Chapter 7
Epigenomic and Noncoding RNA Regulation in Addictive Processes

John S. Satterlee

Abstract The phenotypic effects of drugs of abuse are partially mediated by transcriptional and epigenetic regulatory mechanisms. This chapter will provide a brief overview of substance abuse and then focus on the roles of three epigenetic regulatory mechanisms in addictive processes: histone modifications, DNA modifications, and noncoding RNAs. This chapter will conclude with a focus on three other important areas: (1) the potential for long-lasting epigenetic effects due to drugs of abuse, (2) obstacles and opportunities in this scientific area as they pertain to addiction biology, and (3) the potential for translating epigenomic and noncoding RNA discoveries into improvements in human health and the treatment of substance use disorders.

Keywords Addiction • Chromatin regulation • DNA modifications • Drug abuse • Epigenetic regulation • Gene expression • Histone modifications • MeCP2 • Methylation

Abbreviations

ADAR	Adenosine deaminase
Ago2	Argonaute 2
AML	Acute myeloid leukemia
BDNF	Brain-derived neurotrophic factor
BRD4	Bromodomain-containing protein 4
Brg1	Brahma-related Gene 1
caC	5-carboxylcytosine
CBP	CREB-binding protein

J.S. Satterlee (✉)
National Institute on Drug Abuse, 6001 Executive Boulevard, Bethesda, Maryland 20892-9561
e-mail: satterleej@nida.nih.gov

R.L. Jirtle and F.L. Tyson (eds.), *Environmental Epigenomics in Health and Disease*, Epigenetics and Human Health, DOI 10.1007/978-3-642-36827-1_7,

Cdk5	Cyclin-dependent kinase 5
CHD	Chromodomain helicase DNA-binding protein
ChIA-PET	Chromatin interaction analyses – paired end tags
ChIP-seq	Chromatin immunoprecipitation-sequencing assay
CPP assay	Conditioned place preference assay
CREB	cAMP-response element-binding protein
D1R	Dopamine 1 receptor
DARPP-32	Dopamine- and cyclic-AMP-regulated phosphoprotein 32
DAT	Dopamine transporter
DNMT	DNA methyltransferase
DOHaD	Developmental origins of health and disease
EMX2OS	EMX2 opposite strand
ENCODE	Encyclopedia of DNA elements
eRNA	Enhancer RNA
EWAS	Epigenome-wide association studies
fC	5-formylcytosine
GABA	Gamma-aminobutyric acid
GAD67	Glutamic acid decarboxylase 67
GFP	Green fluorescent protein
GluR2	Glutamate receptor 2
GWAS	Genome-wide association studies
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
Hi-C	Chromatin conformation capture assay
Histone modifications	Example H3K4me3 = histone H3 with trimethylated lysine-4
hmC	5-hydroxymethylcytosine
HOTAIR	HOX antisense intergenic RNA
HOTTIP	HOXA transcript at the distal tip
IGFBP-3	Insulin-like growth factor binding protein 3
JARID1C	Jumonji AT-rich interactive domain 1C protein
lincRNA	Large intervening noncoding RNA
LINE	Long interspersed nuclear element
MBD	Methyl-CpG-binding domain protein
mC	5-methylcytosine
MeCP2	Methyl CpG-binding protein 2
MEG3	Maternally expressed Gene 3 ncRNA
methylC-seq	5-methylcytosine sequencing assay
mGluR5	Metabotropic glutamate receptor 5
MGMT	Methyl guanine DNA methyltransferase
MIAT	Myocardial infarction associated transcript
miRNA	MicroRNA
MOR	Mu opioid receptor
mPFC	Medial prefrontal cortex

MSK1	Mitogen and stress-activated protein kinase 1
NAc	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptor
ncRNA	Noncoding RNA
NEAT1	Nuclear paraspeckle assembly transcript 1 ncRNA
NEAT2	Nuclear paraspeckle assembly transcript 2 ncRNA
PET	Positron emission tomography
PFC	Prefrontal cortex
PHD	Pleckstrin homology domain
piRNA	Piwi-interacting RNA
PRC2	Polycomb repressive complex 2
RIP-seq	RNA immunoprecipitation sequencing assay
SAHA	Suberoylanilide hydroxamic acid
SINE	Short interspersed nuclear element
siRNA	Small interfering RNA
SIRT1	Sirtuin 1
SNP	Single-nucleotide polymorphism
SPRED1	Sprouty-related EVH1 domain containing 1 protein
SUD	Substance abuse disorder
TORC	Transducers of regulated CREB
TPH2	Tryptophan hydroxylase-2
Uhrf1	Ubiquitin-like containing PHD and RING finger domains 1 protein
VTA	Ventral tegmental area
WD40 domain	WD dipeptide-containing domain

7.1 Introduction

7.1.1 The Environment and Epigenomic Regulation

With certain exceptions, an individual's genome is believed to be more or less identical in every cell. However, the epigenomes of different cell types within an individual appear to differ significantly from one another (Hawkins et al. 2010). This is consistent with the distinct phenotypes, functions, and gene expression profiles of particular cell types. There have been a number of reviews indicating that our epigenomes may be sensitive to "environmental" influences which can be broadly defined to include diet, toxins, stressors, and psychosocial influences (Jirtle and Skinner 2007; Zhang and Meaney 2010; Caldji et al. 2011). It has been hypothesized that environmental exposures may lead to changes in signaling in specific cell or tissue types. These changes may in turn impact epigenetic regulation

of gene expression, ultimately leading to transient or long-lasting changes in gene expression and cellular or organismal phenotypes. In some cases, these changes appear to be passed on through mitosis or even to subsequent generations (Youngson and Whitelaw 2008).

7.1.2 Epigenetic Regulation in the Nervous System

Epigenetic regulation has been shown to be important in neurogenesis, neural fate specification, neuronal development, behavioral plasticity, synaptic plasticity, circadian regulation, and learning and memory (Day and Sweatt 2011; Haggarty and Tsai 2011; Ma et al. 2010; Zocchi and Sassone-Corsi 2010; Bellet and Sassone-Corsi 2010; Maze and Nestler 2011; Nelson and Monteggia 2011; Ma et al. 2010; Dulac 2010; Namihira et al. 2008). Additionally, misregulation of epigenetic processes has been implicated in a number of human disorders including neurodevelopmental disorders (e.g., Rett and Prader-Willi syndromes) and psychiatric disorders (e.g., schizophrenia, depression) (Horsthemke and Wagstaff 2008; Graff and Mansuy 2009; Moretti and Zoghbi 2006; Tsankova et al. 2007; Pidsley and Mill 2011; Renthal and Nestler 2009a). Epigenetic regulation has also been implicated in response to early childhood abuse associated with suicide completion (McGowan et al. 2009). For the remainder of this chapter, I will focus on what is known about the role of epigenetic regulation in substance use and abuse.

7.2 Substance Abuse

7.2.1 Substance Use and Abuse

Our brains are inherently plastic, possessing connections and signaling processes that can change in response to distinct environmental exposures. Brains exposed to drugs of abuse on a continuing basis develop changes in particular neuronal regions, including those involved in the reward system. In addicted individuals, these brain changes can lead the individual to seek out drugs of abuse despite serious negative consequences. The definition of drug addiction has evolved over the years. According to Drs. Koob and Volkow: “Drug addiction is a chronically relapsing disorder that has been characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting intake, and (3) emergence of a negative emotional state (e.g. dysphoria, anxiety, irritability) reflecting a motivational withdrawal syndrome when access to drug is prevented” (Koob and Volkow 2010; American Psychiatric Association 2000; Koob and Le 1997). Common addictive substances include nicotine, alcohol, caffeine, cocaine, methamphetamine, opioids, certain prescription medications, inhalants, and cannabis. Food and sex are sometimes

referred to as "natural rewards" and can exhibit effects similar to those caused by drugs of abuse (Olsen 2011; Avena et al. 2008). There are also compulsive "behavioral addictions" such as gambling or internet addiction that have some of the hallmarks of drug addiction (Grant et al. 2010; Ambermoon et al. 2011).

In broad strokes, exposure to addictive substances, such as alcohol, opiates, cannabinoids, and nicotine, leads to increased levels of dopamine within the mesolimbic dopamine system (Sulzer 2011; Justinova et al. 2009). For example, cocaine inhibits dopamine reuptake such that more dopamine remains at the synapse (Newman and Kulkarni 2002; Fleckenstein et al. 2007). Continued drug exposure ultimately leads to adaptations in the strength of circuit connections between different brain regions including the nucleus accumbens (NAc). These changes in circuit strength are mediated in part by alterations in signaling by the neurotransmitters dopamine and glutamate in specific types of neurons with concomitant gene expression changes (Luscher and Malenka 2011; Kalivas et al. 2009; Gardner 2011). A strengthening of the reward connections leads to changes such that an individual craves the drug of abuse more, even if substance use leads to adverse consequences. A weakening of the inhibitory influence of the prefrontal cortex (PFC) on the reward circuitry can also decrease the ability of the individual to resist substance use (Van den Oever et al. 2010).

Not all individuals exposed to drugs of abuse become addicted. Based on heritability measurements, this complex disease appears to have an important genetic component with some individuals particularly susceptible to addiction, while others are resistant to it (Johnson et al. 1996; Kendler et al. 1999; Uhl et al. 2008; Buckland 2008). Individuals that are particularly impulsive or have an enhanced propensity for risk taking are more likely to explore the use of drugs, and this impulsivity phenotype may have a genetic component (Perry and Carroll 2008; Dalley et al. 2011). Environmental influences are important in the development of substance abuse disorder (SUD); access to drugs of abuse, early life adversity, poverty, or exposure to drugs during critical periods such as adolescence can all influence the potential of individuals to develop substance abuse disorder (Caspi et al. 2005; Buka et al. 2003; Hill et al. 2005).

The overall economic cost of substance abuse in the USA has been estimated to be greater than $600 billion per year (Table 7.1). For illicit drugs alone, the cost is estimated to be $193 billion, while for alcohol and tobacco, the costs are estimated to be $235 billion and $193 billion, respectively. In addition to the serious economic cost to society from SUD, the consequences of addiction are extraordinarily destructive to the addicted individuals and their families. Behavioral therapies can be used to improve outcomes in substance abusers, and in some cases, therapy in concert with medication can improve outcomes (Carroll and Onken 2005). Despite ongoing efforts to develop safe and effective medications for the treatment of SUDs, only limited success has been achieved. Currently, approved medications exist to aid in smoking cessation as well as to treat opiate and alcohol dependence, including therapies for the initiation of and maintenance of abstinence (e.g., nicotine replacement therapy, buprenorphine, varenicline, naltrexone), to alleviate symptoms of withdrawal (e.g., varenicline, diazepam), and to prevent relapse

Table 7.1 The estimated yearly economic cost of licit and illicit abused substances

Substance	Estimated economic cost per year	Reference
Illicit drugs	$193 billion	(National Drug Intelligence Center [NDIC] 2010)
Alcohol	$235 billion	(Rehm et al. 2009)
Tobacco	$193 billion	(Centers for Disease Control and Prevention [CDC] 2007)
All	>$600 billion	

(e.g., naltrexone, bupropion). However, with that said, no approved medications exist for the treatment cocaine, methamphetamine, or cannabis addiction even though efforts to develop these are ongoing (Montoya and Vocci 2008; McCann 2008). Unfortunately in the absence of good treatment options for the various SUD-related indications, addicted individuals will continue to struggle with this devastating disease.

In order to advance the development of effective medications and therapies to treat SUDs, it is critical that we understand the molecular and neurobiological mechanisms that lead to the development of addiction. Since exposure to drugs of abuse leads to long-lasting brain changes, it is not surprising that molecular studies exploring changes in gene expression in the nervous system have been very illuminating. For the rest of this chapter, I will focus on the role of transcriptional regulation in substance abuse with a focus on epigenomic regulation.

7.2.2 Transcriptional Regulation and Substance Abuse

Because exposure to drugs of abuse can lead to long-lasting brain changes including changes in neurotransmission in specific brain circuits, it has been proposed that alterations in gene expression via transcriptional regulation play a significant role (Nestler et al. 2001). The role of transcriptional changes in response to drugs of abuse has been well studied and reviewed (Nestler 2008; Nestler and Malenka 2004). Several transcription factors have been shown to play a role in addictive processes. For example, the cAMP-response element-binding protein (CREB), which has a well-established role in learning and memory, can alter drug abuse behaviors (Briand and Blendy 2010; Carlezon et al. 2005). In many ways addiction is an example of learning and memory gone seriously awry, so the identification of CREB is perhaps not so surprising.

Probably the most well-characterized transcription factor involved in drug addiction is the delta-FosB protein. Delta-FosB can be induced in the nucleus accumbens by opiates, cocaine, nicotine, other drugs of abuse, and natural rewards (such as sucrose and sex), and the targets of delta-FosB (e.g., GluR2, dynorphin, Cdk5) are congruent with the signaling molecules previously implicated in addictive processes (Nestler 2008). It has been proposed that significant delta-FosB

induction can lead to "excessive sensitization of the nucleus accumbens circuitry" and ultimately lead to compulsive drug taking (Nestler 2008).

Transcription factors bind to and can alter the properties of chromatin, and conversely chromatin state may impact transcription factor binding and function (Birney 2011; Adrian et al. 2010; Koche et al. 2011). Although the role of transcription factors in mediating long-term changes to gene expression in the brain has been fairly well studied, the role of epigenetic regulation in addictive processes had not been investigated to a significant extent until recently.

7.2.3 Epigenetic Regulatory Mechanisms in Substance Abuse

Of the major epigenetic regulatory mechanisms, histone posttranslational modifications including histone acetylation, histone methylation, and histone phosphorylation have been the best studied in the area of substance abuse. For DNA modifications, DNA methylation has been recently investigated, while the role of the recently discovered DNA hydroxymethylation has not been investigated to date. The roles of ATP-dependent chromatin remodeling and nucleosome position have not been well characterized in substance abuse as yet. Noncoding RNAs can be important regulators of gene expression. While microRNAs have been investigated with respect to substance abuse, other noncoding RNA types including lincRNAs (long intergenic noncoding RNAs) have not been well characterized to date.

7.3 Histone Modifications and Addictive Processes

More than 100 distinct posttranslational histone modifications have been identified and more are likely to be discovered in the future. While some modifications are associated with active chromatin and others are associated with silenced chromatin, the function of the majority of these modifications is currently unknown (Campos and Reinberg 2009). It is also unclear whether histone modifications at particular chromatin regions cause chromatin structural changes or are simply a consequence of these changes (Henikoff and Shilatifard 2011). The enzymes that are responsible for the deposition and removal of posttranslational histone modifications, such as histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methylases, and histone demethylases, are sometimes referred to as the "writers" and "erasers" of the histone code. Additionally, there are proteins that bind to histone posttranslational modifications, which are important for functions relevant to the modification. These molecules are sometimes referred to as "readers" of the histone code. Some of these "readers" contain protein domains such as PHD, Tudor, or WD40 that bind to methylated lysines or arginines, bromodomains that can bind to acetylated lysines, or 14-3-3 domains which can bind to phosphorylated residues (Gardner et al. 2011; Musselman and Kutateladze 2009; Sanchez and Zhou 2011; Kim et al. 2006;

Bonasio et al. 2010). There has been limited work studying histone modification "readers" in the nervous system, although the little information that exists is tantalizing. For example, the JARID1C protein is involved in X-linked mental retardation and in addition to being an H3K4 demethylase (a "writer"); it also appears to bind to H3K9me3 residues (Jensen et al. 2005; Iwase et al. 2007).

One of the most informative assays for detecting histone modifications is chromatin immunoprecipitation (ChIP) in which an antibody specific for a particular histone modification is used to immunoprecipitate cross-linked chromatin. The DNA regions associated with the histone modification can then be analyzed using either microarray analysis (ChIP-chip) or high-throughput sequence analysis (ChIP-seq) (Park 2009). Many genome-wide histone modification datasets for a diversity of cell or tissue types have been generated, enabling one to look at similarities and differences in histone modifications across cell types (Bernstein et al. 2010; Myers et al. 2011; Ernst et al. 2011). These datasets are accessible through a variety of web links including http://www.roadmapepigenomics.org/data and http://www.ncbi.nlm.nih.gov/epigenomics. In addition, a few cell types have been mapped genome-wide for up to 24 different histone modifications to reveal the extent to which histone posttranslational modifications co-occur with one another and with other genomic features as well as to identify chromatin states associated with particular sets of modifications (Heintzman et al. 2009; Hawkins et al. 2010; Ernst and Kellis 2010). Unfortunately, ChIP-quality affinity reagents do not exist for many histone modifications, and so their genomic pattern and function remain mysterious.

Histone acetylation has been the most well-studied histone modification in the nervous system. Histone acetyl marks are covalently attached to histone tails via HATs which include a variety of structurally distinct enzymes including the well-characterized CREB-binding protein (CBP) which has important functions in the nervous system (Dekker and Haisma 2009; Hallam and Bourtchouladze 2006). The HDAC enzymes that can remove these modifications comprise three classes: Class I, Class II, and Class III (sirtuins) (Thiagalingam et al. 2003). Histone acetylation tends to be associated with actively expressed genes, while deacetylated regions tend to be associated with gene silencing (Thiagalingam et al. 2003).

