# Opiate-Induced Epigenetic Changes and Associated Effects on Drug Addiction Behaviors

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## Abstract

Opioid abuse and addiction, in its many forms, poses a threat to the health and the livelihoods of many, with nearly 645,000 deaths occurring as a result of opioid overdose to date. This review focuses on the functions of four neurotransmitters–glutamate, gamma-aminobutyric acid (GABA), dopamine (DA), and opioid receptors–and their role in addiction-related behaviors. In particular, the complex function of epigenetic modifications–specifically DNA methylation and histone acetylation–in mediating the neural changes that underlie opioid addiction are of concern. These epigenetic modifications are linked to the development of common symptoms relating to drug abuse, including craving, loss of control, and withdrawal. Understanding these modifications offers a nuanced view into how opiates disrupt the neurobiological landscape.

*Keywords:* addiction, drug-seeking, drug withdrawal, epigenetics, neurobiology, opiate, opioid

## Introduction

Substance abuse is defined by the DSM-V by the following criteria: use of excessive amounts of the substance or a longer use than intended; failed attempts at controlling its use; intense craving; tolerance; withdrawal; prolonged time obtaining, using, and recovering from the substance; neglecting responsibilities resulting from substance use; continuous use despite the social, physical, and mental issues it causes, and using the substance in risky/dangerous settings (Gateway Foundation, n.d.). Among the substances that are commonly abused by people, including alcohol, cannabis, and psychostimulants, opiates have consistently been observed to be the most diverse and dangerous. While its intent is to be used in a medical setting as a method of pain relief, the sensation of euphoria it creates leads people to recreationally abuse opioids in its many forms, such as morphine, fentanyl, heroin, and many more. Opiates are also one of the most lethal drugs, with nearly 645,000 lives being lost by opioid overdose from 1999 to 2021 (Centers for Disease Control and Prevention, 2024), leading this public health crisis to be termed the “Opioid Epidemic.” The World Health Organization (2023) attributes close to 80% of all drug-related deaths to opioid-related cases. In recent years, the Opioid Epidemic has recently intensified due to the rise in popularity of synthetic opioids, such as fentanyl (State Health Access Data Assistance Center, 2024).

The field of epigenetics and opioid addiction are closely intertwined, with chronic opioid use modifying the four major neurotransmitters involved in opioid addiction: glutamate, gamma-aminobutyric acid (GABA), dopamine (DA), and opioid receptors. “Epigenetics” describes the molecular modifications to the structure of a cell’s DNA (Hamilton & Nestler, 2019). Due to the low number of genes that are expressed in any given cell, epigenetic mechanisms are necessary to provide greater control over genes and thus maintain the great variety of cell functions and types. Consequently, epigenetics becomes an additional source of genetic control, and importantly in this paper’s context, control of gene expression. There are a number of epigenetic changes that regulate different parts of the genome, with the focus of this review being DNA methylation and histone modifications (Robinson & Nestler, 2011).

DNA methylation is an epigenetic mechanism whereby a methyl group (-CH3) is added to a cytosine in the DNA structure to make 5-methyl cytosine (5mC). Rompala et al. (2023) describes methylated cytosines in detail. First, DNA methyl transferases (DNMTs) attach the methyl group to the cytosine, then ten-eleven translocation (TET) enzymes later oxidize 5mC to 5-hydroxymethyl-cytosine (5hmC) and further compounds until the groups are eventually removed from the cytosine entirely. Researchers have discovered 5mC and 5hmC to be relatively stable epigenetic marks (Cui et al., 2020). They are highly enriched in neurons, associated with transcriptional activation, and play a key role in brain development (Wang et al., 2012). Human postmortem brain studies show 5mC and 5hmC's association with a multitude of psychiatric traits, including depression, alcohol use disorders (Gross et al., 2017), and opiate use disorders (Clark et al., 2022).

Another significant epigenetic mechanism is histone modifications, which control the "on/off" nature of DNA using histone proteins. In the "on" state, the histone-DNA interactions are loosened, allowing transcriptional machinery access to the DNA and subsequently activating the gene. In the "off" state, the histone-DNA interactions are tightened, blocking access to the DNA and subsequently deactivating the gene. Therefore, histone modifications target these interactions to either tighten or loosen them in order to suppress or activate the gene activity, respectively (Abcam, 2023).

