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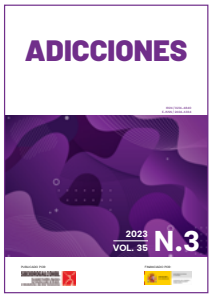

EDITORIAL

Drugs of abuse and epigenetics: Past, present and future

Drogas de abuso y epigenética: Pasado, presente y futuro

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Epigenetic changes

Epigenetic modifications are commonly defined as mitotically or meiotically heritable, and possibly reversible, changes in gene expression that do not entail any direct alteration in the DNA sequence (Dupont et al., 2009). Accumulating evidence suggests that drug use-related environmental (e.g., drug-taking behavior) and social (e.g., stress) factors may alter gene expression in the brain (and other organs) of drug abusers, causing developmental and behavioral changes in these individuals, and likely leading to the onset of substance use disorders (SUDs). Understanding the mechanisms underlying the interaction between these environmental and genetic factors thus assumes critical relevance to ascertaining the development, heredity, and possibly improving the treatment of SUDs.

There are three epigenetics mechanisms that have been mostly associated with substance use in animal models: DNA methylation, histone modifications, and generation of noncoding RNAs (Bastle and Neisewander, 2016). DNA methylation refers to the covalent modification of the fifth carbon in the cytosine base (5-mC), catalyzed by DNA methyltransferases (e.g., DNMT1, DNMT3a, DNMT3b), which mainly occurs in the CpG dinucleotides within the genome, and that is often associated with the repression of transcription (Kouzarides, 2007). Histone modifications relate to post-translational changes (e.g., acetylation, methylation, phosphorylation) in the amino acid residues of these proteins, which can cause transcriptional activation, silencing, and chromatin assembly. For example, histone acetyl transferases catalyze the addition of acetyl groups, usually on a lysine (K) residue, causing chromatin relaxation, which promotes a transcriptionally accessible state. Histone methylation may cause either gene activation or repression, depending on where it occurs. For example, promoter methylation may silence gene expression, whereas methylation occurring at another site of the DNA sequence may trigger the expression of a different gene (D'Addario et al., 2013).

Noncoding RNAs (e.g., miRNAs) comprise a family of small RNAs that post-transcriptionally regulate gene expression in a negative manner, controlling processes like chromosome dynamics, RNA editing or mRNA degradation (Korolev et al., 2018; Liu et al., 2018).

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Abnormal epigenetic patterns and neuronal diseases

Epigenetic signaling has been reported to be a key regulator of several biological processes (e.g., neural stem cell fate) (Szutorisz and Hurd, 2018). As such, dysregulation of such mechanisms may lead to the onset of distinct disorders, with abnormal DNA methylation and histone acetylation patterns in the brain having already been associated with cognitive impairment or childhood brain overgrowth syndromes (Gomes et al., 2020).

For example, mutations in *dnmt3a* (leading to its down-regulation) are known to underlie Tatton-Brown-Rahman syndrome, and methylation of the *ezh2* gene is known to be involved in other overgrowth syndromes such as Sotos or Weaver syndromes. Sotos syndrome has also been correlated with mutations in the gene *nsd1,* coding for the Nuclear Receptor Binding SET Domain Protein 1, a protein displaying histone methyltransferase activity that is responsible for methylating lysine 36 at histone H3 (H3K36) and lysine 20 at histone H4 (H4K20). In addition to the loss of histone methyltransferase activity, the hypermethylation at the 5' regulatory region of this *nsd1* gene in human neuroblastoma and glioma cells has been shown to contribute to Sotos syndrome (Berdasco et al., 2009). A systematic review published by Dall'Aglio et al. (2018) has consistently found methylation at *prrt1*, *c11orf21/tspan32*, and *or2li3* genes (encoding for proline-rich transmembrane protein 1, chromosome 11 open reading frame 21, tetraspanin 32 and olfactory receptor family 2 subfamily L member, respectively) in autism spectrum disorders (Dall'Aglio et al., 2018). Methylation of the *vipr2* gene (which codes for vasoactive intestinal peptide receptor 2) has been detected in attention deficit and hyperactivity disorder (ADHD). Notably, since proteins encoded by *or2li3* (ASD) and *vipr2* (ADHD) are involved in G protein-coupled receptor-mediated neurotransmitter signaling, it seems that the epigenetic dysregulation of neurotransmission could underlie the onset of these disorders. Along with the methylation of *tspan32*, trimethylations and acetylation on lysine residue 27 of histone H3 (H3K27) have been found in Beckwith-Wiedemann syndrome, which is characterized by macrosomia and hemihyperplasia.