In the nervous system, histone modifications are known to have important functions (Miller 2011; Akbarian and Huang 2009; Tsankova et al. 2007; Bredy et al. 2010; Morris et al. 2010; Haggarty and Tsai 2011). For example, disruption of the HAT enzyme CBP leads to memory defects (Korzus et al. 2004). Mutations in CBP are associated with Rubinstein-Taybi syndrome, which has an intellectual disability phenotype (Petrij et al. 1995). Histone deacetylases have been implicated in depression (HDAC5), regulation of dendritic spine density and memory formation (HDAC2), negative regulation of long-term memory formation (HDAC3), cognition (the Class III HDAC SIRT1), and synaptic transmission (Tsankova et al. 2006, 2007; Guan et al. 2009; McQuown et al. 2011; Gao et al. 2010; Morris et al. 2010).

Chromatin modifications, such as histone acetylation, mediate some of the neuronal and behavioral changes induced by cocaine. As can be seen in Table 7.2,

Table 7.2 Selected epigenetic changes associated with exposure to drugs of abuse

Drug class	Drug of abuse	Epigenetic modification	Enzyme/molecule involved	Tissue/brain region	Reference
Stimulants					
	Cocaine	Histone acetylation (H3,H4)	HDAC/HAT?	Striatum	(Kumar et al. 2005)
	Cocaine	Histone acetylation (H3)	HDAC/HAT?	Unknown	(Malvaez et al. 2010)
	Cocaine	Histone acetylation	HDAC Class I/II	Striatum and ventral midbrain	(Schroeder et al. 2008)
	Cocaine	Histone acetylation	HDAC5	Nucleus accumbens	(Renthal et al. 2007)
	Cocaine	Histone acetylation (H3)	HDAC/HAT?	Medial prefrontal cortex	(Sadri-Vakili et al. 2010)
	Cocaine	Histone acetylation	HDAC/HAT	Nucleus accumbens	(Host et al. 2011)
	Cocaine	Histone acetylation?	HDAC/HAT?	Unknown	(Romieu et al. 2008)
	Cocaine	Histone acetylation?	HDAC/HAT?	Unknown	(Sun et al. 2008b)
	Cocaine	Histone acetylation (H3K5ac)	HDAC/HAT?	Dorsal striatum	(Brami-Cherrier et al. 2005)
	Cocaine	Histone acetylation (H4)	HAT (CBP)	Striatum	(Levine et al. 2005)
	Cocaine	Histone acetylation	Sirt1, Sirt2	Nucleus accumbens	(Renthal et al. 2009b)
	Cocaine	Histone methylation (H3K9me2)	Histone methyltransferase G9a	Nucleus accumbens	(Maze et al. 2010)
	Cocaine	Histone methylation (H3K9me3)	H3K9methyltransferase/ demethylase?	Nucleus accumbens	(Maze et al. 2011)
	Cocaine	Histone methylation (H3K4me3)	H3K4methyltransferase/ demethylase?	Hippocampus	
	Cocaine	Histone phosphorylation (H3S10)	Protein phosphatase-1	Striatum	(Stipanovich et al. 2008)
	Cocaine	Histone phosphorylation (H3S10)	MSK1	Dorsal striatum	(Brami-Cherrier et al. 2005)
	Cocaine	DNA methylation	DNMT3a	Nucleus accumbens	(Laplant et al. 2010)
	Cocaine	DNA methylation	DNMTs	Hippocampus	(Novikova et al. 2008)
	Cocaine	DNA methylation	DNMT3A, DNMT3B, MeCP2	Nucleus accumbens	(Anier et al. 2010)

(continued)

Table 7.2 (continued)

Drug class	Drug of abuse	Epigenetic modification	Enzyme/molecule involved	Tissue/brain region	Reference
	Cocaine	DNA methylation?	DNMT1, DNMT3a	Seminiferous tubules	(He et al. 2006)
	Cocaine	DNA methylation?	MeCP2 and microRNA-212	Dorsal striatum	(Im et al. 2010)
	Cocaine	Noncoding RNAs	microRNA-212	Dorsal striatum	(Hollander et al. 2010)
	Cocaine	Noncoding RNAs	miRNAs and Argonaute2	Striatum	(Schaefer et al. 2010)
	Cocaine	Noncoding RNAs	microRNA-124, miR-181a, let-7d	Nucleus accumbens	(Chandrasekar and Dreyer 2011)
	Cocaine	Noncoding RNAs	microRNA-8 family	Nucleus accumbens and striatum	(Eipper-Mains et al. 2011)
	Amphetamine	Histone acetylation?	HDAC/HAT?	Unknown	(Kalda et al. 2007)
	Amphetamine	Histone methylation (H3K9me2)	KMT1A histone methyltransferase	Striatum	(Renthal et al. 2008)
	Amphetamine	DNA methylation?	MeCP2	Nucleus accumbens	(Deng et al. 2010)
	Methamphetamine	Histone methylation (H3K4me3)	H3K4 methyltransferase?	Nucleus accumbens	(Ikegami et al. 2010)
	Methamphetamine	DNA methylation?	DNMT2	Hippocampus	(Numachi et al. 2004)
	Caffeine	Histone methylation (H3K9me2)	Histone methyltransferase G9a/GLP	Striatum	(Schaefer et al. 2009)
	Nicotine	Histone acetylation (H3K9ac)	HDAC2?	Striatum, prefrontal cortex	(Pastor et al. 2011a)
	Nicotine	DNA methylation	DNMT1	Frontal cortex	(Satta et al. 2008b)
	Nicotine	DNA methylation of MAOA	DNMT?	Lymphoblasts, whole blood	(Philibert et al. 2008); (Philibert et al. 2010)
	Nicotine?	Noncoding RNAs	microRNA-504	Cultured cells	(Huang and Li 2009)
	Smoking	DNA methylation	DNMT?	Blood cells	(Launay et al. 2009)
Inhalants					
	Solvents	Histone acetylation	HDAC/HAT?	Fly heads	(Wang et al. 2007b)

Cannabinoids					
	THC	?	?	?	
Opiates					
	Morphine	Histone acetylation (H3)	HDAC/HAT?	Unknown	(Jing et al. 2011)
	Morphine	Histone acetylation?	HDAC	Unknown	(Wang et al. 2010b)
	Morphine	Histone acetylation?	HDAC/HAT	Striatum?	(Sanchis-Segura et al. 2009)
	Morphine?	Histone acetylation	HDAC/HAT?	Neuronal cell culture	(Hwang et al. 2010)
	Morphine?	Histone methylation	Methyltransferase/ demethylases	Neuronal cell culture	(Hwang et al. 2010)
	Morphine	Unknown	Unknown	Not applicable	(Byrnes 2005)
	Heroin/methadone	DNA methylation	DNMT?	Lymphocytes	(Nielsen et al. 2009)
	Heroin	Noncoding RNAs	Long noncoding RNAs (lincRNAs)	Nucleus accumbens	(Michelhaugh et al. 2011)
Depressants					
	Alcohol	Histone acetylation (H3 and H4)	HDAC/HAT?	Amygdala	(Pandey et al. 2008)
	Alcohol	Histone methylation (H3K4me3)	H3K4methyltransferase/ demethylase?	Hippocampus	(Zhou et al. 2011b)
	Alcohol	DNA methylation	DNMT?	Prefrontal cortex	(Taqi et al. 2011)
	Alcohol	DNA methylation	DNMT?	Cultured neurons	(Marutha Ravindran and Ticku 2004)
	Alcohol	DNA methylation of MAOA	DNMT?	Lymphoblasts	(Philibert et al. 2008)
	Alcohol	DNA methylation	DNMT?	Blood	(Muschler et al. 2010)
	Alcohol	DNA methylation	DNMT?	Blood	(Bonsch et al. 2004)
	Alcohol	Noncoding RNAs	microRNA-9	Cultured neurons	(Pietrzykowski et al. 2008)

This table provides an overview of selected studies in the realm of epigenetic regulation and addictive processes. When known, the epigenetic modification, enzymes, or other associated molecules are indicated. Also indicated are the tissue types or brain regions investigated when known

histone acetylation has been the best studied class of histone modifications for addictive processes, although no doubt much more remains to be learned about its functions. Histone acetylation is particularly interesting from a translational point of view since certain medications that inhibit HDAC activity are clinically approved for treating seizure disorders and particular types of cancer (Sharma et al. 2010). The effects of these inhibitors on nervous system function and drug-taking behaviors are discussed in more detail in the Therapeutics section.

The role of histone acetylation and deacetylation in addictive processes in rodents has been recently reviewed; therefore, I will focus on a few of the key studies related to cocaine responses (Renthal and Nestler 2009b; McQuown and Wood 2010; Laplant and Nestler 2011; Wong et al. 2011). This will be followed by descriptions of some recent work on the role of the Class III HDACs (sirtuins) and then studies on tolerance to benzyl alcohol in a *Drosophila* model of inhalant exposure which helps delineate some of the detailed molecular events that may be occurring. I will then touch on several other histone modifications of particular interest including histone dimethylation, histone trimethylation, and histone phosphorylation.

7.3.1 Histone Acetylation and Cocaine Responses

Acute, but not chronic, cocaine exposure is known to induce expression of the mRNA that encodes the cFOS transcription factor. Using ChIP Dr. Eric Nestler and colleagues found that acute, but not chronic, cocaine exposure led to increased acetylation of histone H4 at the cFOS gene promoter, but had no significant effect on histone H3 acetylation (Kumar et al. 2005). Conversely chronic, but not acute, cocaine exposure induces the BDNF and Cdk5 genes. Chronic cocaine exposure led to increased acetylation of histone H3 but had no significant effect on histone H4 acetylation. These data suggest that a chromatin state (acetylation of histones H4 or H3 near the promoters of particular genes) may in part indicate which genes are modulated in response to acute or chronic cocaine administration. Taking these experiments one step further, the investigators showed that administration of the HDAC inhibitor trichostatin A to rodents prior to cocaine administration enhanced the reward response to cocaine, while overexpression of the HDAC4 gene in the striatum using herpes simplex virus decreased the reward response to cocaine.

In a second paper further exploring the roles of HDACs in drug responses, several HDACs were found to be expressed in the NAc, with HDAC3 and HDAC5 having the highest expression levels (Renthal et al. 2007). Viral overexpression of HDAC5 in the NAc led to a reduction in the rewarding properties of cocaine using a conditioned place preference assay, while HDAC5 knockout animals were found to have the converse phenotype, with increased preference for the cocaine-paired chamber. HDAC5 was required specifically in the NAc to regulate this behavioral response, since viral expression of HDAC5 in the NAc of HDAC5 knockout animals reduced the preference for the cocaine-paired chamber.

Interestingly, the investigators also looked at the role of HDAC5 in chronic stress using a social defeat behavioral assay. While acute stress had no effect on HDAC5 levels in the NAc, chronic stress was associated with reduced levels of the HDAC5 mRNA, and HDAC5 knockouts exhibited hypersensitivity to chronic stress. HDAC5 modulation of behavioral responses to cocaine reward and chronic stress responses is quite significant given the important role of stress in drug abuse relapse.

7.3.2 Histone Acetylation, Sirtuins, and Cocaine Responses

In experiments described in the previous section, Dr. Renthal and coworkers characterized genome-wide levels of histone acetylation from the nucleus accumbens (NAc) brain region of rodents treated chronically with cocaine. These studies revealed that many genes previously known to be upregulated by cocaine exposure also have increased acetylation of histone H3 and H4. The investigators then looked genome-wide to identify the binding sites of the cocaine-induced transcription factors delta-FosB and CREB in the NAc of cocaine-treated animals (Renthal et al. 2009a). Cross comparison of these datasets identified many genes that had not previously been implicated in response to cocaine, including the Sirtuin genes (Sirt1 and Sirt2) which function as NAD-dependent histone deacetylases (Vaquero et al. 2007). While these genes function in many biological processes, including circadian and metabolic regulation and aging, their role in the nervous system is not well understood (Haigis and Sinclair 2010; Herranz and Serrano 2010). The investigators used pharmacological activators and inhibitors of sirtuins to look at their function in cocaine responses. Interestingly, systemic pharmacological activation of sirtuins dramatically enhanced the rewarding effect of cocaine, while inhibition of sirtuins had the opposite effect. Pharmacological modulation of sirtuin function may be a fruitful future avenue to explore in the development of therapeutic agents to treat cocaine addiction.

7.3.3 Histone Modifications and Inhalant Exposure in Drosophila

Dr. Nigel Atkinson and coworkers exploited the genetically powerful fruit fly model system to investigate the molecular basis of inhalant tolerance. It has been observed that adult flies become tolerant to sedation by organic solvents (which sometimes are abused as inhalants) and this reduced sensitivity to inhalant requires increased expression of the *slowpoke* potassium channel gene which in turn alters neuronal function (Wang et al. 2007a).

In order to investigate the molecular mechanism behind this observation, Dr. Atkinson and coworkers found that a single exposure to inhalant led to epigenetic changes in regulatory regions of the *slowpoke* gene leading to altered expression of the *slowpoke* gene and reduced sensitivity (tolerance) to additional inhalant exposure. Specifically, they observed that the pattern of acetylation of histone H4 was altered across the *slowpoke* gene, which likely led to a more open localized chromatin structure and subsequent increased expression of the *slowpoke* gene. Exposure of the animals to a pharmacological inhibitor of histone deacetylases, the class of enzymes responsible for the histone H4 acetylation, also led to similar epigenetic and gene expression change as well as tolerance of the animals to inhalant.

Interestingly, Dr. Atkinson and colleagues found DNA elements within the *slowpoke* promoter that could be bound by the CREB transcription factor. A number of labs have shown that the CREB transcription factor is important in the responses of organisms to illicit substances, as well as in other neuroplastic processes such as learning and memory (Han et al. 2007). Using a genetic manipulation to "turn off" CREB, the researchers found that the epigenetic and expression changes to *slowpoke* gene and the development of behavioral tolerance no longer occurred. These results indicated that the CREB transcription factor is required for these processes.

Dr. Atkinson and coworkers also found that sedation with benzyl alcohol leads to increased expression of positively acting CREB isoforms and reduced expression of negatively acting CREB isoforms (including dCREB2). Specifically the dCREB2 isoform shows increased occupancy at the *slowpoke* promoter immediately after benzyl alcohol sedation in a chromatin immunoprecipitation assay. Animals with a knockout in dCREB2 no longer have increased benzyl alcohol-induced *slowpoke* gene expression and also no longer develop tolerance to this organic solvent.

Overall, this work clearly shows that exposure to an organic solvent can alter future sensitivity to the solvent via epigenetic regulatory mechanisms. It also provides insight into the precise mechanisms by which exposure to an inhalant can lead to epigenetic and expression level changes for a single gene, resulting in altered neuronal function and altered behavioral responses of an animal to future inhalant exposure. Although this work was performed using model inhalant, similar mechanisms may well be utilized for responses to other drugs of abuse.

7.3.4 Histone Dimethylation (H3K9me2) and Cocaine Responses

Histone dimethylation (H3K9me2) is normally associated with gene silencing (Wen et al. 2009; Barski et al. 2007). In an article published in *Science*, Dr. Maze and colleagues observed that histone methylation levels are reduced in the nucleus

accumbens (NAc) of rodents (Maze et al. 2010). To further explore this phenomenon, the researchers investigated the gene expression levels of the histone dimethyltransferases and demethylases that regulate this chromatin modification and found that levels of the G9a and GLP histone dimethyltransferases are downregulated upon cocaine administration. The investigators then looked at the effect of G9a manipulation in the NAc on the behavioral effects of cocaine and found that overexpression of G9a decreased the rewarding properties of cocaine, while knockdown of G9a increased the rewarding properties of cocaine. The researchers showed that these behavioral changes were correlated with concomitant changes in G9a levels and global histone dimethylation. Looking upstream of G9a, repeated cocaine exposure increased the levels of the transcription factor delta-FosB, leading to decreased G9a levels. Looking downstream, many of the genomic targets of histone dimethylation are known to play roles in the regulation of dendritic plasticity, and in fact dendritic spine density was shown to be altered by G9a levels. Overall Dr. Nestler and colleagues have elucidated an elegant multistep molecular pathway in which repeated cocaine exposure leads to delta-FosB activation, downregulation of G9a, and reduction in global histone dimethylation levels. Histone dimethylation is normally associated with gene silencing, so decreased histone dimethylation likely leads to increased expression of genes that regulate dendritic plasticity. This change in gene expression leads to increased dendritic spine density and ultimately increased behavioral preference for cocaine. Thus, small molecules that target the activities of histone demethylases or histone dimethyltransferases could have potential efficacy as therapeutic agents for treating cocaine addiction.

7.3.5 Histone Trimethylation (H3K9me3) and Cocaine Effects on Heterochromatin

Histone H3 Lysine 9 trimethylation (H3K9me3) is associated with silencing of heterochromatic regions of the genome (Schotta et al. 2004; Yamada et al. 2005). Work by Dr. Maze and colleagues found that cocaine exposure results in changes in H3K9me3 levels in the NAc but not in brain regions such as the caudate putamen or medial prefrontal cortex (Maze et al. 2011). When ChIP-seq assays were performed on the NAc from cocaine-treated animals, thousands of repetitive elements (e.g., LINEs, SINEs) were associated with increased H3K9me3 binding, while thousands of other sites had decreased binding. Overall, "repeated cocaine decreases H3K9me3 binding and un-silences several specific retrotransposons (e.g. LINE-1)" in the NAc (Maze et al. 2011).

