Activity-Dependent Epigenetic Remodeling in Cocaine Use Disorder

Alberto J. López, Cody A. Siciliano, and Erin S. Calipari

Contents

A. J. López · C. A. Siciliano
Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA

Vanderbilt Center for Addiction Research, Vanderbilt University School of Medicine, Nashville, TN, USA

Vanderbilt Brain Institute, Vanderbilt University School of Medicine, Nashville, TN, USA

E. S. Calipari (✉)
Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA

Vanderbilt Center for Addiction Research, Vanderbilt University School of Medicine, Nashville, TN, USA

Vanderbilt Brain Institute, Vanderbilt University School of Medicine, Nashville, TN, USA

Department of Molecular Physiology and Biophysics, Vanderbilt Institute for Infection, Immunology, and Infection, Vanderbilt University School of Medicine, Nashville, TN, USA

Department of Psychiatry and Behavioral Sciences, Vanderbilt Institute for Infection, Immunology, and Infection, Vanderbilt University School of Medicine, Nashville, TN, USA
e-mail: erin.calipari@vanderbilt.edu

M. A. Nader, Y. L. Hurd (eds.), *Substance Use Disorders*,
Handbook of Experimental Pharmacology 258, https://doi.org/10.1007/164_2019_257

Abstract

Substance use disorder (SUD) is a behavioral disorder characterized by cycles of abstinence, drug seeking, and relapse. SUD is characterized by aberrant learning processes which develop after repeated exposure to drugs of abuse. At the core of this phenotype is the persistence of symptoms, such as craving and relapse to drug seeking, long after the cessation of drug use. The neural basis of these behavioral changes has been linked to dysfunction in neural circuits across the brain; however, the molecular drivers that allow for these changes to persist beyond the lifespan of any individual protein remain opaque. Epigenetic adaptations – where DNA is modified to increase or decrease the probability of gene expression at key genes – have been identified as a mechanism underlying the long-lasting nature of drug-seeking behavior. Thus, to understand SUD, it is critical to define the interplay between neuronal activation and longer-term changes in transcription and epigenetic remodeling and define their role in addictive behaviors. In this review, we discuss the current understanding of drug-induced changes to circuit function, recent discoveries in epigenetic mechanisms that mediate these changes, and, ultimately, how these adaptations drive the persistent nature of relapse, with emphasis on adaptations in models of cocaine use disorder. Understanding the complex interplay between epigenetic gene regulation and circuit activity will be critical in elucidating the neural mechanisms underlying SUD. This, with the advent of novel genetic-based techniques, will allow for the generation of novel therapeutic avenues to improve treatment outcomes in SUD.

Keywords

Circuitry · Dopamine · Epigenome · Genomics · Motivation · Plasticity

1 Introduction

Substance use disorder (SUD) is a chronic relapsing neuropsychiatric disease, characterized by high levels of drug consumption, inability to terminate drug consumption once started, heightened responsivity to drug-associated cues, drug seeking, craving, and relapse even after long periods of abstinence (Aguilar et al. 2009; Le Moal and Koob 2007). This wide range of symptoms, many of which are driven by deficits in learning and memory processes, are difficult to treat, and patients typically experience remittent symptoms for their entire lifetime. Indeed,

one critical characteristic of this disorder is the persistence of symptoms long after the cessation of drug use. For example, there are high rates of relapse in individuals with SUD, even after years of successful abstinence (de Wit 1996; Jaffe et al. 1989). Yet, the long-lasting mechanisms that confer resilience or susceptibility to drug seeking and craving remain only partially identified. Thus, understanding the factors that confer these long-lasting behavioral deficits is critical to understand both the pathology of the disorder and to developing efficacious evidence-based therapies.

To this end, it is paramount to understand the molecular dysfunction that underlies the behavioral dysregulation in SUD. Preclinical models utilizing drug self-administration in animals have recapitulated many of the behavioral phenotypes associated with SUD, and a large body of work has focused on the molecular dysregulation that underlies these behaviors (White et al. 2016; Maze et al. 2010; Stipanovich et al. 2008; Malvaez et al. 2011; Smith et al. 2013). Cocaine-induced changes in neuronal gene expression, molecular and cell signaling, and plasticity have been implicated in driving the behavioral symptomatology of SUD (Beitner-Johnson and Nestler 1991; Kuhar et al. 1991; Anderson and Pierce 2005). However, while each of these factors has been causally linked to particular behavioral phenotypes, compiling these discoveries into a comprehensive framework has been lacking. The development of SUD is controlled by drug-induced alterations in neural circuit activity, which lead to complex changes in transcriptional and receptor-based signaling, which then drive persistent neural plasticity changes that change the brain's subsequent response to drugs and other environmental stimuli (Le Moal and Koob 2007; Hyman et al. 2006; Volkow et al. 2003; Campbell and Wood 2019; Calipari et al. 2019). Here we discuss the current literature and highlight the importance of understanding the bidirectional interaction between neural circuit activity, epigenetic/transcriptional mechanisms, and behavior.

The behavioral symptomology of SUD persists long beyond a transient drug-induced change in neural activity, and even past the turnover of many of the proteins that have been implicated in this disorder; however, our understanding of long-term mechanisms that could drive these seemingly indefinite changes are lacking. Recently, epigenetics has become an avenue of interest with regard to potential mechanisms underlying the long-lasting nature of SUD (Walker et al. 2015; Robison and Nestler 2011). Although historically defined as the heritable interaction between genes and gene products that generate cell fate, as the neuroepigenetic field has developed, the term now refers to changes in gene regulation independent of changes in the DNA sequence itself (Barrett and Wood 2008; Rudenko and Tsai 2014). Epigenetic factors allow for dynamic and stable regulation of gene expression and are emerging as key mechanisms underlying long-lasting changes in neural morphology and function in postmitotic neurons. Several drug-induced changes to neuronal function have been linked to recruitment of various epigenetic mechanisms, including changes in histone posttranslational modifications, nucleosome remodeling, and DNA methylation (White et al. 2016; López et al. 2018; Malvaez et al. 2018; Levine et al. 2011; Vaillancourt et al. 2017).

In this review, we will further assess the epigenetic and circuit-based changes that underlie the alterations in learning and motivation that characterize SUD. We further review the known adaptations that occur at the neural circuit, synaptic, cellular, and epigenetic levels and how these adaptations interface to drive relapse of cocaine-

seeking behavior. The aforementioned avenues of research are often studied independently and in parallel of each other. While this strategy is effective in identifying mechanistic contributors, it fails to encapsulate the long-lasting nature of SUD. As such, SUD is unlikely caused by a single kinase, synapse, receptor, histone mark, or transcription factor. Likely, the resilience and long-lasting nature of drug-seeking behavior is a culmination of changes in synaptic function leading to changes in nuclear processes that ultimately provide a feedback loop to future changes in circuit activity and behavior. Interdisciplinary approaches to understanding the neural control of behavior – where molecular, circuit, and behavioral avenues intersect – will generate a more complete understanding of SUD. Lastly, we evaluate emerging molecular- and circuit-level technologies and their potential to re-commandeer endogenous mechanisms to reverse the drug-induced adaptations which leave individuals susceptible to relapse.

2 Synaptic Mechanisms of Substance Use Disorder

Drug-induced alterations in synaptic function have been the primary focus of neuroscience research into SUDs, and we now have an in-depth understanding of the synaptic changes that occur and how these changes relate to addictive behaviors (Ungless et al. 2001; Jones and Bonci 2005; Thomas et al. 2001; Wolf 2016) (Fig. 1). Perhaps the most studied circuit in this body of literature is the mesolimbic dopamine (DA) pathway, comprising of dopaminergic projections from the ventral midbrain to the ventral striatum [also known as the nucleus accumbens (NAc) (Siciliano et al. 2015; Ferris et al. 2013)]. After cocaine exposure, several groups have reported increased AMPA/NMDA ratios in dopaminergic neurons in the ventral tegmental area (VTA), as well as increased expression of high-conductance GluA2-lacking AMPA receptors in D1-expressing medium spiny neurons (MSNs) in the NAc (Ungless et al. 2001; Thomas et al. 2001; Conrad et al. 2008; Loweth et al. 2014). Paradoxically, while there is increased excitability of DA neurons, DA release probability measured at presynaptic terminals in the NAc is markedly decreased (Siciliano et al. 2015). Together, impaired dopaminergic modulation of postsynaptic activity combined with hyperexcitability of D1-expressing MSNs drives several aspects of addictive behaviors, most notably time-dependent increases in cocaine-conditioned reinforcement, whereby cocaine-associated cues trigger seeking behaviors which become more robust farther into abstinence from cocaine (Calipari et al. 2019; Wolf 2016; Childress et al. 1999).

This effect, termed "incubation of cocaine craving," becomes stronger through 30 days into withdrawal from cocaine and persists for a seemingly indefinite period of time (Grimm et al. 2001). Interesting, the synaptic alterations that underlie these effects persist far past the turnover half-life of any of the specific proteins involved (Calipari et al. 2019; Horikawa and Nawa 1998). These synaptic changes are cell-type specific, suggesting that the epigenetic factors driving these changes do not happen on a global scale (Pascoli et al. 2014; MacAskill et al. 2014), but can be different – even opposite – depending on the neural circuits being altered in these

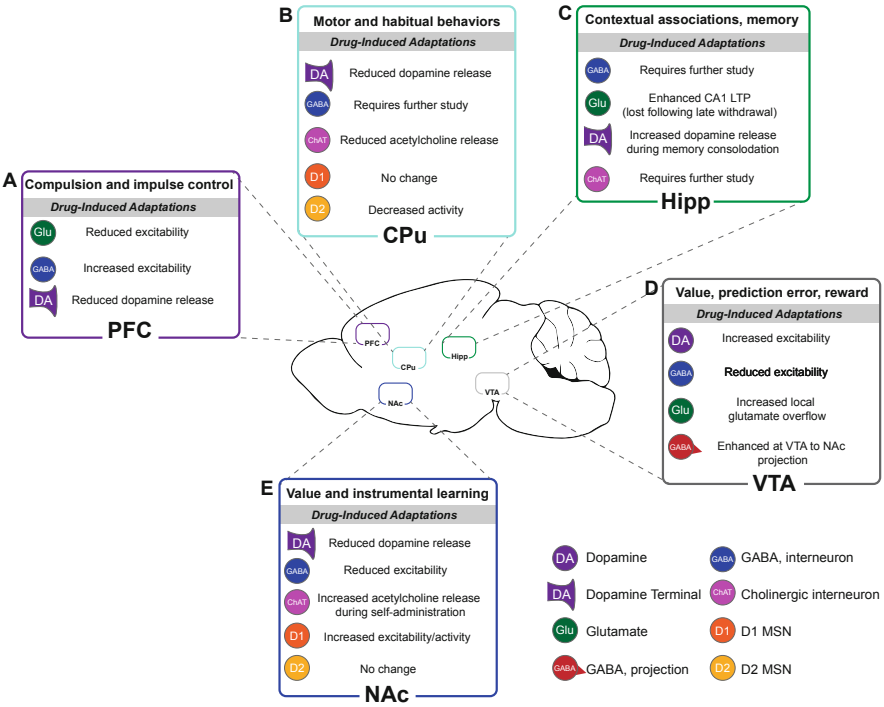

Fig. 1 Drug exposure drives long-lasting alterations in neural circuits in a cell-type-specific fashion. Drug-induced adaptations within defined brain regions are involved in a wide range of behavioral processes including executive control (PFC, **a**) (Sorg et al. 1997; Campanac and Hoffman 2013; Williams and Steketee 2005; Trantham et al. 2002; Cameron et al. 2019); habit formation (CPu, **b**) (Hurd et al. 1990; Park et al. 2013; Yager et al. 2015); drug-associated context and memory (Hipp, **c**) (Kutlu and Gould 2016; Kramar et al. 2014); reward prediction and value (VTA, **d**) (Bocklisch et al. 2013; Kalivas and Duffy 2002; Liu et al. 2005); and reward and contextual integration (NAc, **e**) (Smith et al. 2013; Siciliano et al. 2015; Mark et al. 1999, 2011). The cell-type-specific changes in activity that have been reported across the brain and are outlined in a region-specific fashion. Within each region, there is heterogeneity of cell types, each contributing differentially to behavioral outputs, making understanding the cell-type-specific epigenetic adaptations underlying these alterations critical. A single molecular change within a defined brain region may have opposite effects on behavior. For example, in the NAc, changes that increase the activity of D1 or D2 MSNs (*orange* and *yellow*, respectively), have opposite effects on reward-associated behaviors. Further, within this region, different molecular adaptations have been observed in cholinergic interneurons (*magenta*), D1 MSNs, D2 MSNs, and at dopamine terminals from the VTA – all of which have been implicated in cocaine-seeking behavior. The above circuitry only serves as an overview of the major circuitry linked to cocaine seeking. However, various other brain regions have been linked to the chronic nature of cocaine-associated behaviors, including the medial habenula (López et al. 2018, 2019; McCallum and Glick 2009), the ventral pallidum (Farrell et al. 2019; Mahler et al. 2014; Pardo-Garcia et al. 2019), and lateral hypothalamus (Ahmed et al. 2005; Zhou et al. 2008; Boutrel et al. 2005). Together, while the field has identified epigenetic and transcriptomic changes in these brain regions, it will be critical to characterize the epigenetic adaptations within specific cell types to understand how these changes are linked to the specific molecular changes that underlie the neural control of discrete aspects of motivated behaviors. *CPu*, caudate-putamen; *Hipp*, hippocampus; *MSN*, medium spiny neuron; *NAc*, nucleus accumbens; *PFC*, prefrontal cortex; *VTA*, ventral tegmental area