These modifications lead to presenting symptoms commonly associated with addiction. For example, glutamatergic epigenetic alterations enhance synaptic plasticity, contributing to the development of tolerance and dependence (Van den Oever et al., 2010). Meanwhile, opioid effects on GABA disrupt its inhibitory signaling, leading to increased neuronal excitability (Tang et al., 2023). Dopaminergic pathways, vital to reward and reinforcement, also undergo significant epigenetic changes resulting in heightened sensitivity or expression, which reinforces seeking behaviors (Bossert et al., 2016). Finally, opiate abuse leads to a desensitization of opioid receptors, necessitating higher drug doses and resulting in withdrawal behaviors (Mysels & Sullivan, 2009).

Moreover, it is important to understand the three stages of drug addiction: the binge/intoxication stage, the withdrawal/negative affect stage, and the preoccupation/anticipation stage, also referred to as the "craving" stage (Koob & Volkow, 2010). The binge/intoxication stage is marked by impulsivity and loss of control over drug intake. During the withdrawal/negative affect stage, individuals experience symptoms such as anxiety, dysphoria, and irritability, driving the individual to seek the substance again and reinforcing the addiction cycle. Finally, the preoccupation/anticipation stage involves individuals experiencing intense cravings for substance use, leading to compulsive drug-seeking behavior. Understanding these aligns the various symptoms of opiate abuse to a certain time frame. Given these descriptions of behaviors, it is apparent how each neurotransmitter, and the study of epigenetics in general, is applicable to these stages of drug addiction.

## The Role of Glutamate in Opioid Addiction

To begin, glutamate is an integral factor regarding the expression of drug-seeking behavior. According to Koob and Volkow (2016), while stress-induced reinstatement lacks dependence on glutamate systems, both drug- and cue-induced reinstatement involves

glutamatergic pathways from the prelimbic prefrontal cortex (and basolateral amygdala and ventral subiculum for cue-induced reinstatement) to the nucleus accumbens (NAc), which are modulated by dopamine activity in the prefrontal cortex (PFC) via D1 and D2 receptors in drug-induced reinstatement (McFarland & Kalivas, 2001), and in the basolateral amygdala and dorsal striatum in cue-induced reinstatement (Everitt & Wolf, 2002; Vanderschuren et al., 2005; Vorel et al., 2001). In addition, protracted withdrawal has been linked to overactive glutamatergic systems (De Witte et al., 2005; Valdez et al., 2002). During the preoccupation/anticipation stage of addiction, prefrontal glutamatergic activity increases have been linked to strong glutamatergic responses that mediate craving-like responses. These findings show the key role of glutamate in drug-reinstatement and drug-craving behaviors.

**Epigenetic Changes to Glutamatergic Transmission**

Glutamate signaling and its associated synaptic remodeling pathways have been discovered to be critical targets for opioid-induced epigenetic and transcriptional changes (Browne et al., 2020). Heroin users were found to have these glutamate signaling abnormalities, which caused the behavioral disturbances fundamental to addiction, (Jacobs et al., 2013; Ökvist et al., 2011). Additionally, several studies have identified opioid-induced modifications to glutamatergic transcriptional networks, including enhanced chromatin accessibility surrounding glutamatergic genes (Egervari et al., 2017), DNA methylation at key genes involved in glutamate plasticity (Kozlenkov et al., 2017), and glutamatergic gene expression changes, particularly the GluA1 receptor (Egervari et al., 2017; Hou et al., 2015; Sun et al., 2012). In addition to these findings, Rompala et al. (2023) discusses the many studies examining the relationship between DNA methylation and opioid-related traits in postmortem human brain tissue. In one such study, an individual who died from heroin overdose reported differential 5mC across several gene classes, including those involved in glutamate neurotransmission, axonogenesis, synaptic processes, and the regulation of gene expression (Kozlenkov et al., 2017). In all, this data shows that DNA methylation of glutamatergic genes follow exposure to opioids, which correlates to a decrease in the expression of glutamate.

