Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 7 (2018), Article ID 236039, 27 pages
doi:10.4303/jdar/236039

*Review Article*

# The Developmental Neuroepigenetics of Substance Abuse

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Received 28 August 2017; Revised 2 January 2018; Accepted 3 January 2018



**Abstract** Advances in technology have allowed for the expansion of the field of epigenetics, providing a deeper understanding of gene-environment interactions. Investigations into the neurobiological basis of substance abuse have benefitted from these advances, with findings suggesting that epigenetic mechanisms underlie drug-induced modifications of brain morphology, synaptic plasticity, and behavior. Epigenetic marks likely mediate the long-lasting and potentially transgenerational alterations of neuronal chromatin and subsequent gene expression that may lead to persistent relapse vulnerability and/or offspring vulnerability to addiction. Understanding the epigenetic mechanisms as well as potential sensitive windows for these alterations may provide novel insight into how epigenetics factor into the individual vulnerability and unique time periods for added vulnerability to illicit drug exposure. In the current review, we outline recent literature that provides evidence for early epigenetic changes in several addiction models. We begin the review with an overview of epigenetics as they relate to drug use and abuse, and next focus on findings in the context of prenatal, childhood, and adolescent stages with additional references to adult models of addiction. Further, we also focus on studies that discuss the transgenerational inheritance of epigenetic changes, and how they may affect the individual across development. Lastly, the work presented here and potential future studies focus on demonstrating early disruptions in epigenetic marks following acute or repeated drug exposure that may be of relevance in the broader goal of identifying risk factors and novel targets for addiction treatment.

**Keywords** histone modification; DNA methylation; ncRNA; psychostimulants; opiates; alcohol; cannabinoids

## 1. Epigenetic mechanisms and substance abuse vulnerability

From the initial coinage of the term "epigenetics" in the mid-twentieth century, deriving from the Greek "epigenesis", it was broadly defined across scientific contexts to refer to the transmission of information above the level of the genome [1,2]. Presently, however, epigenetics is used in reference to the study of potentially reversible changes in gene expression and cell phenotype in the absence of inherent deoxyribonucleic acid (DNA) sequence changes. Epigenetics can also be used to reference environmental changes that have an influence on the expression of ribonucleic acid (RNA) from coding sequences [3,4]. Here, we will refer to molecular epigenetics as "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" [5]. Several lines of research suggest that molecular epigenetic mechanisms are associated with the neural plasticity underlying behavioral adaptation and maladaptations observed in mammals, and an important mechanism underlying permanent changes to neural processes across individual systems [6]. From this, it has been generally accepted that epigenetic mechanisms are active and persistent throughout the nervous system and contribute to protracted neurobehavioral effects with potential clinical relevance. For example, epigenetic changes are the basis of rare neurodevelopmental diseases such as Prader-Willi syndrome and Fragile X syndrome, but are also implicated in relatively common diseases such as rheumatoid arthritis and multiple sclerosis [7,8,9,10]. Substance use disorders also seem likely to involve epigenetic mechanisms, given the significant role of both heritable and environmental factors in their etiology [11].

Many epigenetic shifts occur at the beginning of the lifespan and can be introduced or modified by various factors such as levels of environmental toxins, psychological stress, and drug exposure [12,13]. For the latter, maladaptive plasticity within the shell of the nucleus accumbens (NAc), the medial forebrain bundle (MFB), and the ventral tegmental area (VTA) [14,15,16] as well as the prefrontal cortex (PFC), basolateral amygdala (BLA), and hippocampus [11] is often reported in models of addiction. These neuronal modifications following repeated drug use promote the intake of the drug of choice over regular necessities and social obligations, and can exacerbate or initiate already-present forms of mental illness such as schizophrenia and bipolar disorder (as reviewed by Cassidy et al. [17] and Hambrecht and Häfner [18]). Moreover, observed neuroanatomical and epigenetic changes persistent in nascent and long-term substance abusers have been correlated with facilitation of drug craving (as reviewed by Kauer and Malenka [19] and Volkow and Baler [20]).

The heritability of addiction is estimated to be between 30% and 70%, and it is suspected that a large portion of this may be specific to the type of drug utilized [21]. Using heritability indices, the liability for substance abuse transmission has remained relatively high at about 70% between dizygotic twin siblings [22]. Given these data, there were expectations in this field that the identification of specific gene polymorphisms contributing to risk in drug addiction and psychiatric disorders would follow recent revolutions in genomic technologies. Genome-wide association studies have only linked a limited number of genes that explain a fraction of the heritability imputed epidemiologically (reviewed by McCarthy and Hirschhorn [23]). In this context, it is important to remember that the heritability derived from twin studies is not exactly homologous to the presence of changes in protein coding genes [24]. Twins share a maternal uterine environment and often major features of the developmental environment, and both are often underspecified in human and animal studies [25]. Given that germ cells are subject to the effects of uterine environment, it is likely that there are also grandmaternal effects on epidemiologically imputed heritability, as signaled by cases of nicotine exposure [26]. Several plausible explanations for this "missing heritability" exist, including epigenetic information likely responsible for connecting genes and environmental changes [27].

Epigenetic sensitivity during certain developmental windows may also explain adolescents' heightened risk for substance abuse over adults. In adolescence, an imbalance between executive function and self-regulation influence the likelihood of substance use and abuse from initial contact. Multiple negative consequences are associated with adolescent drug use and abuse; in addition to environmental challenges, adolescents face decreased school standing and desire to interact with peers along with increased likelihood of future drug use in adulthood [28,29,30]. A full understanding of adolescent vulnerability to drugs is not completely apparent from clinical research alone (as reviewed by McCutcheon and Marinelli [30]). Findings from epigenetic studies show that patterns of DNA methylation differ globally across neurons and glia prior to adulthood, and centralized epigenetic programming events in the central nervous system (CNS) guide the transition to maturity [31]. Understanding changes to the epigenome caused by drug use prior to adulthood is, therefore, of critical importance to researchers, as the natural processes facilitating maturation of the CNS may be disturbed by the epigenetic modifications introduced by various drugs. Preclinical studies that examine genes, environment, and epigenetic changes over time, with the ability to control varying confounds such as sex, environment, and age, are valuable for highlighting mechanisms of drug vulnerability and potential novel therapeutic intervention.

## 2. Epigenetic mechanisms

Molecular epigenetics includes three main categories of molecular mechanisms: (1) histone modification, (2) covalent DNA modification, typically by methylation and hydroxymethylation of cytosines, and (3) noncoding RNA (ncRNA). To this one might also include the emerging science of "epitranscriptomic" modifications of RNA molecules, though this has been less included. Each of these mechanisms possesses unique subclasses, thus, creating a large assortment of total epigenetic permutations. Contained within every mammalian somatic cell is the organism's entire genome, coded in DNA. A single strand of DNA contains about three billion base pairs coded into gene structure, and may often exceed the physical limits of certain cell types and structures. To overcome this issue, DNA is tightly wound in one hundred and forty-seven base pair turns around nucleosomes, which are octamers composed of four core histones [32,33]. Each nucleosomic core contains two copies of the globular histone proteins H2A, H2B, H3, and H4, and each of these has an unstructured, amino-terminal "tail" [34]. Chromatin is the complex that contains DNA, RNA, and proteins within eukaryotic cells, and is critical for the function of the cell itself [35]. The structure of chromatin may exist in two basic condensation states, euchromatin and heterochromatin [36]. Euchromatin, its open state, is often related to active or on-going gene inactivation transcription processes. Heterochromatin is associated with gene inactivation or repression due to its compacted nature, which renders the DNA inaccessible to the transcriptional machinery. The compaction of the DNA in heterochromatin prevents transcription factors and replication initiators from reaching the promoter and start site of genes [36], thereby inhibiting expression. Figure 1 depicts a cartoon of the epigenetic mechanisms and interactions in neurons emphasized in the following section.

## 3. Histone modifications

Enzymes that interact with the exposed and available terminal tail domains of histones perform these modifications (though modifications within the globular histone core have been observed [37]). It has been suggested that distinct patterns of covalent histone modifications across cell-types have the potential to make up a "histone code" that may help regulate gene transcription on a precise scale—one that exceeds what was previously imagined [38]. Histone alterations may be interrelated or correlated in activation state, demonstrating their complexity [39,40,41]. It is undeniable that histones, therefore, retain a great deal of control over genomic state simply by limiting or increasing the access of transcription factors to exons or regions of interest. The extent of their functional significance is controversial, however, as there are more than one hundred described histone modifications. We will restrict

