drug- and region-specific (Table 48.1).

As mentioned above, an emerging theme developing in the field suggests that inhibition of repressive epigenetic modifying enzymes (e.g., class I and II HDACs) and those histone methyltransferases (HMTs) that mediate gene repression, in the NAc enhances drug-associated behaviors. Studies manipulating these HMTs and associated histone demethylases (HDMs) support this. A recent study investigating mixed-lineage leukemia 1 (MLL1) and histone lysine (K)-specific demethylase 5C (KDM5c), which catalyze the methylation and demethylation of H3K4me3, a permissive mark, respectively, implicate H3K4me3 in drug-elicited behaviors. Methamphetamine administration increases expression of *MLL1* mRNA in NAc when paired with a novel context; however, when exposure occurs in the home cage, expression is decreased. NAc-specific knockdown of MLL1 reduces global H3K4me3 levels and inhibits methamphetamine-induced CPP. Interestingly, NAc-specific knockdown of KDM5c increases global H3K4me3 levels and inhibits methamphetamine CPP if the knockdown occurs after consolidation (between test days 1 and 2) (Aguilar-Valles et al., 2014). This suggests that the methylation state of H3K4 in NAc plays a dynamic role in the temporal- and context-specific neuroplasticity required for development and expression of methamphetamine-induced behavioral adaptations.

Methylation of H3K9 is also critical in regulating drug-induced behavioral responses. Two HMTs for H3K9me2, G9a and G9a-like protein (GLP), are downregulated in the NAc by chronic cocaine (Maze et al., 2010, 2011) or opiates (Sun et al., 2012), which is associated with global decreases in levels of H3K9me2. This effect was not observed for other H3K9/K27 HMTs (Maze et al., 2010; Sun et al., 2012). Pharmacologic inhibition or viral-mediated knockdown of G9a facilitates cocaine- or opiate-mediated CPP, whereas overexpression opposes drug-induced effects (Maze et al., 2010; Sun et al., 2012) (Table 48.3). Indeed, the paradoxical suppression of cocaine-elicited behaviors after prolonged HDAC inhibition in NAc, or local knockout of HDAC1 (see above) appears to be mediated via the induction of G9a and its consequent repression of gene expression (Kennedy et al., 2013) (Table 48.1). Taken together, these data suggest that histone methylation plays an important and complex role in drug-induced behaviors.

As noted above, chronic cocaine administration causes decreases in global levels of H3K9me3 in NAc (Maze et al., 2011). This mark, along with H3K9me2, is thought to contribute to the silencing of genes in heterochromatic regions. As might be expected from the overall depletion of these marks and a reduction in the amount of heterochromatin, we observed an increase in the size of neuronal nuclei in NAc, an effect not seen in other brain regions, after chronic cocaine administration. ChIP-seq (Table 48.2) for H3K9me3 found most of the binding in nongenic regions, where its depletion after cocaine treatment was associated with increased expression of certain repetitive elements (Maze et al., 2011). These data highlight the highly complex genomic changes that a drug of abuse induces in target neurons in the brain and defines further studies of nongenic regions in future years.

Other histone marks—Few studies have investigated the role of other histone modifications in response to drugs of abuse; however, a recent study suggests that polyADP-ribosylation of histones, generally a permissive mark, plays a role in cocaine-induced neuronal plasticity. Expression of polyADP-ribosyltransferase-1 (PARP1), a “writer” of histone polyADP-ribosylation, is increased in the NAc after repeated cocaine exposure, leading to an increase in histone polyADP-ribosylation. Furthermore, overexpression of PARP1 in NAc enhances cocaine locomotor sensitivity and cocaine-induced CPP, as well as cocaine self-administration, while infusion of a PARP1 inhibitor or over-expression of polyADP-ribosylglycohydrolase (PARG), an “eraser” of histone polyADP-ribosylation, yields opposing results. After repeated cocaine, there is a genomewide increase of PARP1 binding across genes, as determined by ChIP-seq (Table 48.2), and a significant correlation of greater PARP1 enrichment with increased gene expression (Scobie et al., 2014). These results are consistent with a permissive transcriptional environment following repeated cocaine exposure identified in previous studies. Further investigation of other histone modifications is under way, but in the future it will be necessary to integrate genome-wide alterations in a multitude of histone modifications in order to identify the complex code that is indicative of altered gene expression and an addicted phenotype.

DNA methylation

An important epigenetic regulator in addition to histone modifications is DNA methylation, which occurs with the addition of a methyl group to cytosine-phosphoguanine (CpG) at the C5 position (5-mC). In development, it plays a pivotal role in tissue-specific gene expression, cellular differentiation, X inactivation, imprinting of parental alleles, and repetitive element silencing. DNA methylation at gene promoters is generally associated with repression, while methylation in gene bodies has been associated with active transcription in several species, including humans (Suzuki and Bird, 2008). Another cytosine modification, 5-hydroxy-mC (5-hmC) appears to be concentrated in gene bodies (Tahiliani et al., 2009), is enriched in brain (Kriaucionis and Heintz, 2009), and associated with gene regulation (Szulwach et al., 2011). While DNA methylation has traditionally been characterized as a stable epigenetic mark, recent evidence suggests that it is much more transient than previously thought (Mikaelsson and Miller, 2011). It is clear that DNA methylation and histone modifications participate in an “epigenetic conversation” to regulate transcription. For example, DNA methylation can recruit methylation-binding proteins, which in turn recruit HDACs, leading to a repressed chromatin state (Hashimoto et al., 2010).

There is a dearth of information regarding the role of DNA methylation in drug addiction but, of the few studies that exist, many are focused on how drugs of abuse regulate expression of DNA methyltransferases (DNMTs), the enzymes that catalyze the addition of methyl groups to DNA. Those studies suggest that there are dynamic expression changes in response to drugs of abuse. In general, both acute exposure to cocaine and prolonged withdrawal (28 days) from chronic exposure increase the expression of *Dnmt3a*, the enzyme regulating de novo DNA methylation in mammals, in the NAc (LaPlant et al., 2010; Anier et al., 2013). Studies investigating repeated exposure to cocaine have produced conflicting results (Anier et al., 2010, 2013; LaPlant et al., 2010). In the PFC, *Dnmt3b* expression is

downregulated 2 hours after cocaine but not morphine CPP, an effect not observed in the NAc (Tian et al., 2012). Additionally, cocaine but not morphine decreases global DNA methylation levels in the PFC, which can be rescued with the methyl donor methionine (Tian et al., 2012). Interestingly, treatment with methionine inhibits cocaine and food CPP, but had no effect on morphine CPP (LaPlant et al., 2010; Tian et al., 2012), thus demonstrating both the drug- and regional specificity of this epigenetic mechanism.