7.3.6 Histone Trimethylation (H3K4me3) Changes Associated with Cocaine and Alcohol Exposure

Histone trimethylation (H3K4me3) tends to be associated with gene promoters (Guenther et al. 2007; Bernstein et al. 2005). Work by Drs. Goldman and Mash and coworkers have shown that histone trimethylation is important in chronic exposure to cocaine and to alcohol (Zhou et al. 2011a). Genome-wide ChIP-seq for H3K4me3 was performed on postmortem hippocampal samples from chronic cocaine addicts, alcoholics, and controls. The investigators found that chronic cocaine use was associated with H3K4me3 changes at >1,100 gene promoters, while chronic alcohol use was associated with changes at >700 promoters. The authors indicate that "there was significant overlap of the changes between the cocaine and alcohol exposure groups" (Zhou et al. 2011a). Interestingly, these H3K4me3 changes did not correlate well with gene expression changes measured in parallel, suggesting that perhaps additional chromatin or transcriptional regulation is important in mediating gene expression changes in response to cocaine and alcohol exposure.

7.3.7 Histone H3S10 Phosphorylation and Cocaine Responses

Phosphorylation of H3S10 has previously been shown to be important in chromatin condensation and transcriptional activation (Nowak and Corces 2004; Crosio et al. 2003). The DARPP-32 protein (dopamine-regulated and cyclic-AMP-regulated phosphoprotein) has been a well-characterized role in responses to cocaine and other drugs of abuse (Svenningsson et al. 2005). Interestingly, Stipanovich and colleagues have identified a regulatory cascade whereby DARPP-32 leads to altered Histone H3 phosphorylation (Stipanovich et al. 2008). Exposure to drugs of abuse via dopamine 1 receptor (D1R) regulation leads to the accumulation of DARPP-32 in the nuclei of D1R-expressing striatal neurons. This nuclear accumulation appears to be regulated by phosphorylation of Ser-97 of DARPP-32, such that when Ser-97 is unphosphorylated, DARPP-32 is primarily nuclear. The dephosphorylation of Ser-97 is mediated by protein phosphatase 2A. The researchers then looked at the effects on histone phosphorylation and found that cocaine exposure led to increased levels of H3S10 phosphorylation. This pathway reveals a mechanism by which drugs of abuse, via dopamine signaling, can influence chromatin and presumably impact gene expression.

7.4 DNA Modifications and Addictive Processes

Methylation of cytosine nucleotides (mC) has long been thought to be the only covalent mammalian DNA modification and is often referred to as the "fifth base" (Lister and Ecker 2009; Miranda and Jones 2007). DNA methyltransferases (DNMTs) are the enzymes that methylate DNA; this methylation frequently occurs at cytosines that occur as dinucleotides followed by guanine (CpG) (Turek-Plewa and Jagodzinski 2005). DNA methylation appears to be a dynamic rather than a static process, especially in the nervous system (Guo et al. 2011; Ma et al. 2009). The enzymes responsible for active DNA demethylation have been difficult to pin down despite significant efforts, and a number of candidate enzymes and mechanisms have emerged (Wu and Zhang 2010; Ooi and Bestor 2008). There have been recent discoveries addressing DNA methylation reversal mechanisms which involve conversion of mC to an intermediate molecular form which can then be excised by thymine DNA-glycosylase (He et al. 2011; Cortellino et al. 2011). DNA methylation has been traditionally associated with gene silencing, but the context of DNA methylation (methylation in CpG islands, CpG island shores, in promoters, or in gene bodies) appears to be important in mediating the functional effects of mC (Ndlovu et al. 2011). Interestingly, genome-wide single-base resolution DNA methylation maps reveal that human embryonic stem cells and reprogrammed induced pluripotent stem cells contain high levels of DNA methylation in a non-CpG context, although the function of non-CpG methylation is not clear (Lister et al. 2009, 2011). As more whole methylome datasets are generated for different cell/tissue types and this information is compared to histone modification, gene expression, and other data, the precise role of mC and the cross talk between DNA methylation and other regulatory mechanisms will become more clear.

Additional covalent DNA modifications were known to occur in other organisms such as plants and bacteriophage (Vanyushin 2006; Fleischman et al. 1976). However, in 2009 a "sixth base" was discovered in mammalian cells: hydromethylcytosine (hmC) (Kriaucionis and Heintz 2009; Tahiliani et al. 2009). Hydroxymethylcytosine was discovered in Purkinje cells in the cerebellum, and an independent paper showed that the TET1 enzyme can convert mC to hmC. Since then, researchers have been working to discover putative functions for hmC and have identified likely roles for hmC in transcriptional regulation and regulation of pluripotency (Pastor et al. 2011b; Ndlovu et al. 2011; Ficz et al. 2011; Wu and Zhang 2011; Stroud et al. 2011; Wossidlo et al. 2011; Szulwach et al. 2011). A very exciting publication indicates that TET proteins can catalyze the in vitro formation of 5-carboxylcytosine (caC) and 5-formylcytosine (fC) from mC (Ito et al. 2011). These new DNA modifications may be intermediates in a TET-mediated DNA demethylation pathway or perhaps could have unexpected regulatory properties of their own. Only time will tell whether these or other novel DNA modifications will be discovered in the genomes of nervous system cells.

As described earlier, specific proteins can bind to different histone modifications and may play an important role in mediating their effects. Similarly for DNA modifications, proteins in the MBD and Kaiso families have been shown to bind to mC, while a recent report indicates that the Uhrf1 protein can bind to hmC (Bogdanovic and Veenstra 2009; Frauer et al. 2011). Methyl CpG-binding protein-2 (MeCP2), a member of the MBD family discussed below, can bind to mC and presumably plays a role in mediating the effects of DNA methylation, perhaps by recruiting additional proteins or protein complexes to a particular region of chromatin.

There are a number of useful assays to determine DNA methylation status including MethylC-seq, which provides genome-wide DNA methylation information at single-base resolution (Lister et al. 2009; Harris et al. 2010). Unfortunately, bisulfite-based sequencing strategies do not distinguish between mC and hmC, but the development of new assays to detect and distinguish between hmC and mC should help us to elucidate the function of hmC in the nervous system (Pastor et al. 2011b). In fact a recently developed Tet-based bisulfite sequencing protocol (TAB-Seq) in combination with traditional bisulfite sequencing enables base resolution mapping of hmC and confirms widespread distribution of 5hmC in embryonic stem cells (Yu et al. 2012).

There is now a substantial body of work showing that DNA methylation has multiple roles in the nervous system, including a significant role in memory formation (Day and Sweatt 2010; Feng and Fan 2009). Mutations in the DNA methyltransferase DNMT1 lead to neurodegeneration (Klein et al. 2011), while the GABAergic neurons of some human schizophrenics show increased DNMT levels (Klein et al. 2011; Zhubi et al. 2009; Mill et al. 2008). In addition, the methyl CpG-binding protein-2 (MeCP2) is a transcriptional regulator originally identified as a protein that can bind to mC (Lewis et al. 1992). Work by Dr. Huda Zogbhi and colleagues have indicated that defects in MeCP2 function are associated with the neurodevelopmental disorder Rett syndrome (Amir et al. 1999).

As can be seen in Table 7.2, there have been a number of studies investigating the effects of drugs of abuse on DNA methylation. I will discuss the role of DNA methylation with respect to cocaine and nicotine exposure as well as the role of the mC-binding protein MeCP2 in addictive processes.

7.4.1 DNA Methylation and Cocaine Responses

Work by Dr. Laplant and coworkers in the Nestler group shows that both chronic cocaine exposure and chronic social defeat stress can lead to changes in the expression of the DNMT3a DNA methyltransferase gene. DNA methylation was found to be required for the cocaine-induced formation of thin dendritic spines in the NAc. To functionally test the role of DNMT3a in cocaine reward, DNMT3a was conditionally knocked down in the NAc, and the treated animals preferred the

cocaine-paired chamber in a CPP assay. In the converse experiment, DNMT3a was overexpressed in the NAc using a herpes simplex virus vector, and the preference of the animal for the cocaine-paired chamber was reduced. NAc injection of the DNA methylation inhibitor RG108 led to increased cocaine CPP, while administration of methionine, which promotes DNA methylation, led to a decrease in cocaine CPP. The authors conclude from these pharmacological and genetic manipulations of DNMT3a that "increased Dnmt3a expression in NAc negatively regulates cocaine reward, whereas decreased Dnmt3a enhances cocaine reward" (Laplant et al. 2010).

7.4.2 DNA Methylation and Nicotine Responses

DNA methylation appears to also play a role in mediating responses to nicotine. Mice injected with nicotine had decreased levels of DNMT1 in the frontal cortex and the hippocampus, but had normal DNMT1 levels in the GABAergic neurons of the striatum (Satta et al. 2008a). Pharmacological experiments were used to show that nicotinic acetylcholine receptor (nAChR) function was required to achieve the change in DNMT levels in the frontal cortex. Further experiments revealed that nicotine exposure led to upregulation of GAD67 (glutamic acid decarboxylase 67) protein in the frontal cortex, but not in the striatum, and that this upregulation was associated decreased CpG methylation in the GAD67 promoter. Overall this study identifies a potential mechanism of action by which nicotine could mediate neuronal gene expression changes via DNA methylation. This study also suggests that therapeutic agents that modulate DNA methylation changes in the appropriate brain regions could be of potential use in treating nicotine addiction or perhaps schizophrenia.

7.4.3 A Role for MeCP2, a Methyl-C-Binding Protein, in Substance Abuse

MeCP2 is known to play a role in the neurodevelopmental disorder Rett syndrome; however, the neurobiological functions of MeCP2 are not completely understood and are under active investigation. MeCP2 can bind to methylated cytosine residues and presumably can recruit additional proteins or protein complexes to a particular region of chromatin to regulate transcription or other molecular processes. Some studies have suggested that MeCP2 regulates alternative splicing, can bind promoters that are not methylated, or may regulate neuronal genome function through histone acetylation in a more global fashion (Yasui et al. 2007; Young et al. 2005; Skene et al. 2010). Neuronal activity is known to stimulate CaMKII phosphorylation of MeCP2 Serine 421 to modulate "dendritic patterning, spine morphogenesis, and the activity-dependent induction of *Bdnf* transcription"

(Zhou et al. 2006; Chen et al. 2003). Functional work indicates that disruption of MeCP2 specifically in GABA-releasing neurons leads to behavioral phenotypes, including compulsive grooming, reminiscent of phenotypes characteristic of Rett syndrome patients (Chao et al. 2010). MeCP2 is not just required during a specific developmental window but is required in adult animals for proper brain function (McGraw et al. 2011). Recent studies suggest that MeCP2 loss stimulates L1 transposition (Muotri et al. 2010).

Several publications point to a role for MeCP2 in regulation of responses to drugs of abuse. Work by Dr. West and colleagues has shown that MeCP2 knockdown in the NAc leads to increased preference of mice for amphetamine using a CPP assay, while animals no longer had preference for the amphetamine-paired chamber when MeCP2 was overexpressed in the NAc (Deng et al. 2010). Interestingly, in a strain of MeCP2 mutant mice, the authors found an almost twofold increase in GABAergic synapses in the NAc as compared to control animals, indicating that MeCP2 is required for the developmental wiring of this brain structure. Furthermore, MeCP2 mutant mice did not show normal amphetamine-induced changes in dendritic spine density of medium spiny neurons, nor did they show normal amphetamine-induced changes in immediate early gene expression in the striatum, both of which correlate with impaired amphetamine-induced behavioral changes in this strain.

In related work Dr. Kenny and colleagues showed that lentiviral knockdown of MeCP2 in the striatum leads to decreased cocaine consumption (Im et al. 2010). Interestingly, MeCP2 was found to repress expression of the miR-212 microRNA involved in regulation of cocaine-taking (discussed in more detail in the microRNA section of this chapter) (Im et al. 2010; Hollander et al. 2010). Furthermore, miR-212 can repress MeCP2 expression, while cross talk between miR-212 and MeCP2 regulates the impact of cocaine on brain-derived neurotrophic factor (*Bdnf*) levels in the striatum. Interestingly, Dr. Sadri-Vakili and colleagues found that cocaine self-administration was associated with decreased MeCP2 binding to brain-derived neurotrophic factor (*Bdnf*) promoter IV in the medial prefrontal cortex (mPFC) (Sadri-Vakili et al. 2010). The medial prefrontal cortex (mPFC) is one of several brain regions that has dopaminergic inputs from the ventral tegmental area (Le et al. 2005). Based on this study and the two publications above, it appears that MeCP2 has an important role in addictive processes and may have special functions in distinct brain regions.

Work by Teresa Reyes and colleagues has shown that in an animal model of diet-induced obesity, animals on a chronic high-fat diet were found to have epigenetic changes in the mu opioid receptor (MOR) promoter in reward areas of the brain (VTA, NAc, PFC). These epigenetic changes included increased H3K9me2, decreased H3ac, increased DNA methylation, and increased MeCP2 binding to the MOR promoter (Vucetic et al. 2011). Related work revealed that high-fat diet influenced dopamine reuptake transporter (DAT) gene expression in the VTA, NAc, and PFC (Vucetic et al. 2010). DNA methylation changes were also observed in DAT and other reward-related genes in the hypothalamus, NAc, and PFC. These molecular changes associated with exposure to high-fat diet suggest that epigenetic

regulation in response to diet and to drugs of abuse could have significant overlap and raises the possibility of whether or not specific dietary regimens, in combination with other therapeutic interventions, could influence addictive processes.

7.4.4 MeCP2 and Epigenomic Regulation of Genomic Structure

As mentioned earlier, MeCP2 was found to play a role in regulation of transposition of L1 retrotransposons (Muotri et al. 2010). Dr. Muotri and colleagues found that there is increased L1 retrotransposition in neural precursor cells derived from patients with MeCP2 mutations. Additionally in a mouse strain designed to detect L1 transposition events using a fluorescent reporter, there were higher numbers of GFP-positive cells in brain tissue from MeCP2 knockout animals as compared to wild type. The results from this paper suggest a possible scenario in which L1 retrotransposons may be methylated and bound by MeCP2, inhibiting retrotransposition. In the absence of MeCP2, retrotransposition becomes more frequent. This study raises the intriguing possibility that our somatic genomes may be much more diverse than we previously expected and that epigenomic regulation may play an important role in regulating somatic genomic diversity.

Could drugs of abuse impact somatic genomic structure via epigenomic regulation? Work by Dr. Maze and colleagues described earlier showed that cocaine exposure leads to changes in Histone H3 Lysine 9 trimethylation (H3K9me3) in the NAc but not two other brain regions (Maze et al. 2011). H3K9me3 is associated with heterochromatin silencing (Schotta et al. 2004; Yamada et al. 2005). The authors indicate that overall "repeated cocaine decreases H3K9me3 binding and unsilences several specific retrotransposons (e.g. LINE-1)" in the NAc (Maze et al. 2011).

LINE-1 retrotransposition during neurodevelopment and neurogenesis could contribute to genomic diversity within the somatic genomes of neurons (Singer et al. 2010). Taken together, the effects of MeCP2 mutation on LINE-1 retrotransposition and the H3K9me3 work suggest the testable hypothesis that repeated cocaine administration could lead to unsilencing and retrotransposition of LINE-1 elements in the NAc leading, in some cases, to permanent gene disruption or long-lasting alterations in gene expression (Muotri et al. 2010). Obviously animal and postmortem brain studies of cocaine-exposed individuals would be needed to assess whether cocaine exposure induces LINE-1 retrotransposition in the NAc and, if so, what the functional consequences of retrotransposition might be.

7.5 Noncoding RNAs and Addictive Processes

Many large and small noncoding RNAs (ncRNAs) have been identified in recent years, and some of these have significant regulatory functions. In particular, small ncRNAs (approximately 20–30 nucleotides) play key roles in gene transcription and translation. For example, Piwi-interacting RNAs (piRNAs) are involved in transposon silencing, and small interfering RNAs (siRNAs) are involved in regulating mRNA levels and chromatin formation (in plants and yeast) and in antiviral responses (in animals), while microRNAs (miRNAs) can simultaneously regulate the mRNA levels and translational efficacy for tens to hundreds of genes (Kaikkonen et al. 2011; Hannon et al. 2006; Czech and Hannon 2011; Marques and Carthew 2007). In the nervous system, a recent paper shows that piRNAs and the piRNA-associated protein MIWI are found in the hippocampus and may play a role in the morphogenesis of spines (Lee et al. 2011). miRNAs have been shown to play diverse roles in processes including neural development, survival, and degeneration, synaptic plasticity, dendritic spine morphology, and memory formation (Olde Loohuis et al. 2011; Saba and Schratt 2010; Davis et al. 2008; Schratt 2009; Schaefer et al. 2007; Schratt et al. 2006; St. Laurent et al. 2009; pp. 81–88; Lin et al. 2011). In mammalian cells miRNAs have important cytoplasmic functions, although as yet they have not been shown as yet to have epigenetic regulatory function (Khraiwesh et al. 2010). Some miRNAs have a profound effect on addictive behaviors and for this reason have been included in this chapter.