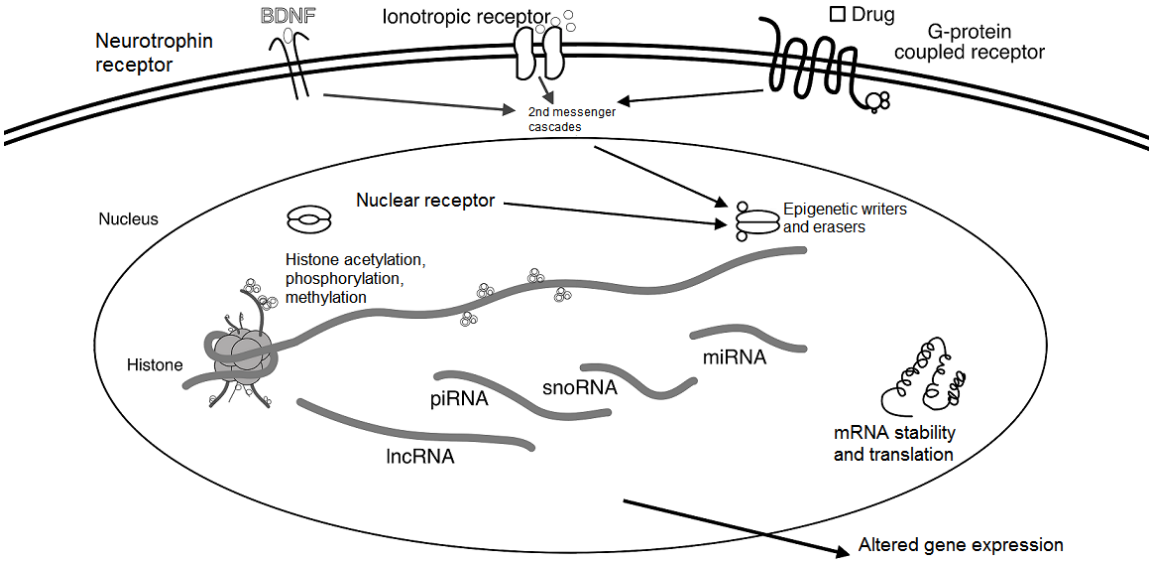


**Figure 1:** A cartoon depicting the potential sites of interaction for acute treatment with multiple classes of drugs of abuse—including ionotropic (e.g., alcohol interacting with NMDA receptors) and metabotropic (e.g., cocaine and amphetamine action on G-protein coupled receptors) receptors. For more repeated drug exposure and chronic models, interaction with neurotrophins and their receptors may also occur (i.e., activation of brain-derived neurotrophic factor (BDNF) interacting with TrKB). Further, many drugs have indirect interactions with nuclear receptors, which play a significant role in chromatin remodeling. Also shown are downstream activation of 2nd messenger cascades that, in turn, stimulate any number of events: epigenetic writers and erasers, DNA methylation, histone modifications (i.e., acetylation, phosphorylation, and methylation), and ncRNAs. These events can lead to longer lasting effects on gene expression and/or messenger RNA (mRNA) stability and translation.

our focus to the best studied regarding substance abuse and related disorders. *Histone acetylation* is one of the most widely studied histone modifications and is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs neutralize positive charges between the histone and wound DNA sequence through the addition of an acetyl group to a basic amino acid such as lysine. The resulting acetyl marks are typically associated with the open chromatin state and gene activation in eukaryotic cells [42,43]. As they remove acetyl marks, HDACs have been associated with gene repression in chromatin [44, 45]. Interestingly, the activity of HATs and HDACs is not definite; the activity of these enzymes may switch or overlap depending on the overall state of chromatin or the requirements of the promoter sequence being modified [46].

In the CNS, regular histone acetylation through HATs appears to be essential for the formation of memories and learning in the hippocampus [47,48,49]. The use of nonselective HDAC inhibitors therefore, increases overall memory capacity in mice, specifically in fear-contextualized learning, and may include additional transcription factors. Histone deacetylation appears to work, in turn, as a process that negatively regulates long-term potentiation (LTP) and memory formation [50,51], and moreover, serve multiple functions that include improving cognition [52].

*Histone phosphorylation* was initially linked to chromatin compaction during mitosis (as reviewed by Gurley et al. [53] and Rossetto et al. [54]), occurring at the protruding histone tails of chromatin that extend from the nucleosome. Primarily occurring at serine (S), threonine (T), and tyrosine (Y) residues, histone phosphorylation has been linked to DNA damage repair and transcriptional regulation as well. Phosphorylation of serine 10 on histone H3 is especially noteworthy, as it is essential for the completion of the mitotic process and is hypothesized to act as a signal for the promotion of transcriptional activation [55,56].

Unlike histone phosphorylation, *histone methylation* is specific to lysine (K) and/or arginine (R) residues along the histone tail and can occur in mono-(me), di-(me2) or tri-(me3) forms [57]. Classes of histone methyltransferase are often grouped according to residue associations and methylation valences (mono-, di- or tri-methyl). Certain methylation states are restricted in eukaryotic organisms. It appears that H3K9me1 and H3K9me2 states, which are primarily associated with transcription, are limited to euchromatic regions of chromatin, and are indeed specialized for eukaryotic organisms. The biological significance of these methyltransferases and changes in histone methylation state are highlighted by their appearance across mechanisms and systems [58].

Occasionally, histone methylation may require multiple enzymes depending on the methylation valence [59], and the most studied in the nervous system include five H3 residues including K4, K9, K27, K36, and K79, along with an additional residue on H4, which is K20 [40], though many other residues can be methylated. The selectivity of these residues is determined by the histone methyltransferase involved, but several enzymes may be required in order to shift one residue from unmethylated to the trimethylated state due to energetic requirements [46]. Classes of histone methyltransferase are often grouped according to their residue associations and methylation abilities, and certain methylation states are restricted in eukaryotic organisms. H3K9me1 and H3K9me2 states, which are primarily associated with transcription, are limited to euchromatic regions of chromatin, and are indeed specialized for eukaryotic organisms. The purpose of histone methylation at gene promoters, much like histone acetylation, may be to activate or inactivate genes required for cellular function; most trimethylations are repressive, though H3K4 trimethyl mark is associated with the promoters of actively transcribed genes [44,59].

## 4. DNA modifications

Covalent modifications of DNA represent one of the best-studied classes of epigenetic marks. Most work on DNA modification has focused on cytosine methylation (mC) or, more recently, hydroxymethylation. Generally, cytosines adjacent to guanosines are methylated, hence the CpG nomenclature is used to describe the mark. Cytosine methylation is usually associated with transcriptional repression and with the binding of methylated DNA binding proteins such as methyl CpG-binding protein 2 (MeCP2), a transcriptional regulatory protein involved in neuronal development. In mammals, cytosine is methylated by three DNA methyltransferases (DNMTs): DNMT1, DNMT3a and DNMT3b. DNMT1 is responsible for maintenance methylation while DNMT3a and DNMT3b are responsible for de novo methylation events [60]. The hydroxymethyl modification of cytosine (hmC) was discovered as a mark enriched in neurons, particularly cerebellar Purkinje cells [61]. The three members of the TET family of dioxengenases (TET 1–3) are responsible for the oxidation of mC to hmC, though TET 3 seems to be the most important in mammalian development [62,63]. The hmC mark is associated with the H3K4me3 mark and positively correlates to gene expression, suggesting that this mark is associated with active chromatin in contrast to the generally repressive nature of the mC mark. While hmC and mC appear to have different functions with regard to the control of gene expression, it is worth noting that the most common method of examining cytosine methylation in the genome, bisulfite sequencing, does not distinguish between the two marks, so results derived from this technique should be interpreted with caution [64]. While a number of other modifications of DNA have been catalogued, few have been examined in the context of the neurosciences [65].

## 5. ncRNAs

Studies of mammalian genomes have shown that most of the DNA is transcribed [66]. A large number of ncRNAs do not code for a protein or a molecule in translation, but are transcribed regardless. The classical molecular genetic view, while initially focused strictly on protein-coding genes, has now been expanded to include these RNA sequences once thought to be "junk" [67]. The few known abilities of these ncRNAs include carrying out a small number of biologically prescribed functions such as ribosomal RNA (rRNA) or transcriptional RNA (tRNA) sequences. New findings have revealed that multiple ncRNAs are able to alter gene expression and chromatin structure. Further, it appears that ncRNA levels increase with the complexity of the organism [68] and are developmentally regulated [69]. These advances in describing the function of ncRNA sequences in humans are exciting for the field of epigenetics specifically and to cell biology more generally.

ncRNAs have been classified based on regulatory properties. For example, small nucleolar RNAs (snoRNAs), Piwi-interacting RNAs (piRNAs), microRNAs (miRNAs), and long noncoding RNAs (lncRNAs) follow this nomenclature [70]. snoRNAs and piRNAs are associated with the "protection" of the genome from the insertion of transposable elements [71] and, until recently, were not thought to be active in the nervous system [72,73, 74]. Further, there are novel ncRNA transcripts that are associated with promoter regions that can enhance the coding ability of enzymes acting in the transcription process [75,76]. miRNA and lncRNA have been extensively studied in humans, as these transcripts are related to stable genomic changes in the individual that persist long after an initial drug exposure. Moreover, given that ncRNAs are conserved from one generation to the next, it is possible that they may contribute to heritable differences in individual susceptibility to different drugs of abuse.

## 6. Epigenetics and metabolic factors

Epigenetic changes are represented in clinical metabolic diseases that were previously considered heritable. For example, common diseases such as Type-II diabetes and hypertension may change susceptibility rate from one generation to the next depending on environmental factors and exposure rates in the parent [77]. The change may be due to epigenetic modifications introduced by nutrition, metabolic factors, and the development of the adipose system in the individual. Theories regarding alterations to metabolic rate and metabolic factors are fairly new and

have yet to be thoroughly analyzed. However, substantial literature suggesting the involvement of epigenetic factors in transgenerational disorders have been described [78]. Metabolic factors and substrates that act as molecular epigenetic precursors control cellular redox reactions and thus, control epigenome status through the production of molecular precursors to HDACs/HATs and histone methyltransferases. These precursors provide feedback for regulation of epigenetic factors in the cell [79]. In turn, epigenetic regulation of the genes that produce these factors may hamper or increase their production, shifting homeostasis in the cell system [78]. For instance, the growth of the cell is dependent on an intracellular burst of acetyl-CoA, which triggers downstream metabolites that enrich genes for growth through histone acetylation [80]. The downstream effects of drugs of abuse on neural systems and physiology such as the mesocorticolimbic system, vasoconstriction, and nutritional decline are well characterized [81,82]. Less information, however, is known regarding how drugs may affect the metabolic pathway and, ultimately, the expression of drug-related genes in the system. It is likely that chronic exposure to drugs over time may result in altered thresholds for metabolic production and decreased system functionality. For example, the methylation donor molecule, S-adenosyl methionine, is produced by the enzyme methionine synthase that, in turn, depends on the levels of folate and vitamin B12 [78, 83]. Metabolic deficiencies that occur upstream in the pathway impact gene expression and chromatin structure, which appears to be the case with high and regular alcohol consumption [84]. In addition, protracted opioid abuse leads to a decrease in the expression of the glutamatergic cell surface receptor EAAT3 [85,86]. EAAT3 is a necessary cytosine transporter and changes in EAAT3 expression are correlated with global methylation patterns [87]. Given these initial findings, it is likely that there may be other molecular-, protein-, and protein receptor-level changes that may be characteristic of the addicted brain.