processes. While the long-lasting nature of these alterations are a hallmark of drug exposure and exemplify the protracted pathology of human SUD, we have a limited understanding of the cellular mechanisms that continue to propagate these alterations as receptors are replaced with newly folded proteins that have not yet interacted with cocaine. The long-lasting changes in synaptic function clearly implicate underlying epigenetic changes that alter receptor expression indefinitely, but linking drug-induced epigenetic changes to specific alterations in synaptic function remains poorly understood. Elucidating these mechanisms may allow for therapeutic interventions that restore normal function without targeting the receptors directly, thus limiting off-target and unwanted effects.

While synaptic remodeling is often discussed in the terms of the mesolimbic DA system, it is important to understand that even within a single brain region, cell-type- and synapse-specific plasticity are critical mediators of motivated behaviors (see Fig. 1). In the context of molecular dysregulation, it is critical to understand how these genetic changes in specific cell types alter the expression of reward-related behaviors. For example, the NAc is a heterogeneous region made up of various cell types that contribute to cocaine-maintained behaviors. Of the total number of cells in the rodent NAc, 95% of them are made up of MSNs, which contain D1 and D2 type dopamine receptors (Kupchik et al. 2015). D1 and D2 MSNs are virtually nonoverlapping cell types that have opposing roles in response to cocaine reward, with D1 encoding reward-based information, and D2 MSNs limiting reward-driven behavior (Le Moine and Bloch 1995). D1 MSN responses to cocaine-associated cues are critical to drug seeking, and optical stimulation of D2 MSNs reduces cocaine self-administration (Bachtell and Self 2008; Kravitz et al. 2012). Moreover, the molecular mechanisms and cocaine response within these two cell populations are often divergent, due to the contrasting effects of D1 vs D2 receptor activation. Specifically, D1- and D2-expressing MSNs have unique basal gene expression patterns that contribute to their respective genetic identity (Chandra et al. 2015). Moreover, acute cocaine and cocaine-associated cues increase activity in D1-expressing MSNs, while leading to hyperpolarization of D2-expressing MSNs (Calipari et al. 2016; Bertran-Gonzalez et al. 2008; Jordi et al. 2013). The downstream molecular adaptations to cocaine within these cell populations also diverge, including DARPP-32 phosphorylation and various CREB-dependent gene expression patterns (Chandra et al. 2015; Bateup et al. 2008).

In addition to these output neurons, there are also GABAergic and cholinergic interneurons that regulate both DA release from presynaptic DA terminals originating in the VTA and modulate the activity of MSNs (Fig. 1) (Collins et al. 2016). Until recently, technical limitations have prevented the identification and characterization of cocaine response in each of these cell types. However, the advent of cell-type-specific assays, such as fluorescence-activated cell sorting (FACS), translating ribosomal affinity purification (TRAP), single-cell RNA sequencing, and Cre-expressing mouse lines (Chandra et al. 2015; Finegersh and Homanics 2016), makes a more thorough assessment of each cell type's contribution to cocaine-seeking behavior possible. Because of the different, often opposing, roles of each of these cell types in drug-associated behavior, it is important to understand

how epigenetic regulation of activity within each population can alter behavioral outputs.

3 The Interface Between Neural Activity and Epigenetic Modifications in Substance Use Disorder

As mentioned above, SUD research has focused on the neural circuit dysfunction induced by drugs of abuse. Pinpointing the interface between neural activity, transcription, and epigenetic processes has been difficult due to the complex technical nature of these studies. SUD is a learning disorder where particular actions and outcomes [i.e., taking drug and the associated high (Hyman et al. 2006; Itzhak and Martin 2002; Mews and Calipari 2017)] are associated with cues or contexts. Enhanced activation of brain reward systems by these cues is a key feature that drives relapse to drug use (MacNiven et al. 2018). Underlying this phenomenon is the ability to learn information about environmental stimuli which relies on experience-dependent plasticity, where experience with a stimulus changes subsequent neural responses to that stimulus. These neural changes need to be plastic, respond quickly to new information in the environment, and be long-lasting to maintain these memories over time (Mews and Calipari 2017). While learning induced by natural reinforcers is critical to survival in mammalian species, dysregulation of these processes by drugs of abuse drives addictive behaviors. Further, while not an emphasis of this review, it is important to consider that when evaluating the molecular and synaptic responses in cocaine-induced behaviors, the route, dose, and schedule of cocaine exposure are critical factors in the subsequent neural response and drug-induced maladaptation, thus adding a layer of complexity into defining the mechanisms driving drug-induced plasticity (Calipari et al. 2013, 2014, 2015; Calipari and Jones 2014; Kawa et al. 2019).

At the heart of experience-dependent plasticity lies the capacity of neural circuits to undergo activity-induced structural and functional changes. In SUD, this process happens in two phases: first, drugs activate or inhibit certain neural circuits which leads to the induction of epigenetic and transcriptional changes within defined neural populations. Second, these epigenetic modifications either serve as a scaffold for more long-lasting changes or act themselves to change the way that these cells respond to subsequent drug-associated stimuli (see Fig. 2). Here, we will primarily focus on the role of the second phase of this process in addictive behaviors. The maintenance of such permanent changes requires efficient posttranslational and transcriptional regulation. A large body of work has defined the importance of both changes in neural circuit dynamics and epigenetic regulation in the expression of reward-related behaviors (Russo et al. 2010; Russo and Nestler 2013; Dudai and Morris 2013). The ability of a cell to efficiently activate transcriptional processes in response to an incoming stimulus is controlled by epigenetic regulation, where the structure of and accessibility to DNA is modified to increase or decrease the probability of gene expression at key genes. This is executed by altering the interactions between the genome and regulatory mechanisms at the level of chromatin. Chromatin is the focal point of transcriptional gene regulation and is comprised

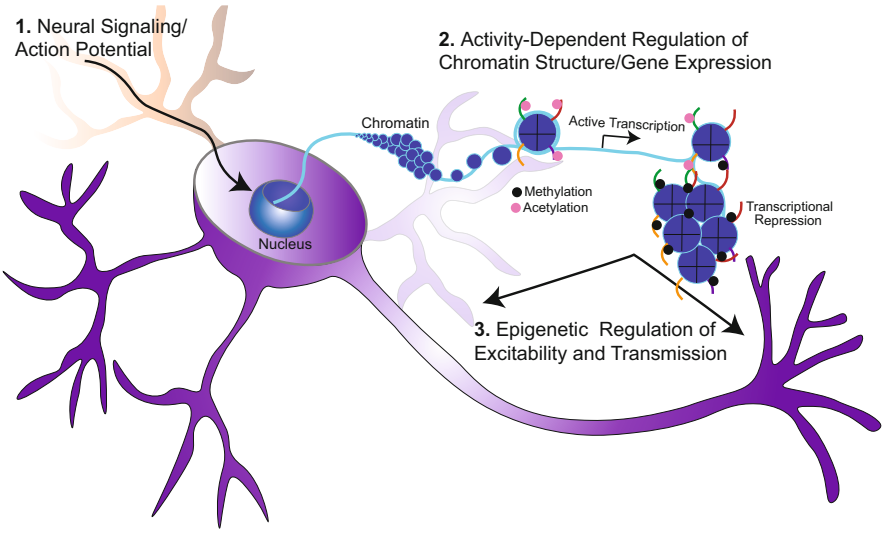

Fig. 2 The bidirectional interaction between epigenetic changes and synaptic function. Neurons are complex information processors that are capable of integrating various inputs to ultimately generate downstream signals. (1) Incoming signals are transmitted from and extracellular signal through receptors and channels and their associated intracellular effectors that converge at the nucleus. (2) These internal molecular cascades trigger activity-dependent changes in transcription within the nucleus. (3) In addition to the acute induction of immediate early genes that leads to stabilized signal processing (e.g., plasticity, LTP), DNA (*teal*) and histone proteins (*blue*) can be modified (*pink* and *black*) to alter future response to activity and drive long-lasting changes in neural excitability and signaling. In the case of drugs of abuse, such as cocaine, repeated drug exposure alters basal gene expression and circuit-specific excitability via widespread epigenetic changes

of a basic repeating unit: the nucleosome. The nucleosome consists of DNA wrapped around a protein octamer, assembled from two molecules each of histone H2A, H2B, H3, and H4 (Fig. 3a). Each histone has tails of amino acids that can be modified allowing for a complex mosaic of chemical modifications – i.e., epigenetic marks – that can dynamically regulate chromatin architecture and subsequent gene transcription (Barrett and Wood 2008; Rivera and Ren 2013). Together these processes are termed the epigenome and serve as the interface between the genome, cellular activity, and the environment.

While basic epigenetic mechanisms controlling transcription have been well described in recent years (see Table 1), how activity-dependent changes within defined cell types interface with epigenetic modifications to guide behaviors remains a major outstanding question in the field. In general, information is transmitted from a synaptic signal, in the form of an action potential or receptor activation, to the nucleus to trigger molecular machinery for epigenetic remodeling on a fast timescale (outlined in Fig. 2). This signal activates a number of immediate early genes as well as other transcriptional processes that alter DNA conformation in order to change the expression levels of key genes (Brami-Cherrier 2005; Ressler et al. 2002). In this