Studies have identified a role for DNA methylation in drug-elicited behaviors by manipulating targets associated with DNA methylation in the brain. Pharmaceutical inhibition or local knockout of DNMT3a in the NAc enhances cocaine reward, whereas overexpression decreases it (LaPlant et al., 2010). Supplementation with methionine, a methyl donor which increases DNA methylation overall, also attenuates behavioral responses to cocaine, including its self-administration (Wright et al., 2015). Additionally, NAc-specific knockout of methyl CpG-binding protein 2 (MeCP2), a methyl-binding protein and important modulator of neural plasticity, enhances amphetamine reward (Deng et al., 2010), whereas cocaine increases expression of MeCP2 specifically in the dorsal striatum and local knockdown of the protein inhibits cocaine self-administration (Im et al., 2010), suggesting again that repression of transcription either through histone modification or DNA methylation generally blunts the actions of drugs of abuse.

Recent work has demonstrated that enzymes involved in the generation of 5-hmC also influence cocaine action (Feng et al., 2015). Chronic cocaine administration down-regulates the expression of ten eleven translocation-1 (TET1) in NAc. TET1 oxidizes 5-mC into 5-hmC and ultimately, with the involvement of additional enzymes, into nonmethylated cytosine. This suppression of TET1 expression serves to enhance behavioral responses to cocaine.

While it is intriguing that the enzymes and other proteins involved in regulating DNA methylation are altered by drugs of abuse, there has still not been a global mapping at single-nucleotide resolution of DNA methylation changes in the reward circuitry in response to drugs of abuse, although a few studies have found gene-specific effects on 5-mC or 5-hmC in the brain (Taqi et al., 2011; Nielsen et al., 2012; Ponomarev et al., 2012; Day et al., 2013; Feng et al., 2015; Massart et al., 2015). Unfortunately, due to technical limitations, it remains very expensive to identify altered DNA methylation on a genomewide scale at single-basepair resolution. Additionally, most genomewide assays focus on the promoter regions of genes and provide little information regarding methylation changes in gene bodies or other genomic regions. Given that methylation is predicted to have different functions in regulating gene expression depending on its location, and different cytosine modifications (5-mC and 5-hmC) are often associated with opposing functional outcomes, it is imperative that genomewide maps of DNA methylation with single-base resolution are generated to develop a comprehensive understanding of the role of this epigenetic mark in addiction.

MicroRNAs and long noncoding RNAs

Recent evidence from the complete sequencing of multiple mammalian genomes has revealed a large number of RNA transcripts that are not translated into protein. Many of these noncoding RNAs are now known to play an important role in cell function.

Specifically, miRNAs are short sequences of RNA (~20 bp) that exert a repressive role on gene expression by binding a target sequence on specific mRNAs and blocking translation or inducing degradation. LncRNAs are longer sequences of RNAs (>200 bp) that have been found to regulate gene expression by forming RNA–protein complexes and modulating chromatin-regulatory proteins or transcription factors (O’Carroll and Schaefer, 2013).

The role of miRNAs is understudied in the field of addiction (Bali and Kenny, 2013). However, cocaine has been shown to increase expression of miR-181a and decrease miR-124 and let-7d in the striatum of rodents (Chandrasekar and Dreyer, 2009; Schaefer et al., 2010). These miRNAs in turn influence cocaine-induced behaviors (Chandrasekar and Dreyer, 2011). Morphine increases expression of miR-339-3p in mouse HC, which then targets and decreases expression of the μ -opioid receptor (Wu et al., 2013). It also increases expression of let-7 in the mouse brain (He et al., 2010) and decreases expression of miR-133b in cultured rat hippocampal neurons (Sanchez-Simon et al., 2010), suggesting that there are drug-specific effects with regard to miRNA regulation as well. Manipulation of specific miRNAs in the brain can affect drug-elicited behaviors. For example, cocaine induces the expression of miR-212 in the dorsal striatum after self-administration, and overexpression of miR-212 inhibits cocaine intake (Chandrasekar and Dreyer, 2011). A recent study found that miR-206 is upregulated in the PFC but not the VTA, amygdala, or NAc of rats self-administering ethanol, and overexpression of miR-206 in the PFC in nondependent animals escalated ethanol consumption. Interestingly, a concomitant decrease in expression of *Bdnf*, a predicted target of miR-206, was observed in these animals (Tapocik et al., 2014). Finally, argonaute-2, which is crucial in the processing of miRNAs, has been shown to control behavioral responses to cocaine via actions in *Drd2*-containing striatal neurons (Schaefer et al., 2010). Taken together, these data provide intriguing evidence that miRNAs play an important role in the acquisition and maintenance of addiction.

LncRNAs have not yet been characterized in addiction models. However, there is promising evidence from mining microarray data indicating that lncRNAs are altered in the brains of heroin-addicted humans (Michelhaugh et al., 2011). Now that lncRNAs are being captured far more completely in RNA-seq (Table 48.2) experiments, it is expected that an improved understanding of their contribution to addiction-related neural and behavioral abnormalities will be forthcoming in the near future.

Conclusions for epigenetic contributions to drug addiction

It is clear that epigenetic mechanisms, including histone modifications, DNA methylation, and noncoding RNAs, all play an important and complex role in the neuroplastic changes associated with addiction. Currently, histone acetylation and methylation are by far the most comprehensively studied histone modifications in the field of addiction. A comprehensive review of the data, which includes numerous drugs of abuse and brain regions, reveals several emergent properties with regard to histone modifications (Table 48.1) suggests that: (1) drugs of abuse generally increase the likelihood of transcription in NAc by increasing histone modifications associated with an “active” chromatin state and reducing those associated with a repressive chromatin state; (2) these activating marks are associated with

immediate early genes and many others that have been implicated generally in neuroplasticity; (3) drugs of abuse likely affect the expression or activation of the “writers” and “erasers” of histone modifications, and manipulation of these enzymes in brain regions associated with reward alters the behavioral response to drugs of abuse; and (4) considerable drug-induced epigenetic regulation occurs in nongenic regions of the genome, which is also likely important in mediating the addiction phenotype. While the general principle of a permissive epigenetic-transcriptional state holds for the majority of regulated genes, it is important to emphasize that hundreds of genes show regulation in the opposite direction.