## 7. Stress, developmental experience, and epigenetic changes

Stressors are environmental events that an organism must adapt to, typically through allostatic mechanisms (i.e., "adaptation through change") [88], and can vary by duration and the animal's response [89]. Thus, stressors are classic examples of environmental events that are likely to recruit epigenetic mechanisms to induce adaptation [25, 89], including the maladaptations that may lead to drug abuse. In animals, stress activates both the hypothalamic pituitary adrenal (HPA) and the sympathetic adrenal medullary (SAM) axes, and significant changes in HPA and SAM functions can eventually alter homeostasis, though the changes are not always coordinated [90]. Stress activation may cause the axes to become hypo- or hyper-responsive to stress, contributing to a range of phenotypes that may characterize vulnerability to drug addiction. For example, a stressor activates the HPA axis beginning with the hypothalamic release of corticotropin releasing factor into the portal system, triggering the release of adrenocorticotropic hormone from the anterior pituitary into the circulatory system. ACTH travels to the adrenal gland to stimulate the release of cortisol in humans and corticosterone in rodents. Circulating cortisol/corticosterone prepares the individual for responding and eventually deactivates the system through negative feedback to the hypothalamus and pituitary. Instances of repeated, uncontrolled stress can result in chronic activation of the HPA axis and increased cortisol, particularly at key developmental periods, creating a vulnerable state for later illnesses including psychiatric decline and drug consumption [91].

Overall, stress contributes to all aspects of the addiction cycle, eliciting first-time use, impacting already-abusing drug users, and increasing risk of relapse in abstinent individuals. For example, increased stress has been shown to lead to an increase in binge drinking behavior in addicts [92], risk of relapse to cocaine use [93], and risk of escalation to move on to more harmful drugs [94]. Elevated amounts of corticosterone have been tied to the hedonic value of abused drugs, and adverse effects on the HPA axis are found in alcoholics and in animal models of chronic alcohol intake, while alcohol escalation can be blocked with glucocorticoid receptor antagonism [95]. However, reports are conflicting as to whether maternal stress may create resiliency in offspring or create lower basal stress activation. These inconsistencies are likely due to variations in design, including timing, type, and duration of stress and testing window(s) for offspring, although a central factor seems to be whether the stressor increases or decreases maternal behavioral investment (for review, see [96,97, 98,99]). The question of the effects of prenatal stress on addiction vulnerability has only been posed in a limited fashion, with results demonstrating increased amphetamine responding and locomotion in offspring [100] as well as greater relapse to cocaine [101].

While many prenatal stress studies have been directed at changes in the late embryonic and fetal period, the changes in circulating blood levels of corticosteroids may also affect future offspring as early as the preimplantation stage [102, 103]. Stress immediately before or towards the beginning of pregnancy has been shown to decrease offspring weight and hinder future social interactions and integration [104]. Early-life stress is common in many clinical populations as well as in the general population, with some data indicating that 21% of men and women have experienced both physical and emotional abuse as children and 37% have experienced

at least one form of abuse [105]. Statistics for childhood sexual abuse are also high, at 16% for men and 32% for women [106]. Witnessing domestic abuse within the home can also be incredibly stressful and increase the likelihood of later life emotional disorders or substance abuse [105, 107]. In clinical studies, use of the adverse childhood experiences (ACE) checklist has yielded findings that negative childhood experiences (i.e., violent, verbal, physical or sexual abuse, witnessing substance abuse, etc.) are correlated with decreased adult self-care and multiple health risks, including substance abuse [108, 109]. Investigations of potential epigenetic mechanisms contributing to this relation between the early environment and later-life wellbeing seem likely to identify targets for intervention.

In utero exposure to glucocorticoids leads to a preference for opiates and ethanol in adult male Wistar rats [110], further implicating stress exposure during early development mapping on to vulnerability to drugs. At the behavioral level, the early environment—including parent-offspring interactions—contributes to the development of the HPA axis. In fact, several lines of research suggest that the quality of dam-pup interactions influences HPA functioning and other neural systems involved in coordinating the response to stress, and this transmission occurs above the genome [111, 112, 113]. Differences in parent-offspring care in rodents that affect later-life HPA-mediated stress response parallel epigenetic changes that influence glucocorticoid receptor gene *Nr3c1* [114] and *BDNF* expression [115]. The epigenetic changes observed following childhood abuse and adversity are similar, where the *GR* and *BDNF* genes show increased DNA methylation over control groups [115, 116, 117]. Witnessing abuse and high chronic stress produces differential DNA methylation patterns that can be predictive of life trajectory as early as infancy and adolescence [118]. Furthermore, chronic stress exposure in adulthood can affect the behavioral response of the following generation, mediated in part by epigenetic marks in the PFC, amygdala, and hippocampus [119, 120].

Early-life stress produces changes in DNA methylation that alters the MeCP2 gene coding in the sperm of male mice [168] and may decrease their reproductive behaviors through miRNA dysregulation [169]. How maternal programming of stress response differs from paternal programming response requires further characterization, though there have been studies that have focused on sexually dimorphic responses [169, 170]. Given that males and females differ by parent-of-origin allele expression [171], miRNA number [169], and circulating hormonal levels in utero and throughout the lifespan, additional work is needed to assess sexually dimorphic epigenetic products of early-life stress. A number of animal studies have been useful for interpreting dynamic, genome-wide changes that may occur from one generation to the next. Given the mounting data providing direct and indirect evidence that epigenetic marks may underlie many later-life behavioral shifts and vulnerability, questions regarding the timing or dosing of stress, the mode of transmission, and sex differences warrant further study.

## 8. Alcohol (see Table 1 for summary of findings presented in the following sections)

Alcohol addiction is a chronic and heritable illness that impacts millions of individuals in the U.S., is linked to increased mortality rates, and creates substantial socioeconomic burden in treatment [172, 173]. Alcohol addiction causes changes to neural networks and alters gene function because of repeated exposure. On the proteomic level, alcohol use is known to modify one-carbon metabolism that controls methylation in cells [174]. Due to this alcohol-induced change, researchers have been able to ascertain that epigenetic regulation is a critical component of alcohol abuse disorders. Indeed, global DNA methylation levels are raised about 10% in abusers relative to nonalcohol consuming peers. This hypermethylation state is contrasted by a decrease in DNMT3b mRNA expression and increased homocysteine, suggesting a balance of methylation and DNMT action [148, 149, 175]. Natural aging is associated with hypermethylation in multiple genes, including the dopamine transporter gene, and as such, one consequence of alcohol dependence may be *exaggeration* of this process.

Alcohol craving and consumption tend to be more stress-related for men [176], and there is a stronger association between striatal activation, alcohol consumption, and craving following stress in male animal models as well [177, 178, 179]. In support of this finding, men are more likely than women to report alcohol craving in response to a stressor, increasing their susceptibility to addictive behaviors and reward-responses from drinking [178]. DNA methylation at the monoamine oxidase-A (*MAOA*) gene sequence is correlated with alcohol and nicotine dependence in women. *MAOA* methylation status determines the suppression of the MAOA enzyme that modifies the oxidation status of dopamine and serotonin, and is increased in alcohol-dependent individuals [180]. In a later study using a similar cohort of adult women, Philibert et al. [181] used lymphoblast DNA to draw a direct relation between genome-wide DNA methylation changes and alcohol use patterns. Methylation of probes in the center of CpG islands were positively related to increased drinking behavior in women; however, this same association is not found in men. Similarly, Ponomarev and colleagues [182] identified upregulation of DNA methylation processes in the promoters of several genes related to chromatin functionality and transcription repression. The authors also showed that the epigenetic alterations in alcoholism were associated with increased transcription of transposon RNA

**Table 1:** This table provides a summary of findings highlighted in the review. Clinical and animal models (in vivo and in vitro) are included here, but are described greater length in the text with exact protocol and findings.

