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Epigenetic Mechanisms of Opioid Addiction

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Abstract

Opioid use kills tens of thousands of Americans each year, devastates families and entire communities, and cripples the healthcare system. Exposure to opioids causes long-term changes to brain regions involved in reward processing and motivation, leading vulnerable individuals to engage in pathological drug-seeking and drug-taking that can remain a lifelong struggle. The persistence of these neuroadaptations is mediated in part by epigenetic remodeling of gene expression programs in discrete brain regions. Although the majority of work examining how epigenetic modifications contribute to addiction has focused on psychostimulants like cocaine, research into opioid-induced changes to the epigenetic landscape is beginning to emerge. This review summarizes our knowledge of opioid-induced epigenetic modifications and their consequential changes to gene expression. Current evidence points towards opioids promoting higher levels of permissive histone acetylation and lower levels of repressive histone methylation, as well as alterations to DNA methylation patterns and non-coding RNA expression, throughout the brain's reward circuitry. Additionally, studies manipulating epigenetic enzymes in specific brain regions are beginning to build causal links between these epigenetic modifications and changes in addiction-related behavior. Moving forward, studies must leverage advanced next-generation sequencing approaches and chromatin purification techniques combined with bioinformatics analyses to identify novel gene networks regulated by particular epigenetic modifications. Improved translational relevance will also require increased focus on volitional drug-intake models and standardization of exposure paradigms. Such work will significantly advance our understanding of how opioids cause persistent changes to brain function, and provide a platform on which to develop interventions for treating opioid addiction.

Keywords

Opioids; Addiction; Epigenetics; Transcription; Histone Acetylation; Histone Methylation

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INTRODUCTION

Opioids claim the lives of nearly 50,000 Americans each year, and leave tens of thousands more struggling with addiction (1). Nevertheless, initiation of opioid use has been increasing at an alarming rate over the last decade (2–4), and users often shift from ingestion of prescription painkillers to intravenous injection of heroin - a significantly more addictive form of drug intake (5). Opioids induce physical dependence, which promotes short-term desire to re-use, in addition to motivational disturbances underlying key aspects of a longer-lasting addiction syndrome. With repeated exposure, positive subjective effects of opioids become integrated with internal states and external cues associated with drug-taking, creating triggers that can instigate drug-seeking even long after terminating use (6–8).

Long-term vulnerability to addiction is mediated by persistent alterations to the function of reward-processing networks in the brain, namely the mesocorticolimbic dopamine system (9). This system is comprised of dopamine neurons projecting from the ventral tegmental area (VTA) that terminate in the nucleus accumbens (NAc) as well as cortical regions including the amygdala, prefrontal cortex (PFC), and hippocampus (HPC). Opioids appear to produce their rewarding and reinforcing effects by activating mu-opioid receptors (MORs) within the VTA, causing disinhibition of dopamine neurons firing and, consequently, elevated dopamine neurotransmission within the NAc (10–16). Opioids also directly activate MORs on neurons of the NAc and other forebrain structures, and elicit distinct neuronal responses throughout the brain via signaling at kappa-ORs and delta-ORs (17). Additionally, these three types of ORs are expressed on non-neuronal cells, the function of which can be altered by opioid exposure (18–22). These diverse actions of opioids can alter various intracellular signaling cascades that drive long-lasting adaptations to cellular function which may promote behavioral and psychological abnormalities underlying addiction.

One persistent adaptation, commensurate with the long-lasting and experience-dependent nature of addiction, involves stable epigenetic modifications to the DNA of affected neurons (23). These modifications modulate transcription, without directly altering the nucleotide sequence, via conformational changes to chromatin structure and accessibility. Consequences of these modifications include altered basal levels of gene transcription, priming/desensitization of particular genes for activation or repression in response to drug or other stimuli, or regulation of splice variant expression (24; 25). Resultant transcriptional changes influence effector genes involved in diverse cellular functions, ultimately causing enduring changes to signaling cascades, cellular structure, and synaptic activity. Epigenetic modifications are critical for the formation and recall of long-term memory (26; 27), and their influence on gene expression programs likely play a critical role in the context of addiction in linking the rewarding experience of drugs with external and internal cues that generate craving and relapse (28).

Although our understanding of how epigenetic modifications promote addiction is growing for psychostimulants such as cocaine (29; 30), opioids have been largely left behind. All drugs of abuse produce some similar changes in reward system function and behavior, but this effect is mediated by divergent mechanisms of action distributed across different brain regions and cell types (31). Thus, we cannot assume transcriptional or epigenetic changes

will generalize from one drug class to another. Advancing our knowledge of how opioids produce persistent epigenetic changes will be crucial to gain a better understanding of how opioid addiction develops, how it is maintained, and how to treat it. Here, we outline current knowledge of opioid-induced alterations to the epigenetic landscape of the brain's reward circuitry, and point to functional consequences. Primary focus is given to studies of human addicts and rodent studies employing long-term experimenter-administered or self-administered opioid exposure paradigms which, although inherently limited in their direct clinical relevance, effectively model several aspects of drug addiction. We conclude by outlining what next steps should be taken to advance our understanding in hopes of fighting the opioid epidemic.