However, there are also significant changes to histones associated with addiction. Egervari et al. (2017) observed hyperacetylation of the histone H3 marker in chronic heroin users, suggesting increased transcriptional activity due to the more open state of chromatin. Studies using Pan-AcH3 and H3K27ac chromatin immunoprecipitation (ChIP) experiments and real-time PCR found histone H3 hyperacetylation at glutamate related genes, specifically around the transcription start site (TSS) of the GRIA1 gene. In addition, higher levels of acetylation of the GRIA1 gene 2kb downstream of the TSS were found to be negatively correlated with blood morphine levels. This data supports the idea that acute morphine exposure has a repressive effect on histone acetylation and gene expression in glutamatergic genes.

These results indicate that histone H3 hyperacetylation associated with chronic heroin use results in an increase of chromatin accessibility along genes involved in glutamatergic neurotransmission, such as GRIA1. This increased accessibility may be of significant importance in understanding transcriptional impairments contributing to drug-induced striatal synaptic plasticity. Consistent with H3K9me2's role in regulating plasticity-associated transcriptional programs, Ingenuity Pathway Analysis identified changes in methylation at genes related to glutamatergic signaling, with one study suggesting that opioid-induced transcriptional

regulation via H3K9 methylation is interestingly not specific to the NAc, but also occurs in the VTA and locus coeruleus as well (Mashayekhi et al., 2012). Compared to the conclusion derived from DNA methylation, histone H3 hyperacetylation following opioid exposure instead implies at the enhancement of glutamatergic gene expression in heroin users.

This contrast of results demonstrates the complex interplay of epigenetic modifications and opiate addiction. The nature of these alterations can change as an individual moves from the acute stage of substance exposure to the chronic stage. This intricacy allows for a nuanced control over gene expression at a given time, balancing the needs of both immediate responses and long-term stability. In the case of opioids, its initial use upregulates glutamatergic genes via histone acetylation, while prolonged use leads to the accumulation of DNA methylation, leading to the long-term suppression of its genes.

**Behaviors Associated With Epigenetic Modifications to Glutamatergic Transmission**

Many studies also observe a strong correlation between the aforementioned glutamatergic modifications and the symptoms of opioid addiction. According to one such study by Van den Oever et al. (2010), acute drug-induced, stress-induced, or cue-induced changes in synaptic plasticity are thought to initiate relapse. In particular, acute changes in glutamatergic transmission in the NA, VTA, and amygdala are theorized to express psychomotor sensitization and relapse to drug-seeking (Brebner et al., 2005; Jones & Bonci, 2005; Kourrich et al., 2007; Lu et al., 2005).

There is further support of glutamate in learning and other adaptive processes in animal drug addiction models (Kauer, 2004). During drug-associated learning, glutamate receptors and glutamatergic transmission are modified, and pharmacological agents act at glutamate receptors during classical Pavlovian and instrumental associative learning (Everitt & Wolf, 2002; Kelley et al., 2003). N-methyl-D-aspartate receptor (NMDAR) antagonists were found to block the development of cocaine-, amphetamine- and morphine-induced sensitization, while a-Amino-3-hydroxy5-methyl-4-isoxazolepropionic acid receptor (AMPAR) antagonists were found to maintain that sensitization (Jackson et al., 2000). This data supports the hypothesis that by recruiting NMDARs and AMPARs, addictive drugs initiate abnormal learning mechanisms. In this case, NMDARs and AMPARs cooperate with glutamate to reinforce drug-seeking behaviors in individuals.

In the context of opioid withdrawal, glutamate has been shown to play a significant role in modulating its severity. According to Dunn et al. (2019), increased glutamate levels lead to exacerbated withdrawal symptoms, while glutamate antagonism leads to an alleviation of those symptoms. For example, withdrawal symptoms such as stretching, wet dog shakes, and teeth chattering increased after an increase in glutamate levels by either direct injection of glutamate into the locus coeruleus (Tokuyama et al., 1998) or pretreatment with the glutamate transporter inhibitor DL-TBOA (Sekiya et al., 2004). Conversely, these symptoms decreased after a decrease in glutamate levels through administration of ceftriaxone, a β-lactam antibiotic (Medrano et al., 2015). After increasing glutamate by blocking ceftriaxone using dihydrokainic acid, withdrawal symptoms increased again. For rats who received H-7, a protein kinase inhibitor to reduce glutamate, in the locus coeruleus, a complete suppression of opioid

withdrawal was observed (Tokuyama et al., 1996), suggesting a direct correlation between glutamate and opioid withdrawal symptoms