| Drug (dose, route) | Model, species or tissue | Developmental age | Effect on the epigenome | References |
|---|---|---|---|---|
| *Cannabinoids* | | | | |
| Δ9-THC (50 mg/ml) | Long-Evans rats ♀ and ♂ | Adolescent | Differential regulation of methylated regions, altered mRNA expressions of glutamate-related genes in the NAc | [121, 122] |
| Δ9-THC (1.5 mg/kg IP, every 3 days) | Cross-generational: Long-Evans rats ♀ and ♂ | Adolescent | ↑PENK in the NAc-shell via H3K9me<br>↑Heroin self-administration vulnerability | [123] |
| Δ9-THC (1.5 mg/kg IP, every 3 days) | Long-Evans rats ♀ | Adolescent | MOR-NAc function correlated with heroin self-administration striatal PENK modified | [124] |
| Δ9-THC (1.5 mg/kg IP, every 3 days) | Long-Evans rats | Prenatal, tested in adulthood | ↑PENK decreased in adolescence, increase MOR coupling following heroin | [125] |
| *Opioids* | | | | |
| High-fat diet (12% fat) | C57BL/6J and DBA/2J Mice, Gestational; offspring tested | Prenatal | ↑PENK and MOR expression in the NAc, PFC, and hypothalamus<br>↑Methylation at DAT, MOR, and PENK gene promoter sits | [126] |
| Heroin, opioid analgesics | Human ♀ and ♂ | Adult | ↑Methylation at OPRM1 exon<br>Global methylation of LINE-1 as correlated with pain sensitivity | [127] |
| Heroin, methadone, various analgesics | Human ♀ gametes | Adult | ↑DNA methylation at the OPRM1 gene in blood and sperm samples, correlated with increased pain | [128, 129] |
| Heroin, methadone, various analgesics | Human ♀; Sprague-Dawley rats ♀; C57BL/J6 mice ♀ | Late adolescence, adult | ↑H3K27me3 in the VTA-NAc circuit<br>↑Dopaminergic signaling<br>↑Downregulation of BDNF expression | [130, 131] |
| *Psychostimulants* | | | | |
| Cocaine (acute, 20 mg/kg IP) (chronic, 20 mg/kg IP daily for 7 days) | Sprague-Dawley rats ♀ | Adult | ↑H4 acetylation, H3 phosphoacetylation at the cFos gene promoter in striatum<br>$\uparrow^{++}$ H3 hyperacetylation after chronic cocaine only | [132] |
| Cocaine (chronic, 4 mg/kg, every 4 h daily for 7 days) | Sprague-Dawley rats ♀ | Adult | ↑H3 and H4 acetylation in the NAc shell positive correlation between CaMKII$\alpha$ and drug motivation | [133] |
| Cocaine (acute, 1.2 mg/kg) (chronic, 5 mg/kg IP for 4 days) | C56B7/J6 mice ♀ | Adult | ↑H3K14 and H4K8 acetylation in the hippocampus | [134] |
| Cocaine (chronic, 5 or 20 mg/kg daily for 7–14 days) | C56B7/J6 mice ♀; rat-originated striatal embryonic neurons | Adult | ↑HDAC5 in the striatum, drug-related modifications in gene expression | [135] |
| Cocaine (acute, 2.5 mg/kg IP) | C57BL/6J mice ♀; genetically modified | Adult | ↑Downregulation of histone acetylation in CBP<br>↑Decrease in cocaine-related behavioral sensitivity | [136] |
| Amphetamine (acute, 2.0 mg/kg IP) | C57BL/J6 mice ♀ | Adult | ↑H4 acetylation<br>$\uparrow^{++}$ H4 acetylation when paired with VPA | [137] |
| Amphetamine (acute, 2.0 mg/kg IP) | C57BL/J6 mice ♀ | Adult | ↑H4 acetylation levels increased<br>$\uparrow^{++}$ H4 acetylation when combined with BA or VPA | [138] |
| Cocaine (self-administration, 0.33 mg/kg on an FR1 schedule) | Wistar rats ♀ | Late adolescence, adult | Combined with HDAC inhibitors (TSA, phenylburtyrate), dose-dependent attenuation of cocaine self-administration | [139] |
| Cocaine (acute, 20 mg/kg IP) (chronic, 20 mg/kg IP for 7 treatments) | C57BL/J6 mice ♀ | Adult | ↑H3K9me2 in the NAc induced by G9a repression | [140] |
| Cocaine (self-administration, 0.33 mg/kg on an FR1 schedule) | Wistar rats ♀ | Late adolescence, adult | ↑Increased synthesis and expression of MeCP2<br>↑HDAC2, HDAC5, and HDAC11 gene expressions | [141] |

**Table 1:** Continued.

| Drug (dose, route) | Model, species or tissue | Developmental age | Effect on the epigenome | References |
|---|---|---|---|---|
| Cocaine (acute, 15 mg/kg IP) (chronic, 15 mg/kg IP for 7 days) | C57BL/J6 mice ♀ | Adult | ↑Increased DNMT3a and DNMT3b expression in response to acute cocaine<br>↑MeCP2 binding<br>↑Global DNA hypermethylation<br>Altered DNMT-1 and DNMT3a profile in the testes | [142] |
| Cocaine (chronic, 2 h exposure for 7 days via inhalation) | C57BL/J6 mice ♀ | Paternal exposure; offspring ♀ and ♂ | Sex-dependent modifications on cognition and memory in offspring | [143] |
| Cocaine (acute, 20 mg/kg IP) (chronic, 20 mg/kg IP for 7 treatments) | C57BL/J6 mice (♀)<br>Long-Evans rats (♀) | Adult | ↑DNMT3a to enhance cocaine reward<br>Regulation of spine density in the NAc | [144] |
| Cocaine (chronic, 20 mg/kg for 11 days) | CD1 mouse (female, gestational) | Maternal exposure; offspring ♀ and ♂ | ↑Elevated methylation across exons in hippocampal neurons<br>Significant fluctuations to DNA methylation levels throughout the lifespan | [145] |
| Cocaine (chronic, 5 mg/kg or 10 mg/kg, 3 times a day for 2 days, then a final injection of 15 mg/kg) | Sprague-Dawley rats (♀) | Adolescent | ↑Reduced H3K4me3 and H3K27me3 levels; differential regulation of $>60$ genes in the mPFC | [146] |
| Cocaine (0.25 mg/kg, self-administration on a FR1 schedule) | Sprague-Dawley rats (♀) | Late adolescence, adult | ↑H3 acetylation associated with BDNF-expression via CREB in the mPFC | [147] |
| Alcohol | Human | Adult | ↑Genomic DNA methylation (10%)<br>↓Global DNMT3a, -3b expressions | [148, 149] |
| Alcohol (chronic) | Human | Adult (gametes) | IG-DMR gene demethylation H19 gene demethylation | [150, 151] |
| Alcohol (chronic, 0.05-0.2%) | Cell line | — | ↑HDAC2 expression | [152] |
| Ethanol | Murine embryonic fibroblasts | — | ↑Degradation DNMTs, methyl CpG binding proteins<br>↓Hippocampal HDAC activity | [153] |
| Alcohol | C57BL/J6 mice ♀ | Adult | ↑H4 acetylation in the hippocampus (dentate gyrus) | [154] |
| Ethanol (chronic, 1.8–8.1% over 8 days) | Sprague-Dawley rats ♀ | Adult | ↑HDAC activity during ethanol withdrawal<br>↓H3K9 and H4K8 acetylation<br>↓Neuropeptide Y (NPY) expression in the amygdala | [155, 156] |
| Alcohol | C57BL/J6 mice ♀ | Adult gametes | ↓Global cytosine methyltransferase mRNA levels | [157] |
| Ethanol (25% in $H_2O$, ad-lib) | CD1 mice | Maternal exposure; ♀ and ♂ | Disruptions in intraneocortical circuitry in sensory and motor areas; aberrant FGF8 and Id2 gene expression | [158] |
| Ethanol (acute, 2.0 g/kg) | C57BL/J6 mice ♀ | Maternal exposure; offspring ♀ and ♂ | ↓Global DNA methylation<br>↓Global methylase activity | [159] |
| Ethanol (acute, 5.8 g/kg) | B6 mice ♀ and ♂ | Maternal exposure; offspring ♀ and ♂ | ↓Igf1 DMR regions | [160] |
| Ethanol (2% w/v, 2000 mg/dL) | Zebrafish embryos | — | ↓Global miR-9 expression downregulated | [161, 162, 163] |
| Ethanol (70 mM/mL over 5 days) | Murine neural stem cells (mNSCs, GD 12.5) | Prenatal | ↓Global miR-9 expression downregulated in the VZ (ventricular zone) | [163] |
| Ethanol (3 g/kg over 2 days) | Wistar rats ♀ | Adolescent, adult | ↑Genomic H3 and H4 acetylation at cFos gene promoters, FosB and BDNF genes<br>Bidirectional H3/H4 modification in the FC, striatum, and NAc<br>↑Genomic increases DRD2 and NMDAR2B expression in the PFC | [164, 165] |
| Ethanol (acute, 6.0 g/kg at 1, 3, and 12 h via IP) | Sprague-Dawley rats ♀ | Late adolescence | ↑G9a H3K9 methylation across varying samples | [166] |
| Ethanol (acute, 1.0 or 2.5 g/kg SC) | C57BL/J6 mice ♀ and ♂; genetically modified ♀ and ♂ $CB_1R$ mice ♀ and ♂ | Adolescent | ↑G9a activity<br>↑H3K9me2 and H3K27me2 | [167] |

and expression of L1 derived proteins. These agents of genomic plasticity have been implicated in both neural plasticity and disease processes [183,184]. This work, and studies employing gene expression profiling targeted at brain regions implicated in alcoholism, points to a role for epigenetic changes in alcohol-related pathology [185,186].

### 8.1. Developmental effects of alcohol

It has long been established that the earlier the exposure to alcohol, the more significant the consequences. Zhou et al. [187] demonstrated that genes associated with neural development—including cut-like 2 (*cutl2*), insulin growth factor-1 (*Igf1*), and SRY-box containing gene 7 (*Sox7*)—were disrupted in expression by changes in H3 lysine 4 trimethylation after a six-hour alcohol exposure in vitro. This model highlights mechanisms contributing to alcohol interference of neural stem cell differentiation for systems involved in long-term neurobehavioral alterations common to fetal alcohol spectrum disorder.

Changes in the epigenetic status of essential genes such as *BDNF* also emerge following repeated alcohol exposure. *BDNF* gene variants are implicated in changes in cortical neuroplasticity, especially as a mediator of LTP, and are critical for neuronal development and survival [188,189]. Alterations to the *BDNF* gene through polymorphisms or gene variants increase the likelihood of ethanol consumption and augment anxiety-like behaviors in rodents [51,155]. At the epigenetic level, histone deacetylase 3 (HDAC3) downregulation to the BDNF promoters (PVI, PII, and PIII) and upregulation of BDNF exons have been noted in C57BL/6J mice exposed to chronic ethanol [154].