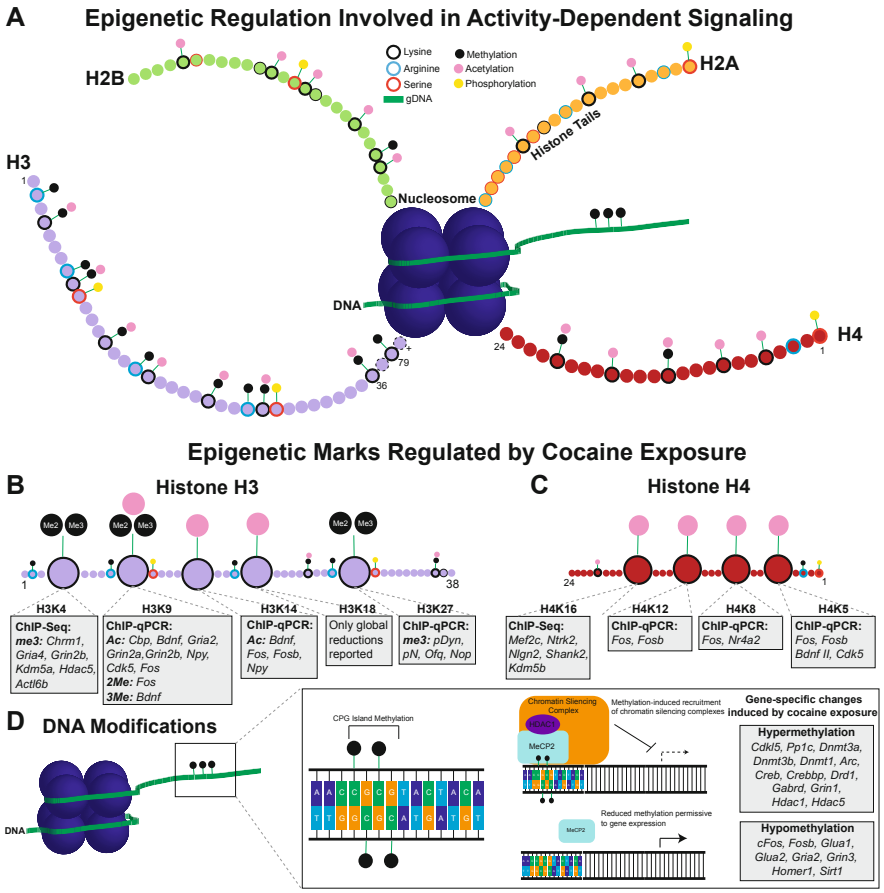

Fig. 3 Epigenetic modifications regulated by cocaine exposure. Cocaine-induced neuronal activation leads to rapid changes in chromatin structure. (**a**) The nucleosome, the functional subunit of chromatin, is composed of 147bp of DNA (*green*) spooled around a histone octamer core (*blue*). In addition to direct DNA methylation (*black*), each histone protein can be modified at stereotyped residues, such as lysine (K, *black circle*), arginine (R, *blue circle*), or serine (S, *red circle*), in an activity-dependent manner to regulate gene expression. Cocaine alters circuit function by generating a unique epigenetic environment at targeted gene loci. Recent work from the neuroepigenetic field has identified cocaine-induced modifications at histone H3 (**b**) (Jordi et al. 2013; Kumar et al. 2005; Wang et al. 2009; Renthal et al. 2009; Farris et al. 2015; Renthal and Nestler 2008; Maze et al. 2011; Feng et al. 2014; Freeman et al. 2008; Caputi et al. 2014), histone H4 (**c**) (Malvaez et al. 2011; Jordi et al. 2013; Kumar et al. 2005; Levine et al. 2005; Renthal et al. 2007, 2009; Rogge et al. 2013; Ferguson et al. 2015), the recruitment of DNA methylation machinery (**d**) (Vaillancourt et al. 2017; Wright et al. 2015; Anier et al. 2010; Massart et al. 2015; Carouge et al. 2010; Pol Bodetto et al. 2013; Baker-Andresen et al. 2013), and the synaptic and plasticity-related gene loci targets. It is critical to note that no single histone mark regulates cocaine-induced gene expression, but it is the overall epigenetic environment that can regulate the suppression or expression of a gene. Similar to neural activity, these various epigenetic modifications are integrated to generate an overall permissive or repressive gene expression environment. Understanding the combinatorial effects of these individual histone marks will be critical in studying the long-lasting nature of cocaine-induced changes to the epigenome, transcriptome, and, ultimately, circuit function. *Me2*, dimethylation; *Me3*, trimethylation

Table 1 Commonly studied epigenetic mechanisms and their respective transcriptional impact

Chromatin modifications in neuronal gene regulation				
Modification	Known regulatory enzyme(s)		Target(s)	Transcriptional effect(s)
DNA methylation	*Writers* DNMT1-3a,3b	*Erasers* TET1-3	CpG DNA	Repressive (proximal promoter) Permissive (gene body)
Histone acetylation	*Writers* CBP/KAT3A p300/KAT3B PCAF/KAT2B GCN5/KAT2A	*Erasers* HDAC1-3 HDAC8 SIRT1-3 SIRT6-7	H1 H2A H2B H3 H4	Permissive
Histone methylation	*Writers* SET-domain containing (14 members) KMT1C/G9a/ EHMT2 KMT1D/GLP/ EHMT1 SUV39H1/ KMT1A ASH1L/KMT2H DOT1L/KMT4	*Erasers* KDM1A/LSD1 JmjC family (17 members, see Agger et al.)	H1 H2A H2B H3 H4	*Mono-methylation:* Permissive *Dimethylation:* H3K4, -K79 permissive H3K9, H3K27 repressive *Trimethylation:* H3K4, H3K79 permissive H3K9, H3K27, H3K79 repressive
Histone phosphorylation	*Writers* MSK1/2 ERK1/2 JAK2	*Erasers* PP1 PP2A	H1 H2A H2B H3 H4	Permissive (indirectly) (enables acetylation prevents methylation)
Histone ribosylation	*Writers* PARP1, PARP3/ ARTD1, RTD3 SIRT2	*Erasers* ARH3 PARG	H1 H2A H2B H3 H4	Permissive
Histone serotonylation	*Writers* TGM2	*Erasers* Unknown	H3	Permissive
Histone SUMOylation	*Writers* SUMO1 SUMO2	*Erasers*[a] SENP2–3 SENP5–6	H1 H2A H2B H3 H4	Repressive
Histone ubiquitination	*Writers* RING1 UBE2D1 UBE2B RNF40 BARD1	*Erasers* USP2, USP3, USP7, USP12/ 46, USP16, USP21,USP22, USP36 BAP1	H1 H2A H2B H3 H4	*UBE2B, RNF40, USP family, BAP1* Permissive *RING1, BARD1* Repressive

(continued)

Table 1 (continued)

Chromatin modifications in neuronal gene regulation				
Modification	Known regulatory enzyme(s)		Target(s)	Transcriptional effect(s)
Nucleosome remodeling	*Complex*	*Relevant subunits*	H1 H2A H2B H3 H4 gDNA	Both repressive and permissive
	nBAF	*nBAF:* BAF53B/ ACTL6B CREST BAF45B/C		
	ISWI-containing complexes	*ISWI:* BAZ1A SMARCA1/ SNF2L, SMARCA2/ SNF2H		
	NuRD	*NuRD:* HDAC1/2 Mi-2a/b, MTA1/2/3 RbAp46/48		

Various epigenetic mechanisms have been identified as critical in neuronal gene expression and are highlighted above, including (1) DNA methylation (Vaillancourt et al. 2017), (2) histone acetylation (Gräff and Tsai 2013; Peixoto and Abel 2012; de Ruijter et al. 2003; Barnes et al. 2019; Fischle et al. 2002), (3) histone methylation (Agger et al. 2008; Roidl and Hacker 2014), (4) histone phosphorylation (Watson and Higgins 2016), (5) histone ribosylation (Messner and Hottiger 2011; Hassa et al. 2006), (6) histone serotonylation (Farrelly et al. 2019), (7) histone SUMOylation (Shiio and Eisenman 2003; Nathan et al. 2003), (8) histone ubiquitination (Cao and Yan 2012), and (9) nucleosome remodeling (López and Wood 2015; Sun et al. 2017; Goodwin and Picketts 2018). Each identified PTM is regulated by a unique family of enzymes, providing a unique profile of transcriptional regulation. See Zhao and Garcia (2015) for a catalog of discovered histone modifications (in neuronal and non-neuronal tissue) (Zhao and Garcia 2015)

[a]Indicates presumptive regulator

view, epigenetic regulation arbitrates acute and transient gene expression in response to upstream neural activity and changes in intrinsic cellular processes (Campbell and Wood 2019). Thus, repeated and sustained firing of cells in a circuit can trigger specific changes to denote the importance of that information. Circuit activity triggers intracellular signaling cascades such as the PKA or MAPK/ERK pathways that are activated by G protein-coupled receptors and calcium and therefore provides a means for information carried in circuit activity to be transmitted to the nucleus (Ménard et al. 2015). In the nucleus, epigenetic signatures demarcate and regulate genes associated with synaptic remodeling, associative processes, and memory formation (Dulac 2010; Sultan and Day 2011). These changes can alter the excitability of the cell and change its ability to respond to subsequent incoming signals. Remarkably, but not surprisingly, the plasticity mechanisms linked to drug addiction correspond to well-described neuronal and circuit plasticity in learning and memory (see Fig. 1). This process is the primary molecular mechanism underlying

learning-related plasticity in postmitotic neurons. Thus, understanding how this process is dysregulated in SUD will shed light onto the basic process, its importance in behavioral control, and provide potential targets for treatment.

4 Epigenetic Regulation of Neural Plasticity

When studying activity-dependent transcription, the key regulators are often immediate early genes, which are activated shortly after stimulation of the cell. It is tempting to attempt to identify a single gene that controls the addictive phenotype; however, the complex nature of a disorder involving learning mechanisms makes it more likely that a complex network of interconnected genes regulates the connectivity of neurons across the brain. In fact, it is likely that many of the immediate early genes that are identified as key players in addictive behaviors are simply the first-in-line transcriptional responses that start a string of events that alters DNA accessibility and neural circuit function (Zhao et al. 2014). The behavioral, circuit, and morphology data point to one important adaptation in drug-addicted individuals. Specific synapses are strengthened, while others are weakened for long periods of time that last far beyond the lifespan of any individual protein (McPherson 2015). These long-lasting changes in synaptic strength are critical mediators of drug seeking and relapse; thus, understanding how they are maintained is of critical importance to our understanding of the brain and how it is dysregulated in SUD (Ungless et al. 2001; Thomas et al. 2001; Siciliano et al. 2015; Conrad et al. 2008; Loweth et al. 2014).

4.1 Histone Posttranslational Modifications, DNA Methylation, and Nucleosome Remodeling

Neurons continually adapt to a changing environment and thus require systems that quickly adjust chromatin structure, transcription, and subsequent cellular excitability. This is done via complex changes in receptor membrane expression, phosphorylation, and epigenetic changes that are transient, such as histone acetylation or phosphorylation, or more long-lived, such as specific histone methylation and DNA methylation, and both of these processes are dysregulated in SUD (Campbell and Wood 2019; Robison and Nestler 2011; Rudenko and Tsai 2014; Fass et al. 2013). Indeed, several types of epigenetic modifications have been associated with learning and memory, including DNA methylation, and posttranslational modification of histone proteins by acetylation, methylation, and phosphorylation (Dulac 2010; Alarcón et al. 2004; Gräff and Tsai 2013; Korzus et al. 2004; Levenson et al. 2004; Wood et al. 2006; Nelson and Monteggia 2011). Importantly, modification of the epigenetic landscape provides a mechanism by which the transcriptional response to stimuli can be permanently altered, thus providing a molecular route to lasting modifications of neuronal and circuit functions such as the expression of SUD.

As mentioned above, central to epigenetic regulation of gene expression is the nucleosome: a complex system upon which posttranslational modifications control rapid or sustained changes in DNA accessibility (Fig. 3). Each histone within the nucleosome core has tails of amino acids that can be modified (Rivera and Ren 2013) (see Fig. 3 and Table 1). The interaction between histone tails and DNA has a profound influence on gene accessibility and transcription. This interaction can be transiently modified via various posttranslational modifications (PTMs), including phosphorylation, acetylation, ubiquitination, serotonylation, methylation, or physical sliding of nucleosomes via nucleosome remodeling complexes (referred to as nucleosome remodeling) (Kouzarides 2007; Berger 2007; Farrelly et al. 2019). In addition, DNA itself can be directly modified through the addition and removal of methyl groups. Each of these modifications can provide a different regulation of gene accessibility, either restricting or enabling transcription to occur (see Table 1). Histone PTMs elicit structural and functional changes within chromatin and regulate various epigenetic processes. Acetylation, for instance, along with methylation, is the most extensively studied histone modification and has broad effects on chromatin function and nuclear signaling pathways (Roth et al. 2003; Shahbazian and Grunstein 2007; Berndsen and Denu 2008). Each of these epigenetic modifications, and their role in transcriptional regulation, is described below and in Table 1.