In addition to small RNAs, some of the longer ncRNA species include enhancer RNAs (eRNAs) and large intergenic noncoding RNAs (lincRNAs). eRNAs are associated with enhancer regions of the genome that likely play a role in transcriptional regulation (Wang et al. 2011). Recent work utilizing a specific chromatin signature identified more than 1,000 mammalian lincRNAs, and some lincRNAs have been shown to be involved in regulation of cellular differentiation (Guttman et al. 2009; Guttman et al. 2011). The lincRNA HOTAIR seems to be able to impact chromatin remodeling by binding to multiple enzymes which are able to modify histones, while the lincRNA HOTTIP seems to be able to "transmit information from higher order chromosomal looping into chromatin modifications" in order to regulate gene expression (Tsai et al. 2010; Wang et al. 2011). Some lincRNAs form complexes with proteins such as polycomb repressive complex 2 (PRC2), and the development of assays such as RIP-seq has allowed the identification of RNAs associated with PRC2 (Zhao et al. 2010). Assays enabling the identification of genomic regions associated with lincRNAs will be an important future tool needed to help uncover regulatory cross talk that may occur between lincRNAs and other epigenetic regulatory mechanisms. Some lincRNAs are expressed in brain and may play a role in the specification of glial and neuronal fates (Mercer et al. 2008, 2010). For example, the RCNR2 RNA has been show to play a role in specification of retinal cell fate (Rapicavoli et al. 2010). Future research is likely to uncover additional roles for lincRNAs in the nervous system.

As shown in Table 7.2, investigations into the role of noncoding RNA regulation in addictive processes have been quite limited to date, and only one study has investigated any role for lincRNAs. This section of this chapter will focus on an emerging role for lincRNAs in heroin abuse followed by a more complete story on microRNA regulation in cocaine-taking behavior.

7.5.1 lincRNAs and Heroin Use

Drs. Bannon and Lipovich and colleagues found that 23 lincRNAs were represented on microarrays being used to characterize gene expression changes in postmortem NAc tissue from heroin and non-heroin users (Michelhaugh et al. 2011). These investigators found that five of these lincRNAs (MIAT, MEG3, NEAT1, NEAT2, and EMX2OS) were expressed in the NAc and further that all five were upregulated in the heroin users as compared to non-heroin users. Thus, a potential lincRNA function may be to regulate widespread gene expression, and this regulation may be disrupted in the NAc of drug-abusing individuals. Further exploration of the role of lincRNAs in neuroplastic and addictive processes is an important area of investigation for the future.

7.5.2 miRNAs and Cocaine

The role of miRNAs in addictive processes has recently been reviewed (Li and van der Vaart 2011; Pietrzykowski 2010). As indicated in Table 7.2, roles have been described for microRNA regulation of the dopamine 1 receptor involved in nicotine dependence and for microRNA regulation of response to alcohol exposure (Huang and Li 2009; Pietrzykowski et al. 2008). There have been several projects that have successfully identified miRNAs involved in cocaine responses (Eipper-Mains et al. 2011; Chandrasekar and Dreyer 2011). Another study shows that rodents with an Argonaute 2 (Ago2) protein deficiency in the dopamine receptor 2 expressing neurons have altered intravenous cocaine self-administration in mice (Schaefer et al. 2010). Ago2 is known to play an important role in miRNA biogenesis and function, supporting a role for miRNAs in cocaine reinforcement (O'Carroll et al. 2007).

A major discovery in this area has been made regarding miRNA regulation of cocaine-taking behavior. Dr. Paul Kenny and coworkers have identified a 21-nucleotide microRNA, miR-212, that is found at higher levels in the dorsal striatal brain region of animals that self-administer cocaine (Hollander et al. 2010). In rats with extended access to cocaine, reduction of miR-212 levels in the striatum leads to increased cocaine intake, while overexpression of miR-212 leads to decreased cocaine intake. Further molecular experiments revealed that miR-212 achieves its effects via simultaneous reduction in expression of several mRNAs encoding

regulatory proteins that impinge upon the Raf1 protein kinase signaling pathway (including the SPRED1 repressor of Raf1). Overall these gene expression changes lead to increased levels of Raf1 protein kinase activity, increased expression of the CREB regulatory protein TORC, and ultimately increased activity of the transcription factor CREB. In a separate publication, miR-212 was found to regulate and be regulated by MeCP2 (see MeCP2 section of this chapter for details) (Im et al. 2010).

The identification of novel miRNA regulatory pathways that control cocaine intake, as well as responses to other drugs of abuse, could reveal new and unexpected targets for the development of potential therapeutic agents to treat addiction. Two key areas for future research in the miRNA arena are the identification and characterization of the target mRNAs regulated by these miRNAs as well as studies investigating whether or not these and/or other miRNAs are associated with addictive behaviors in human populations.

7.6 The Perdurance of Epigenomic Changes

While some chromatin changes are transient and occur as a normal part of transcriptional regulation, others are more long lasting and could be particularly important in the case of terminally differentiated post-mitotic neurons (Miller et al. 2010). There is also evidence for mitotically heritable chromatin changes that may impact progeny cells (Ng and Gurdon 2008). In some cases, epigenomic changes may even be meiotically heritable and affect the next generation (Youngson and Whitelaw 2008). The occurrence and perdurance of some types of epigenetic changes is likely to be influenced by factors including the nature of any environmental exposures involved, the cell type involved, and whether or not that cell type is exposed during a particular developmental window. There is increasing evidence that certain environmental exposures during critical developmental periods such as prenatal development, adolescence, or periods of germline maturation are associated with disease consequences. This concept has been captured in the developmental origins of health and disease (DOHaD) hypothesis which "proposes that during critical periods of prenatal and postnatal mammalian development, nutrition and other environmental stimuli influence developmental pathways and thereby induce permanent changes in metabolism and chronic disease susceptibility" (Waterland and Michels 2007). In the following two sections, we will discuss the limited literature concerning the stability of epigenomic changes associated with exposure to drugs of abuse during particular developmental periods.

7.6.1 Somatic Effects of Drugs of Abuse

The stability of epigenomic changes is a particularly difficult problem to investigate in the brain. For animal studies, individuals need to be sacrificed, and thus, only a

single time point can be generated per animal. Surprisingly few studies have been performed looking at how long epigenomic changes can last within the nervous system. Work by Dr. Courtney Miller and colleagues indicates that fear conditioning-associated DNA methylation changes in the *Calcineurin* gene can last for at least 30 days in the dorsal medial PFC (Miller et al. 2010). Researchers in the Nestler laboratory found H3K9me2 changes in the nucleus accumbens that last for at least 28 days using a social defeat behavioral paradigm (Wilkinson et al. 2009). Investigations into the stability and dynamics of somatic epigenomic changes in response to drugs of abuse and potential drug abuse therapeutics will be important for the future.

7.6.2 *Multigenerational and Transgenerational Effects of Drugs of Abuse*

There is evidence that exposure to certain chemical toxins, social environments, or nutrient levels can, in some cases, lead to specific phenotypes in subsequent generations. These phenotypes can be transmitted without an apparent DNA change through multiple generations even when these generations have not been exposed to the inducing factor (Youngson and Whitelaw 2008; Skinner et al. 2010; Champagne 2008). The phenotypic consequences can in part be dependent upon when the exposure occurred. When an individual encounters an environmental exposure such as a drug of abuse, the exposure could potentially impact the individual, any fetuses present, and any germ cells or gametes present. Effects on progeny derived from the exposed parent can be referred to as multigenerational phenotypic effects, while transgenerational effects usually refer to phenotypes observed in progeny that were not exposed in utero or derived from exposed germ cells (Skinner et al. 2010).

For example, there is evidence that caloric restriction can lead to impaired glucose tolerance in subsequent generations (Zambrano et al. 2005). Exposure to certain chemical toxins or social environments can also impact phenotypes across generations (Skinner et al. 2008; Champagne and Meaney 2007). Several groups of researchers have shown that early life stress or adversity can lead to a number of important phenotypic effects including altered transcription factor binding to and histone acetylation of the glucocorticoid receptor (which plays a critical role in stress responses), altered DNA methylation of the BDNF gene in the adult prefrontal cortex, altered serotonin signaling in the dorsal raphe nucleus, depressive-like phenotypes, and changes to energy metabolism and feeding behavior (Weaver et al. 2004; Roth et al. 2009; Franklin et al. 2010; Dietz et al. 2011; Pankevich et al. 2009; Weiss et al. 2011). The mechanism for phenotypic transmission in all of these cases has not been fully elucidated, although the potential involvement of epigenetic processes is an important avenue of investigation.

There are several lines of evidence suggesting that it would be worthwhile to explore whether or not exposure to drugs of abuse could lead to multigenerational

or transgenerational effects. Human epidemiological studies by Dr. Marcus Pembrey and colleagues indicate that preadolescent paternal smoking is associated with greater body mass indices in sons (at 9 years of age), with no significant effects observed in daughters (Pembrey et al. 2006). There is also preliminary evidence that paternal cocaine exposure in mice could lead to phenotypic consequences in the progeny. In this study, mice were exposed to cocaine via inhalation in an animal model of crack cocaine use (He et al. 2006). Cocaine-exposed males were mated to unexposed females and the progeny characterized morphologically and behaviorally. The progeny had reduced head diameters, perhaps reflecting reduced cerebral volume, and also had impaired spatial working memory and attention. Interestingly, the investigators did not observe significant DNA breaks in the sperm of cocaine-exposed fathers, but did observe "reduced levels of expression of Dnmt1, together with increased levels of expression of Dnmt-3a, in the germ cell-rich seminiferous tubular tissue" of cocaine-exposed males which may mean that cocaine exposure could impact progeny phenotypes through DNA methylation changes in the sperm (He et al. 2006). Related work indicates that cocaine exposure of pregnant female mice can lead to altered DNA methylation patterns in the hippocampus of progeny (Novikova et al. 2008).

In 1972, an article published in Science indicated that maternal morphine exposure prior to fertilization was associated with a decrease in body weight in the progeny (Friedler and Cochin 1972; Friedler 1978). Further studies indicated that male adolescent morphine exposure could impact endocrine responses in offspring (Cicero et al. 1991). More recent work by Dr. Elizabeth Byrnes and colleagues indicates that maternal morphine exposure prior to conception can lead to phenotypic effects on the progeny (Byrnes et al. 2011; Byrnes 2005; Johnson et al. 2011). Female rats were exposed to multiple doses of morphine during adolescent development, drug exposure was halted for at least 20 days, and the animals were mated to males not exposed to morphine (Byrnes et al. 2011). The adult female, but not male, progeny from morphine-exposed mothers had decreased anxiety as measured by the open-field assay. However, adult male, but not female, offspring of morphine-exposed mothers had increased sensitivity to morphine. The potential phenotypic consequences of adolescent or young adult morphine exposure on the next generation are particularly significant given the recent sharp increase in prescription opioid use among adolescents (Sung et al. 2005).

Genomic imprinting is an "epigenetically regulated process that causes genes to be expressed in a parental-origin-specific manner rather than from both chromosome homologues" (Ferguson-Smith 2011). In at least one case, the parental origin of a single genetic variant has been associated with disease protection and disease risk, depending upon whether the variant came from the mother or father (Kong et al. 2009). Imprinting could play a very interesting role in nervous system function and is known to be important in certain neurodevelopmental disorders such as Prader-Willi syndrome (Allen et al. 1995; Gregg et al. 2010; Gurrieri and Accadia 2009). There has been some work suggesting a role for imprinting in alcohol dependence, and it has been reported that the imprinting control region H19-IGF2 may play a role in specification of dopaminergic precursor neurons

(Strauch and Baur 2005; Freed et al. 2008). However, overall, little is known about the role of parental imprinting in drug abuse resilience or susceptibility.

It is clear from this section that additional research, including extremely well-controlled studies, need to be carried out in order to prove unequivocally whether or not any drugs of abuse have authentic transgenerational effects. If substance use/abuse were conclusively shown to lead to deleterious transgenerational phenotypic effects, this new knowledge would have significant public health implications and would likely influence the development of future drug prevention programs. If exposure to drugs of abuse were shown to lead to transgenerational phenotypes, then the mechanism for such transmission would need to be elucidated. There have been a number of mechanisms proposed for transmission of transgenerational effects including (1) viral, microbiome, or prion transmission, (2) neurobehavioral or societal transmission, and (3) altered epigenomic states of germ cells or gametes via altered parental imprinting, or other epigenetic effects (Youngson and Whitelaw 2008).

7.7 Challenges and Opportunities in Epigenomics and Addiction Research

As research proceeds in the area of transcriptional and epigenomic regulation in human disease, there are a number of scientific challenges and opportunities that present themselves. These include investigations into less well-studied chromatin features, renewable affinity reagents, addressing cell-type heterogeneity in the nervous system, epigenomic maps of brain tissues or cell types, epigenome-wide association studies (EWAS), and manipulation of epigenetic changes to understand function and mechanism. These challenges and opportunities are discussed in the following section with an emphasis on their impact on neuroscience and addiction research.

7.7.1 Underexplored Areas: From Novel Modifications to Higher Order Chromatin Structure

There are a number of research areas that have the potential to be quite exciting but have received very limited attention with respect to neuroplasticity and drugs of abuse. Several of these are discussed below including new DNA, histone, and RNA modifications; histone variants; RNA editing; ATP-dependent chromatin remodeling; and higher order chromatin structure.

Histone posttranslational modifications and DNA modifications have both been shown to be important in regulation of gene function, and it is likely that our catalog of these modifications is not complete. Histone variants are known to be important

in a number of biological processes including regulation of transcription; however, little is known about whether they have any special roles in post-mitotic neurons (Talbert and Henikoff 2010). RNAs have a surprising number of posttranscriptional modifications (e.g., 6-methyladenine, 1-methyladenine), but the functional roles of these modifications have not been carefully explored in the nervous system (Cantara et al. 2011; He 2010).

Some mRNAs are modified through the process of A-I RNA editing in which an adenosine in the RNA is converted to inosine by adenosine deaminases (ADARs). The inosine can be translated as a guanosine by the ribosome which can result in the presence of an amino acid in the protein product that was not encoded by the original DNA sequence (Mattick and Mehler 2008). RNA editing may serve to increase the diversity of proteins that can be produced, but it could also enable neurons to modify their properties in response to particular environmental changes. The extent to which RNA editing occurs in noncoding RNAs is poorly characterized but could impact their regulatory functions. mRNAs that have been shown to be edited include the serotonin biosynthetic enzyme TPH2 and the serotonin 2C receptor mRNA (Grohmann et al. 2010; Iwamoto et al. 2009; Dracheva et al. 2008). Little work has been done looking at the role of RNA editing in addictive processes, although Dr. Stella Dracheva and colleagues have found higher serotonin 2C editing in the prefrontal cortex associated with rats that exhibit high locomotor response to novelty (Dracheva et al. 2009).

The ATP-dependent chromatin remodeling proteins, such as members of the SNF2, ISWI, or CHD families, are able to "disrupt nucleosome DNA contacts, move nucleosomes along DNA, and remove or exchange nucleosomes" (Hargreaves and Crabtree 2011; Gkikopoulos et al. 2011). These nucleosome changes regulate access to genomic DNA which can have consequences in terms of gene expression. Some of these chromatin remodeling proteins have been shown to function in neural development and differentiation (Yoo and Crabtree 2009; Brown et al. 2007; Pirotte et al. 2010). When neurons exit from mitosis, there is a switch in the subunit composition of the BAF chromatin remodeling complexes that appears to be important in regulating dendritic morphogenesis, and this switch is regulated by the microRNAs miR-9* and miR-124 (Yoo et al. 2009). For neuroplasticity and substance abuse, investigations into chromatin remodeling has been limited; however, it has been reported that increased levels of the ATP-dependent chromatin remodeling protein Brg1 are found at the Cdk5 promoter in response to chronic cocaine exposure (Kumar et al. 2005).

Higher order chromatin structure within the nucleus may play an extremely important role in regulation of gene expression and in mediating other cellular functions (Eskiw et al. 2010; Li and Reinberg 2011). Technologies have recently been developed that enable the characterization of higher order chromatin (e.g., Hi-C, ChIA-PET) (Lieberman-Aiden et al. 2009; Fullwood et al. 2009; Espinoza and Ren 2011). Studies investigating the role of higher order chromatin structure in the nervous system, or in response to neuroplastic changes or drugs of abuse, are a very interesting area for future investigation.