I. EPIGENETIC MODIFICATIONS PRODUCED BY LONG-TERM OPIOID EXPOSURE

Histone modifications

Gene expression depends on the ability of transcriptional machinery to access DNA, which is tightly packed into chromatin. To condense genetic material, DNA strands are wound around protein octamer spools known as histones. Histones are formed from combinations of four proteins: H2A, H2B, H3, and H4 (32; 33). These proteins' N-terminal "tails" undergo extensive covalent modifications that either loosen or tighten the histone's grip on DNA. Such modifications are the most studied in the context of addiction, and this is particularly true for opioids (Table 1).

Histone Acetylation—Most studies of drug-induced epigenetic modifications have focused on acetylation of lysine residues on histone tails. Acetylation reduces electrostatic tension between histones and wound genes, creating an "open" chromatin state that facilitates gene transcription (Figure 1). In the context of opioids, this has primarily been studied on histone H3 tails. Preclinical work has found that repeated experimenter-administered or self-administered opioids increase global H3 acetylation within the mesolimbic dopamine system (34; 35), and this effect is consistent with findings in postmortem tissue from human heroin users (36). Strikingly, global H3 hyperacetylation in the striatum of heroin users appears to correlate with years of heroin use (36), suggesting that levels of heroin exposure may scale with stabilization of this chromatin modification.

Further dissection of the specific amino acid tail residues acetylated has demonstrated hyperacetylation at H3K9 (37; 38), H3K14 (39), H3K18 (40), and H3K27 (36) following repeated morphine or heroin exposure in either experimenter-administration or self-administration models (see Table 1). Of these marks, H3K27ac has received the most extensive characterization for its role in opioid addiction, predominantly from a single study combining clinical and preclinical approaches. Egervari and colleagues (36) demonstrate elevated H3K27ac levels in striatum of human heroin users as well as heroin self-administering rats, and observe a striking positive correlation between H3K27ac and years of use. Further, chromatin accessibility mapping with ATAC-seq confirmed that this mark induces an open chromatin state. Since H3K27ac is typically enriched at enhancer regions of

DNA (41; 42), these results suggest that higher levels of this mark may facilitate persistent amplification of gene expression systems underlying opioid addiction."

These authors also found H3K23ac to be upregulated in striatum of heroin users, but since this did not correlate with use patterns it was not further examined. One study examined histone H4 and, consistent with studies of H3, showed hyperacetylation at H4K5 and H4K8 within the NAc of rats displaying heroin-seeking behavior (40). Together, these studies provide converging evidence that opioids promote a more open, accessible chromatin state via histone acetylation, ultimately permitting higher levels of transcriptional activity critical for induction of plasticity-related gene expression.

Histone Methylation—Compared to acetylation, far less is known about how opioids alter histone methylation. To date, the few studies examining this epigenetic modification have only identified changes to the methylation state of a specific histone tail residue, H3K9, following opioid treatment (Table 1). Sun and colleagues (41) show that repeated morphine treatment reduces NAc H3K9 di-methylation (H3K9me2), but not mono- or tri-methylation. A similar reduction in H3K9me2 has been observed within the central nucleus of the amygdala following repeated opioid treatment (42). This reduction of H3K9me2 appears to promote transcriptional activity, and depends on the chronic nature of drug exposure (41). Using ChIP-sequencing to assess genome-wide deposition of H3K9me2 within the NAc, Sun and colleagues identified several genic loci showing differential enrichment of H3K9me2 following opioid exposure. Of particular interest was a reduction in enrichment of H3K9me2 throughout the *FosB* gene – a critical transcription factor that promotes drug addiction (see below; 24; 43). This suggests that chronic morphine may release repression of *FosB* by reduced H3K9me2 deposition at the gene. Further, Ingenuity Pathway Analysis identified changes in methylation at genes related to glutamatergic signaling, consistent with a role for H3K9me2 in regulating plasticity-associated transcriptional programs. Interestingly, opioid-induced transcriptional regulation via H3K9 methylation might not be specific to the NAc, as one study has also found reductions in H3K9 tri-methylation within the VTA and locus coeruleus after one week of withdrawal following an escalating dosing regimen (37).

DNA methylation

Methylation of DNA, primarily 5'-methylation of cytosines at cytosine-guanine dinucleotides (5mC), generally silences genes by physically blocking RNA polymerase II and thus gene transcription. Alternative forms of DNA methylation, such as 5-hydroxymethylation of cytosine (5hmC), which is enriched in brain, associate more frequently with transcriptional activation. Studies of DNA methylation in the context of opioid addiction have mostly been limited to (5mC) and to blood from clinical populations, post-mortem human brain tissue, and a small number of rodent opioid exposure paradigms (Table 1).

Genome-wide DNA methylation appears elevated by long-term heroin use based on higher levels of methylation at LINE-1 retrotransposon sites in blood leukocytes from heroin addicts compared to controls (44). Selective analysis of neurons within the frontal cortex of

heroin users has identified differential methylation patterns between intragenic regions (45). Higher levels of methylation also occur at CpG-rich islands within the MOR gene, *OPRM1,* in both blood and brain tissue from heroin addicts (44; 46; 47). Intriguingly, this effect is also observed in patients receiving long-term opioid painkiller treatment compared to un-medicated patients, indicative of a pharmacological influence of chronic opioid exposure (44).