Although data are promising that DNA methylation and noncoding RNAs also play an important role in the establishment of addiction, these endpoints remain understudied such that it is difficult to identify consistent effects of drugs of abuse on these parameters at this time. However, DNA methyltransferases are targets of cocaine and morphine and alterations of these enzymes affect drug-induced behaviors. A better understanding of the temporal and regional alterations of these enzymes, as well as genomewide maps of 5-mC and 5-hmC in brain reward regions, is now necessary. Likewise, we are beginning to identify several specific miRNAs and their targets, which are altered by exposure to drugs of abuse.

The potent influence of epigenetic mechanisms on the addiction process raises the question of whether drugs that target such mechanisms might be useful in the treatment of addictive disorders. The major concern with this approach is that virtually all epigenetic enzymes and related proteins expressed in the brain are also expressed in peripheral tissues, such that drugs that target such mechanisms might be associated with unacceptable sideeffects. However, this is ultimately an empiric question and worthy of direct investigation. Several HDAC and HDM inhibitors are being developed by the pharmaceutical industry for the treatment of cancer. It would be interesting to test tool compounds, known to penetrate the brain, in proof-of-concept studies of addiction. Beyond targeting epigenetic mechanisms directly, there is every expectation that the genomewide characterization of a host of epigenetic modifications in discrete brain regions will point the field toward particular genes and biochemical pathways that should be targeted in future drug discovery efforts.

CONCLUDING REMARKS

It is clear that genetic and environmental factors contribute to drug addiction, and adoption and twin studies have been instrumental in quantifying the contribution of both factors to the syndrome in humans. While there is substantial evidence that genetic factors contribute roughly half of the risk for an addicted phenotype, specific genes and other genomic regions that mediate this risk have been very difficult to identify with certainty. More powerful experimental methods and associated bioinformatic approaches that are agnostic in nature – e.g., GWAS with whole-exome or whole-genome sequencing – are now enabling far more penetrating studies which should reveal gene networks that underlie addiction risk. On the other hand, environmental risk factors are also important and a few vague factors (e.g., child abuse, adverse life experiences) are known, but their mechanisms remain poorly understood, particularly in humans. Epigenetic mechanisms have been studied mainly in animal models of addiction and are beginning to offer an understanding of how environmental stimuli can alter gene expression in the brain on a long-term basis. In the future, it will be necessary to

integrate these studies with individual variations in genome sequences in an effort to understand how an individual's ultimate risk of (or resistance to) an addiction syndrome is mediated. The integration of such data should provide us with the ability to identify biomarkers for susceptibility and resilience to addiction and, we hope, lead to the identification of novel therapeutic targets for improved treatment of addiction syndromes.

References

- Agrawal A, Neale MC, Jacobson KC, et al. Illicit drug use and abuse/dependence: modeling of two-stage variables using the CCC approach. *Addict Behav.* 2005; 30:1043–1048. [PubMed: 15893102]
- Agrawal A, Edenberg HJ, Foroud T, et al. Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. *Behav Genet.* 2006; 36:640–650. [PubMed: 16622805]
- Aguilar-Valles A, Vaissiere T, Griggs EM, et al. Methamphetamine-associated memory is regulated by a writer and an eraser of permissive histone methylation. *Biol Psychiatry.* 2014; 76:57–65. [PubMed: 24183790]
- American Psychiatric Association. The diagnostic and statistical manual of mental disorders. 5. American Psychiatric Publishing; Arlington, VA: 2013.
- Anier K, Malinovskaja K, Aonurm-Helm A, et al. DNA methylation regulates cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology.* 2010; 35:2450–2461. [PubMed: 20720536]
- Anier K, Zharkovsky A, Kalda A. S-adenosylmethionine modifies cocaine-induced DNA methylation and increases locomotor sensitization in mice. *Int J Neuropsychopharmacol.* 2013; 16:2053–2066. [PubMed: 23684129]
- Arora DS, Nimitvilai S, Teppen TL, et al. Hyposensitivity to gamma-aminobutyric acid in the ventral tegmental area during alcohol withdrawal: reversal by histone deacetylase inhibitors. *Neuropsychopharmacology.* 2013; 38:1674–1684. [PubMed: 23474591]
- Bali P, Kenny PJ. MicroRNAs and drug addiction. *Front Genet.* 2013; 4:43. [PubMed: 23717324]
- Bierut LJ. Genetic vulnerability and susceptibility to substance dependence. *Neuron.* 2011; 69:618–627. [PubMed: 21338875]
- Bierut LJ, Dinwiddie SH, Begleiter H, et al. Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. *Arch Gen Psychiatry.* 1998; 55:982–988. [PubMed: 9819066]
- Botia B, Legastelois R, Alaux-Cantin S, et al. Expression of ethanol-induced behavioral sensitization is associated with alteration of chromatin remodeling in mice. *PLoS One.* 2012; 7:e47527. [PubMed: 23110077]
- Cadoret RJ, Troughton E, O'Gorman TW, et al. An adoption study of genetic and environmental factors in drug abuse. *Arch Gen Psychiatry.* 1986; 43:1131–1136. [PubMed: 3778110]
- Cadoret RJ, Yates WR, Troughton E, et al. Adoption study demonstrating two genetic pathways to drug abuse. *Arch Gen Psychiatry.* 1995; 52:42–52. [PubMed: 7811161]
- Cadoret RJ, Yates WR, Troughton E, et al. An adoption study of drug abuse/dependency in females. *Compr Psychiatry.* 1996; 37:88–94. [PubMed: 8654068]
- Chandrasekar V, Dreyer JL. microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. *Mol Cell Neurosci.* 2009; 42:350–362. [PubMed: 19703567]
- Chandrasekar V, Dreyer JL. Regulation of MiR-124, Let-7d, and MiR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. *Neuropsychopharmacology.* 2011; 36:1149–1164. [PubMed: 21307844]
- Clarimon J, Gray RR, Williams LN, et al. Linkage disequilibrium and association analysis of alpha-synuclein and alcohol and drug dependence in two American Indian populations. *Alcohol Clin Exp Res.* 2007; 31:546–554. [PubMed: 17374033]
- D'Addario C, Caputi FF, Ekstrom TJ, et al. Ethanol induces epigenetic modulation of prodynorphin and pronociceptin gene expression in the rat amygdala complex. *J Mol Neuroscience.* 2013; 49:312–319.