4.2 Histone Acetylation

Histone acetylation is one of the most extensively studied PTMs. Addition of acetyl groups to lysine residues on histone tails is generally considered to be permissive for gene expression through relaxation of the histone protein-DNA interaction. The reduced histone-DNA interaction is thought to allow access to subsequent transcriptional regulators (e.g., transcription factors, RNA polymerase II). Activity-dependent histone acetylation is known to be regulated by two competing families of epigenetic writers: histone deacetylases (HDACs) remove acetyl groups from lysine residues, while histone acetyltransferases (HATs) add acetyl groups to histone tails. Generally, HDACs are considered transcriptional repressors, functioning as molecular brake pads to gene expression, whereas HATs are viewed as transcriptional primers, priming transcriptional activity through permissive histone acetylation (Roth et al. 2003; Shahbazian and Grunstein 2007). Additionally, histone acetylation marks can be bound by small protein modules called bromodomains, often referred to as "readers." These domains are conserved within many chromatin-associated proteins – including HATs themselves – that regulate transcription-mediated biological processes (Bannister and Kouzarides 2011; Burdge and Lillycrop 2010; Filippakopoulos and Knapp 2014; De La Cruz et al. 2005). Histone acetylation in particular has spurred considerable interest and is most robustly associated with promoting associative learning and memory formation, which, as discussed previously, is one of the critical learning processes dysregulated in SUD.

4.3 Histone Methylation

Another extensively studied histone PTM critical in regulating gene accessibility is histone methylation. Histone methylation is concentrated on lysine residues of the histone tail (Zhang and Reinberg 2001), positively regulated by lysine methyltransferases (KMTs) and negatively regulated by lysine demethylases (KDMs). However, single residue methylation can occur in mono- (me), di- (me2), or tri-methylated (me3) stages (Kouzarides 2007). Moreover, these multiplexed methylation states are capable of exerting influence of gene expression and are regulated by independent enzymes. For example, G9a (renamed KMT1C) is able to lay mono- and dimethyl marks but appears unable to trimethylate histone residues. In addition, the mechanism by which histone methylation alters chromatin accessibility remains unclear, as methylation-state regulation of gene expression occurs in a residue specific manner. For example, H3K9me2/3 is generally considered to be a repressive mark, whereas H3K4me3 is often associated with gene activation (Santos-Rosa et al. 2002; Baylin and Ohm 2006).

4.4 Nucleosome Remodeling

Nucleosome remodeling is an often-overlooked posttranslational modification, in which large nucleosome remodeling complexes slide or evict nucleosomes to alter large-scale chromatin structure. As such, nucleosome remodeling can be considered both permissive and repressive epigenetic modifications, as nucleosome remodeling complexes (NRCs) can simultaneously increase accessibility of particular genes while decreasing accessibility of others. Neuronal Brg1-/hBrm-associated factor (nBAF) is the primary NRC in the brain and is composed of various proteins containing either nucleosome or DNA-dependent ATPase function (Table 1) (Staahl and Crabtree 2013; López and Wood 2015; Vogel-Ciernia and Wood 2014). While nBAF is the most extensively studied NRC, both ISWI and NuRD NRCs play key roles in neuronal gene regulation.

Critically, these epigenetic mechanisms do not occur in isolation and modify both transcriptional activity and the enzymatic function of other epigenetic modifiers. For example, the NuRD complex consists of HDAC1/HDAC2 and is capable of simultaneously deacetylating histone residues and remodeling nucleosome structure. Interestingly, subunits within nBAF have histone reading bromodomains, and subunits with ISWI complexes carry histone-interactive SANT domains, suggesting both nBAF and ISWI use histone modification states to further regulate gene accessibility. However, a complete understanding of these mechanisms in neuroscience remains lacking.

4.5 DNA Methylation [For More Detailed Review on DNA Methylation in Cocaine Use Disorders, See Vaillancourt et al. (2017)]

DNA methylation is the stable addition of a methyl group to a nucleotide, most often in the form of 5' methylated cytosine (5mC) (Bird 2002). 5mC is typically added by methyl-CpG-binding domain (MBDs) proteins, of which MECP2 has been most extensively studied, whereas demethylation is carried out via DNA methyltransferases (DNMTs). Previously believed to function purely as a gene repressor, the role of DNA methylation in transcriptional regulation has been further defined: when present in the gene promoter, DNA methylation typically represses genes through the recruitment of HDACs to deacetylate nearby histone tails while simultaneously preventing transcription factor binding; however, when present in the gene body, recent data suggests it may function as a gene activator (Jones 2012; Baubec and Schübeler 2014; Wolf et al. 2006).

4.6 Histone Marks Do Not Occur in Isolation

These various epigenetic mechanisms form a powerful system of regulating gene expression. While each modification is often studied in isolation and has been individually linked to changes in transcription, it is critical to be cognizant of the fact that these modifications, and their regulators, function in concert with each other. Similar to how individual neurons are able to integrate various, often conflicting, circuit inputs, the transcriptional machinery within the nucleus must be able to integrate a dynamic epigenetic landscape to ultimately drive or repress gene expression. As no single gene can be used as a readout for the activity and function of a neuron, no single epigenetic modification can provide an accurate readout for the transcriptional landscape for a given gene. As such, recent work has sought to identify specific patterns of histone modifications and the subsequent transcriptional effect at single gene loci (Tweedie-Cullen et al. 2012; Karch et al. 2013). Moving forward the addiction field should begin to link how these epigenetic marks collectively form an epigenetically permissive or repressive environment and how drugs of abuse mediate their transcriptional signatures through collective changes in histone modifications.

The various epigenetic writers and erasers often directly compete for influence, as they often share gene targets and residues for regulating PTMs and other epigenetic marks. More so, their role in activity-dependent gene expression has been extensively reviewed elsewhere and will not be a focus on this review (Barrett and Wood 2008; Sultan and Day 2011; Peixoto and Abel 2012). However, mounting evidence over the past two decades has provided key insights into how drugs of abuse, such as cocaine, are able to recruit or disengage these epigenetic writers to alter gene expression. Together, these epigenetic modifications can regulate various aspects of cellular function and, in neurons, are regulated by activity-dependent processes that alter the neuronal responses to subsequent stimuli. Their role in drug-dependent plasticity that leads to addictive behaviors is critical and underlies the long-lasting synaptic plasticity that is important in

SUD. As mentioned above, these marks allow for persistent upregulation or downregulation of genes involved in neural activity. However, as mentioned previously, at what genes these epigenetic marks occur and which cell types in which they occur are critical determinants in the role they have on behavior. Thus, increases in transcription in a reward-related brain region can alter behavior differentially depending on the cell type in which they are expressed. Understanding how epigenetic modifications that maintain this synaptic plasticity will be critical in understanding how these modifications occur and how they maintain synaptic changes that drive behavior.

5 The Interplay Between Acute Drug Effects and Activity-Dependent Epigenetic Dysregulation in the Transition to Substance Use Disorder

Within epigenetic marks it is important to consider how they were induced and what their ultimate role is on neural activity. Epigenetic modifications serve two major functions in differentiated neurons. First, they act to determine which genes are upregulated on a transient timescale upon cellular activation – for example, after acute drug exposure. Second, they act to control stable gene expression on a timescale that extends beyond the initial transient signal – i.e., changes that are seen during long-term withdrawal. The interplay between these two classes of epigenetic modifications is relatively unstudied. Thus, better insight into how drug-induced transient changes in chromatin structure lead to stable and long-lasting epigenetic regulation of gene expression is needed.

The interplay between quick temporally specific neuronal activation and longer-term changes in transcription is critical in the expression of appropriate, or in the case of SUD, inappropriate behaviors. The first step in drug-induced epigenetic remodeling relies on the actions of drugs on reward pathways within the brain. The reinforcing properties of drugs of abuse, such as cocaine, are attributed to their ability to induce changes in neural activity throughout the central nervous system. In particular, cocaine alters neuronal activity throughout the mesolimbic pathway by blocking the dopamine transporter and thus increasing DA levels (Chen et al. 2006). These acute drug effects are rapid and mediate the "high" induced by the drug and act to promote drug seeking in the future (Volkow et al. 1997). These drug-induced increases in neurotransmitter levels happen on the order of seconds and are faster than any specific transcriptional initiation event and, thus, are the first step in driving drug-induced transcriptional dysregulation (Yorgason et al. 2011). Transient increases in neural activity, neurotransmitter release, and signaling converge to subsequently drive the epigenetic remodeling that occurs following drug exposure. These transcriptional/epigenetic changes are induced by action potential and calcium- or G-protein-dependent signaling cascades. Neural activity signals can trigger chromatin remodeling via the calcium-/calmodulin-dependent kinase II (CaMKII), which becomes activated upon cellular depolarization and influx of calcium. CaMKII stimulates transcription of BDNF, a well-known neurotrophin involved in neuroplasticity, by phosphorylating and thus releasing the DNA methylation "reader" methyl CpG-binding protein 2 (MeCP2), a highly abundant

chromosomal protein within the brain, from its promoter (Im et al. 2010; Nott et al. 2016; Zhou et al. 2006). This process has been shown to be highly involved in addictive behaviors (Bali et al. 2011). The activation of G protein-coupled receptors can induce similar effects via effectors such as cAMP signaling to set off a signaling cascade via the PKA pathway and members of the mitogen-activated protein kinases (MAPKs), which can directly phosphorylate histones to prompt further changes in chromatin structure (Gräff and Tsai 2013; Nestler 2016). This pathway has also been directly linked to cocaine-induced plasticity throughout the ventral midbrain and corticolimbic circuitry.

The acute epigenetic remodeling that occurs in response to cocaine exposure has been primarily studied in regions downstream of VTA dopaminergic projections. For example, acute cocaine exposure increases various acetylation marks throughout the dorsal striatum including increased acH3 and acH4 and increased acH4K5, acH4K8, acH4K12, and acH4K16 at *cFos* and *fosb* promoters (Jordi et al. 2013; Kumar et al. 2005). With regard to the ventral striatum, similar increases in acetylation at sites such as H3K14, H2BK12, and H4K5, H4K8, H4K12, and H4K16 in the NAc in response to acute cocaine have been observed (Malvaez et al. 2011; Levine et al. 2005). Extensive research in the role of striatal MSNs have provided evidence for the divergent roles in D1- and D2-expressing MSNs. Indeed, recent work has demonstrated a likewise divergent epigenetic response in these cell types in response to varying treatments of cocaine. Acute cocaine increases the combinatorial H3 phosphoacetylation in D1 MSNs of the NAc (Bertran-Gonzalez et al. 2008; Jordi et al. 2013), likely mediated by the D1-specific adaptations to DARPP-32 (Stipanovich et al. 2008; Nairn et al. 2004). Moreover, cocaine acutely increases H3K9me2 and H3K9me3 in both D1 and D2 MSNs (Jordi et al. 2013). Lastly, increased MECP2 has also been found throughout the NAc and caudate/putamen following acute cocaine exposure, suggesting induction of DNA methylation (Deng et al. 2011; Mao et al. 2012). However, the gene targets subject to presumptive DNA methylation remain unknown. As mentioned above, it is important to note that these observed changes in histone modifications do not occur in isolation. Any single epigenetic mark is unlikely to induce or repress gene expression on its own. Broad-scale epigenomic changes (such as global changes in acetylation or complete remodeling by nucleosome remodeling complexes) occur in concert with various marks, enzymes, and regulators. For example, cocaine-induced increases in acH3 (particularly at the H3S10 residue) recruit HATs and mediate increased H3K14ac (Jordi et al. 2013; Ciccarelli and Giustetto 2014). Moreover, acute cocaine recruits ARC to pH3S10-tagged transcripts, functioning as a potential feedback mechanism on neuronal gene expression (Salery et al. 2017). Recently, a potential mechanism for how histone modifiers (e.g., HDAC3) may interact with large-scale remodelers (such as nBAF) to regulate activity-dependent gene expression and plasticity has been proposed (Shu et al. 2018). Future work should further elucidate how these various epigenetic modifiers regulate gene expression in concert and in competition with one another.