Compared to work with clinical populations, preclinical studies have had difficulty recapitulating the effects of opioids on DNA methylation, particularly within the reward system. Chronic morphine or heroin treatment with stable or escalating doses did not alter whole-brain DNA methylation (48; 49). Further, neither morphine treatment nor heroin self-administration altered DNA methylation in the mesocorticolimbic dopamine system of rodents (50; 51), which contrasts with evidence that cocaine alters global DNA methylation in the PFC and NAc (51–53). However, one study identified several changes to global or promoter-specific 5mC and 5hmC levels across multiple brain regions following chronic morphine exposure in rats (54). Whether these changes have functional consequences at the level of gene expression or behavior remains to be determined. Unraveling the complexities of DNA methylation in opioid addiction will require more effective and consistent preclinical studies, as well as those focusing on locus- specific changes in DNA methylation. Once identified, genes affected by DNA methylation can be cross-referenced against loci affected by histone modifications to generate a comprehensive understanding of how these opioid-induced epigenetic changes interact to control gene expression.

Non-coding RNA

In addition to histone modifications and DNA methylation, gene expression is regulated by noncoding RNAs at the level of transcription and translation including microRNAs (miRs) and long non-coding RNAs. Although much more work has been done for psychostimulants, especially cocaine (55), some studies have identified changes in miRNA activity following opioid exposure as summarized in Table 1. Region-specific increases have been observed for miR-339–3p and the Let-7 family (56; 57), while decreases have been observed for miR-154, miR-675, and miR-218 following chronic opioid exposure (58; 59). Although no studies have directly examined changes in long non-coding RNAs following opioid exposure, preliminary evidence suggests that these are also regulated in heroin addicts (60). Thus, non-coding RNAs are emerging as important regulators of transcriptional activity in opioid addiction, but more studies are required to understand the functional consequences of these epigenetic changes.

II. ROLE OF EPIGENETIC AND TRANSCRIPTIONAL REGULATORS IN RESPONSE TO OPIOIDS

Most of the evidence for a relationship between opioid use and epigenetic alterations discussed above is correlational. However, studies directly manipulating enzymes responsible for epigenetic modifications are beginning to build causal relationships between specific forms of epigenetic regulation and opioid-induced behavioral abnormalities. Numerous enzymes contribute to establishing the epigenetic landscape at a given locus, and

can serve one of three functions: “writers” which place marks, “readers” which recognize and facilitate transcriptional modulation at marks, and “erasers” which remove marks (Figure 1).

The most common manipulation of epigenetic editors in the context of behavioral responses to opioids has been the use of non-specific pharmacological inhibitors of histone deacetylases (HDACs), which “erase” acetyl groups from histone tails (Table 2). Systemic treatment with different HDAC inhibitors can potentiate morphine-induced locomotor sensitization, and promote the formation of a morphine-conditioned place preference (CPP; 61; 62). Additionally, HDAC inhibitors can blunt reinstatement of a CPP but enhance drug-primed reinstatement of heroin-seeking behavior (40), indicative of a dynamic, stimulus-dependent response. Similar potentiation is observed for morphine-induced locomotor sensitization when the inhibitor is infused into the ventrolateral orbitofrontal cortex (38), and for heroin- or morphine-induced CPP when HDAC inhibitors are infused in the amygdala or NAc (34; 39). Only two studies have suggested opposite changes in behavior following HDAC treatment, but these authors used unconventional approaches to examine behavioral responses to opioids, such as sensitization to a single morphine injection (63), or intracerebroventricular delivery of conditioning doses of morphine (64). Further, intra-striatal infusion of a bromodomain inhibitor, which blocks the ability of “readers” to identify acetylated lysine residues, blunts heroin self-administration and heroin-seeking behavior (36). Taken together, these studies suggest that opioid-induced histone acetylation puts the reward system in a “poised” state, potentiating behavioral responses to opioids. These actions are similar to those observed for cocaine (23).

To our knowledge, only two epigenetic editors have been selectively manipulated in the context of behavioral responses to opioids - exceptionally few when compared to the extensive literature for cocaine and other psychostimulants (30; 65). Ferguson and colleagues (66) examined the sirtuin family of HDACs, and found that repeated morphine treatment selectively upregulates SIRT1 in the NAc, without altering other sirtuins. Overexpression of SIRT1 within the NAc potentiated morphine-induced CPP, while knockdown of SIRT1 has the opposite effect. These results contrast with global HDAC inhibition studies described above and suggest that sirtuins may have a different role from the canonical HDACs. These actions of morphine also contrast with those of cocaine, which induces both SIRT1 and SIRT2 in this brain region (66). The histone methyltransferase G9a, known to be critical for aspects of cocaine reward (67), has also been manipulated in models of opioid addiction. Sun and colleagues showed that G9a is likely responsible for decreased H3K9me2 levels in the NAc following chronic opioid exposure, consistent with other reports in the CeA (42). Indeed, overexpression of G9a within the NAc increased H3K9me2 and blunted both morphine-induced CPP and locomotor sensitization, while knockdown or pharmacological inhibition within the amygdala has the opposite effect (41; 42). These studies parallel results with cocaine and suggest that G9a typically exerts a repressive influence on behavioral responses to drugs of abuse, and repeated exposure to opioids (or cocaine) relinquishes this control. Additionally, some emerging work points to an important role for chromatin remodelers such as BRG1 (SMARCA4) in mediating reinforcing effects of opioids (68). Interestingly, these latter effects may be mediated by altered function of

non-neuronal cells, particularly oligodendrocytes, which exhibit significant gene regulation in response to opioids (69) and appear compromised in human heroin users (70–72).