Mutations in the *mecp2* gene (encoding for methyl-CpG-binding protein, reported to regulate gene expression by binding to methylated DNA) have been correlated to Rett syndrome, a disorder associated with cognitive impairment in children (Good et al., 2021).

There is also accumulating evidence of epigenetic modifications in schizophrenia (SCZ) patients. For example, both hyper- and hypomethylation of different CpG sites have been correlated with reality distortion symptoms in SCZ patients (Liu et al., 2013). Moreover, increased levels of histone methyltransferases and increased acetylation in lysines 9 and 14 of histone H3 (H3K9K14ac) have been reported in patients with SCZ (Jin et al., 2008). Mutations in the *cbp* gene, encoding for the CREB-binding protein (CBP), have been reported as causing the loss of this protein's intrinsic histone acetyltransferase activity, resulting in a deficiency in cAMP response element-binding protein (CREB) recruitment that is typical of Rubinstein-Taybi syndrome (a neurodevelopmental disorder) and that has also been reported to occur in Alzheimer's disease (Caccamo et al., 2010). Interestingly, abnormal histone acetylation patterns have been recently confirmed to occur in patients with Alzheimer's, including the up-regulation of the acetylation of lysines 27 and 9 on histone H3 (H3K9ac and H3K27ac) or the losses of acetylation in lysine 16 on histone H4 (which normally increase with aging) (Nativio et al., 2020).

Drug abuse and epigenetics

Substances of abuse may induce epigenetic modifications, namely DNA methylation and histone modifications, which have already been associated with changes in the reward system, psychomotor activity, craving, and relapse. Indeed, the interaction of these substances with proteins involved in different signaling pathways (e.g., neurotransmitters, transporters), may propagate intracellular signaling into the nucleus, affecting gene expression and subsequently gene transcription (Nielsen et al., 2012). Moreover, the brain region at which such changes occur may determine most of the SUD-related outcomes. For example, DNA methylation and histone modifications occurring in the nucleus accumbens (NAc), a brain region associated with the reward system, may contribute to the regulation of addictive behavior (Flagel et al., 2016). Also, increased gene expression resulting from exposure to substances of abuse is often associated with increased histone acetylation levels, as acetylation usually turns the chromatin into a more relaxed, more transcriptionally active state. Nevertheless, some psychoactive substances have been associated with increased activity of histone deacetylases (HDACs), resulting in decreased histone acetylation, and subsequent transcriptional gene repression. For example, alcohol has been shown to increase HDAC11 and nicotine has been reported to increase the transcription of HDAC1 (Bala et al., 2017; Brooks and Henderson, 2021).

Exposure to substances of abuse (e.g., cocaine, opioids) may also cause a decrease in histone methyltransferase G9a expression (an enzyme responsible for the dimethylation of lysine 9 in histone H3, H3K9me2, in the NAc, which is often associated with transcriptional repression). Notably, decreased G9a has been correlated with a depressive-like behavior in mice (including decreased social interaction and increased anhedonia), and has been detected in the postmortem NAc tissue of clinically depressed individuals (Covington et al., 2011). Interestingly, drug-induced

decreased G9a expression is often associated with ΔFosB expression in that same region, suggesting a link between these two proteins (Maze et al., 2014).

Drug-induced modulation of noncoding RNAs has also been reported to underlie SUDs. For example, upregulation of the miRNA miR-212 in the dorsal striatum of rats, a brain region involved in drug tolerance, has been shown to mimic the compulsive drug-taking behavior observed in drug addicts (Sadakierska-Chudy et al., 2017). Increased miR-206 in the medial prefrontal cortex (e.g., as induced by alcohol) enhances the drug-seeking behavior, most likely by suppressing brain-derived neurotrophic factor (BDNF) expression.

Importantly, it is worth noting that epigenetic marks can be passed on to future generations, provided such modifications occur in the germ cells. For example, it has been observed that male adult rats with a compulsive cocaine self-administration behavior passed an addiction-resistance phenotype onto their male (but not female) descendants, which was most likely mediated by increased H3 acetylation of the BDNF promoter (also known as being associated with resistance to drug effects) (Vassoler et al., 2013). Moreover, male offspring of F0 female adolescent rats repeatedly administered morphine were shown to be more sensitive to the effects of morphine (Byrnes et al., 2011). Similarly, exposure of F0 males to ethanol decreased ethanol intake and increased the sensitivity of their F1 offspring to the inhibitory effects of ethanol on anxiety-like behavior (Bastle and Neisewander, 2016). Some of the drugs most commonly associated with promoting epigenetic changes and respective induced epigenetic changes are summarized below.