- Day JJ, Childs D, Guzman-Karlsson MC, et al. DNA methylation regulates associative reward learning. *Nat Neurosci.* 2013; 16:1445–1452. [PubMed: 23974711]
- Deng JV, Rodriguiz RM, Hutchinson AN, et al. MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. *Nat Neurosci.* 2010; 13:1128–1136. [PubMed: 20711186]
- Drgon T, Montoya I, Johnson C, et al. Genome-wide association for nicotine dependence and smoking cessation success in NIH research volunteers. *Mol Med.* 2009; 15:21–27. [PubMed: 19009022]
- Edenberg HJ, Dick DM, Xuei X, et al. Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet.* 2004; 74:705–714. [PubMed: 15024690]
- Feng J, Nestler EJ. Epigenetic mechanisms of drug addiction. *Curr Opin Neurobiol.* 2013; 23:521–528. [PubMed: 23374537]
- Feng J, Wilkinson M, Liu X, et al. Chronic cocaine-regulated epigenomic changes in mouse nucleus accumbens. *Genome Biol.* 2014; 15:R65. [PubMed: 24758366]
- Feng J, Shao N, Szulwach KE, et al. Role of Tet1 and 5. *Nat Neurosci.* 2015; 18:536–544. [PubMed: 25774451]
- Ferguson D, Koo JW, Feng J, et al. Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. *J Neurosci.* 2013; 33:16088–16098. [PubMed: 24107942]
- Ferguson D, Shao N, Heller E, et al. SIRT1-FOXO3a regulate cocaine actions in the nucleus accumbens. *J Neurosci.* 2015; 35:3100–3111. [PubMed: 25698746]
- Fowler T, Lifford K, Shelton K, et al. Exploring the relationship between genetic and environmental influences on initiation and progression of substance use. *Addiction.* 2007; 102:413–422. [PubMed: 17298649]
- Gelernter J, Panhuysen C, Weiss R, et al. Genomewide linkage scan for nicotine dependence: identification of a chromosome 5 risk locus. *Biol Psychiatry.* 2007; 61:119–126. [PubMed: 17081504]
- Gelernter J, Kranzler HR, Sherva R, et al. Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. *Mol Psychiatry.* 2014a; 19:41–49. [PubMed: 24166409]
- Gelernter J, Kranzler HR, Sherva R, et al. Genome-wide association study of opioid dependence: multiple associations mapped to calcium and potassium pathways. *Biol Psychiatry.* 2014b; 76:66–74. [PubMed: 24143882]
- Gilpin NW, Richardson HN, Lumeng L, et al. Dependence-induced alcohol drinking by alcohol-preferring (P) rats and outbred Wistar rats. *Alcohol Clin Exp Res.* 2008; 32:1688–1696. [PubMed: 18482158]
- Gorwood P, Le Strat Y, Ramoz N, et al. Genetics of dopamine receptors and drug addiction. *Hum Genet.* 2012; 131:803–822. [PubMed: 22350797]
- Guerrini I, Cook CC, Kest W, et al. Genetic linkage analysis supports the presence of two susceptibility loci for alcoholism and heavy drinking on chromosome 1p22.1–11.2 and 1q21.3–24.2. *BMC Genet.* 2005; 6:11. [PubMed: 15740611]
- Hall FS, Drgonova J, Jain S, et al. Implications of genome wide association studies for addiction: are our a priori assumptions all wrong? *Pharmacol Ther.* 2013; 140:267–279. [PubMed: 23872493]
- Hashimoto H, Vertino PM, Cheng X. Molecular coupling of DNA methylation and histone methylation. *Epigenomics.* 2010; 2:657–669. [PubMed: 21339843]
- He Y, Yang C, Kirkmire CM, et al. Regulation of opioid tolerance by let-7 family microRNA targeting the mu opioid receptor. *J Neurosci.* 2010; 30:10251–10258. [PubMed: 20668208]
- Heath AC, Cates R, Martin NG, et al. Genetic contribution to risk of smoking initiation: comparisons across birth cohorts and across cultures. *J Subst Abuse.* 1993; 5:221–246. [PubMed: 8312729]
- Hopfer CJ, Lessem JM, Hartman CA, et al. A genome-wide scan for loci influencing adolescent cannabis dependence symptoms: evidence for linkage on chromosomes 3 and 9. *Drug Alcohol Depend.* 2007; 89:34–41. [PubMed: 17169504]
- Huang YY, Kandel DB, Kandel ER, et al. Nicotine primes the effect of cocaine on the induction of LTP in the amygdala. *Neuropharmacology.* 2013; 74:126–134. [PubMed: 23597510]