Acute and chronic alcohol intake may negatively affect histone configuration and histone-related enzymatic activity as well. Manzardo and colleagues [190] reported large-scale disturbances in histone-related and methylated genes in the frontal cortex of alcoholics. Using a whole genome approach with a combination of immunoprecipitation techniques, both types of genes altered by alcohol exposure were categorized on a scale from “low” to “high”. Genetic information was sampled from frontal cortex tissue taken from subjects who were reported alcoholics. Among the thousands of genes sequenced, the promoter sequences of the genes *GNAS*, *H19*, and *HIST2H2AB* were more likely to be methylated in alcoholics, suggesting that general histone-related modifications may occur in tandem with methylation at alcohol-related genes. These genes are also implicated in critical roles: *GNAS* codes RNA transcripts involved in signaling and imprinting other genes while *H19* codes for a ncRNA transcript and is a gene most commonly associated with imprinting defects in embryogenesis and oncogenic transformation in cancer; *HIST2H2AB* (Histone 2AB) is an intronless sequence that codes for a protein within histone 2A. Modifications at these sites may contribute to the anxiety-related effects caused by acute alcohol exposure, in which decreases in H3K9 and H4K8 acetylation in the central and medial amygdala are found [191,192]. Decreases in HDAC activity lead to anxiolysis, and these benefits are reversed in rats experiencing ethanol withdrawal [155]. Treatment with the HDAC inhibitor, trichostatin A, reduces anxiety-like effects in ethanol withdrawing rats but not controls, suggesting that epigenetic modulation may be a potential target for therapy in abstinent users [156].

### 8.2. Prenatal exposure to alcohol

Alcohol has well-documented teratogenic effects, characterized in a range of severity of developmental, cognitive, and physical birth defects [193]. Fetal alcohol spectrum disorders (FASDs) are estimated to make up one out of every 100 live births, though this number may underestimate the true prevalence (e.g., [194]). FASDs remain a leading cause of nongenetic associated mental retardation [195,196,197]. In animal models of FASD controlling for maternal care, later life poor memory and learning disabilities are apparent thus, implicating in utero exposure to ethanol [198]. Given the lasting effects of alcohol exposure across development, epigenetic marks may be activated in utero. Indeed, the production of DNMTs is disrupted, as alcohol consumption inhibits the production of foliate upstream [199]. The extent of the genetic and epigenetic changes induced by distinct paternal contribution is not yet fully understood, as the physical changes are influenced by infant birth weight in maternal exposure models. However, there is evidence for FASD characteristics in offspring of alcohol-consuming sires [200, 201,202].

Ouko et al. [150] found elevated DNA demethylation as a function of alcohol consumption at two differentially methylated regions, *H19* and *IG-DMR*, in the DNA of sperm taken from male volunteers. The authors hypothesized that new regulation of these genes may be transmitted to offspring with undiscovered consequences for addiction risk. Documented losses in *H19* binding site methylation are correlated with decreased fertility in the sons of alcoholic fathers, as *H19* binding sites are important for the regulation of *Igf2* (insulin-like growth factor 2), a gene implicated in memory and reproduction [203,151]. The challenges during development that the offspring of alcoholic fathers face may ultimately be caused by hyper- and hypomethylated regions of the genome within areas of the brain altered by alcohol consumption, and some of the DNA methylation changes produced may be unique to paternal contribution [151, 204]. Exposing adult male rats to alcohol for several weeks reduced litter size and mean birth weights of the sired offspring [157]. Expression of the mRNA transcript for DNMT was also reduced in alcohol sired offspring [157]. On the positive side, supplementation with methyl donors like choline improves histone and DNA methylation in the

brain [205] and appears protective against animal models of FASD [206]. This demonstrates that in addition to showing face and construct validity, other epigenetics-related studies of rodent models may be valuable for predictive validity—identifying and targeting novel therapeutic interventions for FASDs.

In a study using an in utero rat model, El Shawa and colleagues [158] demonstrated extensive intraneocortical damage and higher levels of anxiety-like behavior in the offspring of female mice administered ethanol from the first day of gestation. Area-specific genes such as *Id2* and *Cadeherin8* were modified in offspring of ethanol-exposed dams, suggesting that epigenetic modifications may influence intergenerational behavioral outcomes. Further evidence for the role of epigenetic programming in ethanol models was provided by disruption studies [159, 207] and prenatal ethanol mouse models that demonstrated elevated DNA methylation in embryonic tissue [160]. An early-life study of the effects of alcohol on the genome of C57BL/6 mice showed a ten-fold increase in DNA methylation during neural development, particularly along chromosomes 7, 10, and the X chromosome in alcohol-defective mouse embryos [195].

miRNA expression is critical for fetal programming, where a small proportion of sequences regulate processes like mitosis and cellular differentiation in developing mammalian embryos [208]. Different miRNAs may become activated depending on the stage of offspring development; neuronal maturation and both positive and negative feedback loops for cell receptors can involve identical miRNA-targets [209,210]. Ethanol exposure can either decrease or suppress the action of specific miRNAs, highlighting miRNAs as "master switches" regulating a complexity of genes involved in ethanol intoxication and neurotoxicity [211]. For example, miR-9 controls a host of cellular mechanisms required for the eventual growth of neuronal progenitor cells and has been linked to synaptic plasticity and circadian rhythm via its downstream effects on other molecules [211, 212]. Since miR-9 is suppressed in the area that will become the brain in fetal stem cells [163] and is upregulated in adult ethanol response [212], it should be noted that these effects might be time-dependent as well (as reported in [213]). This is true of miR-335, which is increased by lower intermittent exposure to alcohol as opposed to miR-335 suppression subsequent to chronic alcohol abuse [214]. These findings demonstrate that early ethanol exposure impacts DNA methylation and miRNA expression patterns that correspond to pleiotropic gene expression profiles and cellular processes that influence in utero development.

### 8.3. Exposure to alcohol during childhood and adolescence

Children and adolescents are more vulnerable to the effects of alcohol relative to adults due to the (1) immaturity of the developing brain, (2) reduced behavioral and social impairments caused by any alcohol consumption, and (3) increased health risks associated with binge drinking [215,216,217]. Children whose parents have a history of protracted alcohol use or heavy episodic intake are more likely to consume alcohol earlier than their peers [218] and experience social and school-related problems that contribute to lowered self-esteem and increased school difficulties [219]. Continued alcohol abuse through adolescence is more likely to map on to sustained drug use and comorbid mental health disorders [220,221]. Therefore, prevention of alcohol use prior to adulthood should be emphasized based on its potential life-long consequences. Alcohol consumption negatively affects brain plasticity in an age-dependent manner by increasing the inflammatory response and decreasing neurogenesis in the dentate gyrus of the hippocampus [222]. Similarly, there is a depression of hippocampal LTP following chronic ethanol exposure in adolescent rats [223]. Abnormal brain plasticity in reward areas has also been described and is associated with specific histone modifications of gene promoters. Using an ethanol binging paradigm in adolescent rats, Pascual and colleagues [165] demonstrated an increase in histone acetylation activity in H3 and H4 in the promoter regions of *cFos*, *FosB*, and *BDNF* genes that was specific to the PFC of adolescent but not adult rats. This corroborates their earlier work [164] in which rats administered ethanol were also found having a striking increase in HAT activity over adults with no differences observed for HDAC activity. In addition, the authors provide direct evidence for the role of HDAC inhibition on gene histone acetylation and reward-related behavior using ethanol conditioned place preference (CPP). Use of the HDAC inhibitor, sodium butyrate, acts synergistically with this change, leading to increased transcription and overall expression of the same genes (*cFos*, *FosB*, and *BDNF*), specifically in the brain of adolescent animals. Given that the adolescent brain is undergoing extensive remodeling [31], repeated alcohol exposure impacting histone modifications (e.g., changes in acetylation and HAT activity) can result in rewiring the brain during this critical period [224]. Further work found that posttranslational histone acetylation of H3 was increased at lysine 9 by ethanol treatment in rat hepatic stellate cells [166]. Ethanol in P7 rats can also increase expression of G9a, a histone methyltransferase, which contributes to elevation in dimethylation of H3 lysine 9 (H3K9me2) and 27 (H3K27me2) [167]. DNA methylation studies that target pre- or peripubescence in animals are rare. The earliest study of methylation changes to offspring was done in a gestational model, in which ethanol was given to a pregnant rat dam in the time leading up to birth [159]. The authors concluded that ethanol contributes to modulation of the general reward system by increasing global DNA

methylation of genes related to alcohol addiction. Fewer studies have looked specifically at how certain regions may compare in overall gene expression. A sharp reduction of DNA methylation is observed in the PFC, following ethanol administration throughout adolescence and similarly in the hippocampus [225].

## 9. Psychostimulants

As their name suggests, psychostimulants have psychoactive properties and can induce increasing levels of behavioral and neurocortical activation with repeated use [226]. Psychostimulants create disruptions of dopaminergic (DA) signaling by occupying dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT-2), leading to increased amounts of synaptic dopamine [227,228]. Epigenetic research, like much basic research focused on psychostimulants, is often divided between the outcomes following acute and chronic use, which may have contradictory effects. For example, both acute and chronic doses of amphetamine cause a marked production of cFos mRNA transcripts in the striatum of rodent models and, thusly, increased *cFos* gene expression in this region. The expression, number, and type of Fos immunoreactive neurons activated in the striatum, however, are modified dependent on acute or chronic exposure [229]. It is important to note that while the appearance of certain proteins and gene outcomes may be similar, the pattern of their expression may not be the same across drug regimens.

Cocaine is the most commonly studied psychostimulant in clinical studies and preclinical models investigating adult acute and/or chronic effects on gene expression. Most research in this area has centered on histone modifications to drug-related genes. *cFos*, *BDNF*, and the cyclin-dependent kinase 5 (*CDK5*) gene which has been implicated in the migration of neuronal migration [230], are often expressed or repressed following psychostimulant use. Kumar and colleagues [132] used chromatin immunoprecipitation (ChIP) sampling on extracted wild-type, modified mouse striatal tissue and found evidence for an increase in acetylation of H4 on the *cFos* gene following acute cocaine treatment; they also reported increases in H3 on the *CDK5* and *BDNF* genes after chronic treatment. H3 acetylation in the NAc shell contributes to the maintenance of cocaine-based drug reinforcement behavior in rats, as evidenced by increased behavioral response to repeated cocaine administration in [133].

