Chapter 7
Neuroscience, Epigenetics, and Psychotropic Substances

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Introduction

The use of psychotropic substances, those with direct effects on the central nervous system and potential for abuse and dependence, has been taking place since the time of ancient civilizations. Currently, the abuse of these substances has become a worldwide problem with the estimate that in the year of 2019, 35 million people worldwide suffered from the disorders associated with the use of substances (SUDs) (UNODC 2019). According to the *National Institute on Drug Abuse*—NIDA (2020), among the main drugs used are alcohol, marijuana, cocaine, opioids, and methamphetamines. It is known that several factors contribute to the abuse of these substances. Biological factors such as genetics, gender, age, ethnicity, and the diagnosis of mental disorders interact with environmental factors with which individuals are inserted, such as economic status, family nucleus, and emotional experiences, making them more prone or not to drug abuse. In the last decade, epigenetic changes have also been proposed as an important factor in the transition from recreational to compulsive use of abusive drugs.

Fortunately, understanding about substance abuse advances significantly every day. Due to the progress of scientific research in genetics and neuroscience and the development of new technologies that provide effective tools for high throughput studies, such as the analysis of molecular changes in specific neuronal populations, every day it is possible to assemble new pieces of the addiction puzzle. In addition, the development of neuroimaging technologies allows access to brain function and neurochemical aspects directly *in* humans diagnosed with SUDs, allowing an in vivo view of the brain aspects of this disorder. In this sense, this chapter aims to provide an overview of the neurobiological aspects of drugs of abuse and how epigenetic changes are involved in this context.

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D. De Micheli et al. (eds.), *Drugs and Human Behavior*,
https://doi.org/10.1007/978-3-030-62855-0_7

Neurobiology of Drugs of Abuse

Clinically, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), SUDs can be defined as a chronically relapsing disorder, characterized by compulsion to seek and take the drug, loss of control in limiting intake, and emergence of a negative emotional state such as anxiety and irritability, in the absence of drug use (APA 2014).

In general, SUDs is characterized by the deregulation of motivational brain circuits, known as reward system, whose role is to regulate motivated behaviors that are evolutionarily important for the perpetuation and maintenance of species, such as food seek and reproduction (Kelley and Berridge 2002). These behaviors are accompanied by an *incentive salience*, a process of integration through which objects and stimuli from outside capture our attention, gain relevance, and influence thoughts and behaviors. Food seeking is an example of motivated behavior, where the individual execute a goal-directed behavior: the search for food to promote satiety and the inherent generation of rewarding feeling. The association between behavior (search for food) and consequence (satiety) is called *positive reinforcement* and is important for the learning process that consolidates the association between action and reward. This process is the result of a balanced brain circuit where the proper functioning of motivation, decision making, inhibitory control, and reward is achieved. Interestingly, the same brain circuits involved in this process are also recruited in the use of drugs of abuse. Contrary to natural behaviors, in the SUDs we observe the deregulation of these circuits that is characterized by the exacerbation of the *incentive salience* and habits formation, deficits in reward, increased stress and the compromise of executive function (Koob 2013).

For a better understanding of the deregulation of these processes and brain circuits, Koob and Volkow (2010) proposed the addiction as a three-stage cycle: (1) *binge/intoxication stage*; (2) withdrawal*/negative affect stage;* and (3) preoccupation*/anticipation stage* (Koob and Volkow 2010). Figure 7.1 represents the processes and regions involved in these cycles.

In the first stage, *binge/intoxication*, it is postulated that the use of the drugs occurs through the search for their pleasurable (rewarding) effects. Studies with rodents and neuroimaging studies in humans point to the Ventral Tegmental Area (VTA) and the Nucleus Accumbens (NAc) as the main regions that regulate *incentive salience*, drug seeking, and reward (Koob and Volkow 2016). At the molecular level, brain reward signaling occurs through activation of the mesolimbic dopaminergic pathway (ML-DA), composed of dopaminergic neurons connecting VTA and NAc (Wise 2008). Alcohol intake, for example, promotes the activation of dopaminergic neurons in VTA, resulting in the rapid and increasing release of dopamine (DA) in NAc, consolidating the rewarding feeling related to its consumption (Volkow and Morales 2015).