- Huang YY, Levine A, Kandel DB, et al. D1/D5 receptors and histone deacetylation mediate the gateway effect of LTP in hippocampal dentate gyrus. *Learn Mem.* 2014; 21:153–160. [PubMed: 24549570]
- Im HI, Hollander JA, Bali P, et al. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nat Neurosci.* 2010; 13:1120–1127. [PubMed: 20711185]
- Itzhak Y, Liddie S, Anderson KL. Sodium butyrate-induced histone acetylation strengthens the expression of cocaine-associated contextual memory. *Neurobiol Learn Mem.* 2013; 102:34–42. [PubMed: 23567105]
- Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet.* 2003; 33(Suppl):245–254. [PubMed: 12610534]
- Jayanthi S, McCoy MT, Chen B, et al. Methamphetamine downregulates striatal glutamate receptors via diverse epigenetic mechanisms. *Biol Psychiatry.* 2014; 76:47–56. [PubMed: 24239129]
- Jenuwein T, Allis CD. Translating the histone code. *Science.* 2001; 293:1074–1080. [PubMed: 11498575]
- Jing L, Luo J, Zhang M, et al. Effect of the histone deacetylase inhibitors on behavioural sensitization to a single morphine exposure in mice. *Neurosci Lett.* 2011; 494:169–173. [PubMed: 21396430]
- Kalda A, Heidmets LT, Shen HY, et al. Histone deacetylase inhibitors modulates the induction and expression of amphetamine-induced behavioral sensitization partially through an associated learning of the environment in mice. *Behavioural brain research.* 2008; 181:76–84.
- Kapoor M, Wang JC, Bertelsen S, et al. Variants located upstream of CHRNA4 on chromosome 15q25.1 are associated with age at onset of daily smoking and habitual smoking. *PLoS One.* 2012; 7:e33513. [PubMed: 22438940]
- Kendler KS, Prescott CA. Cocaine use, abuse and dependence in a population-based sample of female twins. *Br J Psychiatry.* 1998; 173:345–350. [PubMed: 9926041]
- Kendler KS, Karkowski LM, Neale MC, et al. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch Gen Psychiatry.* 2000; 57:261–269. [PubMed: 10711912]
- Kendler KS, Aggen SH, Tambs K, et al. Illicit psychoactive substance use, abuse and dependence in a population-based sample of Norwegian twins. *Psychol Med.* 2006; 36:955–962. [PubMed: 16650346]
- Kennedy PJ, Feng J, Robison AJ, et al. Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci.* 2013; 16:434–440. [PubMed: 23475113]
- Kim WY, Kim S, Kim JH. Chronic microinjection of valproic acid into the nucleus accumbens attenuates amphetamine-induced locomotor activity. *Neurosci Lett.* 2008; 432:54–57. [PubMed: 18164815]
- Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science.* 2009; 324:929–930. [PubMed: 19372393]
- Kumar A, Choi KH, Renthal W, et al. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron.* 2005; 48:303–314. [PubMed: 16242410]
- Kuo PH, Neale MC, Riley BP, et al. Identification of susceptibility loci for alcohol-related traits in the Irish Affected Sib Pair Study of Alcohol Dependence. *Alcohol Clin Exp Res.* 2006; 30:1807–1816. [PubMed: 17067344]
- LaPlant Q, Vialou V, Covington HE 3rd, et al. Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nat Neurosci.* 2010; 13:1137–1143. [PubMed: 20729844]
- Levine AA, Guan Z, Barco 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 U S A.* 2005; 102:19186–19191. [PubMed: 16380431]
- Levine A, Huang Y, Drisaldi B, et al. Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. *Sci Transl Med.* 2011; 3:107ra109.
- Li MD, Sun D, Lou XY, et al. Linkage and association studies in African- and Caucasian-American populations demonstrate that SHC3 is a novel susceptibility locus for nicotine dependence. *Mol Psychiatry.* 2007; 12:462–473. [PubMed: 17179996]

- Liu QR, Drgon T, Walther D, et al. Pooled association genome scanning: validation and use to identify addiction vulnerability loci in two samples. *Proc Natl Acad Sci U S A*. 2005; 102:11864–11869. [PubMed: 16091475]
- Liu QR, Drgon T, Johnson C, et al. Addiction molecular genetics: 639,401 SNP whole genome association identifies many “cell adhesion” genes. *Am J Med Genet B Neuropsychiatr Genet*. 2006; 141B:918–925. [PubMed: 17099884]
- Loh EW, Tang NL, Lee DT, et al. Association analysis of GABA receptor subunit genes on 5q33 with heroin dependence in a Chinese male population. *Am J Med Genet B Neuropsychiatr Genet*. 2007; 144B:439–443. [PubMed: 17440936]
- Malvaez M, Sanchis-Segura C, Vo D, et al. Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. *Biol Psychiatry*. 2010; 67:36–43. [PubMed: 19765687]
- Malvaez M, Mhillaj E, Matheos DP, et al. CBP in the nucleus accumbens regulates cocaine-induced histone acetylation and is critical for cocaine-associated behaviors. *J Neurosci*. 2011; 31:16941–16948. [PubMed: 22114264]
- Malvaez M, McQuown SC, Rogge GA, et al. HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. *Proc Natl Acad Sci U S A*. 2013; 110:2647–2652. [PubMed: 23297220]
- Martin TA, Jayanthi S, McCoy MT, et al. Methamphetamine causes differential alterations in gene expression and patterns of histone acetylation/hypoacetylation in the rat nucleus accumbens. *PLoS One*. 2012; 7:e34236. [PubMed: 22470541]
- Mashayekhi FJ, Rasti M, Rahvar M, et al. Expression levels of the BDNF gene and histone modifications around its promoters in the ventral tegmental area and locus ceruleus of rats during forced abstinence from morphine. *Neurochem Res*. 2012; 37:1517–1523. [PubMed: 22410736]
- Massart R, Barnea R, Dikshtein Y, et al. Role of DNA methylation in the nucleus accumbens in incubation of cocaine craving. *J Neurosci*. 2015; 35:8042–8058. [PubMed: 26019323]
- Maze I, Covington HE 3rd, Dietz DM, et al. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science*. 2010; 327:213–216. [PubMed: 20056891]
- Maze I, Feng J, Wilkinson MB, et al. Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. *Proc Natl Acad Sci U S A*. 2011; 108:3035–3040. [PubMed: 21300862]
- Maze I, Noh KM, Soshnev AA, et al. Every amino acid matters: essential contributions of histone variants to mammalian development and disease. *Nat Rev Genet*. 2014; 15:259–271. [PubMed: 24614311]
- McGue M. Genes, environment, and the etiology of alcoholism. *The Development of Alcohol Problems: Exploring the Biopsychosocial Matrix of Risk*. 1994:1–40.
- Merikangas KR, Avenevoli S. Implications of genetic epidemiology for the prevention of substance use disorders. *Addict Behav*. 2000; 25:807–820. [PubMed: 11125772]
- Merikangas KR, Stolar M, Stevens DE, et al. Familial transmission of substance use disorders. *Arch Gen Psychiatry*. 1998; 55:973–979. [PubMed: 9819065]
- Michelhaugh SK, Lipovich L, Blythe J, et al. Mining Affymetrix microarray data for long non-coding RNAs: altered expression in the nucleus accumbens of heroin abusers. *J Neurochem*. 2011; 116:459–466. [PubMed: 21128942]
- Midanik L. Familial alcoholism and problem drinking in a national drinking practices survey. *Addict Behav*. 1983; 8:133–141. [PubMed: 6613712]
- Mikaelsson MA, Miller CA. The path to epigenetic treatment of memory disorders. *Neurobiol Learn Mem*. 2011; 96:13–18. [PubMed: 21320618]
- Nestler EJ. Epigenetic mechanisms of drug addiction. *Neuropharmacology*. 2014; 76:259–268. [PubMed: 23643695]
- Nichols JR, Hsiao S. Addiction liability of albino rats: breeding for quantitative differences in morphine drinking. *Science*. 1967; 157:561–563. [PubMed: 6067566]
- Nielsen DA, Huang W, Hamon SC, et al. Forced abstinence from cocaine self-administration is associated with DNA methylation changes in myelin genes in the corpus callosum: a preliminary study. *Front Psychiatry*. 2012; 3:60. [PubMed: 22712019]