Histone acetylation within the CA1 of the hippocampus regulates and consolidates memory formation [231] and appears to affect the context-associated properties of drug exposure [134]. Findings show that HDAC4 and HDAC5 in the NAc negatively impact cocaine place preference, likely disrupting brain plasticity around this learning by altering drug-induced brain plasticity [132,135]. HDAC3 is also implicated [232] as the acetylation state of H3 has a strong effect on drug-taking behavior working through not only HATs and HDACs, but also their catalyzing enzymes. For example, adult mice that are generally deficient in CREB-binding protein (CBP)—which possesses HAT activity—have reduced sensitivity to cocaine intake over time. The removal of CBP from the NAc disturbs cocaine-related spatial preference and blocks cocaine CPP [136].

The HDAC inhibitors, butyric acid (BA) and valproic acid (VA), have been used in a number of studies examining epigenetics in drug-related behavior. BA and VA can alter chromatin structure independent of transcriptional events, but are primarily categorized as HDAC inhibitors for their reorganization of chromatin structure. HDAC inhibitors can lead to enhanced, context-specific behavioral sensitization in a mouse model [137]. When administered BA or VPA alone, mice showed no significant change in locomotor response compared to controls; however, marked effects on locomotor sensitization were observed when these animals were given HDAC inhibitors following chronic amphetamine use. This was supported by similar research using a chronic amphetamine model with cotreatment with HDAC inhibitors in C57BL/6 male mice [138].

There are inconsistencies in the literature regarding the mechanisms underlying the neurobehavioral effects of HDAC inhibitors. To address this, attempts have been made to organize HDAC inhibitors into classes (e.g., Classes I, II, and III) and to use more selective targets [138]. Recently, studies have emphasized comparing classes in a single study and noting isoforms that may alter specific responses. For instance, the HDAC inhibitors trichostatin A and phenylbutyrate attenuate cocaine self-administration without impacting sucrose self-administration, suggesting that histone acetylation-based chromatin remodeling did not globally affect motivational responses [139,141]. The use of the Class I HDAC inhibitor, RGFP966, demonstrates that histone acetylation in the hippocampus, infralimbic cortex, and NAc is involved in memory processes important for extinction [233].

ChIP sequencing of rat NAc following cocaine treatment for variable exposure periods has implicated H9K9me2 and -me3 as critical epigenetic marks in models of psychostimulant addiction [140,234]. The histone methyltransferase, G9a, which is regulated by ΔFosB, catalyzes H3K9 dimethylation at specific promoters in the NAc. G9a is downregulated after repeated cocaine exposure, and this, in turn, leads to changes in dendritic spine density in NAc neurons [140]. This is one possible way in which changes in histone modifications may affect overall neural response to drugs, though other studies demonstrate that other histones (e.g., H4) may play similar roles [132]. Changes in DNA methylation levels have also been correlated with behavioral sensitization to psychostimulants as well.

Acute cocaine treatment of adult rats upregulates levels of DNMT3a and DNMT3b in the NAc and the hippocampus, leading to hypermethylation of genes associated with drug behavior such as *FosB* [142]. DNMT3a is the most prevalent methyltransferase in the NAc and has been shown not only to alter spine density in this area by over/underexpression but also to potentiate cocaine place preference when inhibited in the NAc [144]. MeCP2, critical protein, was one of the first epigenetics-related molecules associated with psychostimulant use and contributes to the neurodevelopmental disorder Rett's syndrome [235,236]. MeCP2 levels are increased by DNMT3a action in the NAc, creating changes in the NAc and functionally related reward areas that "set-up" a drug-ready landscape for addiction at a posttranscriptional stage [237,238].

### 9.1. Prenatal exposure to psychostimulants

Among the first generation of functional epigenetic studies, work by He et al. [143] demonstrated that changes to DNMT1 levels contribute to transgenerational genetic transmission. In a chronic cocaine inhalation model in male mice that mimics human crack cocaine binging, sires were bred with drug-naïve dams and cognitive behaviors were recorded in offspring. Cocaine-sired adult female offspring showed disrupted sustained visuospatial attention and spatial working memory relative to male offspring. While there were no significant differences in the DNA structures of spermatozoa between cocaine-sired and control mice, there were alterations in the expression of DNMT-1 and DNMT3a in the seminiferous tubules of the testes. Cocaine-inhaling males had marked decreases in DNMT-1 and increases in DNMT3a. These data imply that in this novel cocaine inhalation model, NAc DNMT3a regulates behavioral and emotional responses to reward [144]. Further, blocking DNMT3a in the NAc leads to increased motivation to continue cocaine intake. Much like MeCP2, DNMT3a can regulate *BDNF* expression and, therefore, may have related effects on learning and memory [239]. In the interpretation of these findings, links have been made between DNMT3a, *BDNF* expression, and methylation as collective markers of early life adversity [240], suggesting potential factors contributing to lifetime vulnerabilities.

Novikova and associates [145] hypothesized that maternal exposure to cocaine would alter offspring behavior through changes in the epigenome. Results indicate that PND3 male offspring prenatally exposed to cocaine demonstrated alterations in DNMT-1 and DNMT3b of pyramidal cells in the hippocampus with some of changes persisting through prepubescence (PND30). Interestingly, both DNA hypomethylation and DNA hypermethylation were found to occur in distinct CpG island regions, and methylation targets not previously observed at PND3 appeared at PND30. An interesting point is that while the authors reported cocaine-induced DNA methylation changes, the changes were reported as irregular over time. They posited that epigenetic status following cocaine exposure might add increased susceptibility to addiction in adulthood. Given this preliminary information, additional animal studies are necessary to evaluate DNA methylation patterns across development and multiple generations following early cocaine insult.

In clinical literature, the changes induced by prenatal cocaine exposure have focused on direct/indirect neurochemical and vascular effects on the fetus. Intrauterine stress was also suggested as a "third pathophysiology" that interacts with fetal development to induce later life changes [82]. Drug abuse and other negative maternal behaviors detrimentally shift the intrauterine environment, and this, combined with cocaine "stress," may interact with epigenetic mechanisms to facilitate vulnerability to substances of abuse and mental health disorders. Prenatal cocaine interferes with the norepinephrine transporter (*NET*), a monoamine transporter gene that plays a role in the intrauterine environment [241,242], such as reducing placental glucocorticoids and catecholamines. *NET* is one gene target that is downregulated following prenatal cocaine working via DNA methylation in the placental genome, leading to an overall decrease of *NET* in the infant [243]. Similarly, the gene that codes for 11$\beta$ hydroxysteroid dehydrogenase type 2 (11$\beta$-*HSD-2*), a metabolic enzyme that catalyzes active glucocorticoids in system, is increasingly methylated with cocaine exposure. Epigenetic repression of this gene is associated with hypercortisolemia, hypertension, and glucose intolerance in both animal and clinical models [244,245]. DNA methylation is associated with the downregulation of *NET* gene expression in the maternal environment following in utero cocaine exposure [82,243]. The challenge in understanding time course and the involvement of the placenta may be critical in future research focused on transgenerational epigenetic mechanisms of stimulant drug effects.

### 9.2. Psychostimulant exposure during childhood and adolescence

Periadolescent mammals tend to be impulsive and seek novel stimulation to a greater degree than animals of other ages [246,247], likely a consequence of high levels of dopaminergic function and immature frontal cortical structures. Impulsivity and novelty seeking are personality traits that increase the likelihood of initiating drug use and can contribute to greater instances of adolescents becoming addicted to substances such as nicotine [248,249] and other psychostimulants [250,251,252]. Nicotine addiction may also prime the brain for later-life addiction to other psychostimulants by increasing transcripts of FosB and inhibiting HDAC [253]. Increased global acetylation may underlie

the behavioral changes in response to drugs of abuse like cocaine; in fact, locomotor sensitization and overall LTP are enhanced following this priming effect [253].

In a binge-like rodent model of adolescent addiction where cocaine was administrated in increasing amounts over twelve days, there was a decrease in H3 methylation in the mPFC of the adult rats compared to nonexperienced controls [146]. Chromatin remodeling in this period may be partially responsible for behavioral effects associated with cocaine addiction, such that, for example, epigenetic repression of BDNF increases cocaine seeking behavior [147]. Cocaine-induced changes to gene expression may occur via transcriptional activation by H3K4 trimethylation and transcriptional repression by H4K27me3 in the mPFC [146]. Many studies have focused on stimulant-induced locomotor activity and epigenetic marks in young adulthood, but further time-dependent epigenetic studies that model the acute effects of cocaine/psychostimulant exposure are warranted given the inconsistencies reported in literature [254,255].

Studies using HDAC/HAT inhibition add to our understanding of the complexity of epigenetic mechanisms involved in adolescent psychostimulant exposure. Shen and colleagues [138] found that coadministration of amphetamine and either of the HDAC inhibitors VA or BA augmented H4 acetylation. In fact, there was a synergistic effect in striatal regions, regardless of the substance dosing order. Coadministration of VA and amphetamine upregulated CREB phosphorylation and *Fos* activation and attenuated locomotor activity, indicating a cascade of intracellular effects that may change the activation patterns of the neurons involved [256]. Microinjection of BA into other areas of the brain, including the amygdala and striatum, also attenuated the drug-induced locomotor activity associated with psychostimulant use [257].