Acute cocaine exposure recruits mechanisms critical to early phases of circuit plasticity and drug-seeking behavior. Typically, these changes to chromatin occur at and have been studied with regard to immediate early genes (such as *cFos*, *Bdnf*, *Arc*,

and *Fosb*) (Zhao et al. 2014; Miller and Marshall 2005). However, repeated cocaine intake generates a novel epigenetic landscape at not only immediate early genes but also various gene targets linked to plasticity and synaptic function. These changes occur throughout the mesolimbic and mesocortical pathways and are believed to underlie the persistence of cocaine-seeking behavior. In the VTA, Schmidt et al. identified increased H3K9 and H3K14 acetylation at the *Bdnf I* promoter, coinciding with cocaine-induced increases in *Bdnf I* expression (Schmidt et al. 2012). Similarly, striatal BDNF transmission is known to increase the motivation to self-administer cocaine (Im et al. 2010; Graham et al. 2007, 2009; Grimm et al. 2003; Hall et al. 2003; Lu et al. 2004; Horger et al. 1999; Schoenbaum et al. 2007), and increases have been linked to the increased spine changes that are characteristic of cocaine exposure (Zhou et al. 2006). BDNF activates the enzyme nitric oxide synthase, leading to nitrosylation and dismissal of chromatin-bound HDAC2, thus ultimately increasing histone acetylation at genes involved in neural plasticity for LTP and learning (Nott et al. 2008). However, while these transient immediate early genes and growth factors are a critical component of synaptic plasticity induced by drugs, they are just the first step in a line of epigenetic modifications and synaptic remodeling that ultimately solidifies information about drugs and associated stimuli in the brain. Thus, it is important to understand how these initial changes lay the groundwork for the activity-dependent circuit remodeling that ultimately underlies addiction.

Yet, the major question is whether these changes are seen following repeated cocaine exposure and whether they are long-lasting. Whereas activity-induced gene expression and protein synthesis is transient, the circuit rewiring linked to associative learning and memory storage is long-lasting (Tonegawa et al. 2015). Notably, histone acetylation is known as a highly dynamic modification that rapidly turns over. Equally, even the extended half-life of channel proteins such as AMPA and NMDA receptors – whose expression is manipulated by drugs of abuse – is transitory when compared to timescales of pathological states of addiction, as drug relapse can occur even after years of abstinence and clinical intervention. Therefore, persistent changes in transcriptional regulation caused by drugs of abuse are likely maintained by the complex interplay of short-lived epigenetic marks – e.g., transient histone acetylation with dramatic effects on gene expression – that regulate synaptic and circuit strengths and permanent epigenetic aberrations that preserve transcriptional dysregulation in concert with alterations at the synapse and cell signaling. Recently, gene-specific enrichment of H3K4me3 was identified in the hippocampus of chronic cocaine users (Zhou et al. 2011). Yet, these changes to histone methylation did not correlate with changes in gene expression. Similarly, chronic cocaine self-administration induces long-lasting changes in acetylation states in the prefrontal cortex that correspond with increases in *Dot1l/Kmt4*, *Kdm5a*, *Kdm6a*, *Kdm6b*, and *Kdm7a* (Sadakierska-Chudy et al. 2017). However, despite these changes in KDM expression, no subsequent changes in global histone methylation state was observed, demonstrating that changes in any given histone mark are unable to dictate gene expression alone (and vice versa). However, it is possible that these changes to histone methylation (and its regulatory enzymes) do not alter baseline levels of

particular genes but do leave the transcriptome in a permissive or repressive state for subsequent challenges (such as withdrawal and cue- and drug-induced relapse).

6 Transient Changes as a Scaffold for Long-Term Epigenetic Changes

All of the aforementioned mechanisms rely on acute changes that are transient in nature and are likely involved in quick and adaptive responses of cellular circuits to environmental information. But the question is how these precisely timed processes ultimately lead to permanently altered epigenetic landscapes that underlie dysregulated transcription in addiction. In addition to the long-lasting circuit changes induced by cocaine (see Fig. 2), cocaine exposure induces long-lasting changes in gene expression via targeted alterations in epigenetic structure. While there are various residues on histone tails susceptible to posttranslational modifications, it appears that cocaine induces stereotyped marks to generate long-lasting gene expression (Fig. 3). With regard to the striatum, repeated cocaine exposure drastically alters the epigenetic landscape. Both experimenter-administered chronic cocaine and repeated cocaine self-administration have led to increased acH3 and acH4 in the NAc (Malvaez et al. 2011; Wang et al. 2009; Renthal et al. 2007), particularly at plasticity-related genes, such as *Fosb*, *cFos*, *Bdnf I*, and *Cdk5*. These changes in histone marks are partially explained by cocaine's effects on various epigenetic writers, such as HDACs and KMTs. For example, chronic cocaine has been shown to cause export of HDAC5 from the nucleus in MSNs of the NAc (Renthal et al. 2007), alter HDAC expression (Renthal et al. 2009), and misregulate G9a/KMT1C function and subsequent histone methylation (Maze et al. 2010). Again, it is critical to emphasize that these epigenetic adaptations do not occur in isolation and are often competitive with one another. While previous studies have indicated simultaneous increases in H3 acetylation and methylation (Jordi et al. 2013), recent ChIP-seq studies identified gene-specific increases in both acH3 and acH4 in the NAc. Further, gene targets depleted with acH3/H4 correspond to gene targets which show enrichment for meH3 (Renthal et al. 2009; LaPlant and Nestler 2011). Moreover, gene-specific changes in nucleosome remodelers have been seen at the *Cdk5* promoter (Kumar et al. 2005), further suggesting the interaction between various epigenetic mechanisms to ultimately generate loci-specific transcription. Cocaine has been demonstrated to alter HDAC-mediated regulation of other histone modifiers – repeated cocaine disengages HDAC1 at KMT1C, leading to enhanced H3K9me2 in the NAc (Kennedy et al. 2013).

DNA methylation and several of its key regulatory enzymes appear sensitive to repeated cocaine exposure. Following self-administration, there is an increase in *Dnmt3a/b* expression and alterations of methylation at the *cFos* promoter (Wright et al. 2015) that persists following a period of withdrawal (Laplant et al. 2010). MECP2 levels have been demonstrated to increase in the striatum and hippocampus following self-administration (Im et al. 2010). Repeated cocaine is able to generate pervasive changes to the epigenome. These changes occur not only in a brain region-

specific fashion but also in a cell-type-specific fashion. Accordingly, results support an emerging view that rapid changes in DNA methylation – traditionally viewed as a permanent and immutable mark in postmitotic cells – are involved in activity-dependent regulation of neuronal gene transcription. DNA methyltransferase, DNMT1, is highly expressed across the brain, and transient increases in DNMT1 expression are not only seen with Pavlovian learning but have been reported after administration of drugs of abuse (Goto et al. 1994; Numachi et al. 2007). In fact, following chronic exposure to drug, increases in DNA methylation in the striatum are persistent and evident even after extended periods of withdrawal (Mychasiuk et al. 2013). Notably, in the case of Pavlovian learning – a critical process involved in SUD – acquisition and extinction of memory have been linked to alterations in the methylation machinery in the prefrontal cortex, including changes to the TET family of enzymes, targeted DNA methylation, and recruitment of MECP2, suggesting that these DNA modifications are critical to the maintenance of long-term memories associated with addiction (Alaghband et al. 2016; Bredy et al. 2007; Bredy 2013; Li et al. 2014; Viola et al. 2016). These findings highlight the dynamic nature of the neuronal DNA methylome and suggest an important role for DNA methylation in the stabilization of epigenetic change that is instigated by drugs of abuse (Feng et al. 2015). In fact, both acute and chronic cocaine have been shown to cause hypomethylation of the *FosB* promoter in the striatum, linked to decreased binding of MeCP2 and upregulation of FosB expression (Anier et al. 2010). Thus, it is possible that acute histone changes allow for changes in DNA conformation and subsequent DNA methylation that stabilizes long-term memory and persistent changes in cellular function, as seen through the long-lasting nature of DNA methylation and targeted recruitment of DNA methylation in a gene-specific fashion following cocaine self-administration (Massart et al. 2015; Baker-Andresen et al. 2015).

As outlined earlier, the aforementioned changes in chromatin structure produce plasticity at the synaptic and circuit level, including alterations of the AMPA and NMDA receptor levels and their subunit composition. Just like with dendritic spines where thin spines serve as a scaffold to create more mature spines, transient epigenetic marks can set a series of events in place that help to consolidate information permanently only if the stimulus is incredibly salient or encountered repeatedly over long periods of time. This can serve as a gating mechanism, so that the long-term changes would only happen after repeated exposure.

7 Causally Linking Epigenetic Modification to Substance Use Disorder

Cocaine-associated behaviors have been linked with change in function of various epigenetic modifiers (Malvaez et al. 2011; Renthal et al. 2007; Kennedy et al. 2013; Rogge et al. 2013). For example, the associative processes that occur with cocaine exposure and cocaine-associated behaviors can be altered through targeting of specific epigenetic mechanisms. Bidirectional manipulation of histone acetylation in the NAc has profound effects on cocaine-associated behaviors. Genetic and

pharmacological loss of HDAC3 function in the NAc enhances the acquisition of cocaine-conditioned place preference (CPP), with predictive increases to cocaine-induced acetylation (Rogge et al. 2013; Malvaez et al. 2013). However, this is unlikely through a mediation of the rewarding properties of cocaine but more likely regulating the associative processes, as HDAC3 inhibition during extinction not only accelerates extinction but also blunts cocaine-primed reinstatement of CPP. Cocaine-primed reinstatement has also been demonstrated to enhance H4K8Ac and alter HDAC3-dependent gene regulation in the medial habenula, an epithalamic region strongly linked to drug-seeking and drug-associated behaviors (López et al. 2018, 2019). Conversely, NAc-specific loss of CBP (a histone acetyltransferase) led to a hypoacetyl state in response to acute and chronic cocaine and blocked cocaine-induced CPP (Malvaez et al. 2011). Additionally, DARPP-32-mediated decreases in pH3S10 are also able to block cocaine-induced CPP (Stipanovich et al. 2008). Similarly, overexpression of KMT1C in the NAc led to increases in H3K9me2 which corresponded with decreased cocaine-CPP. Pharmacological inhibition of KMT1C was able to reverse the effects on H3K9me2 and enhance cocaine-CPP (Maze et al. 2010). Lastly, mutations to nBAF blunt cocaine-induced CPP that is restored through local overexpression of BDNF into the NAc (White et al. 2016; Alaghband et al. 2018). These epigenetic mechanisms have been demonstrated to play an important role in learning and memory and appear to mediate the associative properties of SUD.

Two major aspects in cocaine seeking are the reinforcing properties of the drug and the enhanced motivation to seek drug; each aspect is regulated by various molecular and circuit pathways. For example, recent work has characterized the formation of habitual cocaine seeking via the recruitment of MSNs in the dorsal lateral striatum (DLS) during long-access cocaine self-administration (Malvaez et al. 2018; Fouyssac et al. 2017; Murray et al. 2015). This DLS recruitment is mediated via disengagement of HDAC3 – pharmacological or genetic inhibition of HDAC3 in the DLS accelerated habit formation, whereas overexpression of HDAC3 in the DLS suppresses habit formation (Malvaez et al. 2018). Moreover, systemic HDAC inhibition suppresses acquisition of cocaine self-administration (Romieu et al. 2008). Similarly, HDAC3 inhibition enhanced extinction and blocked cue-induced reinstatement of cocaine seeking (Hitchcock et al. 2018). So while similar findings in CPP may not provide insights into HDAC-mediated regulation of reinforcing properties of cocaine, these findings in self-administration studies suggest HDACs may regulate motivational aspects of cocaine seeking as well as the changes that occur following repeated volitional exposure. This body of work demonstrates that HDACs function as molecular brake pads to not only acute gene expression but also recruitment of neural pathways in behavior. Drugs of abuse, such as cocaine, alter these molecular mechanisms leading to long-term alteration in circuit function.

While histone acetylation is a well-studied mechanism in the addiction field, recent work has further defined a role for KMT1C-mediated histone methylation in cocaine seeking. Overexpression of KMT1C in the NAc shell and subsequent increases in H3K9me2 enhance cocaine self-administration and generate increased susceptibility of stress-induced reinstatement (Anderson et al. 2017). Furthermore,

while loss of nBAF function (through deletion of nBAF-specific subunit CREST) had no effect on the initial motivation to respond for cocaine, CREST deletions in the NAc slow the acquisition of cocaine self-administration and alter the acquisition of cocaine-associated memories (Alaghband et al. 2018). Overall, an elaborate interplay of epigenetic mechanisms regulates the various circuit and molecular mechanisms of drug seeking.