7.7.2 *Renewable Affinity Reagents for Epigenomic Research*

Chromatin immunoprecipitation (ChIP) assays can provide extremely valuable information concerning the chromatin landscape of particular cells and tissues. These assays have only become possible due to the development of very low-cost and very high-throughput sequencing (Zhang and Pugh 2011; Park 2009). The continued reduction in the cost of sequencing DNA will improve the ability of researchers to apply this technique to important biological processes and disease studies. However, one of the key needs for successful ChIP assays is a high-quality antibody or other affinity reagent that binds specifically to the target of interest enabling chromatin enrichment of DNA regions in the vicinity of that particular epitope. Unfortunately some commercially available antibodies do not have appropriate specificity or are not useful for ChIP assays (Egelhofer et al. 2011). Efforts have been made to begin validating commercially available antibodies through Western blot, dot blot, or ChIP-seq analyses (http://compbio.med.harvard.edu/antibodies/). Even if an antibody is found to be useful for ChIP assays, the supplies are finite unless the antibody is monoclonal. Thus, there is a great need to develop a renewable resource of ChIP-grade antibodies (such as monoclonal antibodies) or affinity reagents (using recombinant affinity technologies) so that the scientific community has an unlimited supply of these reagents. A ChIP affinity reagent resource would allow researchers to compare ChIP experiments performed in different labs using identical antibodies, which is not always possible when the ChIP assays are performed with polyclonal antibodies. The development of a panel of one or more renewable ChIP-grade affinity reagents for each posttranslational histone modification, DNA modification, and ultimately each transcriptional regulatory protein would be an extremely valuable resource for the scientific community as a whole.

7.7.3 *Addressing Cell-Type Heterogeneity in the Nervous System*

One of the major barriers impeding epigenetic studies in the nervous system, as well as other organ systems, is cellular heterogeneity. The mixture of neurons, glia, microglia, and cardiovascular tissue in different brain regions may mask or confound epigenomic changes that may be taking place. One strategy to address this problem includes the sorting of labeled nuclei from specific brain regions or other clinically relevant tissue to enrich for cell types of interest while preserving the relevant epigenomic information (Cheung et al. 2010). Genetically tractable systems have been used to label ribosomes from specific cell types for purification and molecular identification of cell-specific mRNAs (Heiman et al. 2008; Doyle et al. 2008). In recent years, our ability to epigenomically characterize smaller and smaller numbers of cells has improved significantly, and in the future it might even

be possible to assay the epigenomes of single cells (Gu et al. 2010; Goren et al. 2010; Geng et al. 2011; Cipriany et al. 2010).

7.7.4 Epigenomic Maps of Brain Cell/Tissue Types

Scientific consortia such as the NIH Roadmap Epigenomics program are generating comprehensive maps of chromatin from a wide variety of "normal" human cell and tissue types (Bernstein et al. 2010). These maps typically include DNA methylation information, ChIP assays for several highly informative histone modifications (H3K4me1, H3K4me3, H3K9me3, H3K27me3, H3K36me3, H3K9ac, or H3K27ac), chromatin accessibility information using the DNAse1 hypersensitivity assay, and gene expression information. At the moment there are 65 epigenomic maps of cells that have all of these data types, while partial datasets are available for around 180 additional cell or tissue types. For brain researchers there are currently partial datasets for fetal brain from six time points between 85 and 142 days, as well as postmortem adult brain from anterior caudate, cingulate gyrus, hippocampus, inferior temporal lobe, midfrontal lobe, and substantia nigra (http://www.roadmapepigenomics.org/). Other epigenomic datasets for human and model organisms cells/tissues can be found at the NCBI Epigenomics gateway (http://www.ncbi.nlm.nih.gov/epigenomics) or produced by the ENCODE consortium (http://www.genome.gov/10005107).

One goal for the future would be to develop a comprehensive atlas of chromatin maps for a wider variety of brain regions and brain-specific cell types for both human and mouse. It will be important to link these epigenomic features with other molecular phenotypes such as mRNA and ncRNA expression, transcription factor binding sites, and higher order chromatin structure information. It will also be important to link molecular phenotypes to other cellular phenotypes such as morphology, connections with other neuronal or support cells, and the electrophysiological properties of the cells. These maps would be an important aid to researchers studying neuropsychiatric, neurodevelopmental, and cognitive disorders and may also yield neuronal cell type-specific targets for developing small molecular probes and therapeutic compounds.

For drug abuse researchers, the systematic generation of an "addiction epigenomics" data resource cataloging molecular phenotypes for drug abuse relevant brain regions with and without exposure to different drugs of abuse would provide an invaluable resource. Researchers would be able to mine this data to identify novel candidate loci to test for their involvement in addictive processes. They would also be able to compare profiles of molecular phenotypes responses for different drugs of abuse to begin to identify loci and networks common to addictive processes in general as well as those that might be unique for a particular drug of abuse.

Genome-wide datasets for DNA methylation, histone modifications, chromatin accessibility, ncRNAs, and transcription factor binding sites can be harnessed to

interpret data from genome-wide association studies (GWAS) for various diseases. Of particular interest, recent investigations reveal that disease SNPs identified by GWAS frequently fall in regions of accessible chromatin or in enhancer elements of cell types relevant to the disease (Maurano et al. 2012; Pennisi 2011; Ernst et al. 2011). Overall these studies "can facilitate the interpretation of GWAS data sets by predicting specific cell types and regulators related to specific diseases and phenotypes" (Ernst et al. 2011). Application of this strategy to polymorphisms associated with addictive behaviors could help to shed light on the function of some of these SNPs, particularly those in noncoding genomic regions, help flesh out the gene networks involved, as well as to confirm or generate new hypotheses concerning the brain regions and cell types involved in addiction to particular drugs of abuse.

7.7.5 *Epigenome-Wide Association Studies (EWAS) for Drug Abuse Research*

Although GWAS have been valuable in identifying unexpected genes and loci involved in particular human diseases, some diseases have a significant environmental component. If certain epigenomic states are indeed influenced by environmental exposures, then EWAS, which look at the epigenomic states of disease-relevant tissues in a case/control design, could be of great value in identifying loci involved in particular environmental exposures (Rakyan et al. 2011). Identification of genes and loci using EWAS approach could point the way to new therapeutic targets to treat disease. As scientists begin to perform EWAS using readily accessible human tissues, it will be interesting to see how the genes and loci identified compare and contrast with those identified in GWAS. At this time, EWAS for an addiction phenotype would be difficult to implement since this would necessitate the ability to monitor brain epigenomic regulation either through in vivo imaging techniques or through the use of an accessible surrogate tissue type that accurately reflects epigenomic changes that occur within the relevant brain regions.

7.7.6 *Pharmacological and Molecular Manipulation of Epigenetic Changes to Understand Function*

As correlative hypotheses are generated, it becomes essential to determine the functional role of a particular epigenetic or ncRNA regulatory pathway on a phenotype. Small-molecule probes that activate or inhibit specific epigenetic regulators provide an invaluable resource for testing the function of specific regulation for substance abuse phenotypes. As interest in epigenomic and ncRNA regulation unfolds, more small-molecule modulators are being made available to

the scientific community. The NIH Molecular Libraries Program has several projects to identify epigenetic modulators underway (http://mli.nih.gov/mli/) as does the Structural Genomics Consortium (http://www.thesgc.org/chemical_probes/epigenetics/) (Austin et al. 2004). These small-molecule modulators could be used as probes to confirm whether or not a given pathway is functionally important and should be further investigated. These small molecules could also serve as the foundation for developing therapeutic agents. As small-molecule reagents become more readily available, it will become easier to determine which epigenetic regulatory pathways have the most impact on drug abuse phenotypes and might be useful to target therapeutically.

Molecular genetic approaches can also be useful for investigating the function of epigenomic and noncoding RNA regulation. Unfortunately most small molecules and molecular genetic manipulations impact epigenetic function on a global level. Efforts have been made to manipulate chromatin states in a locus-specific manner, typically by using fusion proteins to target epigenetic modifying enzymes to particular DNA loci (Hansen et al. 2008). These techniques will need further development to enable robust locus-specific manipulation of chromatin states in the future.

7.8 Translating Epigenomic Discoveries into Improvements in Human Health

Although a deeper understanding of the biological mechanisms of drug abuse is of great significance, this understanding has the potential to be translated into improvements in human health. Most substance abuse studies to date have investigated epigenomic regulation in the brain regions of rodents since the level of drug exposure can be readily quantitated and tissues from the exposed brains are accessible to the investigator. However, it will be important to pursue epigenomic studies on postmortem brain samples from substance users and abusers to begin to determine the extent to which the elegant discoveries in rodents are recapitulated in humans. In addition to these types of studies, some of the fundamental discoveries that have been made in addiction epigenomics could have future impact on addiction diagnosis, prevention, and therapy.

7.8.1 Future Substance Use Disorder Diagnostics?

Epigenetic changes have been identified that could serve as potentially useful cancer biomarkers or diagnostics. For example, promoter methylation of a panel of genes may be useful for early detection of colorectal cancer, a DNA methylation phenotype has been used to identify a glioma subgroup, DNA methylation of the

promoter of the MGMT DNA repair enzyme can be used as a biomarker to predict glioblastoma chemotherapy outcome, and promoter methylation of the IGFBP-3 growth factor binding protein may predict cisplatin chemotherapy outcome in non-small lung cancer (Lind et al. 2011; Noushmehr et al. 2010; Weller et al. 2010; Ibanez et al. 2010). In the realm of brain disorders, DNA methylation information can be useful for predicting the efficacy of treatment of Fragile X using an mGluR inhibitor, suggesting the potential for epigenomically informed personalized medicine (Jacquemont et al. 2011).

A significant barrier to developing diagnostic tools for substance abuse based on epigenomic changes is our current inability to assess the epigenomic state of tissue types within the human brain. Unlike genomic studies which can readily be carried out using blood samples, different cell types within the brain express different suites of genes and are thus expected to have epigenomes that differ from one another (Doyle et al. 2008). Thus, to study epigenomic dysregulation in disease, one would ideally investigate the cell or tissue type of most relevant to the disease. In the case of substance abuse, epigenomic studies would thus focus on postmortem human or animal brain tissue. There has been speculation that epigenomic changes in human samples such as specific blood cell types, olfactory neurons, or other more accessible tissues could serve as a surrogate for epigenomic changes in particular brain regions, but to date there has been little compelling evidence that surrogate tissues are of significant utility in studying epigenomic processes in psychiatric diseases.

The ability to image epigenomic processes or changes within the nervous system in a noninvasive manner would be a major technological advance that could help bring epigenomic studies of substance abuse and other psychiatric diseases into living humans. One could imagine using this technology in future clinical settings for diagnosis, monitoring of disease progression, or monitoring of therapeutic efficacy. To date, only limited efforts have been made to image epigenomic processes in vivo. As a good first step, Dr. Joanna Fowler and colleagues have generated reagents to visualize the levels of histone deacetylases in vivo using positron emission tomography (PET) (Hooker et al. 2010; Reid et al. 2009). These and related strategies could eventually be used to image gross changes in the levels and/or activity of epigenetic modifying enzymes relevant to substance abuse and other diseases in vivo.

Measurement of epigenomic regulatory changes in brain using in vivo imaging techniques, or perhaps through assay of more accessible tissues that serve as a surrogate for the brain, might one day be used to help predict susceptibility to substance use disorder, to diagnose disease progression, or perhaps to provide biomarkers that accurately reflect levels and duration of chronic drug use. Future development of substance use disorder diagnostics will require us to more fully understand what epigenomic changes truly mean with respect to addictive processes as well as how long these changes persist.

7.8.2 Preventing Substance Use Disorder?

As mentioned earlier, there is evidence that exposure to certain chemical toxins, social environments, or nutrient levels can occasionally lead to organismal phenotypes in subsequent generations. Whether or not this phenomenon is also true for any drugs of abuse remains unclear. However, if particular drugs of abuse were shown to have phenotypic effects on subsequent generations, then this scientific information could be used to strengthen public health messages documenting the known adverse health consequences of drug abuse for public dissemination to facilitate scientifically informed choices on the use of licit and illicit drugs.

7.8.3 New Therapeutics for Substance Use Disorder?

Epigenetic changes are fundamentally more plastic than genetic changes and thus appear to be more amenable to therapeutic intervention (Haberland et al. 2009). Epigenetic therapeutics have shown great potential in cancer and other diseases. For example, DNA methyltransferase inhibitors have been approved by the FDA to treat myelodysplastic syndromes and may be useful for treating certain leukemias (Sharma et al. 2010). There are also HDAC inhibitors such as SAHA that have been approved to treat T-cell lymphoma (Sharma et al. 2010). Other HDAC inhibitors have been previously approved to treat urea cycle disorders, while the HDAC inhibitor valproic acid has been used to treat seizures, migraines, and bipolar disorder (Mack 2006; Bialer and Yagen 2007). HDAC inhibitors have also shown very promising effects in certain animal models of neurodegeneration, depression, and cognitive disorders (Fischer et al. 2010).

There is interest in testing FDA approved compounds for efficacy in treatment of a wider variety of diseases. As one example, clinicians have been investigating whether HDAC inhibitors can be used to activate latent HIV within the genome making the cells susceptible to antiretroviral therapy (Margolis 2011). If successful for all the tissue reservoirs that contain the latent virus, this strategy could point the way to a possible cure for HIV/AIDS. In another very exciting example, the HDAC inhibitor SAHA was used to successfully treat a patient with seizure disorder likely due to a genetic mutation, suggesting that in some cases epigenetic therapies may have the potential to treat genetic diseases (Almeida et al. 2007).

Scientists are also developing new compounds that impact epigenetic targets other than HDACs and DNMTs, such as histone methyltransferases and histone demethylases (Grant 2009; Hamada et al. 2010; Fiskus et al. 2009). There have even been efforts to target proteins that bind to histone modifications. For example, a molecule that can inhibit the BRD4 protein, which can bind to acetylated lysines, has potential for treating acute myeloid leukemia (AML) (Zuber et al. 2011).

The effects of small-molecule modulators of HDACs and other epigenetic regulatory enzymes suggest an important role for histone posttranslational

regulation in the nervous system (Haggarty and Tsai 2011). For example, Class I HDAC inhibitors have been shown to ameliorate cognitive defects in an Alzheimer's rodent model system, while environmental enrichment and HDAC inhibitors have been shown to enable "the recovery of impaired learning and lost long-term memories after animals had developed severe neurodegeneration and synaptic loss" (Kilgore et al. 2010; Fischer et al. 2007).

Histone acetylation is particularly interesting from a translational point of view since, as described above, certain medications based on inhibition of HDAC activity are clinically approved for treating seizure disorders and particular types of cancer (Sharma et al. 2010). Administration of nonspecific inhibitors of HDACs has yielded mixed results with respect to responses to drugs of abuse (Table 7.2). In some cases these inhibitors led to an increase in the rewarding properties of cocaine or an increase in cocaine intake (Kumar et al. 2005; Renthal et al. 2007; Sun et al. 2008a; Wang et al. 2010a). In other cases, these compounds have led to decreased cocaine intake (Romieu et al. 2008). The precise timing of HDAC inhibitor administration may play a crucial role in determining the effects of the compound. For example, in work by Dr. Marcelo Wood and colleagues, the HDAC inhibitor sodium butyrate was found to "facilitate extinction and prevent reinstatement of drug-induced behavioral changes" (Malvaez et al. 2010). In aggregate, these studies suggest that epigenetic therapies should be further explored as a potential treatment for addictive disorders.

As described earlier, despite ongoing efforts to develop safe and effective medications for the treatment of substance use disorders (SUDs), only limited success has been achieved, and no approved medications exist for the treatment of cocaine, methamphetamine, or cannabis addiction even though efforts are ongoing. It is possible that future epigenetic therapies could serve to complement current gaps in the treatment of individuals who are addicted to these drugs of abuse. In this chapter, several possible new avenues of inquiry for possible therapeutic intervention are indicated, including the development of isoform-specific HDAC inhibitors, sirtuin modulators, H3K9me2 demethylase inhibitors, DNA methylation inhibitors, and MeCP2 modulators. In addition, targeting of miRNAs or components of the pathways they regulate (Raf1 protein kinase, SPRED1, TORC, and CREB) could be of therapeutic value.

Acknowledgments I would like to acknowledge suggestions and input from Nigel Atkinson, Elizabeth Byrnes, Paul Kenny, Courtney Miller, Eric Nestler, Lisa Onken, Jonathan Pollock, Joni Rutter, Anne Schaefer, Susan Volman, Anne West, David White, and Marcelo Wood, as well as excellent assistance on the manuscript by Dena Procaccini. The author is solely responsible for any errors that may be found in this chapter.