Although each type of drug present a different mechanism of action, the final effect for all of them is the activation of the ML-DA pathway (Volkow and Morales 2015). For example, heroin and morphine act as antagonists to opioid

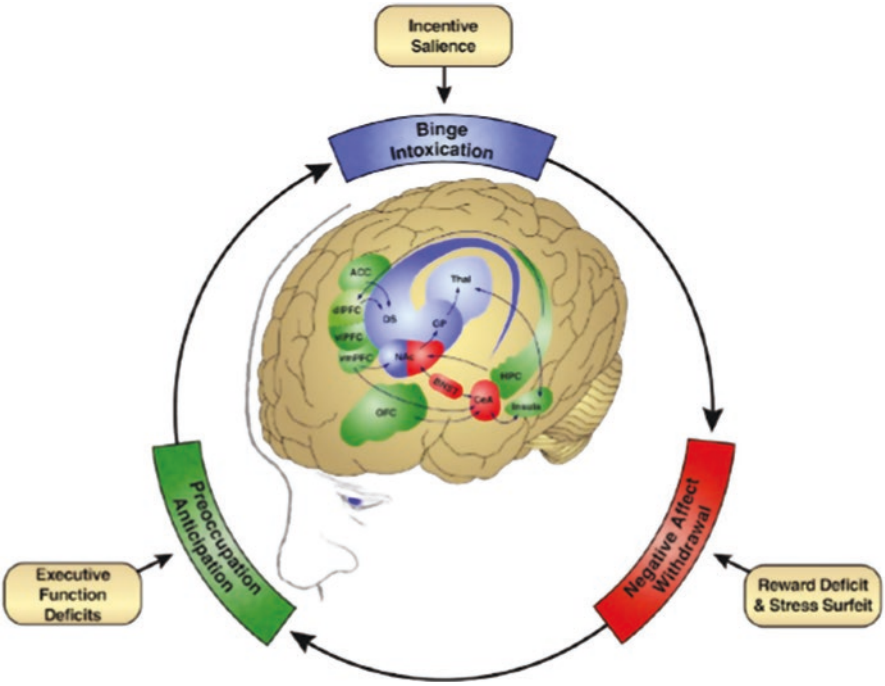

Fig. 7.1 Schematic representation of the neural substrates involved in the different cycles of addiction: binge/intoxication, in blue; abstinence/negative affect, in red; preoccupation/anticipation, in green. Source: Reproduced from Koob and Schulkin (2019). *DS* Dorsal Striated, *GP* Pale Globe, *NAC* Accumbens Nucleus, *Thal* Thalamus, *BNST* Amygdala Terminal Stria, *CeA* Amigdala Central Core, *ACC* Anterior Cingulate Cortex, *dlPFC* Pre-Frontal Dorsolateral Cortex, *vlPFC* Pre-Frontal Ventrolateral Cortex, *vmPFC* Pre-Frontal Ventromedial Cortex, *OFC* Orbitofrontal Cortex, *HPC* Hippocampus

receptors and activate the ML-DA through the disinhibition of GABA receptors. Cocaine increases the concentrations of DA by blocking its transporter and preventing its reuptake in the synaptic cleft. Alcohol, on the other hand, interacts with a variety of receptors, such as opioids and cannabinoids, in addition to GABA and glutamate receptors of the AMPA type, triggering the activation of ML-DA and the release of DA (Abrahao et al. 2017; Adinoff 2004).

In the second stage of addiction, withdrawal*/negative affect stage*, the consumption of the drug is no longer directed by positive reinforcement, but by negative reinforcement (Koob and Volkow 2010). In this context, negative reinforcement can be understood as actions that seek to relieve negative emotional states (e.g. anxiety, discomfort, and tensions). These emotional states emerges during withdrawal temptatives, and it is a result of neurobiological adaptations triggered by chronic use of a drug (Koob and Volkow 2016). At this stage, a loss of function of the reward system is followed by a hyporegulation of the dopaminergic response and the recruitment of brain systems involved in stress modulation, such as the

hypothalamic-pituitary-adrenal axis (HPA), the system mediated by the corticotropin release factor (CRF), and the amygdala (Koob 2015). Thus, the combination of the loss of function of the reward system and the recruitment of brain systems involved in stress leads to the drug seeking and consumption, triggering the third cycle of addition.