Transcription Factors

Both epigenetic changes and regulation of editing enzymes rely on drug-induced activation of intracellular signaling pathways, which couple synaptic activity with transcriptional regulation through downstream activation of transcription factors – proteins that bind directly to DNA in a sequence-specific manner. Thus, the development and expression of epigenetic changes depend on iterative interactions between intracellular signaling cascades and the marks themselves (73).

One critical regulator of transcription is cyclic AMP response element-binding protein (CREB). CREB is activated downstream of multiple signaling pathways and integrates the transcriptional response to a broad range of cellular stimuli (74). Once activated, CREB promotes transcriptional activation by binding to cyclic AMP response elements (CREs) throughout the genome, many of which activate gene expression programs relevant to addiction (75–78). In the context of opioids, CREB has primarily been studied for its role in the aversive state produced by withdrawal (79), but some work suggests a dynamic regulation of CREB at different stages of drug-taking in a region-specific manner. For example, chronic morphine treatment enhances CREB signaling and activity in the NAc (80; 81). Overexpression of CREB specifically within the NAc reduces morphine reward (81), which is similar to effects observed with cocaine (82). Thus, persistent CREB signaling within the NAc may be a mechanism of tolerance to the rewarding effects of drugs of abuse. However, whole-brain knockdown or systemic pharmacological inhibition of CREB reduces morphine CPP and heroin-seeking, respectively (83; 84). These effects are opposite to findings with cocaine, and suggest that brain regions other than NAc have a significant impact on CREB-mediated transcriptional responses to opioids.

Another important transcription factor mediating responses to drugs of abuse is activator protein- 1 (AP-1). AP-1 is comprised of heterodimers of Fos family (c-Fos, FosB, Fral, and Fra2) and Jun family (c-Jun, JunB, JunD) members, which are expressed rapidly and transiently following acute drug exposure. AP-1 mediated gene expression promotes plasticity within reward regions via transcriptional activation or repression of genes involved in the behavioral and pharmacological response to cocaine (75). While typically short-lived, AP-1 activity can be extended by drug-induced expression of ΔFosB – a truncated splice variant of FosB. ΔFosB is exceptionally stable, enabling it to persist long after induction and accumulate with repeated drug exposure (85). While most work to date has focused on cocaine-induced potentiation of ΔFosB expression, particularly within the NAc and dorsal striatum (86), parallel changes are observed with opioids. Treatment with a sensitizing regimen of morphine increases ΔFosB in the NAc and ventral pallidum, and persists for over 3d into withdrawal (87). Global knockout of ΔFosB blunts morphine reward (88), while this is potentiated by upregulating ΔFosB within the NAc or striatum (89). Thus, ΔFosB may be an important mediator of prolonged transcriptional activity that sets the stage for long-term epigenetic modifications induced by opioids that promote addiction. Induction of ΔFosB by drugs of abuse shows an interesting pattern of cell-type specificity: all drugs of abuse induce

ΔFosB solely within the D1 subtype of NAc medium spiny neuron, whereas opioids alone induce it in both D1 and D2 subtype neurons (90). This holds for both investigator-administered as well as self-administered drug, and points to some unique effects of opioids on gene expression in this brain region.

III. TRANSCRIPTIONAL CONSEQUENCES OF LONG-TERM OPIOID EXPOSURE

Exposure to drugs of abuse or to drug-associated stimuli elicits multiple waves of transcription. The rapid and transient induction of multiple immediate early genes (IEGs) sets the stage for persistent changes to the expression of effectors genes critical for long-term plasticity. These waves of gene expression regulate, and are regulated by, epigenetic modifications.

Immediate early genes

IEG induction couples rapid synaptic activation and intracellular signaling with long-term changes in neurons. Many IEGs encode transcription factors and contribute to epigenetic depositions onto chromatin (91; 92). Reciprocally, epigenetic modifications alter the pattern of IEG expression upon drug exposure. Like other drugs of abuse, opioids cause rapid induction of many IEGs after acute treatment (93). However, regulation patterns of IEG induction following chronic opioid exposure are still unclear. Some studies suggest increases in Arc expression in the PFC and striatum following repeated experimenter-administered morphine (94; 95). Self-administration studies have identified increases in expression of Fos, Arc, Egr1, and Egr2 in the PFC that persist for at least 24 hr (96), while Egr1 and Egr2 are reported to be decreased within the NAc, possibly mediated by changes in methylation state of these genes (50). Drug-seeking behavior is also associated with changes in IEG expression. For example, Egr1 is differentially regulated between the NAc and PFC following cue-induced heroin-seeking (97). This same paradigm also elicits distinct IEG profiles specifically within PFC neurons that are tagged as “activated” by cue and context re-exposure compared to surrounding non-activated neurons, with increases in FosB, Arc, Egr1, and Egr2 (98). The pattern of IEG expression produced specifically in neurons responsive to drug-associated stimuli suggests that there may be a specific molecular signature defining a population of neurons that drive craving and contribute to relapse.