8 Linking Changes in Epigenome to Changes in Synaptic Function

To date, the field has identified various pathways, individual transcription factors, and altered epigenetic mechanisms engaged by cocaine exposure. But we, as a field, have yet to fully characterize how these individual levels of analysis combine to alter behavior. It is unlikely that any single gene, transcription factor, or epigenetic writer is responsible for the various aspects of SUD, nor will any single molecular mechanism provide an avenue for effective long-lasting therapeutics. Moreover, the heterogeneity that exists across cell types (e.g., D1 vs D2 MSNs) and brain regions (e.g., dorsal vs ventral striatum), although not fully elaborated or emphasized in this review, provides a further complication in understanding how the identified epigenetic changes ultimately lead to long-lasting changes in circuit function and behavior. Moving forward, it will be important to identify the mechanisms within defined cellular populations – based on genetic identity, circuit connectivity, or functional recruitment – that alter subsequent cocaine response and cocaine-associated behaviors.

Changes in the resting membrane potential, gene priming, and stable receptor expression levels can all alter the probability that a specific cell will fire and thus can increase the incorporation of these neurons into memory ensembles and strengthen synaptic connections. Maintenance of these tonic levels of neurotransmitter, changes in transporter function, and postsynaptic receptor content have been shown to be regulated by epigenetic modifications at the chromatin level. Specific methyl and acetyl marks can act to change stable expression levels of proteins involved in this process, such as AMPA and NMDA receptors, which can change the speed and efficiency with which new synapses can be formed and destabilized. This can also change the response magnitude of these cells and circuits to salient stimuli in the environment, thus driving maladaptive behaviors. These specific processes have been shown to be dysregulated in both human subjects with cocaine use disorder and rodent models of cocaine-associated behaviors (Volkow et al. 2003; Calipari et al. 2016; Breiter et al. 1997; Dackis and O'Brien 2005). (While the focus of this chapter has been specifically the cocaine-induced adaptations in plasticity, circuitry, and epigenetic mechanisms, other drugs of abuse are likewise able to induce unique molecular and neural circuit signatures to drive drug seeking as well). Thus, basal epigenetic regulation of membrane-associated proteins can alter the excitability of neurons and concomitant behavioral processes associated with addiction. For example, long-term potentiation induced by theta-burst stimulation is impaired as a result

of nBAF loss of function in the NAc (White et al. 2016; Vogel-Ciernia et al. 2013). Maze et al. demonstrated altered synaptic pruning in the NAc following KMT1C overexpression (Maze et al. 2010). Moreover, HDAC inhibition or deletion leads to increased synaptic plasticity in both the hippocampus and NAc, likely mediated by enhanced expression of various immediate early genes, such as *Nr4a2*, *cFos*, and *Bdnf* (Malvaez et al. 2013; Barrett et al. 2011). Yet, how cocaine-induced changes in nuclear chromatin structure feed into altered circuits remains an elusive question. Nevertheless, while immediate early genes are critical in generating LTP, learning, memory, and various associative processes, it remains unclear how epigenetic adaptations at these loci ultimately generate differential function in neural circuits. Kennedy et al. linked changes in HDAC-mediated repression in histone methylation to altered expression of various GABA-receptor subunits, including *Gabra1* and *Gabra2* that is linked to altered synaptic function in the NAc (Kennedy et al. 2013). Altered acetylation at other various synaptic proteins have also been reported, including increased acH3 at *Gria2*, *Grin1*, and *Grin2b* in the NAc (Wang et al. 2009). Although increased AMPA/NMDA ratios are a hallmark feature of cocaine exposure, how epigenetic dysfunction at these individual gene loci are linked to altered NAc function is a key open question.

Conversely, repeated stimulation of strengthened synapses can result in activity-dependent epigenetic remodeling via calcium-dependent signaling (Nestler 2013). This increase in the activity level of neurons can lead to the activation of immediate early genes and concomitant wide-scale nuclear changes in the accessibility of DNA and transcriptional processes. In addition, these changes can lead to a feedforward loop in which activity-dependent epigenetic changes lead to enhanced sensitivity to subsequent inputs. If these inputs are in neuronal pathways driving reinforcement learning, this can act to increase self-administration and drug seeking. Thus, it is the communication between the nuclear changes in DNA conformation/transcription and the precise changes in membrane excitability that allows for the refinement of information at the level of each individual neuron. Inflexibility in both the behavioral adaptations and related neural circuitry is what underlies drug addiction in a way that results in the strong and stable storage and expression of drug-associated memories over all others.

9 Defining Causal Links Between Epigenetic Factors and Neural Activity in Substance Use Disorder

As discussed throughout this review, long-term cocaine exposure and cocaine-seeking behavior generate widespread changes to the epigenome, ultimately leading to not only targeted changes in single genes but recruitment of a cocaine-specific transcriptional network, in a cell-type- and circuit-specific manner. Moreover, cocaine-repressed genes provide an equally critical component to SUD as cocaine-induced transcripts and should not be overlooked. Moving forward, it will be crucial to generate an encompassing perspective on the full transcriptional and molecular adaptations induced by repeated cocaine. A full acknowledgment and understanding

of how drugs of abuse engage these networks will provide a more promising avenue of success for treating such disorders. In line with this view, work from Walker et al. has identified a full gene network induced by repeated cocaine self-administration and reinstatement of cocaine seeking (Walker et al. 2018). Similarly, transcriptional dysregulations in the PFC over various periods of forced abstinence from repeated cocaine exposure have been identified (Li et al. 2017). However, the molecular mechanisms which regulate these transcriptional adaptations remain unknown. Moreover, how induction of the identified gene network leads to changes in circuit activity and behavior has not been defined. Yet, the advent of new viral and molecular strategies now allows researchers the ability to target these epigenetic modifications to particular loci within particular subsets of cell populations. For example, both engineered zinc-finger proteins (ZFPs) and CRISPR/dCas9 systems allow for gene-specific targeting of transcriptional regulators and have previously been used to selectively increase or decrease the expression of single genes. However, combined with novel viral and Cre-dependent approaches, these technologies can now be adapted to target specific epigenetic writers or erasers in cell-type-, pathway-, and gene-loci-specific manners (Savell et al. 2018; Heller et al. 2014, 2016; Kwapis et al. 2018). Paired with in vivo techniques for circuit monitoring [such as Miniscopes (Silva 2017), in vivo fiber photometry (Calipari et al. 2017), and fast scan cyclic voltammetry (Willuhn et al. 2014)], the field of neuroepigenetics now has the potential to directly link epigenetic regulation and circuit activity to behavioral outcomes. Ultimately, these insights will lead to the development of effective therapeutics for the varying aspects of SUD.

Acknowledgments A.J.L. is supported by NIH Grant K00 DA048436. C.A.S. is supported by NIH grant K99 DA045103 (NIDA) and a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation. E.S.C is supported by NIH grants R00 DA042111 and DP1 DA048931 (NIDA), a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation, and funding from the Whitehall Foundation and Edward Mallinckrodt Jr. Foundation.

References

Agger K, Christensen J, Cloos PA, Helin K (2008) The emerging functions of histone demethylases. Curr Opin Genet Dev 18:159–168

Aguilar MA, Rodríguez-Arias M, Miñarro J (2009) Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. Brain Res Rev 59:253–277

Ahmed SH et al (2005) Gene expression evidence for remodeling of lateral hypothalamic circuitry in cocaine addiction. Proc Natl Acad Sci 102:11533–11538

Alaghband Y, Bredy TW, Wood MA (2016) The role of active DNA demethylation and Tet enzyme function in memory formation and cocaine action. Neurosci Lett. https://doi.org/10.1016/j.neulet.2016.01.023

Alaghband Y et al (2018) CREST in the nucleus accumbens core regulates cocaine conditioned place preference, cocaine-seeking behavior, and synaptic plasticity. J Neurosci 38:9514–9526

Alarcón JM et al (2004) Chromatin acetylation, memory, and LTP are impaired in CBP+/− mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron 42:947–959

Anderson SM, Pierce RC (2005) Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. Pharmacol Ther 106:389–403
Anderson EM et al (2017) Overexpression of the histone dimethyltransferase G9a in nucleus accumbens shell increases cocaine self-administration, stress-induced reinstatement, and anxiety. J Neurosci 38:1657–1617
Anier K, Malinovskaja K, Aonurm-Helm A, Zharkovsky A, Kalda A (2010) DNA methylation regulates cocaine-induced behavioral sensitization in mice. Neuropsychopharmacology 35:2450
Bachtell RK, Self DW (2008) Renewed cocaine exposure produces transient alterations in nucleus accumbens AMPA receptor-mediated behavior. J Neurosci 28:12808–12814
Baker-Andresen D, Ratnu VS, Bredy TW (2013) Dynamic DNA methylation: a prime candidate for genomic metaplasticity and behavioral adaptation. Trends Neurosci 36:3–13
Baker-Andresen D et al (2015) Persistent variations in neuronal DNA methylation following cocaine self-administration and protracted abstinence in mice. Neuroepigenetics 4:1–11
Bali P, Im H-I, Kenny PJ (2011) Methylation, memory and addiction. Epigenetics 6:671–674
Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21:381–395
Barnes CE, English DM, Cowley SM (2019) Acetylation & Co: an expanding repertoire of histone acylations regulates chromatin and transcription. Essays Biochem 63:97–107
Barrett RM, Wood MA (2008) Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. Learn Mem 15:460–467
Barrett RM et al (2011) Hippocampal focal knockout of CBP affects specific histone modifications, long-term potentiation, and long-term memory. Neuropsychopharmacology 36:1545–1556
Bateup HS et al (2008) Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. Nat Neurosci 11:932–939
Baubec T, Schübeler D (2014) Genomic patterns and context specific interpretation of DNA methylation. Curr Opin Genet Dev 25:85–92
Baylin SB, Ohm JE (2006) Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction? Nat Rev Cancer 6:107–116
Beitner-Johnson D, Nestler EJ (1991) Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J Neurochem 57:344–347
Berger SL (2007) The complex language of chromatin regulation during transcription. Nature 447:407
Berndsen CE, Denu JM (2008) Catalysis and substrate selection by histone/protein lysine acetyltransferases. Curr Opin Struct Biol 18:682–689
Bertran-Gonzalez J et al (2008) Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 28:5671–5685
Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16(1):6–21. https://doi.org/10.1101/gad.947102.6
Bocklisch C et al (2013) Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area. Science 341:1521–1525
Boutrel B et al (2005) Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. Proc Natl Acad Sci U S A 102:19168–19173
Brami-Cherrier K (2005) Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J Neurosci 25:11444–11454
Bredy T (2013) The 6Th base: TET3-mediated DNA hydroxymethylation regulates the formation of memory for fear extinction. Biol Psychiatry 73:5S
Bredy TW et al (2007) Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem 14(4):268–276. https://doi.org/10.1101/lm.500907.lation
Breiter HC et al (1997) Acute effects of cocaine on human brain activity and emotion. Neuron 19:591–611
Burdge GC, Lillycrop KA (2010) Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease. Annu Rev Nutr 30:315–339