References

Adrian J, Farrona S, Reimer JJ, Albani MC, Coupland G, Turck F (2010) cis-Regulatory elements and chromatin state coordinately control temporal and spatial expression of FLOWERING LOCUS T in Arabidopsis. Plant Cell 22:1425–1440

Akbarian S, Huang HS (2009) Epigenetic regulation in human brain-focus on histone lysine methylation. Biol Psychiatry 65:198–203

Allen ND, Logan K, Lally G, Drage DJ, Norris ML, Keverne EB (1995) Distribution of parthenogenetic cells in the mouse brain and their influence on brain development and behavior. Proc Natl Acad Sci U S A 92:10782–10786

Almeida AM, Murakami Y, Baker A, Maeda Y, Roberts IA, Kinoshita T, Layton DM, Karadimitris A (2007) Targeted therapy for inherited GPI deficiency. N Engl J Med 356:1641–1647

Ambermoon P, Carter A, Hall WD, Dissanayaka NN, O'Sullivan JD (2011) Impulse control disorders in patients with Parkinson's disease receiving dopamine replacement therapy: evidence and implications for the addictions field. Addiction 106:283–293

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington, DC

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188

Anier K, Malinovskaja K, Aonurm-Helm A, Zharkovsky A, Kalda A (2010) DNA methylation regulates cocaine-induced behavioral sensitization in mice. Neuropsychopharmacology 35:2450–2461

Austin CP, Brady LS, Insel TR, Collins FS (2004) NIH molecular libraries initiative. Science 306:1138–1139

Avena NM, Rada P, Hoebel BG (2008) Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci Biobehav Rev 32:20–39

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Bellet MM, Sassone-Corsi P (2010) Mammalian circadian clock and metabolism – the epigenetic link. J Cell Sci 123:3837–3848

Bernstein BE, Kamal M, Lindblad-Toh K, Bekiranov S, Bailey DK, Huebert DJ, McMahon S, Karlsson EK, Kulbokas EJ III, Gingeras TR, Schreiber SL, Lander ES (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 120:169–181

Bernstein BE, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, Kellis M, Marra MA, Beaudet AL, Ecker JR, Farnham PJ, Hirst M, Lander ES, Mikkelsen TS, Thomson JA (2010) The NIH roadmap epigenomics mapping consortium. Nat Biotechnol 28:1045–1048

Bialer M, Yagen B (2007) Valproic acid: second generation. Neurotherapeutics 4:130–137

Birney E (2011) Chromatin and heritability: how epigenetic studies can complement genetic approaches. Trends Genet 27:172–176

Bogdanovic O, Veenstra GJ (2009) DNA methylation and methyl-CpG binding proteins: developmental requirements and function. Chromosoma 118:549–565

Bonasio R, Lecona E, Reinberg D (2010) MBT domain proteins in development and disease. Semin Cell Dev Biol 21:221–230

Bonsch D, Lenz B, Reulbach U, Kornhuber J, Bleich S (2004) Homocysteine associated genomic DNA hypermethylation in patients with chronic alcoholism. J Neural Transm 111:1611–1616

Brami-Cherrier K, Valjent E, Herve D, Darragh J, Corvol JC, Pages C, Arthur SJ, Girault JA, Caboche J (2005) Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J Neurosci 25:11444–11454

Bredy TW, Sun YE, Kobor MS (2010) How the epigenome contributes to the development of psychiatric disorders. Dev Psychobiol 52:331–342

Briand LA, Blendy JA (2010) Molecular and genetic substrates linking stress and addiction. Brain Res 1314:219–234

Brown E, Malakar S, Krebs JE (2007) How many remodelers does it take to make a brain? Diverse and cooperative roles of ATP-dependent chromatin-remodeling complexes in development. Biochem Cell Biol 85:444–462

Buckland PR (2008) Will we ever find the genes for addiction? Addiction 103:1768–1776

Buka SL, Shenassa ED, Niaura R (2003) Elevated risk of tobacco dependence among offspring of mothers who smoked during pregnancy: a 30-year prospective study. Am J Psychiatry 160:1978–1984

Byrnes EM (2005) Transgenerational consequences of adolescent morphine exposure in female rats: effects on anxiety-like behaviors and morphine sensitization in adult offspring. Psychopharmacology 182:537–544

Byrnes JJ, Babb JA, Scanlan VF, Byrnes EM (2011) Adolescent opioid exposure in female rats: transgenerational effects on morphine analgesia and anxiety-like behavior in adult offspring. Behav Brain Res 218:200–205

Caldji C, Hellstrom IC, Zhang TY, Diorio J, Meaney MJ (2011) Environmental regulation of the neural epigenome. FEBS Lett 585:2049–2058

Campos EI, Reinberg D (2009) Histones: annotating chromatin. Annu Rev Genet 43:559–599

Cantara WA, Crain PF, Rozenski J, McCloskey JA, Harris KA, Zhang X, Vendeix FA, Fabris D, Agris PF (2011) The RNA modification database, RNAMDB: 2011 update. Nucleic Acids Res 39:D195–D201

Carlezon WA Jr, Duman RS, Nestler EJ (2005) The many faces of CREB. Trends Neurosci 28:436–445

Carroll KM, Onken LS (2005) Behavioral therapies for drug abuse. Am J Psychiatry 162:1452–1460

Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A, Arseneault L, Williams B, Braithwaite A, Poulton R, Craig IW (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57:1117–1127

Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health (2007) Best practices for comprehensive tobacco control programs. GPO, Atlanta

Champagne FA (2008) Epigenetic mechanisms and the transgenerational effects of maternal care. Front Neuroendocrinol 29:386–397

Champagne FA, Meaney MJ (2007) Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. Behav Neurosci 121:1353–1363

Chandrasekar V, Dreyer JL (2011) Regulation of MiR-124, Let-7d, and MiR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. Neuropsychopharmacology 36:1149–1164

Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, Neul JL, Gong S, Lu HC, Heintz N, Ekker M, Rubenstein JL, Noebels JL, Rosenmund C, Zoghbi HY (2010) Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 468:263–269

Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME (2003) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 302:885–889

Cheung I, Shulha HP, Jiang Y, Matevossian A, Wang J, Weng Z, Akbarian S (2010) Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. Proc Natl Acad Sci U S A 107:8824–8829

Cicero TJ, Adams ML, Giordano A, Miller BT, O'Connor L, Nock B (1991) Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of their offspring. J Pharmacol Exp Ther 256:1086–1093

Cipriany BR, Zhao R, Murphy PJ, Levy SL, Tan CP, Craighead HG, Soloway PD (2010) Single molecule epigenetic analysis in a nanofluidic channel. Anal Chem 82:2480–2487

Cortellino S, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, Le CM, Devarajan K, Wessels A, Soprano D, Abramowitz LK, Bartolomei MS, Rambow F, Bassi MR, Bruno T, Fanciulli M, Renner C, Klein-Szanto AJ, Matsumoto Y, Kobi D, Davidson I, Alberti C, Larue L, Bellacosa A (2011) Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. Cell 146:67–79

Crosio C, Heitz E, Allis CD, Borrelli E, Sassone-Corsi P (2003) Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. J Cell Sci 116:4905–4914

Czech B, Hannon GJ (2011) Small RNA sorting: matchmaking for Argonautes. Nat Rev Genet 12:19–31

Dalley JW, Everitt BJ, Robbins TW (2011) Impulsivity, compulsivity, and top-down cognitive control. Neuron 69:680–694

Davis TH, Cuellar TL, Koch SM, Barker AJ, Harfe BD, McManus MT, Ullian EM (2008) Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. J Neurosci 28:4322–4330

Day JJ, Sweatt JD (2010) DNA methylation and memory formation. Nat Neurosci 13:1319–1323

Day JJ, Sweatt JD (2011) Epigenetic mechanisms in cognition. Neuron 70:813–829

de Ibanez CI, Cortes-Sempere M, Moratilla C, Machado-Pinilla R, Rodriguez-Fanjul V, Manguan-Garcia C, Cejas P, Lopez-Rios F, Paz-Ares L, de CastroCarpeño J, Nistal M, Belda-Iniesta C, Perona R (2010) IGFBP-3 hypermethylation-derived deficiency mediates cisplatin resistance in non-small-cell lung cancer. Oncogene 29:1681–1690

Dekker FJ, Haisma HJ (2009) Histone acetyl transferases as emerging drug targets. Drug Discov Today 14:942–948

Deng JV, Rodriguiz RM, Hutchinson AN, Kim IH, Wetsel WC, West AE (2010) MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. Nat Neurosci 13:1128–1136

Dietz DM, Laplant Q, Watts EL, Hodes GE, Russo SJ, Feng J, Oosting RS, Vialou V, Nestler EJ (2011) Paternal transmission of stress-induced pathologies. Biol Psychiatry 70:408–414

Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Bupp S, Shrestha P, Shah RD, Doughty ML, Gong S, Greengard P, Heintz N (2008) Application of a translational profiling approach for the comparative analysis of CNS cell types. Cell 135:749–762

Dracheva S, Patel N, Woo DA, Marcus SM, Siever LJ, Haroutunian V (2008) Increased serotonin 2C receptor mRNA editing: a possible risk factor for suicide. Mol Psychiatry 13:1001–1010

Dracheva S, Lyddon R, Barley K, Marcus SM, Hurd YL, Byne WM (2009) Editing of serotonin 2C receptor mRNA in the prefrontal cortex characterizes high-novelty locomotor response behavioral trait. Neuropsychopharmacology 34:2237–2251

Dulac C (2010) Brain function and chromatin plasticity. Nature 465:728–735

Egelhofer TA, Minoda A, Klugman S, Lee K, Kolasinska-Zwierz P, Alekseyenko AA, Cheung MS, Day DS, Gadel S, Gorchakov AA, Gu T, Kharchenko PV, Kuan S, Latorre I, Linder-Basso D, Luu Y, Ngo Q, Perry M, Rechtsteiner A, Riddle NC, Schwartz YB, Shanower GA, Vielle A, Ahringer J, Elgin SC, Kuroda MI, Pirrotta V, Ren B, Strome S, Park PJ, Karpen GH, Hawkins RD, Lieb JD (2011) An assessment of histone-modification antibody quality. Nat Struct Mol Biol 18:91–93

Eipper-Mains JE, Kiraly DD, Palakodeti D, Mains RE, Eipper BA, Graveley BR (2011) microRNA-Seq reveals cocaine-regulated expression of striatal microRNAs. RNA 17:1529–1543

Ernst J, Kellis M (2010) Discovery and characterization of chromatin states for systematic annotation of the human genome. Nat Biotechnol 28:817–825

Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, Zhang X, Wang L, Issner R, Coyne M, Ku M, Durham T, Kellis M, Bernstein BE (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473:43–49

Eskiw CH, Cope NF, Clay I, Schoenfelder S, Nagano T, Fraser P (2010) Transcription factories and nuclear organization of the genome. Cold Spring Harb Symp Quant Biol 75:501–506

Espinoza CA, Ren B (2011) Mapping higher order structure of chromatin domains. Nat Genet 43:615–616

Feng J, Fan G (2009) The role of DNA methylation in the central nervous system and neuropsychiatric disorders. Int Rev Neurobiol 89:67–84

Ferguson-Smith AC (2011) Genomic imprinting: the emergence of an epigenetic paradigm. Nat Rev Genet 12:565–575

Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, Marques CJ, Andrews S, Reik W (2011) Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature 473:398–402

Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH (2007) Recovery of learning and memory is associated with chromatin remodelling. Nature 447:178–182

Fischer A, Sananbenesi F, Mungenast A, Tsai LH (2010) Targeting the correct HDAC(s) to treat cognitive disorders. Trends Pharmacol Sci 31:605–617

Fiskus W, Wang Y, Sreekumar A, Buckley KM, Shi H, Jillella A, Ustun C, Rao R, Fernandez P, Chen J, Balusu R, Koul S, Atadja P, Marquez VE, Bhalla KN (2009) Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. Blood 114:2733–2743

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007) New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47:681–698

Fleischman RA, Cambell JL, Richardson CC (1976) Modification and restriction of T-even bacteriophages. In vitro degradation of deoxyribonucleic acid containing 5-hydroxymethylctosine. J Biol Chem 251:1561–1570

Franklin TB, Russig H, Weiss IC, Graff J, Linder N, Michalon A, Vizi S, Mansuy IM (2010) Epigenetic transmission of the impact of early stress across generations. Biol Psychiatry 68:408–415

Frauer C, Hoffmann T, Bultmann S, Casa V, Cardoso MC, Antes I, Leonhardt H (2011) Recognition of 5-hydroxymethylcytosine by the Uhrf1 SRA domain. PLoS One 6:e21306

Freed WJ, Chen J, Backman CM, Schwartz CM, Vazin T, Cai J, Spivak CE, Lupica CR, Rao MS, Zeng X (2008) Gene expression profile of neuronal progenitor cells derived from hESCs: activation of chromosome 11p15.5 and comparison to human dopaminergic neurons. PLoS One 3:e1422

Friedler G (1978) Pregestational administration of morphine sulfate to female mice: longterm effects on the development of subsequent progeny. J Pharmacol Exp Ther 205:33–39

Friedler G, Cochin J (1972) Growth retardation in offspring of female rats treated with morphine prior to conception. Science 175:654–656

Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y (2009) An oestrogen-receptor-alpha-bound human chromatin interactome. Nature 462:58–64

Gao J, Wang WY, Mao YW, Graff J, Guan JS, Pan L, Mak G, Kim D, Su SC, Tsai LH (2010) A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 466:1105–1109

Gardner EL (2011) Addiction and brain reward and antireward pathways. Adv Psychosom Med 30:22–60

Gardner KE, Allis CD, Strahl BD (2011) Operating on chromatin, a colorful language where context matters. J Mol Biol 409:36–46

Geng T, Bao N, Litt MD, Glaros TG, Li L, Lu C (2011) Histone modification analysis by chromatin immunoprecipitation from a low number of cells on a microfluidic platform. Lab Chip 11:2842–2848

Gkikopoulos T, Schofield P, Singh V, Pinskaya M, Mellor J, Smolle M, Workman JL, Barton GJ, Owen-Hughes T (2011) A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. Science 333:1758–1760

Goren A, Ozsolak F, Shoresh N, Ku M, Adli M, Hart C, Gymrek M, Zuk O, Regev A, Milos PM, Bernstein BE (2010) Chromatin profiling by directly sequencing small quantities of immunoprecipitated DNA. Nat Methods 7:47–49

Graff J, Mansuy IM (2009) Epigenetic dysregulation in cognitive disorders. Eur J Neurosci 30:1–8

Grant S (2009) Targeting histone demethylases in cancer therapy. Clin Cancer Res 15:7111–7113

Grant JE, Potenza MN, Weinstein A, Gorelick DA (2010) Introduction to behavioral addictions. Am J Drug Alcohol Abuse 36:233–241

Gregg C, Zhang J, Butler JE, Haig D, Dulac C (2010) Sex-specific parent-of-origin allelic expression in the mouse brain. Science 329:682–685

Grohmann M, Hammer P, Walther M, Paulmann N, Buttner A, Eisenmenger W, Baghai TC, Schule C, Rupprecht R, Bader M, Bondy B, Zill P, Priller J, Walther DJ (2010) Alternative splicing and extensive RNA editing of human TPH2 transcripts. PLoS One 5:e8956

Gu H, Bock C, Mikkelsen TS, Jager N, Smith ZD, Tomazou E, Gnirke A, Lander ES, Meissner A (2010) Genome-scale DNA methylation mapping of clinical samples at single-nucleotide resolution. Nat Methods 7:133–136

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60

Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA (2007) A chromatin landmark and transcription initiation at most promoters in human cells. Cell 130:77–88

Guo JU, Su Y, Zhong C, Ming GL, Song H (2011) Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell 145:423–434

Gurrieri F, Accadia M (2009) Genetic imprinting: the paradigm of Prader-Willi and Angelman syndromes. Endocr Dev 14:20–28

Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458:223–227

Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L, Yang X, Amit I, Meissner A, Regev A, Rinn JL, Root DE, Lander ES (2011) lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 477:295–300

Haberland M, Montgomery RL, Olson EN (2009) The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 10:32–42

Haggarty SJ, Tsai LH (2011) Probing the role of HDACs and mechanisms of chromatin-mediated neuroplasticity. Neurobiol Learn Mem 96:41–52

Haigis MC, Sinclair DA (2010) Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol 5:253–295

Hallam TM, Bourtchouladze R (2006) Rubinstein-Taybi syndrome: molecular findings and therapeutic approaches to improve cognitive dysfunction. Cell Mol Life Sci 63:1725–1735

Hamada S, Suzuki T, Mino K, Koseki K, Oehme F, Flamme I, Ozasa H, Itoh Y, Ogasawara D, Komaarashi H, Kato A, Tsumoto H, Nakagawa H, Hasegawa M, Sasaki R, Mizukami T, Miyata N (2010) Design, synthesis, enzyme-inhibitory activity, and effect on human cancer cells of a novel series of jumonji domain-containing protein 2 histone demethylase inhibitors. J Med Chem 53:5629–5638

Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, Neve RL, Guzowski JF, Silva AJ, Josselyn SA (2007) Neuronal competition and selection during memory formation. Science 316:457–460

Hannon GJ, Rivas FV, Murchison EP, Steitz JA (2006) The expanding universe of noncoding RNAs. Cold Spring Harb Symp Quant Biol 71:551–564

Hansen KH, Bracken AP, Pasini D, Dietrich N, Gehani SS, Monrad A, Rappsilber J, Lerdrup M, Helin K (2008) A model for transmission of the H3K27me3 epigenetic mark. Nat Cell Biol 10:1291–1300

Hargreaves DC, Crabtree GR (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res 21:396–420

Harris RA, Wang T, Coarfa C, Nagarajan RP, Hong C, Downey SL, Johnson BE, Fouse SD, Delaney A, Zhao Y, Olshen A, Ballinger T, Zhou X, Forsberg KJ, Gu J, Echipare L, O'Geen H, Lister R, Pelizzola M, Xi Y, Epstein CB, Bernstein BE, Hawkins RD, Ren B, Chung WY, Gu H, Bock C, Gnirke A, Zhang MQ, Haussler D, Ecker JR, Li W, Farnham PJ, Waterland RA, Meissner A, Marra MA, Hirst M, Milosavljevic A, Costello JF (2010) Comparison of sequencing-based methods to profile DNA methylation and identification of monoallelic epigenetic modifications. Nat Biotechnol 28:1097–1105