- Noble EP, Zhang X, Ritchie T, et al. D2 dopamine receptor and GABA(A) receptor beta3 subunit genes and alcoholism. *Psychiatry Res.* 1998; 81:133–147. [PubMed: 9858031]
- O'Carroll D, Schaefer A. General principals of miRNA biogenesis and regulation in the brain. *Neuropsychopharmacology.* 2013; 38:39–54. [PubMed: 22669168]
- Parikshak NN, Gandal MJ, Geschwind DH. Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders. *Nat Rev Genet.* 2015; 16:441–458. [PubMed: 26149713]
- Pena CJ, Bagot RC, Labonte B, et al. Epigenetic signaling in psychiatric disorders. *J Mol Biol.* 2014; 426:3389–3412. [PubMed: 24709417]
- Pergadia ML, Heath AC, Martin NG, et al. Genetic analyses of DSM-IV nicotine withdrawal in adult twins. *Psychol Med.* 2006; 36:963–972. [PubMed: 16749946]
- Pomerleau OF, Pomerleau CS, Chu J, et al. Genome-wide linkage analysis for smoking-related regions, with replication in two ethnically diverse populations. *Nicotine Tob Res.* 2007; 9:955–958. [PubMed: 17763112]
- Ponomarev I, Wang S, Zhang L, et al. Gene coexpression networks in human brain identify epigenetic modifications in alcohol dependence. *J Neurosci.* 2012; 32:1884–1897. [PubMed: 22302827]
- Prescott CA, Kendler KS. Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. *Am J Psychiatry.* 1999; 156:34–40. [PubMed: 9892295]
- Renthal W, Maze I, Krishnan V, et al. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron.* 2007; 56:517–529. [PubMed: 17988634]
- Renthal W, Carle TL, Maze I, et al. Delta FosB mediates epigenetic desensitization of the cfos gene after chronic amphetamine exposure. *J Neurosci.* 2008; 28:7344–7349. [PubMed: 18632938]
- Renthal W, Kumar A, Xiao G, et al. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron.* 2009; 62:335–348. [PubMed: 19447090]
- Rhee SH, Hewitt JK, Young SE, et al. Genetic and environmental influences on substance initiation, use, and problem use in adolescents. *Arch Gen Psychiatry.* 2003; 60:1256–1264. [PubMed: 14662558]
- Rogge GA, Wood MA. The role of histone acetylation in cocaine-induced neural plasticity and behavior. *Neuropsychopharmacology.* 2013; 38:94–110. [PubMed: 22910457]
- Rogge GA, Singh H, Dang R, et al. HDAC3 is a negative regulator of cocaine-context-associated memory formation. *J Neurosci.* 2013; 33:6623–6632. [PubMed: 23575859]
- Romieu P, Host L, Gobaille S, et al. Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *J Neurosci.* 2008; 28:9342–9348. [PubMed: 18799668]
- Saccone SF, Pergadia ML, Loukola A, et al. Genetic linkage to chromosome 22q12 for a heavy-smoking quantitative trait in two independent samples. *Am J Hum Genet.* 2007; 80:856–866. [PubMed: 17436240]
- Saccone NL, Culverhouse RC, Schwantes-An TH, et al. Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. *PLoS Genet.* 2010; 6 pii e1001053.
- Sanchez-Simon FM, Arenzana FJ, Rodriguez RE. vivo effects of morphine on neuronal fate and opioid receptor expression in zebrafish embryos. *Eur J Neurosci.* 2010; 32:550–559. [PubMed: 20646065]
- Schaefer A, Im HI, Veno MT, et al. Argonaute 2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction. *J Exp Med.* 2010; 207:1843–1851. [PubMed: 20643829]
- Schmidt HD, Sangrey GR, Darnell SB, et al. Increased brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area during cocaine abstinence is associated with increased histone acetylation at BDNF exon I-containing promoters. *J Neurochem.* 2012; 120:202–209. [PubMed: 22043863]
- Schroeder FA, Penta KL, Matevosian A, et al. Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. *Neuropsychopharmacology.* 2008; 33:2981–2992. [PubMed: 18288092]
- Scobie KN, Damez-Werno D, Sun H, et al. Essential role of poly(ADP-ribosyl)ation in cocaine action. *Proc Natl Acad Sci U S A.* 2014; 111:2005–2010. [PubMed: 24449909]