## The Role of Gamma-Aminobutyric Acid (GABA) in Opioid Addiction

Alongside glutamatergic systems, GABA plays an important role in mediating addiction-related behaviors. George et al. (2012) and Volkow et al. (2011) found in human imaging studies that disrupted GABAergic activity in the PFC correlates to deficits in executive function that are related to addiction. For example, impairments in spatial information and behavioral inhibition, and decision-making disruptions have been shown in alcoholics. Additionally, both Goldstein and Volkow (2011) and Volkow et al. (2003) have shown that drug addiction leads to excessive emphasis on drug-paired cues for individuals, reduced responsiveness to non-drug rewards, and failure to inhibit maladaptive behavior. These results reveal the role of GABAergic systems in controlling the behavioral aspects of the brain, especially regarding decision-making.

### Epigenetic Changes to GABAergic Transmission

Tang et al. (2023) indicates that opioid use disorder consistently led to the hypermethylation of the GAD2 gene, which is responsible for the release of GABA. The duration and dosage of opioid use were associated with the level of methylation in the GAD2 gene promoter. Because gene expression is inhibited following methylation in the promoter region (Hoffman & Hu, 2006; Zhang et al., 2010), it can be concluded that compared to non-abusing individuals, hypermethylation in the promoter region of the GAD2 gene in opiate-abusing individuals leads to the lower expression of the GAD2 gene, resulting in decreased GABA levels.

On the other hand, Abdulmalek and Hardiman (2023) explains the role of opioids on the histone modifications of GABA, particularly in the ventral tegmental area (VTA). The VTA is one of the central brain regions of reward circuitry, and is populated with a diverse array of neurons, amongst which GABAergic interneurons consist the second majority (Cooper et al., 2017). From the VTA originates dopaminergic projections which innervate key mesolimbic and mesocortical structures such as the nucleus accumbens, the PFC, the amygdala, and the hippocampus (Hearing, 2019). The VTA receives significant GABAergic input from the rostromedial tegmental nucleus (RMTg), located at the tail of rodents' VTA. By modulating the inhibition of this GABAergic input, the dopaminergic projections have been found to mediate the reinforcing effects of morphine (Jalabert et al., 2011). In addition, opioid exposure has been reported to increase H3 acetylation within the mesolimbic system. Specifically, morphine exposure correlates to increased acetylation at H3K9 in the VTA (Mashayekhi et al., 2012). This data shows that morphine and opioid exposure leads to an increase in histone acetylation in the VTA, suggesting that opioid abuse results in GABAergic histone acetylation, meaning an increase in GABA levels. Similarly to glutamate, the conflicting results of DNA methylation and histone acetylation can be explained by the complex intersection of epigenetic mechanisms and drug addiction.

### Behaviors Associated With Epigenetic Modifications to GABAergic Transmission

Furthermore, Petrella et al. (2024) explains the connection between opioid seeking behaviors and GABAergic processes. Both VTA dopamine and GABA neurons express nociceptin/orphanin FQ peptide (NOP) receptors. In VTA DA neurons, treatment with the NOP receptor antagonist LY2817412 increases the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) without affecting miniature inhibitory postsynaptic currents (mIPSCs). This observation suggests that NOP antagonists control VTA DA neuronal activity via the increase or decrease of presynaptic, action potential-dependent GABA. In addition, the inhibitory effect of N/OFQ on VTA DA neuron activity is not prevented by blocking both the GABAA and GABAB receptors, matching previous findings that DA neuron activity is reduced by their direct hyperpolarization by NOP receptor activation (Zheng et al., 2002). On the other hand, the inhibitory effect of LY2817412 is prevented by blocking both GABAA and GABAB receptors. This data suggests that an enhancement of GABA transmission inhibits VTA DA activity, concluding that conversely, inhibition of GABA leads to a higher level of DA firing, which is responsible for the increase of drug-seeking/taking behaviors.