Calipari ES, Ferris MJ, Zimmer BA, Roberts DC, Jones SR (2013) Temporal pattern of cocaine intake determines tolerance vs sensitization of cocaine effects at the dopamine transporter. Neuropsychopharmacology 38(12):2385–2392
Calipari ES, Jones SR (2014) Sensitized nucleus accumbens dopamine terminal responses to methylphenidate and dopamine transporter releasers after intermittent-access self-administration. Neuropharmacology 82:1–10
Calipari ES, Ferris MJ, Siciliano CA, Zimmer BA, Jones SR (2014) Intermittent cocaine self-administration produces sensitization of stimulant effects at the dopamine transporter. J Pharmacol Exp Ther 349(2):192–198
Calipari ES, Siciliano CA, Zimmer BA, Jones SR (2015) Brief intermittent cocaine self-administration and abstinence sensitizes cocaine effects on the dopamine transporter and increases drug seeking. Neuropsychopharmacology 40(3):728–735
Calipari ES et al (2016) In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward. Proc Natl Acad Sci 113:2726–2731
Calipari ES et al (2017) Dopaminergic dynamics underlying sex-specific cocaine reward. Nat Commun 8:13877
Calipari ES et al (2019) Synaptic microtubule-associated protein EB3 and SRC phosphorylation mediate structural and behavioral adaptations during withdrawal from cocaine self-administration. J Neurosci. pii: 0024-19. https://doi.org/10.1523/JNEUROSCI.0024-19.2019
Cameron CM, Murugan M, Choi JY, Engel EA, Witten IB (2019) Increased cocaine motivation is associated with degraded spatial and temporal representations in IL-NAc neurons. Neuron. https://doi.org/10.1016/J.NEURON.2019.04.015
Campanac E, Hoffman DA (2013) Repeated cocaine exposure increases fast-spiking interneuron excitability in the rat medial prefrontal cortex. J Neurophysiol 109:2781–2792
Campbell RR, Wood MA (2019) How the epigenome integrates information and reshapes the synapse. Nat Rev Neurosci 20:133–147
Cao J, Yan Q (2012) Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Front Oncol 2:1–9
Caputi FF et al (2014) Dynorphin/KOP and nociceptin/NOP gene expression and epigenetic changes by cocaine in rat striatum and nucleus accumbens. Prog Neuropsychopharmacol Biol Psychiatry 49:36–46
Carouge D, Host L, Aunis D, Zwiller J, Anglard P (2010) CDKL5 is a brain MeCP2 target gene regulated by DNA methylation. Neurobiol Dis 38:414–424
Chandra R et al (2015) Opposing role for Egr3 in nucleus accumbens cell subtypes in cocaine action. J Neurosci 35:7927–7937
Chen R et al (2006) Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. Proc Natl Acad Sci 103:9333–9338
Childress AR et al (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11–18
Ciccarelli A, Giustetto M (2014) Role of ERK signaling in activity-dependent modifications of histone proteins. Neuropharmacology 80:34–44
Collins AL, Aitken TJ, Greenfield VY, Ostlund SB, Wassum KM (2016) Nucleus accumbens acetylcholine receptors modulate dopamine and motivation. Neuropsychopharmacology 41:2830
Conrad KL et al (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118–121
Dackis C, O'Brien C (2005) Neurobiology of addiction: treatment and public policy ramifications. Nat Neurosci 8:1431–1436
De La Cruz X, Lois S, Sánchez-Molina S, Martínez-Balbás MA (2005) Do protein motifs read the histone code? Bioessays 27:164–175
de Ruijter AJM, van Gennip AH, Caron HN, Kemp S, van Kuilenburg ABP (2003) Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J 370:737–749

de Wit H (1996) Priming effects with drugs and other reinforcers. Exp Clin Psychopharmacol 4:5–10
Deng JV et al (2011) Behavioral responses to psychostimulants. Nat Neurosci 13:1128–1136
Dudai Y, Morris RGM (2013) Memorable trends. Neuron 80:742–750
Dulac C (2010) Brain function and chromatin plasticity. Nature 465:728–735
Farrell MR et al (2019) Ventral pallidum is essential for cocaine reinstatement after voluntary abstinence. bioRxiv:653741. https://doi.org/10.1101/653741
Farrelly LA et al (2019) Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. Nature 567:535–539
Farris SP, Harris RA, Ponomarev I (2015) Epigenetic modulation of brain gene networks for cocaine and alcohol abuse. Front Neurosci 9:1–10
Fass DM et al (2013) Crebinostat: a novel cognitive enhancer that inhibits histone deacetylase activity and modulates chromatin-mediated neuroplasticity. Neuropharmacology 64:81–96
Feng J et al (2014) Chronic cocaine-regulated epigenomic changes in mouse nucleus accumbens. Genome Biol 15:R65
Feng J et al (2015) Role of Tet1 and 5-hydroxymethylcytosine in cocaine action. Nat Neurosci 18:536–544
Ferguson D et al (2015) SIRT1-FOXO3a regulate cocaine actions in the nucleus accumbens. J Neurosci 35:3100–3111
Ferris MJ, Calipari ES, Yorgason JT, Jones SR (2013) Examining the complex regulation and drug-induced plasticity of dopamine release and uptake using voltammetry in brain slices. ACS Chem Neurosci 4:693–703
Filippakopoulos P, Knapp S (2014) Targeting bromodomains: epigenetic readers of lysine acetylation. Nat Rev Drug Discov 13:337–356
Finegersh A, Homanics GE (2016) Chromatin immunoprecipitation and gene expression analysis of neuronal subtypes after fluorescence activated cell sorting. J Neurosci Methods 263:81–88
Fischle W et al (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell 9:45–57
Fouyssac M, Everitt BJ, Belin D (2017) Cellular basis of the intrastriatal functional shifts that underlie the development of habits: relevance for drug addiction. Curr Opin Behav Sci 13:144–151
Freeman WM et al (2008) Persistent alterations in mesolimbic gene expression with abstinence from cocaine self-administration. Neuropsychopharmacology 33:1807–1817
Goodwin LR, Picketts DJ (2018) The role of ISWI chromatin remodeling complexes in brain development and neurodevelopmental disorders. Mol Cell Neurosci. https://doi.org/10.1016/j.mcn.2017.10.008
Goto K et al (1994) Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. Differentiation 56:39–44
Gräff J, Tsai L-H (2013) Histone acetylation: molecular mnemonics on the chromatin. Nat Rev Neurosci 14:97
Graham DL et al (2007) Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nat Neurosci 10:1029–1037
Graham DL et al (2009) Tropomyosin-related kinase B in the mesolimbic dopamine system: region-specific effects on cocaine reward. Biol Psychiatry 65:696–701
Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Incubation of cocaine craving after withdrawal. Nature 412:141–142
Grimm JW et al (2003) Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 23:742–747
Hall FS, Drgonova J, Goeb M, Uhl GR (2003) Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. Neuropsychopharmacology 28:1485
Hassa PO, Haenni SS, Elser M, Hottiger MO (2006) Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? Microbiol Mol Biol Rev 70:789–829

Heller EA et al (2014) Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. Nat Neurosci 17:1720–1727
Heller EA et al (2016) Targeted epigenetic remodeling of the Cdk5 gene in nucleus accumbens regulates cocaine- and stress-evoked behavior. J Neurosci 36:4690–4697
Hitchcock LN, Raybuck JD, Wood MA, Lattal KM (2018) Effects of a histone deacetylase 3 inhibitor on extinction and reinstatement of cocaine self-administration in rats. Psychopharmacology (Berl). 3. doi: https://doi.org/10.1007/s00213-018-5122-2
Horger BA et al (1999) Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 19:4110–4122
Horikawa HPM, Nawa H (1998) Turnover rates of the AMPA-type glutamate receptor GluR1 measured by transient gene expression. J Neurosci Methods 84:173–179
Hurd YL, Weiss F, Koob G, Ungerstedt U (1990) The influence of cocaine self-administration on in vivo dopamine and acetylcholine neurotransmission in rat caudate-putamen. Neurosci Lett 109:227–233
Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598
Im HI, Hollander JA, Bali P, Kenny PJ (2010) MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat Neurosci 13:1120–1127
Itzhak Y, Martin JL (2002) Cocaine-induced conditioned place preference in mice: Induction, extinction and reinstatement by related psychostimulants. Neuropsychopharmacology 26:130–134
Jaffe JH, Cascella NG, Kumor KM, Sherer MA (1989) Jaffe_COC_Craving_Psychopharm_1989.pdf
Jones PA (2012) Functions of DNA methylation: Islands, start sites, gene bodies and beyond. Nat Rev Genet 13:484–492
Jones S, Bonci A (2005) Synaptic plasticity and drug addiction. Curr Opin Pharmacol 5:20–25
Jordi E et al (2013) Differential effects of cocaine on histone posttranslational modifications in identified populations of striatal neurons. Proc Natl Acad Sci U S A 110:9511–9516
Kalivas PW, Duffy P (2002) Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. J Neurochem 70:1497–1502
Karch KR, DeNizio JE, Black BE, Garcia BA (2013) Identification and interrogation of combinatorial histone modifications. Front Genet 4:1–15
Kawa AB, Allain F, Robinson TE, Samaha AN (2019) The transition to cocaine addiction: the importance of pharmacokinetics for preclinical models. Psychopharmacology (Berl). https://doi.org/10.1007/s00213-019-5164-0
Kennedy PJ et al (2013) Class i HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. Nat Neurosci 16:434–440
Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42:961–972
Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705
Kramar CP, Chefer VI, Wise RA, Medina JH, Barbano MF (2014) Dopamine in the dorsal hippocampus impairs the late consolidation of cocaine-associated memory. Neuropsychopharmacology 39:1645–1653
Kravitz AV, Tye LD, Kreitzer AC (2012) Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816–818
Kuhar MJ, Ritz MC, Boja JW (1991) The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci 14:299–302
Kumar A et al (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314
Kupchik YM et al (2015) Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. Nat Neurosci 18:1230–1232

Kutlu MG, Gould TJ (2016) Effects of drugs of abuse on hippocampal plasticity and hippocampus-dependent learning and memory: contributions to development and maintenance of addiction. Learn Mem 23:515–533
Kwapis JL et al (2018) Epigenetic regulation of the circadian gene Per1 contributes to age-related changes in hippocampal memory. Nat Commun 9
LaPlant Q, Nestler EJ (2011) CRACKing the histone code: cocaine's effects on chromatin structure and function. Horm Behav 59:321–330
Laplant Q et al (2010) Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. Nat Neurosci 13:1137–1143
Le Moal M, Koob GF (2007) Drug addiction: pathways to the disease and pathophysiological perspectives. Eur Neuropsychopharmacol 17:377–393
Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAS in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426
Levenson JM et al (2004) Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 279:40545–40559
Levine AA et al (2005) 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 102:19186–19191
Levine A et al (2011) Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. Sci Transl Med 3:107ra109
Li X et al (2014) Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. Proc Natl Acad Sci U S A 111:7120–7125
Li M et al (2017) Dynamic expression changes in the transcriptome of the prefrontal cortex after repeated exposure to cocaine in mice. Front Pharmacol 8:1–10
Liu Q, Pu L, Poo M (2005) Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. Nature 437:1027
López AJ, Wood MA (2015) Role of nucleosome remodeling in neurodevelopmental and intellectual disability disorders. Front Behav Neurosci 9:1–10
López AJ et al (2018) Medial habenula cholinergic signaling regulates cocaine-associated relapse-like behavior. Addict Biol. https://doi.org/10.1111/adb.12605
López AJ et al (2019) Epigenetic regulation of immediate-early gene Nr4a2/Nurr1 in the medial habenula during reinstatement of cocaine-associated behavior. Neuropharmacology 153:13–19
Loweth JA et al (2014) Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. Nat Neurosci 17:73–80
Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y (2004) A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. J Neurosci 24:1604–1611
MacAskill AF, Cassel JM, Carter AG (2014) Cocaine exposure reorganizes cell type- and input-specific connectivity in the nucleus accumbens. Nat Neurosci 17:1198–1207
MacNiven KH et al (2018) Association of neural responses to drug cues with subsequent relapse to stimulant use. JAMA Netw Open 1:e186466
Mahler SV et al (2014) Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. Nat Neurosci 17(4):577–585. https://doi.org/10.1038/nn.3664
Malvaez M, Mhillaj E, Matheos DP, Palmery M, Wood MA (2011) CBP in the nucleus accumbens regulates cocaine-induced histone acetylation and is critical for cocaine-associated behaviors. J Neurosci 31:16941–16948
Malvaez M et al (2013) HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. Proc Natl Acad Sci U S A 110:2647–2652
Malvaez M et al (2018) Habits are negatively regulated by histone deacetylase 3 in the dorsal striatum. Biol Psychiatry 84(5):383–392. https://doi.org/10.1016/j.biopsych.2018.01.025
Mao L et al (2012) In vivo: a differential role of NMDA receptors. Neurochem Int 59:610–617