Effector genes

Early studies into opioid-induced gene expression have typically taken a candidate gene approach, noting significant alterations to genes encoding opioid receptors, transcriptional regulators, and proteins that control structural and functional aspects of the cell, as reviewed extensively elsewhere (17; 79). However, candidate gene studies have limited potential to harvest a comprehensive understanding of the transcriptional landscape associated with opioid addiction. To assess this, studies employ whole-genome sampling techniques such as microarray profiling and next-generation RNA sequencing (RNA-seq). Although these studies are emerging for opioids, the results are difficult to synthesize. Comparisons between studies are challenging due to variability of exposure paradigms (e.g., drug treatment schedules, doses, self-administration designs), and the surprising lack of overlap in

differentially regulated genes (see Table 3). Although this work suggests that several distinct pathways contribute to the development of opioid addiction, the lack of consistency must be addressed through standardization of experimental design and greater use of comprehensive RNA-seq approaches combined with advanced bioinformatics pipelines. These strategies are being extensively employed to identify transcriptomic and epigenomic changes to the reward system in cocaine addiction (53; 67; 99–104). Considering evidence from humans that cocaine and heroin elicit drastically distinct transcriptional profiles (105; 106), contrasting cocaine and opioid datasets will be critical to pinpoint unique or common patterns that promote addiction to these two classes of abused drugs.

Glutamate signaling and synaptic remodeling—Despite inconsistencies within the transcriptional literature, glutamate signaling and associated synaptic remodeling pathways have emerged as critical targets for opioid-induced epigenetic and transcriptional changes. Abnormalities in glutamate signaling support behavioral disturbances underlying addiction (107–109), and such changes may be present in heroin users (110; 111). Correspondingly, several studies have identified opioid-induced epigenetic modifications to glutamatergic transcriptional networks in human heroin addicts and preclinical models. These include enhanced chromatin accessibility surrounding glutamatergic genes (36), DNA methylation at key genes involved in glutamate plasticity (45), and changes to glutamatergic gene expression, particularly the GluA1 receptor (36; 41; 112). Thus, epigenetic modifications to glutamatergic signaling may be a critical mechanism that supports opioid addiction.

Conclusions and Future Directions

Our understanding of how opioids induce persistent neuroplastic changes within the brain's reward circuitry is growing. Several epigenetic changes have been identified and linked to changes in gene expression programs that interact with the physiology of neurons, including higher levels of permissive histone acetylation and lower levels of repressive histone methylation. Manipulations of epigenetic editors suggests that these modifications potentiate behavioral responses to opioids. Complex changes in DNA methylation state have been identified in humans, and additional preclinical work is required to determine consequences of these changes. Emerging evidence for a role of non-coding RNAs in opioid addiction will be important to identify post-transcriptional regulation of gene expression induced by opioids. An array of transcriptional changes are induced by opioid exposure, but systematic patterns are currently unclear. Overall, current evidence suggests opioid-induced epigenetic modifications switch the reward system into a hyperresponsive state promoting future drug-seeking and drugtaking. However, it is difficult at this stage to build a conclusive narrative as to how opioids reprogram the epigenetic and transcriptional landscape of the brain.

To elaborate the epigenetic mechanisms underlying opioid addiction, several experimental aims and technological advancements are necessary. Much more consistency in opioid exposure paradigms is required, and studies should employ human-relevant dosing regimens, or shift to volitional models of opioid exposure, to promote translational relevance. To determine the stability of opioid-induced epigenetic modifications, changes to these marks should be characterized following both short- and long-term withdrawal periods. Most studies have also focused on exposure to a single opioid compound. Considering differences

in their potency, signaling properties, and use patterns, systematic comparisons of multiple opioids and their epigenetic consequences should be performed. Additionally, front-line treatment of opioid addiction with MOR agonists or partial agonists, such as methadone or buprenorphine, are known to produce epigenetic modifications (113), and future work should explore how such changes interact with those induced by abused opioids. Preclinical studies have also examined male rodents almost exclusively, and greater efforts are needed to understand how sex differences influencing opioid-induced epigenetic modifications (114).

Furthermore, the opioid field must embrace cutting-edge epigenomic and transcriptomic techniques to generate the richest datasets possible that tell conclusive stories as to how opioids fundamentally alter the brain's reward system. Opioid models should leverage next-generation sequencing technologies more extensively. Examples include RNA-seq to identify novel gene targets regulated by opioids, ATAC-seq to search the genome for regions of opened or closed chromatin following long-term opioid exposure, and ChIP-seq to link epigenetic changes with affected gene loci. Further, functional consequences of epigenetic modifications should be identified by manipulating these targets in a cell-type specific manner using recently developed locus-specific epigenetic editing tools (115). Work in the cocaine field is beginning to examine transcriptional regulation in a cell-type-specific manner (116), and parallel studies should be performed with opioids for both neuronal and non-neuronal cells. These novel approaches should be combined with bioinformatics analyses to identify differentially regulated gene networks and novel targets for opioid addiction. Concerted effort on these fronts will form a strong foundation on which to generate more effective treatment strategies and preventative measures in the fight against opioid addiction.