The third and last stage of addiction, named of *preoccupation/anticipation* is characterized by the emergence of the fissure (or *craving*), defined as an uncontrollable desire to consume the drug, which originates even after long periods of abstinence, leading the individual to relapse episodes (Koob and Volkow 2010). It is proposed that the pre-frontal cortex (PFC) is the main brain substrate of this stage, due to its role in controlling the cognitive and emotional processes that drive the *incentive salience*, and the decision making over drug seeking and consumption (Koob and Volkow 2016). Specifically, it is suggested that the glutamatergic neurons projections between the medial PFC and the ventral striatum are modulated by dopamine activity (via mesocortical dopamine) through dopamine receptors (DRD1 and DRD2), being one of the mechanisms involved in relapse episodes. Additionally, the connectivity between PFC and basolateral amygdala seems to play an important role in the cue-induced relapse (Koob and Volkow 2016).

In summary, the transition from controlled to compulsive drug consumption and eventually to addiction involves neurological adaptations in ML-DA. Although the VTA, NAc, and PFC regions are described as the main regions involved in the above stages, it is important to note that these regions receive and send projections to other brain areas involved, for example, with the regulation of mood (Amygdala, Hypothalamus, and Habenula) and with interoception (Insula and Anterior Cingulate Cortex), which allows, for example, the awareness of negative states, such as anxiety and irritability, which end up leading to relapse.

Epigenetics and Drugs of Abuse

As explained in the previous section, the chronic use of drugs of abuse leads to neurological adaptations in several brain regions, with the reward system being one of the major targets. In the scientific literature on addiction, the term "neuroadaptation" is used to refer to brain neuroplasticity triggered by drug use (Seo and Sinha 2015). Neuroplasticity, in turn, also referred to as brain plasticity or neural plasticity, characterizes the brain's ability to change and adapt in response to environmental stimuli. During this process, synapses are strengthened or weakened, resulting in the increase or decrease of their activities. Finally, these adaptations can be consolidate into long-term changes due to changes in gene expression (Gulyaeva 2017; Kalivas and O'Brien 2008).

It is proposed that the use of drugs of abuse leads to changes in gene expression, directly or indirectly, via increased release of DA and the consequent activation of its receptors and their *downstream* signaling cascades. The stimulation of these cascades in turn causes activation or inhibition of transcription factors (e.g. ΔFOSB