Another form of GABAergic seeking comes via hyperalgesia. A chronic symptom of opioid withdrawal marked by heightened sensitivity to pain (Carcoba et al., 2011), hyperalgesia is believed to cause continued drug use through negative reinforcement as a method to alleviate pain (Tsui et al., 2016). Li et al. (2019) reported that in morphine-treated mice, a decreased inhibition of dorsal raphe-GABAergic (DR-GABAergic) neurons during spontaneous withdrawal was shown. In addition to this, Alvarez-Bagnarol et al. (2023) found that in mice, DR-GABAergic neurons expressing mu-opioid receptors, located in the lateral subregions of the rostral DR, were activated during spontaneous withdrawal. Furthermore, a chemogenetic repression of DR-GABAergic neurons during spontaneous withdrawal led to the inhibition of hyperalgesia. These results demonstrate the crucial link between DR-GABAergic neurons and hyperalgesia, and thus demonstrates the role of GABA in opioid seeking.

Emotions and mood are also affected by opiate use and are related to GABA-ergic activity. After acute fentanyl withdrawal, Wang et al. (2023) discovered that animals developed negative emotions, such as anxiety-like behaviors, and other physical symptoms as a result of reduced DA activity. Spontaneous withdrawal symptoms, linked to heightened GABA release to midbrain DA neurons, typically occur 16 to 24 hours following the last drug exposure (Madhavan et al., 2010; McDevitt et al., 2021). A decrease in GABAergic output to DA neurons following chemogenetic inhibition of striatal MOR+ neurons was found to reduce physical symptoms. In addition, reduced DA activity is also associated with anxiety-related symptoms during acute opioid withdrawal (McKendrick et al., 2020; Nguyen et al., 2021; Zweifel et al., 2011). Following fentanyl withdrawal, inhibition of striatal MOR+ neurons was found to relieve anxiety-like behaviors. This is thought to be because of the inhibition of striatal GABAergic outputs leading to the disinhibition of SNc DA neurons. In all, these findings show the correlation between GABA and withdrawal symptoms, in which an increase in GABA release leads to the reduction of DA neuron activity, which in turn heightens physical and anxiety-like withdrawal behaviors.

## The Role of Dopamine in Opioid Addiction

In addition, dopamine systems are the major components of the rewarding aspects of nearly all drugs of abuse. Human studies show that intoxicating levels of drugs release dopamine into the ventral striatum, where that fast and steep release of dopamine corresponds

to the sensation of being “high” (Volkow et al., 2003). This correlation occurs due to the activation of low-affinity dopamine D1 receptors, which are necessary for drug reward (Caine et al., 2007) and conditioned responses (Zweifel et al., 2009) by the fast and steep dopamine increases. On the other hand, high-affinity D2 receptors are not sufficient for (Caine et al., 2002; Norman et al., 2011), or even block (Durieux et al., 2009), drug reward. In this sense, drugs imitate the bursts of dopamine triggered by phasic dopamine firing, which is associated with rewarding stimuli (Covey et al., 2014).

Incentive salience, defined as the motivational or arousing properties of stimuli such as drugs (Berridge, 2012), provides the foundation for cue-induced drug-seeking, drug self-administration, and the transition to habit-like compulsive drug-seeking. Neurobiological studies have found that midbrain dopamine cells initially fire in response to a new reward. After repeated exposure, the neurons shift from firing in response to the reward itself to stimuli that are predictive of the reward (Schultz et al., 1997). This combination of reward and cues trigger phasic dopamine firing and dopamine D1 receptor activation, which are essential for conditioning to occur (Ungless et al., 2001). This process creates a strong motivation to seek a reward via incentive salience.

**Epigenetic Changes to Dopaminergic Transmission**

The dopaminergic mesolimbic pathway, located within the ventral tegmental area (VTA), is highly concentrated with dopaminergic neurons and is considered pivotal in addiction. Barrow et al. (2017) observed that morphine significantly decreased DNA methylation in the superior colliculus and 5hmC content in this brain area, demonstrating the significant epigenetic changes morphine exposure produces. Additionally, it was identified that morphine exposure resulted in the methylation of the brain derived neurotrophic factor (BDNF) and catechol-O-methyltransferase (COMT) genes in the pons region of the brain. This region regulates respiration and sleep, which morphine use commonly disturbs. Other studies demonstrate the reduction of acetylcholine in the pons and medulla oblongata following morphine exposure (Ge et al., 1990), further correlating morphine-induced response with the pons region. Dopamine-dependent response to opiates is assisted by BDNF expression, and reports show in the VTA, an increase of BDNF expression follows morphine usage (Bolanos & Nestler, 2004). On the other hand, COMT assists in dopamine elimination, and it is observed that COMT activity reduces following morphine exposure (Kambur et al., 2008). In all, these findings show that opioids such as morphine result in the methylation of BDNF and COMT, leading to the increase of overall dopamine levels.