- Shen HY, Kalda A, Yu L, et al. Additive effects of histone deacetylase inhibitors and amphetamine on histone H4 acetylation, cAMP responsive element binding protein phosphorylation and DeltaFosB expression in the striatum and locomotor sensitization in mice. *Neuroscience*. 2008; 157:644–655. [PubMed: 18848971]
- Sheng J, Lv Z, Wang L, et al. Histone H3 phosphoacetylation is critical for heroin-induced place preference. *Neuroreport*. 2011; 22:575–580. [PubMed: 21734607]
- Sun J, Wang L, Jiang B, et al. The effects of sodium butyrate, an inhibitor of histone deacetylase, on the cocaine- and sucrose-maintained self-administration in rats. *Neurosci Lett*. 2008; 441:72–76. [PubMed: 18599214]
- Sun H, Maze I, Dietz DM, et al. Morphine epigenomically regulates behavior through alterations in histone H3 lysine 9 dimethylation in the nucleus accumbens. *J Neurosci*. 2012; 32:17454–17464. [PubMed: 23197736]
- Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet*. 2008; 9:465–476. [PubMed: 18463664]
- Szulwach KE, Li X, Li Y, et al. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. *Nat Neurosci*. 2011; 14:1607–1616. [PubMed: 22037496]
- Szutorisz H, DiNieri JA, Sweet E, et al. Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. *Neuropsychopharmacology*. 2014; 39:1315–1323. [PubMed: 24385132]
- Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*. 2009; 324:930–935. [PubMed: 19372391]
- Tapocik JD, Barbier E, Flanigan M, et al. microRNA-206 in rat medial prefrontal cortex regulates BDNF expression and alcohol drinking. *J Neurosci*. 2014; 34:4581–4588. [PubMed: 24672003]
- Taqi MM, Bazov I, Watanabe H, et al. Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. *Addict Biol*. 2011; 16:499–509. [PubMed: 21521424]
- Tian W, Zhao M, Li M, et al. Reversal of cocaine-conditioned place preference through methyl supplementation in mice: altering global DNA methylation in the prefrontal cortex. *PLoS One*. 2012; 7:e33435. [PubMed: 22438930]
- True WR, Xian H, Scherrer JF, et al. Common genetic vulnerability for nicotine and alcohol dependence in men. *Arch Gen Psychiatry*. 1999; 56:655–661. [PubMed: 10401514]
- Tsuang MT, Lyons MJ, Eisen SA, et al. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. *Am J Med Genet*. 1996; 67:473–477. [PubMed: 8886164]
- Uhl GR, Liu QR, Walther D, et al. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *Am J Hum Genet*. 2001; 69:1290–1300. [PubMed: 11704927]
- Uhl GR, Liu QR, Drgon T, et al. Molecular genetics of nicotine dependence and abstinence: whole genome association using 520,000 SNPs. *BMC Genet*. 2007; 8:10. [PubMed: 17407593]
- Uhl GR, Drgon T, Johnson C, et al. “Higher order” addiction molecular genetics: convergent data from genome-wide association in humans and mice. *Biochem Pharmacol*. 2008a; 75:98–111. [PubMed: 17764662]
- Uhl GR, Drgon T, Liu QR, et al. Genome-wide association for methamphetamine dependence: convergent results from 2 samples. *Arch Gen Psychiatry*. 2008b; 65:345–355. [PubMed: 18316681]
- Vassoler FM, White SL, Schmidt HD, et al. Epigenetic inheritance of a cocaine-resistance phenotype. *Nat Neurosci*. 2013; 16:42–47. [PubMed: 23242310]
- Vialou V, Feng J, Robison AJ, et al. Epigenetic mechanisms of depression and antidepressant action. *Annu Rev Pharmacol Toxicol*. 2013; 53:59–87. [PubMed: 23020296]
- Walker DM, Cates HM, Heller EA, et al. Regulation of chromatin states by drugs of abuse. *Curr Opin Neurobiol*. 2015; 30:112–121. [PubMed: 25486626]
- Wang L, Lv Z, Hu Z, et al. Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. *Neuropsychopharmacology*. 2010; 35:913–928. [PubMed: 20010550]

- Wang JC, Kapoor M, Goate AM. The genetics of substance dependence. *Annu Rev Genomics Hum Genet.* 2012; 13:241–261. [PubMed: 22703173]
- Wang Y, Lai J, Cui H, et al. Inhibition of histone deacetylase in the basolateral amygdala facilitates morphine context-associated memory formation in rats. *J Mol Neurosci.* 2015; 55:269–278. [PubMed: 24829091]
- Wheeler JM, Reed C, Burkhart-Kasch S, et al. Genetically correlated effects of selective breeding for high and low methamphetamine consumption. *Genes Brain Behav.* 2009; 8:758–771. [PubMed: 19689456]
- Wright KN, Hollis F, Duclot F, et al. Methyl upplementation attenuates cocaine-seeking behaviors and cocaine-induced cFos activation in a DNA methylation-dependent manner. *J Neurosci.* 2015; 35:8948–8958. [PubMed: 26063926]
- Wu Q, Hwang CK, Zheng H, et al. MicroRNA 339 downregulates mu-opioid receptor at the post-transcriptional level in response to opioid treatment. *FASEB J.* 2013; 27:522–535. [PubMed: 23085997]
- Zhong X, Zhang H. Linkage analysis and association analysis in the presence of linkage using age at onset of COGA alcoholism data. *BMC Genet.* 2005; 6(Suppl 1):S31. [PubMed: 16451641]

Fig. 48.1.

The reward circuitry of the brain is similar across species and is activated by drugs of abuse. The major brain regions involved in the mesolimbic reward pathway are depicted in the human (**A**) and rodent (**B**) brain: dopaminergic neurons (green) in the ventral tegmental area (VTA) project to the nucleus accumbens (NAC), prefrontal cortex (PFC), amygdala (AMY), and hippocampus (HPC). The NAC also receives glutamatergic (red) innervation from the PFC, AMY, and HPC. While the mechanisms of action are specific for each drug, most drugs of abuse increase dopaminergic signaling from the VTA to other regions of the reward circuitry. Studies investigating the contribution of genetic factors to the addicted phenotype have focused on identifying markers in vulnerable human subjects that presumably result in altered sensitivity and function of the mesolimbic dopamine system. On the other hand, studies investigating epigenetic mechanisms of drug abuse have focused on the NAC in animal models of addiction, as it is a major region of integration for rewarding stimuli. (Modified with permission from Pena CJ, Bagot RC, Labonte B, et al. (2014) Epigenetic signaling in psychiatric disorders. *J Mol Biol* 426: 3389–3412.)

Fig. 48.2.

Addiction is a complex phenotype that is regulated by both genetic and environmental factors. Information from the environment is recognized by the brain or body and in turn elicits a response, which often involves changes in gene expression, as indicated by the blue arrows. These gene–environment interactions are relayed by epigenetic mechanisms, including chromatin modifications, DNA methylation, and the expression of noncoding RNAs. Vulnerability to substance abuse has both genetic and environmental risk factors that act in concert to produce the phenotype but exposure to drugs of abuse (indicated with the red arrow) is necessary for the behavioral phenotype to emerge. The details of gene–environment interactions through the full life cycle of addiction are highly iterative and remain incompletely understood. *AMY*, amygdala; *HPC*, hippocampus; *PFC*, prefrontal cortex; *SNPs*, single-nucleotide polymorphisms; *VTA*, ventral tegmental area.

Fig. 48.3 and Table 48.1.