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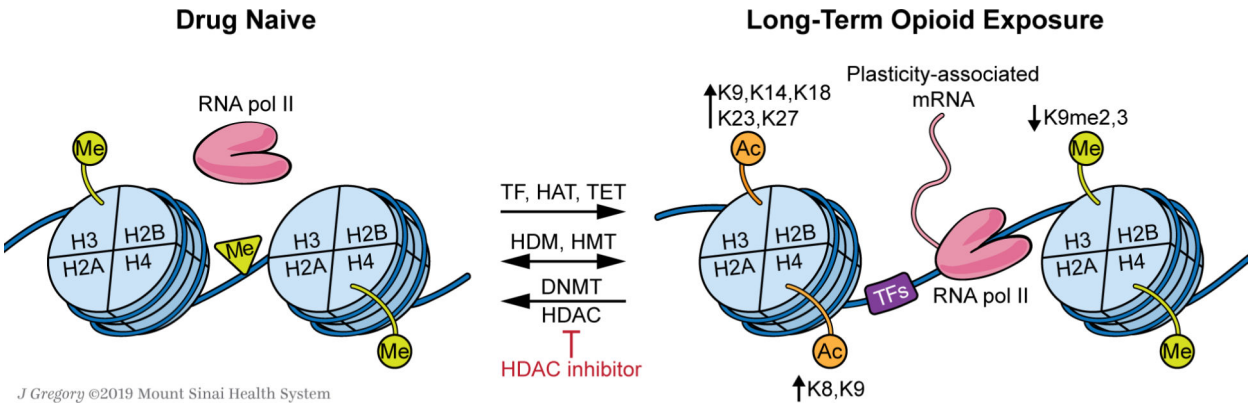

Figure 1. Repeated opioid exposure alters the epigenetic landscape of brain cells, causing persistent changes to transcriptional activity and cellular physiology.

Opioids induce a host of changes to the function of epigenetic editors and transcription factors (TFs) which remodel chromatin structure and DNA accessibility within various regions of the brain's reward circuitry. Acetylation of histones H3 and H4 at several N-terminal tail lysine (K) residues is enhanced by opioid exposure (listed), promoting a more open, accessible chromatin state. Additionally, the repressive influence of histone methylation appears to be relieved by opioid exposure (listed). Opioids also induce complex changes to DNA methylation in a gene locus-specific manner. These epigenetic modifications are mediated by diverse interactions between TFs and epigenomic "editors" including histone acetyltransferases (HATs), histone methyltransferases (HMTs), DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone demethylases (HDMs), and DNA oxidases or demethylases (e.g., ten eleven translocation proteins, TETs). Several classes of non-coding RNAs are also involved (not shown in the figure). Studies manipulating the function of these enzymes, particularly HDACs or G9a (an H3K9me2 HMT), suggest that opioid-induced histone acetylation or reduced repressive histone methylation drives heightened behavioral responses to opioids, indicative of a "poised" state within the reward circuitry. Such changes are likely mediated by greater levels of transcriptional activity at genes critical for neuroplasticity and synaptic physiology which promote behavioral abnormalities underlying addiction.

Table 1.
Epigenetic marks induced by opioid exposure.

Current clinical and preclinical evidence for changes to histone acetylation, histone methylation, DNA methylation, and expression of non-coding RNAs following repeated opioid exposure are summarized. Abbreviations: BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CPP, conditioned reinforcement; dStri, dorsal striatum; FC, frontal cortex; HPC, hippocampus; IVSA, intravenous self-administration; LC, locus coeruleus; NAc, nucleus accumbens; PFC, prefrontal cortex; VLO, ventolateral orbitofrontal cortex; VTA, ventral tegmental area.

Epigenetic Mark	Change	Brain Region	Model Examined	Gene targets identified?	Reference
Pan-H3ac	↑	Striatum	Human heroin addicts Rat IVSA		(36)
Pan-H3phosphoac	↑	NAc (not PFC)	Mouse, repeated heroin in CPP		(34)
H3K9ac	↑	LC, VLO	Rat, repeated morphine	*Bdnf*	(37; 38)
H3K14ac	↑	BLA	Mouse, repeated morphine in CPP, normalized during extinction	*Bdnf, FosB, Creb*	(39)
H3K18ac	↑	NAc	Rat, drug-primed reinstatement of heroin-seeking		(40)
H3K23ac	↑	NAc/dStri	Human heroin addicts (not correlated with use history)		(36)
H3K27ac	↑	NAc/dStri	Human heroin addicts Rat IVSA	Glutamate signaling, *GRIA1*	(36)
H4K5ac	↑	NAc	Rat, drug-primed reinstatement of heroin-seeking		(40)
H4K8ac	↑	NAc	Rat, drug-primed reinstatement of heroin-seeking		(40)
H3K9me1	No Δ	NAc	Mouse, repeated morphine		(41)
H3K9me2	↓	dStri	Mouse, repeated morphine	*FosB*, *Bdnf*, glutamate signaling genes	(41)
	↓	CeA	Mouse, repeated morphine in CPP	*Bdnf*, glutamate signaling genes	(42)
H3K9me3	No Δ	NAc	Mouse, repeated morphine		(41)
	↓	VTA, LC	Mouse, 7d withdrawal from escalating morphine	*Bdnf*	(37)
H3K27me3	No Δ	NAc	Mouse, repeated morphine		(41)
DNA Methylation	No Δ	Whole-brain	Mouse, repeated heroin or morphine		(48; 49)
	No Δ	VTA, NAc, PFC	Rat, heroin IVSA Mouse, repeated morphine in CPP		(50; 51)
	↓ or No Δ	*In vitro*	Cell Lines		(117; 118)