Regarding histone acetylations, the preclinical and postmortem findings of Egervari et al. (2017), Sheng et al. (2011), and Wang et al. (2014) demonstrated an increase in global H3 acetylation within the mesolimbic dopamine system following self-administration or experimenter-administration of opioids. In addition, a correlation seems to exist between the length of heroin use and global H3 hyperacetylation in the striatum of heroin users (Egervari et al., 2017). This information suggests that opioid abuse causes histone hyperacetylation of dopaminergic genes, which, additionally to DNA methylation studies, suggests an increase in dopamine levels.

**Behaviors Associated with Epigenetic Modifications to Dopaminergic Transmission**

In the context of opioid-seeking behavior, Bossert et al. (2016) explored the impact of glutamatergic projections from the ventral subiculum (vSub) to the nucleus accumbens and the ventral medial prefrontal cortex (vmPFC). Researchers found that in the vSub neurons projecting to both the NAc shell and vmPFC were significant increases in neuronal activity, marked by elevated Fos expression. However, a reduction of heroin-seeking reinstatement followed the interruption of only the vSub→NAc shell pathway. These results underscore the vSub→NAc shell projection's unique role in contributing to context-induced reinstatement. The disruption of this pathway is caused by a combination of vSub neuron inactivation using GABA receptor agonists, and a block of dopamine D1-family receptors in the NAc shell. In conclusion, this study highlights the critical interaction between dopamine D1 receptors and the vSub-NAc shell glutamatergic projections, ultimately connecting dopamine to drug-seeking behaviors.

Additionally, the link between dopamine and opioid withdrawal has been established by much preclinical behavioral evidence, and drugs acting on the dopamine system exerts varying effects based on dosage (Dunn et al., 2019). For example, DA elevating substances such as amphetamine, cocaine, and the DA precursor L-DOPA show a dose-dependent duality: both increasing symptoms and decreasing others based on the dose (el-Kadi & Sharif, 1998; Herz et al., 1974). Studies report that low doses of the D2 agonist apomorphine intensify withdrawal symptoms, demonstrated by increased behaviors such as jumping, wet dog shakes, burrowing, and hyperthermia. On the other hand, higher doses reduce these behaviors (Cox et al., 1976; el-Kadi & Sharif, 1998; Herz et al., 1974). Furthermore, D2 receptor antagonists such as domperidone, flupenthixol, and pimozide alleviate several withdrawal symptoms, while conversely the D2 receptor agonist sulpiride exacerbates them (Cox et al., 1976, el-Kadi & Sharif, 1998). These findings highlight the critical yet varying role of dopamine on drug addictions such as opioids, where the dosage and type of drug can either alleviate or intensify opioid withdrawal symptoms.

## The Role of Opioid Receptors in Opioid Addiction

Opioid receptors play a key role in mediating addiction-related behaviors. Schlosburg et al. (2013), Walker and Koob (2008), and Whitfield et al. (2015) observed that kappa-opioid receptor antagonists, when injected into the nucleus accumbens shell, can block compulsive drug-seeking development. In addition, dopamine release in the nAC and activity of CRF in the VTA can be affected by adjusting the activity of the dynorphin-kappa opioid receptor system in the ventral striatum, which contributes to the negative emotional state associated with withdrawal and prolonged abstinence (Koob et al., 2014). Several genes have been identified in vivo as crucial to drug responses, whose modifications strongly affect drug self-administration. In particular, mice without mu-opioid receptors are found to not express the rewarding effects of opioids (Matthes et al., 1996). These results indicate opioid receptors are fundamental regarding both drug-seeking and drug withdrawal behaviors.