Chromatin modifications regulated by drugs of abuse. The illustration (top) indicates histone octamers in a repressive (left) or permissive (right) state. Enzymes involved in maintaining these states and associated transcription factors are indicated and histone tails with specific residues are highlighted as targets of modification: H3 residues subject to methylation or acetylation are indicated in green on the left and H4 residues subject to acetylation are indicated in purple on the right. Table 48.1 lists histone tail modifications of specific residues that are altered in response to drugs of abuse. Arrows indicate an increase (yellow), decrease (blue), or no effect (gray) in specific modifications; Ø indicates that no information is available. *2Me*, dimethylation; *3Me*, trimethylation; *Ac*, acetylation; *Amphet*, amphetamine; *AMY*, amygdala; *Bdnf*, brain-derived neurotrophic factor; *CaMKII*, calcium/calmodulin-dependent kinase II alpha; *Cbp*, CREB-binding protein; *cFos*, FBJ murine osteosarcoma viral oncogene homolog; *CPP*, conditioned place preference; *DG*, dentate gyrus; *Egr1*, early growth response protein 1; *Ehmt2*, euchromatic histone-lysine *N*-methyltransferase 2; *Gria1*, glutamate receptor, ionotropic, AMPA 1; *Gria2*, glutamate receptor, ionotropic, AMPA 2; *Grin1*, glutamate receptor, ionotropic, *N*-methyl-D-aspartate 1; *Grin2b*, glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2B; *LC*, locus coeruleus; *Meth*, methamphetamine; *mPFC*, medial prefrontal cortex; *NAc*, nucleus accumbens; *Nr4a2*,

nuclear receptor subfamily 4, group A, member 2; *Otxr*, oxytocin receptor; *Pdyn*, prodynorphin; *Pnoc*, pronociceptin; *Dlg4*, discs, large homolog 4; postsynaptic density protein 95, PSD-95; *Suv39h1*, suppressor of variegation 3–9 homolog 1; *VTA*, ventral tegmental area; *WD*, withdrawal. (Modified with permission from Vialou et al. (2013).)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 48.2

Methods for studying histone modifications

Method	
ChIP	Involves lightly fixing tissue to cross-link DNA to associated proteins; followed by fragmenting the fixed chromatin by sonication or micrococcal nuclease digestion; and finally, immunoprecipitating the fragmented chromatin with an antibody specific for a given modification of interest (e.g., acetylated or methylated histone)
qChIP	Involves quantifying the amount of immunoprecipitated chromatin at a single genomic region using qPCR analysis
ChIP-chip	Involves quantifying the amount of immunoprecipitated chromatin at thousands of promoter regions using a promoter-specific DNA microarray
ChIP-seq	Involves quantifying the amount of immunoprecipitated chromatin for all genomic regions by high-throughput sequencing ChIP-seq has the additional advantage over ChIP-chip of providing higher basepair resolution for each genomic region
RNA-seq	Involves isolating RNA and converting it to cDNA, followed by high-throughput sequencing RNA-seq has several advantages over older microarray methods, including higher basepair resolution, detection of noncoding RNAs, and the ability to identify alternative splice variants

ChIP, chromatin immunoprecipitation; qChIP, quantitative ChIP; seq, high-throughput sequencing.

Reproduced from Walker et al. (2015).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Histone Acetylation						
Histone Acetyl Transferases (HATs)		Expression changes	Time of Exposure	Behavioral Effect of Inhibition		Behavioral Effect of Overexpression
				inhibition / knockout	activation / overexpression	
Class IV	Sirt 7					
	HDAC 11	↓ mRNA				
General HDAC Inhibitors						
TSA	Expression changes	# of doses HDACi	Behavioral Effect	# of doses agonist	Region	Reference
						Drug
VPA	Expression changes	# of doses HDACi	Behavioral Effect	# of doses agonist	Region	Reference
						Drug
NaB	Expression changes	# of doses HDACi	Behavioral Effect	# of doses agonist	Region	Reference
						Drug
Histone Methylation						
Histone Methyltransferases (HMTs)		Expression changes		activation / overexpression		Reference
				inhibition / knockout		
				Behavioral Effect		
					Drug	

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Histone Acetylation							
Histone Acetyl Transfrases (HATs)							
Expression changes	Time of Exposure	inhibition / knockout	Behavioral Effect of Inhibition	activation / overexpression	Behavioral Effect of Overexpression	Region	Drug
G9a GLP Miti (Mixed- lineage leukemia I)	↓ mRNA expression	Pre-treatment	viral mediated / pharmacological	↑ CPP acquisition		NAc	cocaine/morphine
	↓ mRNA expression	Pre-treatment				NAc	cocaine/morphine
	↑ mRNA in CPP ↓ mRNA in home cage	Pre-treatment	viral mediated	↓ CPP acquisition		NAc	Meth
Histone Demethylases (HDMs)							
Expression changes	Pre-treatment	viral mediated	Behavioral Effect	activation / overexpression		Region	Drug
histone lysine (K)-specific demethylase 5C (KDM5c)	↔ mRNA expression		↓ CPP consolidation			NAc	Meth
Reference							
							Aguilar-Valles, A et al., 2013

The expression changes, brain region, drug of abuse, timing of exposure, type of manipulation, and behavioral outcome are listed for each enzyme. Only those enzymes with available information are included. Pre-treatment indicates that the manipulation occurred at least 24 hours before exposure to drug of abuse. Concurrent indicates that the manipulation occurred between 60 and 0 min before exposure to drug. Post-treatment indicates that the manipulation occurred after exposure to drug. Arrows indicate an increase (yellow), decrease (blue), or no effect (gray).

Amphet, amphetamine; BLA, basolateral amygdala; CBP, CREB-binding protein; CPP, conditioned place preference; G9a, euchromatic histone-lysine N-methyltransferase 2; GLP, G9a-like protein; HC, home cage; KDM5c, lysine (K)-specific demethylase 5C; Meth, methamphetamine; MLL1, myeloid/lymphoid mixed lineage leukemia I; NAb, sodium butyrate; NAc, nucleus accumbens; TSA, trichostatinA; VPA, valproic acid; VTA, ventral tegmental area.

Reproduced from Walker DM, Cates HM, Heller EA, et al. (2015) Regulation of chromatin states by drugs of abuse. Curr Opin Neurobiol 30: 112–121.