Mark GP, Hajnal A, Kinney AE, Keys AS (1999) Self-administration of cocaine increases the release of acetylcholine to a greater extent than response-independent cocaine in the nucleus accumbens of rats. Psychopharmacology (Berl) 143:47–53
Mark GP, Shabani S, Dobbs LK, Hansen ST (2011) Cholinergic modulation of mesolimbic dopamine function and reward. Physiol Behav 104:76–81
Massart R et al (2015) Role of DNA methylation in the nucleus accumbens in incubation of cocaine craving. J Neurosci 35:8042–8058
Maze I et al (2010) Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. Science 327:213–216
Maze I et al (2011) Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. Proc Natl Acad Sci U S A 108:3035–3040
McCallum SE, Glick SD (2009) 18-Methoxycoronaridine blocks acquisition but enhances reinstatement of a cocaine place preference. Neurosci Lett 458:57–59
McPherson PS (2015) Eating locally: microautophagy and protein turnover at the synapse. Neuron 88:619–621
Ménard C, Gaudreau P, Quirion R (2015) Signaling pathways relevant to cognition-enhancing drug targets. J Dement Care 13:59–98
Messner S, Hottiger MO (2011) Histone ADP-ribosylation in DNA repair, replication and transcription. Trends Cell Biol 21:534–542
Mews P, Calipari ES (2017) Cross-talk between the epigenome and neural circuits in drug addiction. Prog Brain Res 235:19–63
Miller CA, Marshall JF (2005) Altered Fos expression in neural pathways underlying cue-elicited drug seeking in the rat. Eur J Neurosci 21:1385–1393
Murray JE et al (2015) Basolateral and central amygdala differentially recruit and maintain dorsolateral striatum dependent cocaine-seeking habits. Nat Commun 6:1–9
Mychasiuk R, Muhammad A, Ilnytskyy S, Kolb B (2013) Persistent gene expression changes in NAc, mPFC, and OFC associated with previous nicotine or amphetamine exposure. Behav Brain Res 256:655–661
Nairn AC et al (2004) The role of DARPP-32 in the actions of drugs of abuse. Neuropharmacology 47:14–23
Nathan D, Sterner DE, Berger SL (2003) Histone modifications: now summoning sumoylation. Proc Natl Acad Sci 100:13118–13120
Nelson ED, Monteggia LM (2011) Epigenetics in the mature mammalian brain: effects on behavior and synaptic transmission. Neurobiol Learn Mem 96:53–60
Nestler EJ (2013) Cellular basis of memory for addiction. Dialogues Clin Neurosci 15:431–443
Nestler EJ (2016) Reflections on: "A general role for adaptations in G-Proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function". Brain Res 1645:71–74
Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A (2008) S-nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. Nature 455:411
Nott A et al (2016) Histone deacetylase 3 associates with MeCP2 to regulate FOXO and social behavior. Nat Neurosci 19:1497
Numachi Y et al (2007) Methamphetamine-induced hyperthermia and lethal toxicity: role of the dopamine and serotonin transporters. Eur J Pharmacol 572:120–128
Pardo-Garcia TR et al (2019) Ventral pallidum is the primary target for accumbens D1 projections driving cocaine seeking. J Neurosci 39:2041–2051
Park K, Volkow ND, Pan Y, Du C (2013) Chronic cocaine dampens dopamine signaling during cocaine intoxication and unbalances D1 over D2 receptor signaling. J Neurosci 33:15827–15836
Pascoli V et al (2014) Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature 509:459
Peixoto L, Abel T (2012) The role of histone acetylation in memory formation and cognitive impairments. Neuropsychopharmacology 38:62–76

Pol Bodetto S et al (2013) Cocaine represses protein phosphatase-1Cβ through DNA methylation and methyl-CpG binding protein-2 recruitment in adult rat brain. Neuropharmacology 73:31–40
Renthal W, Nestler EJ (2008) Epigenetic mechanisms in drug addiction. Trends Mol Med 14:341–350
Renthal W et al (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron 56:517–529
Renthal W et al (2009) Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron 62:335–348
Ressler KJ, Paschall G, Zhou X, Davis M (2002) Regulation of synaptic plasticity genes during consolidation of fear conditioning. J Neurosci 22:7892–7902
Rivera CM, Ren B (2013) Mapping human epigenomes. Cell 155:39–55
Robison AJ, Nestler EJ (2011) Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci 12:623–637
Rogge GA, Singh H, Dang R, Wood MA (2013) HDAC3 is a negative regulator of cocaine-context-associated memory formation. J Neurosci 33:6623–6632
Roidl D, Hacker C (2014) Histone methylation during neural development. Cell Tissue Res 356:539–552
Romieu P et al (2008) Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. J Neurosci 28:9342–9348
Roth SY, Denu JM, Allis CD (2003) Histone acetyltransferases. Annu Rev Biochem 70:81–120
Rudenko A, Tsai L-H (2014) Epigenetic modifications in the nervous system and their impact upon cognitive impairments. Neuropharmacology 80:70–82
Russo SJ, Nestler EJ (2013) The brain reward circuitry in mood disorders. Nat Rev Neurosci 14:609–625
Russo SJ et al (2010) The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. Trends Neurosci 33:267–276
Sadakierska-Chudy A et al (2017) Cocaine administration and its withdrawal enhance the expression of genes encoding histone-modifying enzymes and histone acetylation in the rat prefrontal cortex. Neurotox Res 32:141–150
Salery M et al (2017) Activity-regulated cytoskeleton-associated protein accumulates in the nucleus in response to cocaine and acts as a brake on chromatin remodeling and long-term behavioral alterations. Biol Psychiatry. https://doi.org/10.1016/j.biopsych.2016.05.025
Santos-Rosa H et al (2002) Active genes are tri-methylated at K4 of histone H3. Nature 419:407–411
Savell KE et al (2018) A neuron-optimized CRISPR/dCas9 activation system for robust and specific gene regulation. bioRxiv:371500. https://doi.org/10.1101/371500
Schmidt HD et al (2012) 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 120:202–209
Schoenbaum G, Stalnaker TA, Shaham Y (2007) A role for BDNF in cocaine reward and relapse. Nat Neurosci 10:935–936
Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. Annu Rev Biochem 76:75–100
Shiio Y, Eisenman RN (2003) Histone sumoylation is associated with transcriptional repression. Proc Natl Acad Sci 100:13225–13230
Shu G et al (2018) Deleting HDAC3 rescues long-term memory impairments induced by disruption of the neuron-specific chromatin remodeling subunit BAF53b. Learn Mem 25(3):109–115. https://doi.org/10.1101/lm.046920.117.25
Siciliano CA, Calipari ES, Ferris MJ, Jones SR (2015) Adaptations of presynaptic dopamine terminals induced by psychostimulant self-administration. ACS Chem Nerosci 6:27–36
Silva AJ (2017) Miniaturized two-photon microscope: seeing clearer and deeper into the brain. Light Sci Appl 6:e17104

Smith RJ, Lobo MK, Spencer S, Kalivas PW (2013) Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). Curr Opin Neurobiol 23:546–552
Sorg BA, Davidson DL, Kalivas PW, Prasad BM (1997) Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine in the medial prefrontal cortex. J Pharmacol Exp Ther 281:54–61
Staahl BT, Crabtree GR (2013) Creating a neural specific chromatin landscape by npBAF and nBAF complexes. Curr Opin Neurobiol 23:903–913
Stipanovich A et al (2008) A phosphatase cascade by which rewarding stimuli control nucleosomal response. Nature 453:879–884
Sultan FA, Day JJ (2011) Epigenetic mechanisms in memory and synaptic function. Epigenomics 3:157–181
Sun HS et al (2017) Regulation of BAZ1A and nucleosome positioning in the nucleus accumbens in response to cocaine. Neuroscience 353:1–6
Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. Nat Neurosci 4:1217–1223
Tonegawa S, Pignatelli M, Roy DS, Ryan TJ (2015) Memory engram storage and retrieval. Curr Opin Neurobiol 35:101–109
Trantham H, Szumlinski KK, McFarland K, Kalivas PW, Lavin A (2002) Repeated cocaine administration alters the electrophysiological properties of prefrontal cortical neurons. Neuroscience 113:749–753
Tweedie-Cullen RY et al (2012) Identification of combinatorial patterns of post-translational modifications on individual histones in the mouse brain. PLoS One 7:e36980
Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature 411:583–587
Vaillancourt K, Ernst C, Mash D, Turecki G (2017) DNA methylation dynamics and cocaine in the brain: progress and prospects. Genes (Basel) 8(5). pii: E138. doi: https://doi.org/10.3390/genes8050138
Viola TW et al (2016) Increased cocaine-induced conditioned place preference during periadolescence in maternally separated male BALB/c mice: the role of cortical BDNF, microRNA-212, and MeCP2. Psychopharmacology (Berl) 233:3279–3288
Vogel-Ciernia A, Wood MA (2014) Neuron-specific chromatin remodeling: a missing link in epigenetic mechanisms underlying synaptic plasticity, memory, and intellectual disability disorders. Neuropharmacology 80:18–27
Vogel-Ciernia A et al (2013) The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. Nat Neurosci 16:552–561
Volkow ND et al (1997) Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 386:827
Volkow ND, Fowler JS, Wang G-J (2003) The addicted brain: insights from imaging studies. J Clin Invest 111(p):1444–1451
Walker DM, Cates HM, Heller EA, Nestler EJ (2015) Regulation of chromatin states by drugs of abuse. Curr Opin Neurobiol 30:112–121
Walker DM et al (2018) Cocaine self-administration alters transcriptome-wide responses in the brain's reward circuitry. Biol Psychiatry. https://doi.org/10.1016/j.biopsych.2018.04.009
Wang L et al (2009) Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKII a in the nucleus accumbens is critical for motivation for drug reinforcement. Neuropsychopharmacology 35:913–928
Watson NA, Higgins JMG (2016) Histone kinases and phosphatases. Chromatin Signal Dis:75–94. https://doi.org/10.1016/B978-0-12-802389-1.00004-6
White AOAO et al (2016) BDNF rescues BAF53b-dependent synaptic plasticity and cocaine-associated memory in the nucleus accumbens. Nat Commun 7:11725
Williams JM, Steketee JD (2005) Effects of repeated cocaine on the release and clearance of dopamine within the rat medial prefrontal cortex. Synapse 55:98–109

Willuhn I, Burgeno LM, Groblewski PA, Phillips PE (2014) Excessive cocaine use results from decreased phasic dopamine signaling in the striatum. Nat Neurosci 17(5):704–709. https://doi.org/10.1038/nn.3694

Wolf ME (2016) Synaptic mechanisms underlying persistent cocaine craving. Nat Rev Neurosci 17:351–365

Wolf SF, Jolly DJ, Lunnen KD, Friedmann T, Migeon BR (2006) Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: implications for X-chromosome inactivation. Proc Natl Acad Sci 81:2806–2810

Wood MA, Hawk JD, Abel T (2006) Combinatorial chromatin modifications and memory storage: a code for memory? Learn Mem 13:241–244

Wright KN et al (2015) Methyl supplementation attenuates cocaine-seeking behaviors and cocaine-induced c-Fos activation in a DNA methylation-dependent manner. J Neurosci 35:8948–8958

Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: role in drug addiction. Neuroscience 301:529–541

Yorgason JT, Jones SR, España RA (2011) Low and high affinity dopamine transporter inhibitors block dopamine uptake within 5 sec of intravenous injection. Neuroscience 182:125–132

Zhang Y, Reinberg D (2001) Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev 15(18):2343–2360. https://doi.org/10.1101/gad.927301.vealed

Zhao Y, Garcia BA (2015) Comprehensive catalog of currently documented histone modifications. Cold Spring Harb Perspect Biol 7

Zhao C, Eisinger BE, Driessen TM, Gammie SC (2014) Addiction and reward-related genes show altered expression in the postpartum nucleus accumbens. Front Behav Neurosci 8:388

Zhou Z et al (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 52:255–269

Zhou Y et al (2008) Effects of cocaine place conditioning, chronic escalating-dose "binge" pattern cocaine administration and acute withdrawal on orexin/hypocretin and preprodynorphin gene expressions in lateral hypothalamus of Fischer and Sprague–Dawley rats. Neuroscience 153:1225–1234

Zhou Z, Yuan Q, Mash DC, Goldman D (2011) Substance-specific and shared transcription and epigenetic changes in the human hippocampus chronically exposed to cocaine and alcohol. Proc Natl Acad Sci 108:6626–6631