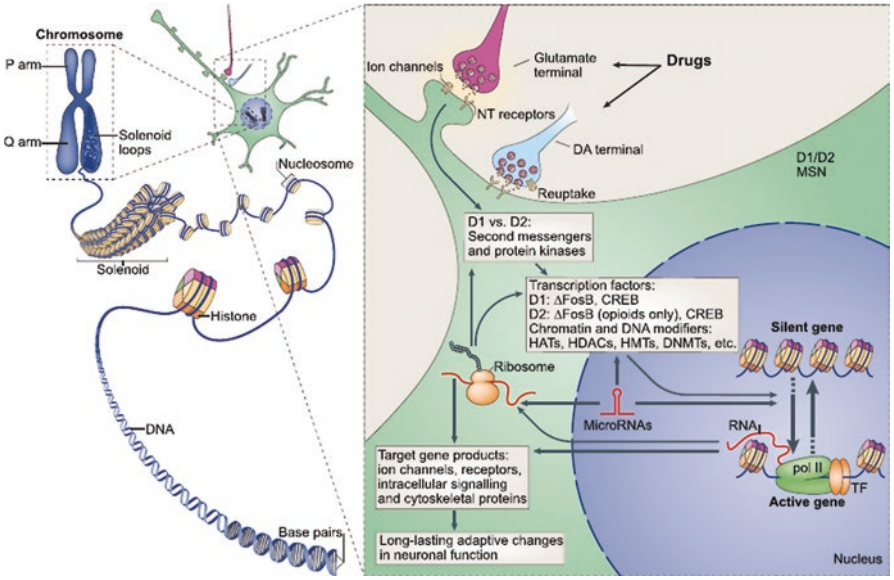

Fig. 7.2 Schematic representation of the mechanisms involved in the changes in the regulation of gene transcription, triggered by drugs of abuse. Source: Reprinted from Nestler and Lüscher (2019). *ΔFOSB* FOS transcription factor, *CREB* cAMP responsive binding protein, *DNMTs* DNA methyltransferase, *HATs* histone acetyltransferase, *HDACs* histone acetylase, *HDMs* histone demethylase, *HMT* histone methyltransferase, *D1 and D2* dopamine receptor 1 and 2

and CREB) and other targets such as chromatin structure modifying enzymes (HDACs, HAT, HMT) that regulate the induction or suppression of genes involved in addiction (Ron and Barak 2016). In this context, epigenetics is considered as one of the probable mechanisms involved in changes in gene transcription regulation (Fig. 7.2). While many of these modifications are transient, some may be stable and inheritable, thus contributing to cellular plasticity and the emergence of maladaptive behaviors observed in addiction (Nestler and Lüscher 2019).

In a broad definition, epigenetics describes a series of biochemical processes that lead to alteration of gene expression, but without causing changes in DNA nucleotide sequence (Jaenisch and Bird 2003). To recall, our DNA is organized and compressed into a structure called chromatin where the fundamental unit is the nucleosome. This in turn is composed of the histone octamers (two copies of each histone H2A, H2B, H3, and H4) in which approximately 147 pairs of DNA bases are wrapped (Fig. 7.3). Basically, epigenetic mechanisms control the space between the nucleosomes and the levels of their condensation, determining the activity of genes. The control of this conformation involves several types of histone modifications, the DNA methylation, and the activity of non-coding RNAs (*microRNAs (*miRNA) and *long non-coding* RNAs (lncRNAs) (Allis and Jenuwein 2016).

The existence of a wide range of post-translational modifications of histones and their combinations makes this epigenetic mechanism extremely complex. Different

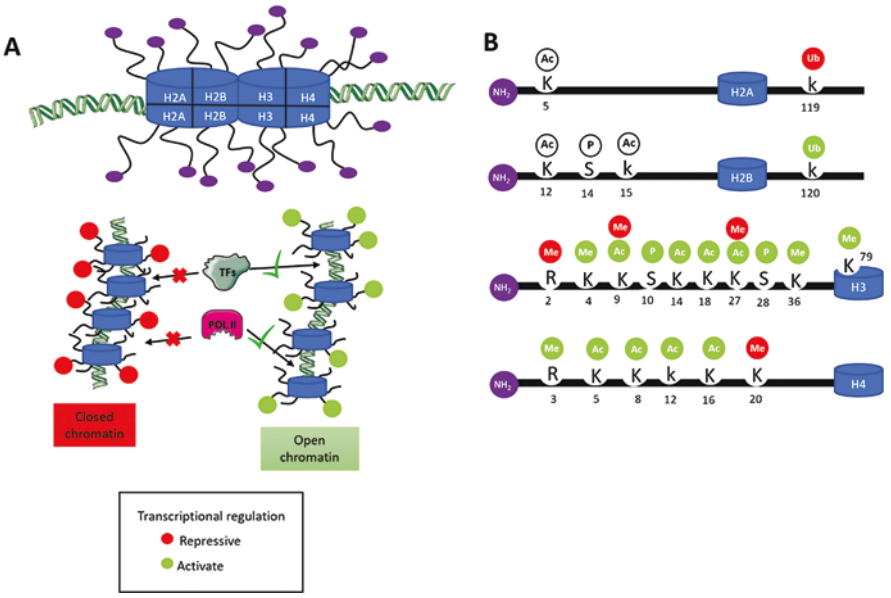

Fig. 7.3 Representation of the structure of the nucleosome (**a**) and the modifications in the amino acid residues of the histones (**b**). Modifications: acetylation (Ac), methylation (Me), phosphorylation (P), and ubiquitination (Ub). Amino acid residues: lysine (K); serine (S), and arginine (R). Histone octomers: H2a, H2b, H3, and H4. The function of each of these modifications in the transcriptional regulation of genes is represented by the colors green (transcription activation) and orange (transcription repression)