## 10. Opioids

Opioid abuse is currently escalating at a higher rate than psychostimulant abuse, because of the increasing availability of prescription opioids [258]. Initial exposure to prescription medications is linked to heroin use [259], adversely contributing to increased rates of dependency with a reported 2.1 million U.S. adults and younger users [260], and elevated mortality rates [261,262]. Morphine and codeine are active ingredients in the opium poppy. Synthetic analogs, including oxycodone, are used in prescription pain medication and are also known as drugs of abuse [259,263]. Morphine is the most commonly studied opiate in investigations of genetic and epigenetic changes, but all-natural opiates and synthetic opioids act as agonists at mu, delta, and kappa opiate receptors in the periphery and CNS.

The rewarding effects of opioids are mediated primarily through mu-opiate receptors (MORs) located on the surface of neurons throughout the brain and spinal cord. These inhibitory G-protein coupled receptors indirectly modulate mesolimbic dopamine neurons, but they are also located in regions important for pain, memory, and contextual conditioning such as the hippocampus and the amygdala [264]. In fact, endogenous opiate signaling, MOR levels, mRNA transcript production, and polymorphisms have been implicated in several classes of drugs of abuse in addition to opioids, including cocaine (reviewed by Nestler [265]) and alcohol [266,267]. Chronic and long-term use of opioids often results in a general hyperalgesia as a primary withdrawal symptom [268]. The individual differences in clinical response to opioids and their use are hypothesized to be a direct result of genetic polymorphisms of the *OPRM1* gene that produces MORs [269,270]. The HAT and HDAC balance is disrupted in mice with opiate dependence [127], however, as the authors noted, the study did not provide direct evidence for repeated pain medications altering epigenetic marks, and so insights into earlier triggers for epigenetic changes are needed.

DNA methylation in CpG-rich islands at the *OPRM1* gene promoter is associated with increased chronic opiate use, especially in the VTA [127,271]. Following a decrease in MORs, silencing via hypermethylation likely contributes to increased pain sensitivity following opiate abuse [127]. However, questions remain regarding the timing of these effects of chronic opioid abuse as well as any other consequences.

### 10.1. Prenatal exposure to opioids

While endogenous opioids mediate pain tolerance and rewarding behaviors in both sexes, there are notable sex differences in opioid effects on fertility. For males, fertility, sex drive [272], and the DNA methylation of genes such as *OPMR1* in sperm are affected by repeated opioid exposure [128,129]. In females, opioids influence parturition, lactation, and maternal behaviors [273]. Ovariectomized female rats treated with exogenous estrogens show increases in MOR internalization in neurons of both the pre-optic nucleus and the amygdala [274]. As of yet, DNA methylation patterns on the *OPMR1* gene relative to estrogen and progesterone receptor changes have not been identified across the lifespan [275]. Interestingly, fluctuations in endogenous opiates are observed at key points in the development of maternal behavior, including at the end of pregnancy, during parturition, and following ingestion of afterbirth [276]. These data suggest that there are epigenetic regulating effects that are likely to occur during prenatal development. In a study by Sarkaki et al. [277], male and female rats were administered morphine orally and bred in order to assess the effect of parental addiction on offspring brain plasticity. LTP was effectively reduced in both male and female offspring in comparison to control

groups, demonstrating that shifts in plasticity may be a mechanism for transmission of offspring drug vulnerability and/or learning deficits. Further work will be necessary to investigate these changes, intergenerational transmission, and the associated, causal effect on the epigenome.

Prenatal morphine exposure causes robust changes in offspring: disruptions to the HPA axis and modification of the stress response [278], decreases in long-term potentiation in males [279], changes in male and female sex behavior, and alterations in pain sensitivity [280,281]. Animals exposed to morphine in the perinatal period also experience disruptions in the brain's endocrine signaling pathway, changing overall central nervous system levels of norepinephrine as well as ovarian cycling and sexual receptivity to wild-type males [282]. While there is not yet any direct evidence linking early insult with opioids to epigenetic changes that are transmitted across generations, many of the aforementioned systems impacted by prenatal opioids have been shown to induce a variety of epigenetic changes. In fact, opioid exposure (as well as other licit and illicit substances) during pregnancy leads to neonatal abstinence syndrome (NAS) in the infant that may adversely affect development and create physiological vulnerability to later life disease and developmental challenges [283]. Jansson and colleagues [284] suggest that these NASs may be a consequence of the infant's adaptation to the repeated methadone insult in utero. Interestingly, epigenetic alteration of MORs in the PFC, NAc, and VTA following prenatal high-fat diet exposure [126] implicates a role for the adipose system in programming susceptibility to opioid abuse.

### 10.2. Opioid exposure during childhood and adolescence

Childhood exposure to morphine or other exogenous opioids negatively affects individual health, and later, reproductive habits. Until recently, youth opioid abuse was under represented in scientific literature and poorly documented outside of case studies [285]. Outcomes following opioid abuse range from mild cognitive disorders to severe respiratory issues [286], resulting in increased hospitalizations and deaths in recent decades [287,288]. Rising numbers of adolescents exposed to opioids warrant further research on opioid alterations to the developing brain, in addition to public health prevention efforts [287].

Opioid use has noticeable effects on cognitive, social, and emotional behaviors in the individual and across generations. Male rats administered morphine show increased, independently driven play behavior with a partner [289,290]. Chronic morphine administration alters the VTA such that it becomes 25% smaller [291], specifically through reduction of dopaminergic synapses and neuronal branching in pyramidal cells in this region [292]. The pyramidal cells of the PFC and NAc are also changed by initial and subsequent exposure to opioids [293] and these neurons undergo long-lasting modifications that outlast the drug exposure itself [294].

Both male and female adolescent animals are developmentally delayed in spatial memory, but only females are delayed in memory recall [280]. Drug-naïve adolescent female animals that have had a maternal dam exposed to morphine also show increased anxiety-like behavior in the open field, implying that these changes are generationally conserved [295]. Findings from Byrnes and colleagues [296] also indicate that elevated mRNA expression of MOR-1 in the NAc may mark addiction susceptibility in morphine-exposed offspring. Therefore, this may suggest transmission through epigenetic regulation of opioid-related genes in gametes or maternal/paternal germline as observed in other substances of abuse such as alcohol and psychostimulants. Interestingly, modifications in vulnerability status may be conserved from adolescence in the generation prior [297].

Adolescent opioid use has the potential to affect the *BDNF* gene across its exon variants through epigenetic repression in the VTA. Transcriptional events, specifically through RNA polymerase II, are downregulated with morphine use, and have lowered activity at specific promoter regions of the *BDNF* gene in adult mice [131]. Blockade of this gene and its products enhances overall locomotor and rewarding properties of chronic morphine administration in animals by increasing excitability and action potential response of dopaminergic neurons [130]. BDNF levels are initially low early in development and rise in late adolescence into adulthood. Thus, it is likely that opioid use may introduce epigenetic modifications to additional neurotrophic factor-related genes to heighten susceptibility to addiction through MOR mechanisms [298]. With MOR transcripts detectable in the developing embryonic mouse model as early as gestational day 9, even acute exposure to opioids may modify the system [299].

Interactions with inflammatory system have also been described in mediating the long-term and stable vulnerability to morphine exposure. Astroglial activation through bacteria inflammation has been shown to selectively increase MOR mRNA transcripts in reward-related brain regions, most likely through the interleukins [300,301]. Further, the reduction of *IL-10* gene expression throughout glial cells in the NAc decreases overall potential for morphine-related behaviors, like conditioned place preference [302], leading to the conclusion that illness or neuroinflammation may create vulnerability to later drug use. Opioid dependence has also been linked to inflammatory activation in the VTA, as spontaneous withdrawal after escalation to chronic morphine exposure causes increased prevalence of the microglial factor Iba-1 [303]. Further work is needed to examine whether these changes also correspond to disruptions in epigenetic marks that persist throughout life and/or can be transmitted across generations.

## 11. Cannabinoids

Cannabinoids, most often inhaled marijuana treatments, are used in medical therapies for pain reduction, anti-emesis for cancer patients, stimulating appetite for HIV/AIDS patients, and relieving intraocular pressure in glaucoma sufferers [304]. There is also widespread recreational use of marijuana in many populations across the world. $\Delta^9$-tetrahydrocannabinol (THC) is the major psychoactive component of *Cannabis* derived from the *Cannabis sativa* and *C. indica* plants. Recreational users report feelings of relaxation and greater sociability (see [305]), and the rate of use among young adults has increased within recent years [262]. Despite evidence that suggests chronic THC exposure is linked to disruptive physical (i.e., increased incidence of bronchitis) and cognitive health (e.g., memory deficits), U.S. national surveys indicate that marijuana is the most commonly used illicit substance for adults and adolescents over the age of twelve [306,262]. Several lines of research link illicit cannabis use and disruptions of the endocannabinoid system to psychological disorders like schizophrenia [307,308], bipolar disorder [309], and anhedonia/major depression [310]. Debate exists as to whether this exposure leads to later-life *harsher* drug-taking tendencies, though reports of the early use of marijuana do highlight a need for understanding how it may affect the brain at this critical stage of life [311]. Repeated marijuana use causes dynamic changes in CB1 receptor expression and increases in MOR expression in the mesocorticolimbic and opiate systems in the offspring of the exposed parental line, contributing to the conservation of drug-induced modifications in the genome and epigenome across generations [306,312].

Similar effects are noted in adult populations, with chronic exposure to exogenous cannabinoids producing unique impairments in emotionality, serotoninergic and GABAergic transmission [313,314,315]. Given these behavioral effects and alterations in multiple neural systems, investigations of the epigenetic mechanisms underlying the effects of cannabinoids have been reported and discussed at length (for review, see [316]). Further work should focus on identifying the window(s) of early life exposure and the associated epigenetic changes.