Hawkins RD, Hon GC, Lee LK, Ngo Q, Lister R, Pelizzola M, Edsall LE, Kuan S, Luu Y, Klugman S, Antosiewicz-Bourget J, Ye Z, Espinoza C, Agarwahl S, Shen L, Ruotti V, Wang W, Stewart R, Thomson JA, Ecker JR, Ren B (2010) Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. Cell Stem Cell 6:479–491

He C (2010) Grand challenge commentary: RNA epigenetics? Nat Chem Biol 6:863–865

He F, Lidow IA, Lidow MS (2006) Consequences of paternal cocaine exposure in mice. Neurotoxicol Teratol 28:198–209

He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, Ding J, Jia Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, Xu GL (2011) Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science 333:1303–1307

Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE, Suarez-Farinas M, Schwarz C, Stephan DA, Surmeier DJ, Greengard P, Heintz N (2008) A translational profiling approach for the molecular characterization of CNS cell types. Cell 135:738–748

Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, Ching KA, Antosiewicz-Bourget JE, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Crawford GE, Kellis M, Ren B (2009) Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459:108–112

Henikoff S, Shilatifard A (2011) Histone modification: cause or cog? Trends Genet 27:389–396

Herranz D, Serrano M (2010) SIRT1: recent lessons from mouse models. Nat Rev Cancer 10:819–823

Hill KG, Hawkins JD, Catalano RF, Abbott RD, Guo J (2005) Family influences on the risk of daily smoking initiation. J Adolesc Health 37:202–210

Hollander JA, Im HI, Amelio AL, Kocerha J, Bali P, Lu Q, Willoughby D, Wahlestedt C, Conkright MD, Kenny PJ (2010) Striatal microRNA controls cocaine intake through CREB signalling. Nature 466:197–202

Hooker JM, Kim SW, Alexoff D, Xu Y, Shea C, Reid A, Volkow N, Fowler JS (2010) Histone deacetylase inhibitor, MS-275, exhibits poor brain penetration: PK studies of [C]MS-275 using positron emission tomography. ACS Chem Neurosci 1:65–73

Horsthemke B, Wagstaff J (2008) Mechanisms of imprinting of the Prader-Willi/Angelman region. Am J Med Genet A 146A:2041–2052

Host L, Dietrich JB, Carouge D, Aunis D, Zwiller J (2011) Cocaine self-administration alters the expression of chromatin-remodelling proteins; modulation by histone deacetylase inhibition. J Psychopharmacol 25:222–229

Huang W, Li MD (2009) Differential allelic expression of dopamine D1 receptor gene (DRD1) is modulated by microRNA miR-504. Biol Psychiatry 65:702–705

Hwang CK, Kim CS, Kim dK, Law PY, Wei LN, Loh HH (2010) Up-regulation of the mu-opioid receptor gene is mediated through chromatin remodeling and transcriptional factors in differentiated neuronal cells. Mol Pharmacol 78:58–68

Ikegami D, Narita M, Imai S, Miyashita K, Tamura R, Narita M, Takagi S, Yokomizo A, Takeshima H, Ando T, Igarashi K, Kanno J, Kuzumaki N, Ushijima T, Suzuki T (2010) Epigenetic modulation at the CCR2 gene correlates with the maintenance of behavioral sensitization to methamphetamine. Addict Biol 15:358–361

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

Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y (2011) Tet proteins can convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. Science 333:1300–1303

Iwamoto K, Bundo M, Kato T (2009) Serotonin receptor 2C and mental disorders: genetic, expression and RNA editing studies. RNA Biol 6:248–253

Iwase S, Lan F, Bayliss P, Torre-Ubieta L, Huarte M, Qi HH, Whetstine JR, Bonni A, Roberts TM, Shi Y (2007) The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. Cell 128:1077–1088

Jacquemont S, Curie A, Des Portes V, Torrioli MG, Berry-Kravis E, Hagerman RJ, Ramos FJ, Cornish K, He Y, Paulding C, Neri G, Chen F, Hadjikhani N, Martinet D, Meyer J, Beckmann JS, Delange K, Brun A, Bussy G, Gasparini F, Hilse T, Floesser A, Branson J, Bilbe G, Johns D, Gomez-Mancilla B (2011) Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. Sci Transl Med 3:64ra1

Jensen LR, Amende M, Gurok U, Moser B, Gimmel V, Tzschach A, Janecke AR, Tariverdian G, Chelly J, Fryns JP, Van EH, Kleefstra T, Hamel B, Moraine C, Gecz J, Turner G, Reinhardt R, Kalscheuer VM, Ropers HH, Lenzner S (2005) Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am J Hum Genet 76:227–236

Jing L, Luo J, Zhang M, Qin WJ, Li YL, Liu Q, Wang YT, Lawrence AJ, Liang JH (2011) Effect of the histone deacetylase inhibitors on behavioural sensitization to a single morphine exposure in mice. Neurosci Lett 494:169–173

Jirtle RL, Skinner MK (2007) Environmental epigenomics and disease susceptibility. Nat Rev Genet 8:253–262

Johnson EO, van den Bree MB, Uhl GR, Pickens RW (1996) Indicators of genetic and environmental influences in drug abusing individuals. Drug Alcohol Depend 41:17–23

Johnson NL, Carini L, Schenk ME, Stewart M, Byrnes EM (2011) Adolescent opiate exposure in the female rat induces subtle alterations in maternal care and transgenerational effects on play behavior. Front Psychiat 2:29

Justinova Z, Panlilio LV, Goldberg SR (2009) Drug addiction. Curr Top Behav Neurosci 1:309–346

Kaikkonen MU, Lam MT, Glass CK (2011) Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc Res 90:430–440

Kalda A, Heidmets LT, Shen HY, Zharkovsky A, Chen JF (2007) Histone deacetylase inhibitors modulates the induction and expression of amphetamine-induced behavioral sensitization partially through an associated learning of the environment in mice. Behav Brain Res 181:76–84

Kalivas PW, LaLumiere RT, Knackstedt L, Shen H (2009) Glutamate transmission in addiction. Neuropharmacology 56(Suppl 1):169–173

Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA (1999) A population-based twin study in women of smoking initiation and nicotine dependence. Psychol Med 29:299–308

Khraiwesh B, Arif MA, Seumel GI, Ossowski S, Weigel D, Reski R, Frank W (2010) Transcriptional control of gene expression by microRNAs. Cell 140:111–122

Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, Rumbaugh G (2010) Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology 35:870–880

Kim J, Daniel J, Espejo A, Lake A, Krishna M, Xia L, Zhang Y, Bedford MT (2006) Tudor, MBT and chromo domains gauge the degree of lysine methylation. EMBO Rep 7:397–403

Klein CJ, Botuyan MV, Wu Y, Ward CJ, Nicholson GA, Hammans S, Hojo K, Yamanishi H, Karpf AR, Wallace DC, Simon M, Lander C, Boardman LA, Cunningham JM, Smith GE, Litchy WJ, Boes B, Atkinson EJ, Middha S, B Dyck PJ, Parisi JE, Mer G, Smith DI, Dyck PJ (2011) Mutations in DNMT1 cause hereditary sensory neuropathy with dementia and hearing loss. Nat Genet 43:595–600

Koche RP, Smith ZD, Adli M, Gu H, Ku M, Gnirke A, Bernstein BE, Meissner A (2011) Reprogramming factor expression initiates widespread targeted chromatin remodeling. Cell Stem Cell 8:96–105

Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, Jonasdottir A, Sigurdsson A, Kristinsson KT, Jonasdottir A, Frigge ML, Gylfason A, Olason PI, Gudjonsson SA, Sverrisson S, Stacey SN, Sigurgeirsson B, Benediktsdottir KR, Sigurdsson H, Jonsson T, Benediktsson R, Olafsson JH, Johannsson OT, Hreidarsson AB, Sigurdsson G, Ferguson-Smith AC, Gudbjartsson DF, Thorsteinsdottir U, Stefansson K (2009) Parental origin of sequence variants associated with complex diseases. Nature 462:868–874

Koob GF, Le MM (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52–58

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217–238

Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42:961–972

Kriaucionis S, Heintz N (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324:929–930

Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314

Laplant Q, Nestler EJ (2011) CRACKing the histone code: cocaine's effects on chromatin structure and function. Horm Behav 59:321–330

Laplant Q, Vialou V, Covington HE III, Dumitriu D, Feng J, Warren BL, Maze I, Dietz DM, Watts EL, Iniguez SD, Koo JW, Mouzon E, Renthal W, Hollis F, Wang H, Noonan MA, Ren Y, Eisch AJ, Bolanos CA, Kabbaj M, Xiao G, Neve RL, Hurd YL, Oosting RS, Fan G, Morrison JH, Nestler EJ (2010) Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. Nat Neurosci 13:1137–1143

Launay JM, Del PM, Chironi G, Callebert J, Peoc'h K, Megnien JL, Mallet J, Simon A, Rendu F (2009) Smoking induces long-lasting effects through a monoamine-oxidase epigenetic regulation. PLoS One 4:e7959

Le FB, Diaz J, Sokoloff P (2005) A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning. Neuroreport 16:175–178

Lee EJ, Banerjee S, Zhou H, Jammalamadaka A, Arcila M, Manjunath BS, Kosik KS (2011) Identification of piRNAs in the central nervous system. RNA 17:1090–1099

Levine AA, Guan Z, Barco A, Xu S, Kandel ER, Schwartz JH (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

Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69:905–914

Li G, Reinberg D (2011) Chromatin higher-order structures and gene regulation. Curr Opin Genet Dev 21:175–186

Li MD, van der Vaart AD (2011) MicroRNAs in addiction: adaptation's middlemen? Mol Psychiatry 16:1159–1168

Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J (2009) Comprehensive

mapping of long-range interactions reveals folding principles of the human genome. Science 326:289–293
Lin Q, Wei W, Coelho CM, Li X, Baker-Andresen D, Dudley K, Ratnu VS, Boskovic Z, Kobor MS, Sun YE, Bredy TW (2011) The brain-specific microRNA miR-128b regulates the formation of fear-extinction memory. Nat Neurosci 4:1115–1117
Lind GE, Danielsen SA, Ahlquist T, Merok MA, Andresen K, Skotheim RI, Hektoen M, Rognum TO, Meling GI, Hoff G, Bretthauer M, Thiis-Evensen E, Nesbakken A, Lothe RA (2011) Identification of an epigenetic biomarker panel with high sensitivity and specificity for colorectal cancer and adenomas. Mol Cancer 10:85
Lister R, Ecker JR (2009) Finding the fifth base: genome-wide sequencing of cytosine methylation. Genome Res 19:959–966
Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462:315–322
Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O'Malley R, Castanon R, Klugman S, Downes M, Yu R, Stewart R, Ren B, Thomson JA, Evans RM, Ecker JR (2011) Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 471:68–73
Luscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69:650–663
Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H (2009) Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science 323:1074–1077
Ma DK, Marchetto MC, Guo JU, Ming GL, Gage FH, Song H (2010) Epigenetic choreographers of neurogenesis in the adult mammalian brain. Nat Neurosci 13:1338–1344
Mack GS (2006) Epigenetic cancer therapy makes headway. J Natl Cancer Inst 98:1443–1444
Malvaez M, Sanchis-Segura C, Vo D, Lattal KM, Wood MA (2010) Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. Biol Psychiatry 67:36–43
Margolis DM (2011) Histone deacetylase inhibitors and HIV latency. Curr Opin HIV AIDS 6:25–29
Marques JT, Carthew RW (2007) A call to arms: coevolution of animal viruses and host innate immune responses. Trends Genet 23:359–364
Marutha Ravindran CR, Ticku MK (2004) Changes in methylation pattern of NMDA receptor NR2B gene in cortical neurons after chronic ethanol treatment in mice. Brain Res Mol Brain Res 121:19–27
Mattick JS, Mehler MF (2008) RNA editing, DNA recoding and the evolution of human cognition. Trends Neurosci 31:227–233
Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, Reynolds AP, Sandstrom R, Qu H, Brody J, Shafer A, Neri F, Lee K, Kutyavin T, Stehling-Sun S, Johnson AK, Canfield TK, Giste E, Diegel M, Bates D, Hansen RS, Neph S, Sabo PJ, Heimfeld S, Raubitschek A, Ziegler S, Cotsapas C, Sotoodehnia N, Glass I, Sunyaev SR, Kaul R, Stamatoyannopoulos JA (2012) Systematic localization of common disease-associated variation in regulatory DNA. Science 337(6099):1190–1195. doi:10.1126/science.1222794, Sep 7, Epub 2012 Sep 5
Maze I, Nestler EJ (2011) The epigenetic landscape of addiction. Ann N Y Acad Sci 1216:99–113
Maze I, Covington HE III, Dietz DM, Laplant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovsky A, Schaefer A, Nestler EJ (2010) Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. Science 327:213–216
Maze I, Feng J, Wilkinson MB, Sun H, Shen L, Nestler EJ (2011) Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. Proc Natl Acad Sci U S A 108:3035–3040

McCann DJ (2008) Potential of buprenorphine/naltrexone in treating polydrug addiction and co-occurring psychiatric disorders. Clin Pharmacol Ther 83:627–630
McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 12:342–348
McGraw CM, Samaco RC, Zoghbi HY (2011) Adult neural function requires MeCP2. Science 333:186
McQuown SC, Wood MA (2010) Epigenetic regulation in substance use disorders. Curr Psychiatry Rep 12:145–153
McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, Alenghat T, Mullican SE, Jones S, Rusche JR, Lazar MA, Wood MA (2011) HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci 31:764–774
Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS (2008) Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci U S A 105:716–721
Mercer TR, Qureshi IA, Gokhan S, Dinger ME, Li G, Mattick JS, Mehler MF (2010) Long noncoding RNAs in neuronal-glial fate specification and oligodendrocyte lineage maturation. BMC Neurosci 11:14
Michelhaugh SK, Lipovich L, Blythe J, Jia H, Kapatos G, Bannon MJ (2011) Mining Affymetrix microarray data for long non-coding RNAs: altered expression in the nucleus accumbens of heroin abusers. J Neurochem 116:459–466
Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, Jia P, Assadzadeh A, Flanagan J, Schumacher A, Wang SC, Petronis A (2008) Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. Am J Hum Genet 82:696–711
Miller CA (2011) Forgot your HAT? CBP might be to blame. Neuropsychopharmacology 36:1543–1544
Miller CA, Gavin CF, White JA, Parrish RR, Honasoge A, Yancey CR, Rivera IM, Rubio MD, Rumbaugh G, Sweatt JD (2010) Cortical DNA methylation maintains remote memory. Nat Neurosci 13:664–666
Miranda TB, Jones PA (2007) DNA methylation: the nuts and bolts of repression. J Cell Physiol 213:384–390
Montoya ID, Vocci F (2008) Novel medications to treat addictive disorders. Curr Psychiatry Rep 10:392–398
Moretti P, Zoghbi HY (2006) MeCP2 dysfunction in Rett syndrome and related disorders. Curr Opin Genet Dev 16:276–281
Morris MJ, Karra AS, Monteggia LM (2010) Histone deacetylases govern cellular mechanisms underlying behavioral and synaptic plasticity in the developing and adult brain. Behav Pharmacol 21:409–419
Muotri AR, Marchetto MC, Coufal NG, Oefner R, Yeo G, Nakashima K, Gage FH (2010) L1 retrotransposition in neurons is modulated by MeCP2. Nature 468:443–446
Muschler MA, Hillemacher T, Kraus C, Kornhuber J, Bleich S, Frieling H (2010) DNA methylation of the POMC gene promoter is associated with craving in alcohol dependence. J Neural Transm 117:513–519
Musselman CA, Kutateladze TG (2009) PHD fingers: epigenetic effectors and potential drug targets. Mol Interv 9:314–323
Myers RM, Stamatoyannopoulos J, Snyder M, Dunham I, Hardison RC, Bernstein BE, Gingeras TR, Kent WJ, Birney E, Wold B, Crawford GE (2011) A user's guide to the encyclopedia of DNA elements (ENCODE). PLoS Biol 9:e1001046
Namihira M, Kohyama J, Abematsu M, Nakashima K (2008) Epigenetic mechanisms regulating fate specification of neural stem cells. Philos Trans R Soc Lond B Biol Sci 363:2099–2109
National Drug Intelligence Center. United States Department of Justice (2010) National drug threat assessment. GPO, Washington
Ndlovu N, Denis H, Fuks F (2011) Exposing the DNA methylome iceberg. Trends Biochem Sci 36:381–387