chemical groups can be covalently added to the different amino acid residues of the N-terminal tails of each of the histones, naming the changes in chromatin structure—acetylation, methylation, phosphorylation, ubiquitination, sumoylation, citrullination, among others (Fig. 7.3) (Allis and Jenuwein 2016). Such epigenetic marks are added or removed by a group of enzymes known as "*writers*" or "*erasers,*" making the epigenetic modifications reversible and dynamic (Allis and Jenuwein 2016). Although all these modifications contribute to the acquisition and maintenance of addiction, acetylation and methylation of histones are the most studied post-translational modifications in the field of addiction (Mews et al. 2018).

Acetylation involves the participation of the histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes, which are responsible for adding and removing, respectively, acetyl groups from histones. The HDACS are divided into 4 classes, where classes I (HDAC1–3 and 8), II (HDAC 4–7 and 9–10), and IV (HDAC 11) are zinc dependent and class III (HDACS sirtuins—Sirt1–7) needs the NAD+ protein as cofactor (Gräff and Tsai 2013). Acetylation of lysine residues (represented by the letter K) removes positive charges reducing affinity between histones and DNA. This process takes chromatin to a permissive state, where access to transcription factors and RNA polymerase to DNA is facilitated. For this reason,

acetylation, for example, of histone 3 in lysine residues 9 and 14 (H3K9 and H3K14) is associated with transcriptional gene activation (Fig. 7.3) (Allis and Jenuwein 2016).

The process of methylation of the histones occurs in a similar way, in which the lysine and arginine residues (represented by the letter R) are methylated by the lysine's (KMTs) and arginine's (PRMTs) methyltransferases, respectively, also known as methyltransferase histones (HMT). These enzymes can catalyze the transfer of 2 or even 3 methyl groups to the same histone residue. However, demethylase enzymes (KMD) whose function is to catalyze the removal of methyl groups from these histones may reverse this process. Unlike acetylation, histone methylation can result in both activation and transcriptional repression of genes depending on the histone residue to which the methyl groups have been added. For example, the trimethylation of histone 3 in lysine residue 4 (H3K4), as well as in H3K36 and H3K79 residues, is associated with activation of the gene transcription. In contrast, methylation of the H3K9, H3K27, and H4K20 residues are associated with transcriptional repression (Fig. 7.3) (Alam et al. 2015).

Most studies investigating the epigenetic modifications related to the use of drugs of abuse are conducted in animal models, mainly in rodents, which allows the study of the effects of drugs under controlled conditions of genetic and phenotypic variability (Nestler 2014). These studies focus on brain regions important for reward processing, with PFC, VTA, and NAc being the main regions studied. While some studies examine epigenetic changes in individual candidate genes using, for example, microarray technologies, others use methodologies such as chromatin immunoprecipitation (ChIP) followed by high-performance sequencing to access global changes in chromatin (Maze et al. 2014a, b).

Changes in Histone Acetylation and Methylation

Studies in animal models show that exposure to cocaine results in an overall increase in acetylation, regulating the transcription of genes related to neuroplasticity (De Sa Nogueira et al. 2019; Kumar et al. 2005; Wang et al. 2010). For example, intraperitoneal injections (IP) of cocaine (20 mg/kg) in Sprague-Dawley rats lead to hyperacetylation of H3 and H4 histones in the promoters of *cFos*, *BDNF,* and *Cdk5* genes in striatal neurons (Kumar et al. 2005). A second study showed that self-administration of cocaine (0.5 mg/kg for 12 days) in Wistar rats followed by a short abstinence period (3 days) causes changes in the expression of chromatin remodeling genes (e.g. *Kdm6a, Smarcc2, Dot1l, Brd1)* in the PFC. However, these genes return to their baseline transcription levels after a longer period of abstinence (10 days), confirming the hypothesis that epigenetic changes may be reversible (Sadakierska-Chudy et al. 2017). Also in the same study, although an increase in acetylation of H3K9 and H4K8 histones was observed, no significant global change in methylation levels of histone 3 in lysine residues 4, 9, 27, and 79 were observed (Sadakierska-Chudy et al. 2017).

