Hormones and Behavior 59 (2011) 321–330

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Review

CRACKing the histone code: Cocaine's effects on chromatin structure and function

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ARTICLE INFO

Article history:
Received 10 April 2010
Revised 14 May 2010
Accepted 22 May 2010
Available online 4 June 2010

Keywords:
Addiction
Cocaine
Nucleus accumbens
Chromatin remodeling
Epigenetics
Histone acetylation
Histone methylation
DNA methylation
Gene priming
Desensitization

ABSTRACT

Epigenetics, the nongenetic component of how chromatin structure influences gene expression, is amazingly complex, and linking how environmental stimuli can influence epigenetic 'gene programs' in specific nerve cells to ultimately control behavior is a seemingly insurmountable puzzle. Cocaine is a highly potent stimulus capable of influencing behavior for the lifetime of an organism. Not surprisingly, psychostimulant-induced epigenetic regulation of gene expression has thus been identified as key to understanding the pathology of addiction. In addition to identifying this essential role of epigenetics in addiction, several important concepts have emerged such as the importance of global, temporal, and spatial control of mRNA expression in considering any given histone modification's influence on a given gene. Adding to this complexity, one has to account for the cumulative influence of other epigenetic modifications on a gene's transcription in addition to the interplay between transcription factors and chromatin structure. This review highlights how bioinformatic, molecular, and behavioral studies on addiction provide new insight into these concepts and outlines two distinct psychostimulant-induced patterns of chromatin regulation which are thought to underlie unique programs of gene expression that contribute importantly to the addicted state.

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Introduction

Drug addiction is a debilitating psychiatric disorder characterized by compulsive drug seeking and taking despite severe adverse consequences (Hyman et al., 2006; Kalivas et al., 2005; Koob and Kreek, 2007). Once a person succumbs to addiction, few effective therapies exist. Even when former addicts remain abstinent for long durations of time, they often find themselves in a lifelong struggle—always vulnerable to drug relapse. Therefore, the two major questions on which basic science research focuses relate to understanding the molecular events that occur during (1) the transition to the addicted state and (2) the maintenance of the addicted state. A better understanding of these mechanisms would provide insight into how we can block or perhaps reverse the neuroplastic changes that define addiction.

Drug-induced changes in gene expression in key brain reward regions, such as the nucleus accumbens (NAc), prefrontal cortex (PFC), and ventral tegmental area (VTA), represent one mechanism thought to contribute to both of these key questions. A multitude of microarray studies under different experimental conditions have found drug-induced alterations in the expression levels of hundreds of mRNAs in these target regions (Freeman et al., 2010; Heiman et al.,

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0018-506X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.yhbeh.2010.05.015

2008; Maze et al., 2010; McClung and Nestler, 2003; McClung et al., 2005; Renthal et al., 2007; Winstanley et al., 2007; Yao et al., 2004). In response to psychostimulants, many genes, such as those encoding c-Fos, FosB, ΔFosB, ATF2 (activating transcription factor 2), ATF3, and ATF4, are rapidly and transiently induced in response to initial drug exposures. Chronic exposure differentially affects the steady state levels of these various mRNAs as well as their degree of induction upon re-exposure to the same drug dose, with some genes showing sensitized responses and others desensitized responses (Alibhai et al., 2007; Green et al., 2008; Hope et al., 1994; Renthal et al., 2008). In contrast, numerous other genes, such as those encoding CDK5 (cyclin-dependent kinase 5), several NFκB (nuclear factor κB) subunits, SIRT2 (sirtuin 2), PSD-95 (postsynaptic density protein of 95 kDa), and BDNF (brain-derived neurotrophic factor), are consistently induced only by chronic drug experience, and some even increase further over several weeks of withdrawal after the last drug experience (Alibhai et al., 2007; Bibb et al., 2001; Green et al., 2008; Grimm et al., 2003; Hope et al., 1994; Renthal et al., 2008, 2009; Russo et al., 2009; Yao et al., 2004).

These complex patterns of transcriptional regulation point to the need to identify the underlying mechanisms responsible for altering a gene's 'inducibility' and those capable of stably influencing transcription for prolonged periods. In focusing on psychostimulant-induced changes in the NAc, recent evidence has suggested that epigenetics—a molecular translator that interprets diverse environmental stimuli into changes in gene expression via the regulation of chromatin structure—contributes to drug-induced transcriptional and behavioral changes (Kumar et al., 2005; Levine et al., 2005; Maze et al., 2010; Renthal et al., 2007; Wang et al., 2010). This review takes an integrative approach in discussing current progress being made toward understanding how epigenetic mechanisms are regulated by cocaine and other psychostimulant drugs of abuse in the NAc to influence specific gene expression programs and how such mechanisms might contribute to addiction-related behaviors.