Epigenetic Mark	Change	Brain Region	Model Examined	Gene targets identified?	Reference
	↑ exons ↑ gene body ↓ promoters	OFC neurons (nuclei isolated by FACS)	Human heroin addicts	Differential effects in gene ontology analysis	(45)
	↑ LINE-1, *OPRM1* CpG islands	Thalamus, somato-sensory cortex, blood	Human heroin addicts		(44; 46; 47)
	Region-specific changes	10 brain regions	Rat, acute and chronic morphine	*Bdnf, Nr3c1, Il6, Il1b*	(55)
Let-7	↑	Whole-brain	Mouse, morphine pellets		(56)
miR-339–3p	↑	HPC	Mouse, repeated morphine or fentanyl	*Oprm1* mRNA	(57)
miR-133b	No Δ	VTA, NAc	Mouse, morphine pellets Mouse, morphine IVSA		(58; 119)
miR-218	↓	NAc (not PFC, HPC)	Mouse, repeated (not acute) heroin	*Gabrb3, Mecp2, Nrxn1, Gng3, Ube3a*	(59)
H19, miR-675	↓	vStri	Mouse, morphine IVSA		(58)
Mirg, miR-154	↓	vStri	Mouse, morphine IVSA	*Fxyd4, Grm3, Odf21, Slc4a4; Oprm1*	(58)

Table 2.

Opioid-induced behavioral outcomes following manipulation of epigenetic editors.

Summary of studies examining behavioral responses to opioids following pharmacological or genetic manipulations of epigenetic enzyme function. Abbreviations: BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CPP, conditioned reinforcement; dStri, dorsal striatum; HDAC, histone deacetylase; HMT, histone methyltransferase; HPC, hippocampus; ICV, intracereboventricular; IVSA, intravenous self-administration; NaBut, sodium butyrate; NAc, nucleus accumbens; PFC, prefrontal cortex; TSA, trichostatin A; VLO, ventolateral orbitofrontal cortex; VPA, valproic acid; VTA, ventral tegmental area.

Chromatin State	Manipulation	Location of Treatment	Result	Reference
Open	Pan-HDAC inhibitor (NaBut)	Systemic	↑ Morphine-induced locomotor sensitization, CPP	(61)
	Pan-HDAC inhibitor (NaBut)	Systemic	↑ Morphine-induced CPP ↓ Reinstatement of CPP	(62)
	Pan-HDAC Inhibitor (VPA + NaBut)	Systemic	↓ Morphine-induced locomotor sensitization (acute, single-dose)	(63)
	Pan-HDAC inhibitor (NaBut)	Both systemic and ICV	No Δ, Heroin IVSA ↑ Drug-primed reinstatement of heroin-seeking	(40)
	Pan-HDAC inhibitor (VPA)	ICV	↓ Morphine-induced CPP (morphine delivered ICV)	(64)
	Pan-HDAC inhibitor (TSA)	NAc (but not PFC)	↑ Heroin-induced CPP	(34)
	Pan-HDAC inhibitor (TSA)	BLA	↑ Morphine-induced CPP, ↓ CPP extinction	(39)
	Pan-HDAC inhibitor (TSA)	VLO	↑ morphine-induced locomotor sensitization	(38)
	G9a (HMT) knockdown	NAc	↑ Morphine-induced CPP, locomotor sensitization	(41)
	G9a inhibitor	CeA	↑ Morphine-induced CPP	(42)
	SIRT1 (HDAC) KO	NAc (but not dstri)	↓ Morphine-induced CPP	(66)
Closed	G9a overexpression	NAc	↓ Morphine-induced CPP, locomotor sensitization	(41)
	SIRT1 overexpression	NAc (but not dstri)	↑ morphine-induced CPP	(66)

Table 3.

Summary of studies examining transcriptome-wide changes in gene expression following long-term opioid exposure.

Microarrays and RNA-seq approaches are beginning to be employed to identify opioid-induced changes to gene regulation within the brain's reward circuitry. However, few studies have performed network analyses using advanced bioinformatics approaches, and commonalities between experimental design are lacking. Abbreviations: CPu, caudate/putamen; dStri, dorsal striatum; FC, frontal cortex; GO, gene ontology; HPC, hippocampus; IVSA, intravenous self-administration; NAc, nucleus accumbens; OFC, orbitofrontal cortex; PFC, prefrontal cortex; qPCR, quantitative polymerase chain reaction; vMB, ventral midbrain; vStri, ventral striatum; VTA, ventral tegmental area.