The use of other psychostimulant drugs such as amphetamine and methamphetamine can also trigger epigenetic changes (Godino et al. 2015). Specifically, a single injection of methamphetamine (20 mg/kg) in rats can induce global changes in acetylation, increasing the acetylation of H4K5 and H4K8 histones and reducing the acetylation of H3K9, H3K18, and H4K16 histones in the NAc of these animals (Martin et al. 2012). These changes are accompanied sequentially by increases and decreases in HDAC1 and HDAC2 proteins levels, and consequently by changes in the transcriptional regulation of genes involved in the rewarding effects of acute and chronic exposure to psychostimulants (*c-fos, phosB, Crf, Cck, and Npas4*) (Martin et al. 2012). Another study using the conditioned place preference test for methamphetamine showed the importance of methylation levels of histone H3K4 for the acquisition and consolidation of methamphetamine-associated memory, one of the main aspects that increase relapse vulnerability (Aguilar-Valles et al. 2014). While the KO of the methyltransferase Mll1 in the NAc of C57BL/J6 mice led to a reduction in methylation in histone H3K4me3 and stopped the acquisition of drug-related memory, the KO of lysine-specific demethylase 5C (Kdm5c) resulted in hypermethylation of histone H3K4 and blocked drug-related memory expression. These results highlight the enzymes "writers" and "erasers" as potential therapeutic targets to be considered for the treatment of SUDs (Aguilar-Valles et al. 2014).

Morphine, a potent opioid used for pain relief, is a drug with great potential for abuse, and like other drugs its abuse can also lead to epigenetic changes. A study with class III HDACs, sirtuins (Sirt), showed that chronic (7 days) administration of morphine (20 mg/Kg) causes a specific increase in Sirt1 expression in NAc of C57BL/J6 mice. Additionally, virus-induced over-expression of Sirt1 increases the rewarding effects of morphine in these animals, while the KO of this protein decreases these effects. Such changes also lead to changes in the regulation of several synaptic proteins, indicating the role of Sirt1 as a possible mediator of molecular and cellular plasticity triggered by morphine abuse (Ferguson et al. 2013).

Studies investigating the effects of alcohol exposure on the genome have shown that its consumption is able to change the chromatin structure in two directions. Through gene activation or repression, alcohol can induce anxiety in addition to other negative symptoms, contributing to an increase in drug use and dependence (Pandey et al. 2017). In this sense, clinical and pre-clinical studies have demonstrated that alcohol consumption can trigger changes in the transcriptional regulation of HDAC coding genes (López-Moreno et al. 2015; Pascual et al. 2012; Sakharkar et al. 2014; Warnault et al. 2013). For example, both alcohol binge drinking in humans and daily alcohol self-administration in rats increases the gene transcription of HDACs class I, II and IV, in peripheral blood in both species (López-Moreno et al. 2015).

A second study using alcohol-preferring rats (P rats) versus nonpreferring rats (NP rats), showed that, at baseline, P rats had an upregulation of HDAC2 and a higher nuclear activity of this enzyme in the central nucleus of the amygdala when compared to NP rats. However, since these P rats were subjected to acute exposure to alcohol (1 g/kg of alcohol via IP), the results observed were opposite: hyporegulation of HDAC2 levels and inhibition of its nuclear activity. Additionally, the KO

of HDAC2 in the amygdala of these animals led to a reduction in the anxiety-like behavior and in the voluntary consumption of alcohol, when compared to NP animals. Together, these results suggest that the innate anxious-like behavior observed in P rats may be related to high nuclear activity of HDAC2 in the amygdala, and that the anxiolytic effects obtained with acute alcohol exposure may be related to inhibition of this activity (Moonat et al. 2013). Another study, also on the investigation of histone modifications and the development of anxiety triggered by alcohol exposure, showed that intermittent administration of alcohol (2 mg/kg) in rats during adolescence triggers anxious-like behaviors, increase nuclear and cytosolic activity of HDAC, increase protein levels of HDAC2 and HDAC4 and decrease levels of acetylation of H3K9 histones in the central and medial amygdala of these animals. Interestingly, some of these changes persist until the adult life of the animal, contributing to the increase of alcohol consumption in this phase (Pandey et al. 2015).