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 (2008) Review. Transcriptional mechanisms of addiction: role of DeltaFosB. Philos Trans R Soc Lond B Biol Sci 363:3245–3255
Nestler EJ, Malenka RC (2004) The addicted brain. Sci Am 290:78–85
Nestler EJ, Barrot M, Self DW (2001) DeltaFosB: a sustained molecular switch for addiction. Proc Natl Acad Sci U S A 98:11042–11046
Newman AH, Kulkarni S (2002) Probes for the dopamine transporter: new leads toward a cocaine-abuse therapeutic–A focus on analogues of benztropine and rimcazole. Med Res Rev 22:429–464
Ng RK, Gurdon JB (2008) Epigenetic inheritance of cell differentiation status. Cell Cycle 7:1173–1177
Nielsen DA, Yuferov V, Hamon S, Jackson C, Ho A, Ott J, Kreek MJ (2009) Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. Neuropsychopharmacology 34:867–873
Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17:510–522
Novikova SI, He F, Bai J, Cutrufello NJ, Lidow MS, Undieh AS (2008) Maternal cocaine administration in mice alters DNA methylation and gene expression in hippocampal neurons of neonatal and prepubertal offspring. PLoS One 3:e1919
Nowak SJ, Corces VG (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet 20:214–220
Numachi Y, Yoshida S, Yamashita M, Fujiyama K, Naka M, Matsuoka H, Sato M, Sora I (2004) Psychostimulant alters expression of DNA methyltransferase mRNA in the rat brain. Ann N Y Acad Sci 1025:102–109
O'Carroll D, Mecklenbrauker I, Das PP, Santana A, Koenig U, Enright AJ, Miska EA, Tarakhovsky A (2007) A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. Genes Dev 21:1999–2004
Olde Loohuis NF, Kos A, Martens GJ, Van Bokhoven H, Nadif Kasri N, Aschrafi A (2012) MicroRNA networks direct neuronal development and plasticity. Cell Mol Life Sci 69(1):89–102. doi:10.1007/s00018-011-0788-1, Epub 2011 Aug 11
Olsen CM (2011) Natural rewards, neuroplasticity, and non-drug addictions. Neuropharmacology 61:1109–1122
Ooi SK, Bestor TH (2008) The colorful history of active DNA demethylation. Cell 133:1145–1148
Pandey SC, Ugale R, Zhang H, Tang L, Prakash A (2008) Brain chromatin remodeling: a novel mechanism of alcoholism. J Neurosci 28:3729–3737
Pankevich DE, Mueller BR, Brockel B, Bale TL (2009) Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. Physiol Behav 98:94–102
Park PJ (2009) ChIP-seq: advantages and challenges of a maturing technology. Nat Rev Genet 10:669–680
Pastor V, Host L, Zwiller J, Bernabeu R (2011a) Histone deacetylase inhibition decreases preference without affecting aversion for nicotine. J Neurochem 116:636–645
Pastor WA, Pape UJ, Huang Y, Henderson HR, Lister R, Ko M, McLoughlin EM, Brudno Y, Mahapatra S, Kapranov P, Tahiliani M, Daley GQ, Liu XS, Ecker JR, Milos PM, Agarwal S, Rao A (2011b) Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. Nature 473:394–397
Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J (2006) Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14:159–166
Pennisi E (2011) The biology of genomes. Disease risk links to gene regulation. Science 332:1031

Perry JL, Carroll ME (2008) The role of impulsive behavior in drug abuse. Psychopharmacology 200:1–26

Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, Tommerup N, van Ommen GJ, Goodman RH, Peters DJ (1995) Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 376:348–351

Philibert RA, Gunter TD, Beach SR, Brody GH, Madan A (2008) MAOA methylation is associated with nicotine and alcohol dependence in women. Am J Med Genet B Neuropsychiatr Genet 147B:565–570

Philibert RA, Beach SR, Gunter TD, Brody GH, Madan A, Gerrard M (2010) The effect of smoking on MAOA promoter methylation in DNA prepared from lymphoblasts and whole blood. Am J Med Genet B Neuropsychiatr Genet 153B:619–628

Pidsley R, Mill J (2011) Epigenetic studies of psychosis: current findings, methodological approaches, and implications for postmortem research. Biol Psychiatry 69:146–156

Pietrzykowski AZ (2010) The role of microRNAs in drug addiction: a big lesson from tiny molecules. Int Rev Neurobiol 91:1–24

Pietrzykowski AZ, Friesen RM, Martin GE, Puig SI, Nowak CL, Wynne PM, Siegelmann HT, Treistman SN (2008) Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. Neuron 59:274–287

Pirotte D, Wislet-Gendebien S, Cloes JM, Rogister B (2010) Neuregulin-1 modulates the differentiation of neural stem cells in vitro through an interaction with the Swi/Snf complex. Mol Cell Neurosci 43:72–80

Rakyan VK, Down TA, Balding DJ, Beck S (2011) Epigenome-wide association studies for common human diseases. Nat Rev Genet 12:529–541

Rapicavoli NA, Poth EM, Blackshaw S (2010) The long noncoding RNA RNCR2 directs mouse retinal cell specification. BMC Dev Biol 10:49

Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J (2009) Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. Lancet 373:2223–2233

Reid AE, Hooker J, Shumay E, Logan J, Shea C, Kim SW, Collins S, Xu Y, Volkow N, Fowler JS (2009) Evaluation of 6-([(18)F]fluoroacetamido)-1-hexanoicanilide for PET imaging of histone deacetylase in the baboon brain. Nucl Med Biol 36:247–258

Renthal W, Nestler EJ (2009a) Chromatin regulation in drug addiction and depression. Dialogues Clin Neurosci 11:257–268

Renthal W, Nestler EJ (2009b) Histone acetylation in drug addiction. Semin Cell Dev Biol 20:387–394

Renthal W, Maze I, Krishnan V, Covington HE III, Xiao G, Kumar A, Russo SJ, Graham A, Tsankova N, Kippin TE, Kerstetter KA, Neve RL, Haggarty SJ, McKinsey TA, Bassel-Duby R, Olson EN, Nestler EJ (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron 56:517–529

Renthal W, Carle TL, Maze I, Covington HE III, Truong HT, Alibhai I, Kumar A, Montgomery RL, Olson EN, Nestler EJ (2008) Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. J Neurosci 28:7344–7349

Renthal W, Kumar A, Xiao G, Wilkinson M, Covington HE III, Maze I, Sikder D, Robison AJ, Laplant Q, Dietz DM, Russo SJ, Vialou V, Chakravarty S, Kodadek TJ, Stack A, Kabbaj M, Nestler EJ (2009) Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron 62:335–348

Romieu P, Host L, Gobaille S, Sandner G, Aunis D, Zwiller J (2008) Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. J Neurosci 28:9342–9348

Roth TL, Lubin FD, Funk AJ, Sweatt JD (2009) Lasting epigenetic influence of early-life adversity on the BDNF gene. Biol Psychiatry 65:760–769

Saba R, Schratt GM (2010) MicroRNAs in neuronal development, function and dysfunction. Brain Res 1338:3–13

Sadri-Vakili G, Kumaresan V, Schmidt HD, Famous KR, Chawla P, Vassoler FM, Overland RP, Xia E, Bass CE, Terwilliger EF, Pierce RC, Cha JH (2010) Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine. J Neurosci 30:11735–11744

Sanchez R, Zhou MM (2011) The PHD finger: a versatile epigenome reader. Trends Biochem Sci 36:364–372

Sanchis-Segura C, Lopez-Atalaya JP, Barco A (2009) Selective boosting of transcriptional and behavioral responses to drugs of abuse by histone deacetylase inhibition. Neuropsychopharmacology 34:2642–2654

Satta R, Maloku E, Zhubi A, Pibiri F, Hajos M, Costa E, Guidotti A (2008) Nicotine decreases DNA methyltransferase 1 expression and glutamic acid decarboxylase 67 promoter methylation in GABAergic interneurons. Proc Natl Acad Sci U S A 105:16356–16361

Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M, Llinas R, Greengard P (2007) Cerebellar neurodegeneration in the absence of microRNAs. J Exp Med 204:1553–1558

Schaefer A, Sampath SC, Intrator A, Min A, Gertler TS, Surmeier DJ, Tarakhovsky A, Greengard P (2009) Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex. Neuron 64:678–691

Schaefer A, Im HI, Veno MT, Fowler CD, Min A, Intrator A, Kjems J, Kenny PJ, O'Carroll D, Greengard P (2010) Argonaute 2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction. J Exp Med 207:1843–1851

Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D, Jenuwein T (2004) A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Genes Dev 18:1251–1262

Schratt G (2009) microRNAs at the synapse. Nat Rev Neurosci 10:842–849

Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME (2006) A brain-specific microRNA regulates dendritic spine development. Nature 439:283–289

Schroeder FA, Penta KL, Matevossian A, Jones SR, Konradi C, Tapper AR, Akbarian S (2008) Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. Neuropsychopharmacology 33:2981–2992

Sharma S, Kelly TK, Jones PA (2010) Epigenetics in cancer. Carcinogenesis 31:27–36

Singer T, McConnell MJ, Marchetto MC, Coufal NG, Gage FH (2010) LINE-1 retrotransposons: mediators of somatic variation in neuronal genomes? Trends Neurosci 33:345–354

Skene PJ, Illingworth RS, Webb S, Kerr AR, James KD, Turner DJ, Andrews R, Bird AP (2010) Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell 37:457–468

Skinner MK, Anway MD, Savenkova MI, Gore AC, Crews D (2008) Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. PLoS One 3:e3745

Skinner MK, Manikkam M, Guerrero-Bosagna C (2010) Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab 21:214–222

Stipanovich A, Valjent E, Matamales M, Nishi A, Ahn JH, Maroteaux M, Bertran-Gonzalez J, Brami-Cherrier K, Enslen H, Corbille AG, Filhol O, Nairn AC, Greengard P, Herve D, Girault JA (2008) A phosphatase cascade by which rewarding stimuli control nucleosomal response. Nature 453:879–884

St Laurent G 3rd, Faghihi MA, Wahlestedt C (2009) Non-coding RNA transcripts: sensors of neuronal stress, modulators of synaptic plasticity, and agents of change in the onset of Alzheimer's disease. Neurosci Lett 466(2):81–88, Dec 4

Strauch K, Baur MP (2005) Parent-of-origin, imprinting, mitochondrial, and X-linked effects in traits related to alcohol dependence: presentation Group 18 of Genetic Analysis Workshop 14. Genet Epidemiol 29(Suppl 1):S125–S132

Stroud H, Feng S, Morey KS, Pradhan S, Jacobsen SE (2011) 5-hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. Genome Biol 12: R54

Sulzer D (2011) How addictive drugs disrupt presynaptic dopamine neurotransmission. Neuron 69:628–649

Sun J, Wang L, Jiang B, Hui B, Lv Z, Ma L (2008) The effects of sodium butyrate, an inhibitor of histone deacetylase, on the cocaine- and sucrose-maintained self-administration in rats. Neurosci Lett 441:72–76

Sung HE, Richter L, Vaughan R, Johnson PB, Thom B (2005) Nonmedical use of prescription opioids among teenagers in the United States: trends and correlates. J Adolesc Health 37:44–51

Svenningsson P, Nairn AC, Greengard P (2005) DARPP-32 mediates the actions of multiple drugs of abuse. AAPS J 7:E353–E360

Szulwach KE, Li X, Li Y, Song CX, Han JW, Kim S, Namburi S, Hermetz K, Kim JJ, Rudd MK, Yoon YS, Ren B, He C, Jin P (2011) Integrating 5-hydroxymethylcytosine into the epigenomic landscape of human embryonic stem cells. PLoS Genet 7:e1002154

Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324:930–935

Talbert PB, Henikoff S (2010) Histone variants–ancient wrap artists of the epigenome. Nat Rev Mol Cell Biol 11:264–275

Taqi MM, Bazov I, Watanabe H, Sheedy D, Harper C, Alkass K, Druid H, Wentzel P, Nyberg F, Yakovleva T, Bakalkin G (2011) Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. Addict Biol 6:499–509

Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF (2003) Histone deacetylases: unique players in shaping the epigenetic histone code. Ann N Y Acad Sci 983:84–100

Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY (2010) Long noncoding RNA as modular scaffold of histone modification complexes. Science 329:689–693

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9:519–525

Tsankova N, Renthal W, Kumar A, Nestler EJ (2007) Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 8:355–367

Turek-Plewa J, Jagodzinski PP (2005) The role of mammalian DNA methyltransferases in the regulation of gene expression. Cell Mol Biol Lett 10:631–647

Uhl GR, Drgon T, Johnson C, Fatusin OO, Liu QR, Contoreggi C, Li CY, Buck K, Crabbe J (2008) "Higher order" addiction molecular genetics: convergent data from genome-wide association in humans and mice. Biochem Pharmacol 75:98–111

Van den Oever MC, Spijker S, Smit AB, De Vries TJ (2010) Prefrontal cortex plasticity mechanisms in drug seeking and relapse. Neurosci Biobehav Rev 35:276–284

Vanyushin BF (2006) DNA methylation in plants. Curr Top Microbiol Immunol 301:67–122

Vaquero A, Sternglanz R, Reinberg D (2007) NAD + −dependent deacetylation of H4 lysine 16 by class III HDACs. Oncogene 26:5505–5520

Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM (2010) Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. Endocrinology 151:4756–4764

Vucetic Z, Kimmel J, Reyes TM (2011) Chronic high-fat diet drives postnatal epigenetic regulation of mu-opioid receptor in the brain. Neuropsychopharmacology 36:1199–1206

Wang Y, Krishnan HR, Ghezzi A, Yin JC, Atkinson NS (2007) Drug-induced epigenetic changes produce drug tolerance. PLoS Biol 5:e265

Wang L, Lv Z, Hu Z, Sheng J, Hui B, Sun J, Ma L (2010a) Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. Neuropsychopharmacology 35:913–928

Wang R, Zhang Y, Qing H, Liu M, Yang P (2010b) The extinction of morphine-induced conditioned place preference by histone deacetylase inhibition. Neurosci Lett 483:137–142

Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA, Wysocka J, Lei M, Dekker J, Helms JA, Chang HY (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 472:120–124

Waterland RA, Michels KB (2007) Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr 27:363–388

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854

Weiss IC, Franklin TB, Vizi S, Mansuy IM (2011) Inheritable effect of unpredictable maternal separation on behavioral responses in mice. Front Behav Neurosci 5:3

Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME (2010) MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol 6:39–51

Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP (2009) Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. Nat Genet 41:246–250

Wilkinson MB, Xiao G, Kumar A, Laplant Q, Renthal W, Sikder D, Kodadek TJ, Nestler EJ (2009) Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models. J Neurosci 29:7820–7832

Wong CC, Mill J, Fernandes C (2011) Drugs and addiction: an introduction to epigenetics. Addiction 106:480–489

Wossidlo M, Nakamura T, Lepikhov K, Marques CJ, Zakhartchenko V, Boiani M, Arand J, Nakano T, Reik W, Walter J (2011) 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. Nat Commun 2:241

Wu SC, Zhang Y (2010) Active DNA demethylation: many roads lead to Rome. Nat Rev Mol Cell Biol 11:607–620

Wu H, Zhang Y (2011) Tet1 and 5-hydroxymethylation: a genome-wide view in mouse embryonic stem cells. Cell Cycle 10:2428–2436

Yamada T, Fischle W, Sugiyama T, Allis CD, Grewal SI (2005) The nucleation and maintenance of heterochromatin by a histone deacetylase in fission yeast. Mol Cell 20:173–185

Yasui DH, Peddada S, Bieda MC, Vallero RO, Hogart A, Nagarajan RP, Thatcher KN, Farnham PJ, LaSalle JM (2007) Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range interaction with active genes. Proc Natl Acad Sci U S A 104:19416–19421

Yoo AS, Crabtree GR (2009) ATP-dependent chromatin remodeling in neural development. Curr Opin Neurobiol 19:120–126

Yoo AS, Staahl BT, Chen L, Crabtree GR (2009) MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460:642–646

Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, Kang D, Richman R, Johnson JM, Berget S, Zoghbi HY (2005) Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc Natl Acad Sci U S A 102:17551–17558

Youngson NA, Whitelaw E (2008) Transgenerational epigenetic effects. Annu Rev Genomics Hum Genet 9:233–257

Yu M, Hon GC, Szulwac KE, Song C-X, Zhang L, Kim A, Li X, Dai Q, Shin Y, Park B, Min J-H, Jen P, Ren B, He C (2012) Base-resolution analysis of 5-Hydroxymethylcytosine in the mammalian genome. Cell 149(6):1368–1380

Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, Guzman C, Larrea F, Nathanielsz PW (2005) Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J Physiol 566:225–236

Zhang TY, Meaney MJ (2010) Epigenetics and the environmental regulation of the genome and its function. Annu Rev Psychol 61:439–466

Zhang Z, Pugh BF (2011) High-resolution genome-wide mapping of the primary structure of chromatin. Cell 144:175–186

Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, Song JJ, Kingston RE, Borowsky M, Lee JT (2010) Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol Cell 40:939–953

Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, Chen WG, Lin Y, Savner E, Griffith EC, Hu L, Steen JA, Weitz CJ, Greenberg ME (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 52:255–269

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 U S A 108:6626–6631

Zhubi A, Veldic M, Puri NV, Kadriu B, Caruncho H, Loza I, Sershen H, Lajtha A, Smith RC, Guidotti A, Davis JM, Costa E (2009) An upregulation of DNA-methyltransferase 1 and 3a expressed in telencephalic GABAergic neurons of schizophrenia patients is also detected in peripheral blood lymphocytes. Schizophr Res 111:115–122

Zocchi L, Sassone-Corsi P (2010) Joining the dots: from chromatin remodeling to neuronal plasticity. Curr Opin Neurobiol 20:432–440

Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR (2011) RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 478:524–528