Papers with gene lists	Paradigm	Regions	Genes/pathways differentially regulated
(105)	Human heroin addicts, microarray	NAc	1050 transcripts altered, 10x more than cocaine observed in previous companion paper, with only 25 overlapping differentially regulated transcripts, and only 10 oppositely regulated No change in myelin-related genes (unlike cocaine; potentially region specific) ↓ Presynaptic machinery genes (neurotransmitter release: vesicle storage, release, recycling; not observed for cocaine), synaptic function genes ↑ TRKB (opposite to cocaine), ↑ FAS, ↓ prodynorphin (opposite to cocaine)
(45)	Human heroin addicts, microarray	Isolated nuclei from OFC neurons	GO terms associated with hypermethylation: axons, synaptic compartments, synaptic membrane, transmission of nerve impulse, axonogenesis, cell-cell-signaling Networks associated with hypomethylation: gene expression and regulation, regulation of neuron differentiation Differentially methylated genes: *SLC17A7, OPRL1, TET3, ARC*
(120)	Oxycodone IVSA, RNA-seq	vStri, dStri	Inflammation/immune pathways: vStri: 126 ↑, 15 ↓; dStri: 54 ↑, 1 ↓ Linked to glial responses to morphine
(121)	Oxycodone IVSA, RNA-seq	vStri, dStri	Opioid signaling, stress pathways, neurotransmission, serotonin signaling, kinases and TFs vStri qPCR confirmation: *Pomc* ↑, *Htr1b* ↑, *Fkbp3* ↑, *Htr7* ↓, *Grin3a* ↓, dStri qPCR confirmation: *Gabr2b* ↓, *Gabra1* ↓
(122)	Oxycodone IVSA, RNA-seq	NAc, CPu	NAc: 6 ↑, 8 ↓, CPu: 3 ↑, 2 ↓. Focus on structural markers: integrins, axon guidance factors.
(58)	Morphine IVSA, long-access, yoked design	vStri, vMB	21000 differentially regulated genes, large GO lists across exposure paradigms Gene regulation due to morphine exposure: cell differentiation, cell-cell signalling, immune response, oxidative stress signaling Gene regulation specific to morphine-reinforced behavior: neuroplasticity, axonal guidance, miRNA pathways
(123)	Repeated morphine, microarray	vStri, PFC	Large list of altered genes, ingenuity pathway analysis to identify GO networks affected; Identified chromatin remodeling genes pFc, plasticity-related genes in NAc Ingenuity pathway analyses: neuroadaptive processes (long-term potentiation, axonal guidance, ephrin, and neuregulin pathways)
(124)	Repeated heroin, microarray	NAc	Comparison with methamphetamine treatment, 21 genes differentially regulated by heroin Focus on circadian genes (*Gm129*, *Dbp*, *Per1*, *Per2*)
(125)	Heroin IVSA rat, yoked design subtractive hybridization	NAc core and shell subregions	Active vs. passive drug intake causes major transcriptional differences in the NAc shell, with minimal differences in NAc core NAc shell pathways affected: transcription, translation, and cell metabolism 25 ↓, including TFs (H*nrp*, *Tbp-1*), signaling (*Chn1*, *Limk1*, *14–3–3*),

Papers with gene lists	Paradigm	Regions	Genes/pathways differentially regulated
(61)	Repeated morphine, with challenge, microarray	dStri	Morphine: *Arc, Nfkbia, Ttr, Kcnj13* Potentiated by co-administration of HDAC inhibitor: rhythm genes (*Per1*, Rev-erba, *Cry1*), addiction genes (*Fos*, *Nr4a1*, *Zbtb16*, *FosB*)
(126)	Acute morphine, heroin microarray	dStri	Extensive gene lists, hierarchical clustering Identify different patterns of induction based on gene identities and timing of tissue extraction following injection
(95)	Escalating morphine treatment, microarray	FC	Heat shock pathways (Hsp70, Hsp27, Hsp40, Hsp105, Cryab, BiP), circadian rhythm, synaptic activity pathways, Arc, nucleoporin p2
(127)	Heroin IVSA, yoked design, microarray	PFC	Intracellular signaling genes; physiology related genes; GO pathways included developmental processes
(128)	Oxycodone IVSA, microarray	HPC	Microarray for synaptic plasticity (84 genes examined) Compared adolescent vs. adult, many age-dependent changes ↑ Cadherin2 (*Cdh2*), ↑ *CREM*,
(129)	Repeated morphine, subtractive hybridization	HPC	6 ↑: vesicular transport, heat shock, steroid synthesis, oxidoreductase activity 5 ↓: cellular processes (cytoskeletal organization, vesicular transport, cell adhesion, iron transport, growth receptor binding, transaminase activity)
(130)	Repeated morphine, cocaine, RNA-seq	VTA	152 ↑, 35 ↓ for morphine 5 ↑ 28 ↓ for cocaine Focused on *Sgk1*