The use of HDAC inhibitors has been an important pharmacological tool for the functional validation of the different classes of HDACs in the addiction and maladaptive behaviors that characterize this disorder (Bourguet et al. 2018). For example, the use of HDAC inhibitors has been shown to be effective in preventing changes in the expression of HDAC enzymes and consequently of genes involved in the processing of alcohol rewarding effects, such as the Gabra receptor1 (Bohnsack et al. 2018). In addition, the use of these inhibitors is able to attenuate and/or block anxious-like behaviors related to alcohol consumption and reduce voluntary consumption in rats (Bohnsack et al. 2018; Pandey et al. 2008). For other drugs, such as morphine, the use of the HDAC inhibitor SAHA attenuates tolerance to the use of this drug in animal bone cancer models (He et al. 2018). For nicotine, the administration of the sodium butyrate inhibitor (Nab), besides extinguishing the preference for this drug, analyzed by the conditioned place preference test, is also efficient in blocking the reestablishment of its self-administration in rats (Castino et al. 2015). Similarly, for cocaine, the use of the RGFP966 inhibitor, specific for HDAC3, is also effective in extinguishing the behavior of preference for the drug as well as in blocking the reestablishment of its use (Hitchcock et al. 2019).

Final Considerations

In summary, it can be concluded that most of the drugs of abuse are capable of modulating target genes by modifying histones in different brain regions involved in addiction. Many of these drugs have common targets, such as transcription factors (e.g., *FosB*) and neurotrophic factors (e.g., *Bdnf*) (Mews et al. 2018). Interestingly, while some studies show that these changes can be reversible, others show that they can be persistent, lasting, for example, the entire adult life cycle of the animal. Although the studies used here as examples have reported results in the same direction, for example, the increase in global acetylation congruent with transcriptional activation of specific genes, it is important to note that these changes can be observed in opposite directions. Thus, although the transcriptional effects of most histone

modifications are known, it is still difficult to predict the effects of each type of modification on gene expression and behavior (Lawrence et al. 2016).

Much work has been done so far contributing to the understanding of the epigenetic modifications triggered by the different drugs of abuse. However, considering the different brain areas and networks of gene expression recruited and involved in the development of addiction, as well as the specific mechanisms of action of each drug, we are still far from a detailed understanding of the mechanisms involved in these changes. Thus, it is of extreme importance the development of studies that access the different types of epigenetic modifications in different animal models of addiction. All variations included in these models, such as the time and method of drug administration, the species and strains of animals used, age and sex, are factors that can influence the epigenetic modifications and the results interpretation (Becker and Chartoff 2019).

As already mentioned, the several brain areas involved in the development of addition make the study of the genesis of this disorder complex. However, even focusing on a single region it is important to take into consideration that this specific region is formed by several types of neural and non-neuronal cells that perform specific functions, and at some moments, opposite effects in the addiction. For example, the activation of dopaminergic neurons D1 and D2 in NAc results in different actions in the reward processing of cocaine (Lobo et al. 2010). Therefore, it is important to consider the use of technologies that allow the investigation of molecular mechanisms in isolated cells to avoid non-specific results during data analysis.

Finally, the results obtained so far strengthen the role of epigenetic changes in addiction and their consequences for emotional states and the perpetuation of maladaptive behaviors related to drug use. Additionally, the use of HDAC inhibitors and their results in the manipulation of drug seeking and consumption behaviors in animal models suggest epigenetic modulation as a possible therapeutic target for the treatment of SUDs.

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