### Epigenetic Changes to Opioid Receptors

Similarly to the aforementioned neurotransmitters, opioid receptors demonstrate clear epigenetic changes that occur following opioid use. Sandoval-Sierra et al. (2020) studied the effects of opioid use on opioid receptor-related DNA methylation. In opioid-naive patients, they demonstrated that following opioid abuse, increased methylation occurred at the promoter of the

opioid receptor mu 1 (OPRM1) gene, which controls the expression of mu-opioid receptors. Nielsen et al. (2012) observed that this hypermethylation is likely a response to, not a cause of, opioid use, and that these epigenetic modifications are a form of early responses to the drug. Reid et al. (2022) confirmed the hypermethylation of the OPRM1 promoter following acute and chronic exposure to opiates of opioid-naive patients, where out of ten selected CpG sites in the OPRM1 promoter, nine showed increased methylation following morphine treatment (Sandoval-Sierra et al., 2020). Collectively, these studies highlight the hypermethylation of the OPRM1 gene, and thus the decrease in mu-opioid receptor expression, following opiate use. Similarly, the opioid receptor delta 1 (OPRD1) and opioid receptor kappa 1 (OPRK1) gene promoters are also subject to methylation by opioids (Sun et al., 2017; Wang et al., 2003), thus leading to the downregulation of both delta-opioid receptors and kappa-opioid receptors, suggesting the decreased expression of opioid receptors following opioid use.

In contrast to DNA methylation, histone acetylation of opioid receptor genes demonstrates the increase in expression of opioid receptors resulting from opiate abuse. One key histone modification involved in this process is the acetylation of histone H3 at lysine 9 (H3K9) of the OPRD1 gene. Normally, acetylation of H3K9 occurs only in sites where the lysine is not methylated. Treatment using nerve growth factor (NGF) resulted in a significant reduction (approximately 40%) of methylated H3K9 levels, while simultaneously increasing acetylated H3K9 levels (Chen et al., 2006, Chen et al., 2008). This shift opens up the chromatin structure of the OPRD1 gene, thus boosting the expression of the delta-opioid receptor (Chen et al., 2007). The OPRK1 gene also showed increased acetylation, this time in its H4 histone, further demonstrating the acetylation of opioid receptor genes following opioid use. In all, these findings demonstrate the duplexity of the epigenetic changes regarding opioid receptor genes following opioid abuse.

**Behaviors Associated With Epigenetic Modifications to Opioid Receptors**

Furthermore, many studies have observed the link between mu- and delta-opioid receptors and drug-seeking behaviors. According to Shippenberg et al. (2008), mu-opioid receptor (MOPr) agonists, which include opioids such as morphine, are well known for their conditioned rewarding effects, causing its self-administration from both humans and experimental animals. Rewarding effects are not limited to MOPr agonists as well, as the ICV administration of β -END and delta-opioid receptor (DOPr) agonists indicates that the activation of either MOPr or DOPr can elicit these rewarding effects (Shippenberg & Elmer, 1998). Studies show the critical role of meso-accumbal opioid receptors, especially in the Acb and the VTA, in contributing to these effects (Bals-Kubik et al., 1993; Devine & Wise, 1994; Goeders et al., 1984). In these regions, the interactions between opioid receptors and DA is essential, as a decrease in meso-accumbal DA transmission was shown to reduce the rewarding effects of MOPr agonists. In all, opioid receptors via DA have a great influence over the rewarding aspects of opiates, thus impacting drug-seeking behaviors.

On the other hand, Mysels and Sullivan (2009) demonstrate the effects kappa-opioid receptor agonism and upregulation have on human opioid withdrawal symptoms (“abstinence syndrome”), including dysphoria, shaking, rhinorrhea, and diarrhea. This relationship has been proven through many animal models involving opiates and other addictive substances (Acquas & Di Chiara, 1992; Di Chiara & Imperato, 1988). Further animal studies also demonstrate the

inverse, as kappa-opioid receptor antagonism relieves withdrawal symptoms (Beardsley et al., 2005).

## Conclusion

The neural mechanisms underlying opioid addiction is controlled by a complex interplay between neurotransmitters and its epigenetic modifications. These alterations are found to influence the wide variety of addiction symptoms, especially regarding craving and withdrawal. Given this understanding, we not only enhance our knowledge of opioid addiction and its effects, but also highlight the potential for targeted solutions to alleviate the devastating effects of the opioid epidemic.

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