### 11.1. Prenatal exposure to cannabinoids

Early longitudinal studies that follow children prenatally exposed to cannabinoids report disrupted sleep in the exposed infant [317]. By three years of age, children exposed to cannabis in utero already show poor cognitive performance such as impaired visual-spatial reasoning relative to their nonexposed peers. These children also show significantly greater behavioral problems and heightened distractibility [318,319]. As they aged, the children also show decreased intellectual scores and heightened likelihood of developing schizophrenia [320,321,322]. In prenatal cannabinoid study, using male and female rats placed on a chronic schedule of THC exposure and then mated with similarly exposed rodents, researchers observed distinct behavioral effects in drug-naïve offspring [121]. F1 offspring of THC exposed parental lines developed increased stereotypies and approach behavior towards a novel stimulus following heroin self-administration and withdrawal. These animals also demonstrated morphological changes with striatal NMDA receptors significantly decreased in comparison to control offspring. Long-term depression of synaptic plasticity was also altered in the NAc and dorsal striatum in offspring of THC-experienced parents. These changes implicate epigenetic programming in mediating this intergenerational transmission. Using the offspring of rat dams exposed to THC during adolescence, genome-wide DNA methylation analysis identified over one thousand differentially methylated regions in comparison to wild-type rats. Many of these alterations were in genes associated with the regulation of the glutamatergic synapse [122].

It is likely that maternal programming interacts with the endocannabinoid system for separate programming, as exogenous corticosterone administrated to pregnant mice has been shown to decrease overall body weight while increasing anxiety and reducing overall CB1-receptor expression in the cerebellum of the offspring [323]. THC is retained in the plasma of dams at a rate of about 10% of total dose concentration and can cross the placenta [324, 325]. The endocannabinoid system places a critical role in the conception, implantation, and development, and THC may disrupt these mechanisms epigenetically in the CNS (as reviewed by Taylor et al. [326] and Szutorisz and Hurd [316]). This may explain vulnerabilities observed later in life to other drugs of abuse such as alcohols and opioids [327,328]. The ongoing importance of the maternal environment in epigenetic regulation of the endocannabinoid system of the offspring is further highlighted by the immunosuppressive effects of THC. Through epigenetic marks like DNA methylation or miRNAs like miR-690 [329], inherent systems may undergo permanent changes prior to birth (as reviewed by Zumbrun et al. [330]).

THC exposure resulted in decreases in DNA methylation in promoter regions within the NAc, as well as hypermethylation within gene bodies at exogenic and intronic sequences. These changes may lead to a "network" of epigenetic marks in CpG islands that creates risk for drug abuse. Functional analyses of these marks revealed modifications at glutamate-related genes that mediate synaptic plasticity, as well as genes that code for ionic receptors and scaffolding proteins [122,331]. Observed behavioral and morphological changes caused by THC exposure should be related to their genic regions, though an

obvious emphasis should be made for region specificity in these studies. It will be important to describe the emergent changes in these epigenetic marks from early drug exposure, as this work may offer insight into potential windows for treatment and/or intervention.

### 11.2. Exposure to cannabinoids during childhood and adolescence

Given that adolescent populations are among the most frequent users of cannabis as a recreational drug [332], uncovering the lasting changes associated with repeated use at this age remains an important topic to researchers. The maturation of the endocannabinoid system occurs during the adolescent period [333]. Accordingly, childhood and adolescent use of endocannabinoids produces changes that may have enduring effects on the developing system. Studies that review outcome to long-term endocannabinoid use in young clinical populations report increased risk for later life depression and lack of motivation [313], which may contribute to drug vulnerability [334]. Rodent models match this modification in emotionality. Using an adolescent cohort of male and female Sprague-Dawley rats tested and sacrificed in adulthood, Rubino and colleagues [335] found sex-dependent neuron morphological changes and shifts in CB1 receptor number. These sexually differentiated responses were associated with alterations in behavior, where males and females demonstrated anhedonia in a sucrose preference test, but only females presented with comorbid with depression-like symptoms. Poor emotional regulation is often met with cannabis-induced impairments in working memory [336] and susceptibility to psychiatric disorders like schizophrenia [337].

Interestingly, several adolescent exposure models to cannabinoids (e.g., cannabinoid agonist (HU-210), $\Delta^9$-THC) alterations have been observed in adult NAc histone methylation (i.e., H3K4me3, H3K9me2), miRNAs, promoter, and gene body (for review, see [316]). The plasticity of dopaminergic neurons, responding to opioid exposure, may increase individual vulnerability through this overlap of reward system activation [338]. Further, the epigenetic modifications made by cannabinoid exposure in the adolescent brain overlap with those observed within the opioid system. Adolescent male rats exposed intraperitoneally to THC increase heroin self-administration significantly faster than controls and have larger neuronal opioid receptor populations overall as compared to controls [124]. Exposure in this period is likely mediated by epigenetic alteration to the proenkephalin (*Penk*) gene, which codes for opiate neuropeptide enkephalin in the NAc. Adolescent rats treated with THC and then made to overexpress *Penk* by viral recombination and stereotaxic infusion showed an increase in heroin-seeking as well as heroin self-administration [123]. This same study found that adolescent THC dosing was strongly associated with histone methylation, one of the more stable and dynamic epigenetic alterations. Increases in *Penk* gene expression were related to decreases in H3K9me2 marks upstream from the *Penk* gene in the NAc shell over a period of thirty days [123]. Distinct *Penk*-gene related profiles may even explain the differences in adolescent vulnerability to other classes of drugs as a consequence of THC use considering that *Penk* mRNA did not vary between adolescents and adults. This is supported by evidence that earlier—prenatal—exposure to THC may alter *Penk* profiles in adulthood [125].

## 12. Expanded lifetime view

Given that there are enduring changes to the epigenome that are caused by chronic drug taking, and that several lines of research implicate critical developmental periods of vulnerability, greater attention should be paid to molecular epigenetic changes that may occur during fetal development and adolescence to better understand later-life drug susceptibility. Again, changes in epigenetic patterns may also vary by age, and so additional work should examine multiple changes associated with early drug insult over long periods as well as at intervening time points. For example, a naturally occurring genome-wide decrease in DNA methylation has been associated with aging in vitro [339,340] and in vertebrate tissues [341]. Specifically, there is hypomethylation along normally hypermethylated, repetitive element sequences [342], and the reason for this is unknown. Immunoprecipitation assays at multiple points or at varying ages in clinical populations may add insight into these developmental variations. If the process is a consequence of or a cause of the global aging of somatic cells, then it is likely that lasting epigenetic changes from prenatal, early-life, and young adolescent exposure may also have the potential to interact with these epigenetically programmed events. Indeed, it is plausible that the decline in global DNA methylation might reverse some of the adolescent imprinting caused by drug exposure or stress thus, reducing the burden of addiction with age. Understanding this process would, therefore, be of benefit not only towards understanding brain aging but also with a potential for reversing the effects of drug risk patterns acquired in early life. The mechanisms and research covered discussed point to evidence of lasting changes “above the genome” related to drug use and abuse that may be transmitted across generations and certainly persist across the individual lifespan. Given the available technologies and the likelihood of their rapid improvement over the near term, these questions are answerable and may offer hope for innovative preventative therapies.

## 13. Future directions

This review highlights a number of novel areas of research that remain open with regard to the epigenetic component

of developmental vulnerabilities to substance abuse. Transgenerational studies in addiction models will be of value in examining unanswered questions as to how drug use affects the germline and future generations both behaviorally and physiologically. Even without direct exposure to an addictive substance in the offspring, epigenetic changes can appear in the following generation due to parental substance use—some of which are persistent through adolescence and may contribute to risk of use and abuse. Several of the empirical papers presented here focus on the filial 1 generation of offspring, but studies that continue for additional generations can contribute a great deal of knowledge for the field. Moreover, given the vast amount of information implicating biological sex differences across generations, and the clear interactions between the organizational and activational effects of sex steroids and the epigenome, future studies need to include both sexes in investigations of epigenetic changes related to drug exposure. Studies that focus on cross-sensitization between distinct classes of drugs would also provide important insights into addiction pathways in the brain and further substantiate the mechanisms by which initial exposure to one class of drug increases responses to a separate class of drug by epigenetic reprogramming.

On a clinical level, individual differences in sex, ethnicity, and socioeconomic level may need to be parsed out for further study as well. For example, while *OPRM1* gene expression and subsequently, receptor activation, are important factors in opiate use disorders, there are ethnicity-dependent genetic changes via DNA methylation that occur in the promoter region of the *OPRM1* gene [343]. DNA methylation state is also variable by ethnicity from birth, and the individual differences in ethnic groups within clinical populations can be statistically accounted for [344, 345, 346]. Liu et al. [345] specifically suggested that ethnic differences may be better captured by future improvements in technology in the same manner that genome wide analyses advanced knowledge of individual differences in single nucleotide polymorphisms. Defining the population stratification will impact genetic and epigenetic influences and these changes are mediated through complex and unique biological processes. Though ethnic differences cannot be directly translated to an animal model, the continuation of the work reviewed here will highlight epigenetic mechanisms that may be relevant for understanding ethnic variations.

Drug use and abuse clearly impact epigenetic changes in the brain and may have a more pronounced effect at select windows such as prenatal development and adolescence due to the increased epigenetic plasticity present during these periods. However, little is known about regionally specific epigenetic changes during development, and still less about how drug use or exposure might perturb those changes and canalize the brain towards an abuse prone phenotype. Challenges also exist in the development of drugs targeting epigenetic mechanisms, while such drugs have made it to the clinic already, for instance DNA methyltransferase inhibitors, many have significant side effects which make their use for the treatment of substance abuse difficult from both a compliance and cost-benefit perspective. Others, such as valproate, are better tolerated, though it remains unclear the extent to which this drug acts on epigenetic or other mechanisms with regard to substance use disorders. Nonetheless, epigenetics represents a significant frontier for substance abuse research and has already clarified many of the molecular mechanisms by which the environment can have lasting effects on the brain and behavior.

**Acknowledgment** The authors are grateful for the technical and graphical assistance of Keith D. Donaldson.

**Conflict of interest** The authors declare that they have no conflict of interest.

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