Epigenetics overview

Historically, the word 'epigenetic' refers to a heritable phenotype not coded by DNA itself, but by a cellular process 'above the genome'. More recently, epigenetics is used to refer to the extremely complex processes of organizing the genome in a manner that allows for regulated gene expression in the appropriate cell type upon appropriate cellular stimuli. On a molecular level, the fundamental unit that accomplishes this feat is chromatin, which is composed of DNA wrapped around histone octomers made up of two copies each of H2A, H2B, H3, and H4. In the past decade, it has been appreciated that the structure of chromatin is highly regulated by posttranslational modifications (PTMs) that occur on histones and DNA itself. Importantly, these modifications—via regulation of chromatin structure—profoundly influence gene expression in different ways, and since multiple PTMs can occur on a given histone octomer, it is hypothesized that the combination of these modifications summate to influence gene expression, also known as the histone code hypothesis (Borrelli et al., 2008; Kouzarides, 2007; Lee and Mahadevan, 2009; Strahl and Allis, 2000). Highlighted in Table 1 are just a few well-characterized examples of such PTMs, their associated effect on gene transcription, as well as the enzymes that 'write' and 'erase' such modifications (reviewed in greater detail in Stolzenberg et al., 2011; Renthal and Nestler, 2008; Strahl and Allis, 2000).

There are several important caveats to consider when studying epigenetics in brain. First, a close look at Table 1 illustrates that many enzymes each have multiple histone substrates and, in fact, although not listed in the table, it is highly likely that many have non-histone substrates as well. For example, in addition to deacetylating H4K16 (histone H4 on Lys16), SIRT2 is well known to also deacetylate tubulin as well as several major transcription factors (Renthal and Nestler, 2009). Therefore, to understand the cellular and behavioral roles of a given enzyme and histone modification, a multifaceted approach is absolutely crucial. A second major caveat is that most of the discovered PTMs and their ascribed influence on transcription have been derived from *in vitro* work in nonneuronal cells. Therefore, it is possible that the presumed functions of known PTMs in influencing transcription in cultured cells may not necessarily be the same in brain. Moreover, it is likely that unique PTMs exist in brain. In fact, a recent proteomic study on whole brain tissue identified 196 novel histone modifications (Tweedie-Cullen et al., 2009), and a study analyzing brain DNA identified hydroxy-methylation as a novel brain-specific DNA modification (Kriaucionis and Heintz, 2009; Tweedie-

Table 1
Examples of major chromatin modifications and their function and enzyme mediators.

Modification	Effect on gene expression	"Writers" (enzymatic addition)	"Erasers" (enzymatic removal)
Histone phosphorylation		**kinases**	**Phosphatases**
H3pS10	↑	AURKB, **MSK1**	**PP1, DARPP32 (indirectly via PP1 regulation)**
Histone acetylation		**KATs**	**HDACs (1-11), HDAC4,5, 9**
H3K9ac	↑	2a (GCN5), 2b, 12	"
H3K14ac	↑	2a-b, 3a **(CBP)**, 3b (p300), 6a, 6b	"
H4K16ac	↑	5, 8	**Sirt2**
Histone lysine methylation		**KMTs**	**KDMs**
H3K4me3	↑	2a-h, 7	1, 2a, 5a-d
H3K9me1/2/3	↑ **(me1)**; ↓ (me2/3)	1a-f (1c=**G9a/EHMT2**), 8	1, 3a-b, 4a-d
H3K27me3	↓	6	6a-b
H3K36me3	↑	3a-c	2a-b, 4a-c
H3K79me3	↑	4	?
Histone arginine methylation	**?**	**PRMTs (1-8)**	**JMJD6**
Histone ubiquitination	↑	**Ub ligases** (RING2)	**Ub protease** (USP16)
Histone sumolyation	↑	**SUMO E2s/E3s? (UBC9)**	**SUMO protease (SUSP17)**
DNA methlyation		**DNMT1, DNMT3a, DNMT3b**	**Gadd45a,b,g ?**

List of histone modifications, their known effects on transcription, and examples of enzymes that catalyze their "writing" (enzymatic addition) or "erasing" (enzymatic removal). ↑ = increased transcription, ↓ = decreased transcription; **Blue** = examples of enzymes in which cocaine-induced biochemical regulation has been identified and its behavioral significance has been assessed.