Alcohol

Alcohol breakdown in the liver increases blood acetate levels, which in turn may contribute to increased histone acetylation levels in the brain (Mews et al., 2019). In fact, acute and chronic alcohol intake has been shown to promote epigenetic modifications. For example, chronic ethanol exposure has been shown to cause the demethylation of CpG islands in the NR2B subunit of the N-methyl-D-aspartate (NMDA) receptor, as well as increased H3K9 acetylation in *nr2b*'s gene promoter in primary cortical neurons of mice administered alcohol (Marutha Ravindran et al., 2005). Acute ethanol administration decreased HDAC activity, and subsequently increased H3 and H4 acetylation in rats' amygdala (Gapp et al., 2014), whereas withdrawal from chronic ethanol treatment produced the opposite effects (i.e., increased HDAC activity, decreased H3/H4 acetylation) in the same brain region, and was associated with anxiety-like behavior in the animals.

Interestingly, data from different epigenome-wide analysis studies have identified several genes (e.g., *hnrnpa1*, *lrrc20*, *plekhg4b*, *lmf1*) methylated following alcohol exposure that were associated with immune regulation, suggesting the importance of immune modulation in alcohol use disorders (Mews et al., 2019). Specifically, hypermethylation of the *herp* (coding for homocysteine), *snca* (coding for a-synuclein) and *avp* (coding for vasopressin) genes has been observed in alcoholics (Bastle and Neisewander, 2016).

Moreover, it has been reported that alcohol increases the sensivity of Kupffer cells of alcohol-treated mice to pro-inflammatory agents (e.g., lipopolysaccharide) by inducing the inflammatory miR-155 and regulating HDAC11 levels (Bala et al., 2017). Also, alcohol-mediated increase of miR-206 levels in the medial prefrontal cortex of alcohol-dependent rats was shown to contribute to the onset of alcohol dependence in the treated animals (Tapocik et al., 2014).

Cannabinoids

Epigenetic modifications have also been observed following cannabinoid exposure. For example, higher methylation status in the promoter of the *cnr1* gene, coding for cannabinoid type-1 receptor (CB1R), has been observed in THC-dependent subjects, compared to control groups, an alteration that was negatively correlated with CB1R mRNA expression and that suggests the involvement of *cnr1* methylation in regulating THC dependence (Rotter et al., 2013). Tomasiewicz et al. (2012) observed a THC-mediated alteration in the transcriptional and epigenetic state of the dopaminergic D_2 receptor (*drd2*) and the opioid neuropeptide proenkephalin (*penk*) genes as a result of reduced histone H3K9 methylation, namely the trimethylation of lysine 4 on histone H3 (H3K4me3) and dimethylation of lysine 9 on histone H3 (H3K9me2) in the nucleus accumbens of THC-exposed male rats, suggesting that cannabis use could increase its user's vulnerability to the effects of opiates. Moreover, Prini et al. observed that chronic administration of THC to adolescent female rats resulted in transcriptional repression immediately after exposure, mediated by increased trimethylation of H3K9 (H3K9me3), followed by transcriptional activation at later timepoints (i.e., 48h) via increased acetylation of H3K9 (H3K9ac) (Prini et al., 2018). Notably, increased H3K9me3 levels were shown to downregulate the expression of several genes, including *Homer1*, *Mgll*, and *Dlg4*.

Synthetic cannabinoids (SCs) have also been reported to promote epigenetic modifications. For example, both specific CB1R and CB2R cannabinoid receptor agonists (HU-210 and JWH-133, respectively) have been shown to regulate the differentiation of glioma cells by increasing trimethylated H3K9 (H3K9me3) levels in a cannabinoid receptor activation-dependent manner (Aguado et al., 2007). Moreover, adolescent rats exposed to the SC WIN55212.2 showed increased DNA hypermethylation in the *rgs7* gene (coding for a protein involved in the acceleration of GTP hydrolysis on G protein) (Tomas-Roig

et al., 2017). Additionally, JWH-133-exposed male mice have shown decreased expression of all *tet* genes (coding for the TET enzymes) in sperm cells. Since TET enzymes promote DNA demethylation, this SC-induced effect was associated with increased DNA methylation, specifically in *dio3*, *dlk1*, *hymai,* or *igf2*, which are mostly associated with cell growth and differentiation (Innocenzi et al., 2019).

Cocaine

Cocaine use has been shown to promote histone modifications in male mice, including the trimethylation of lysine residues 9 and 27 in the silent chromatin of histone H3 (H3K9me3 and H3K27me3), and decreased active marks H3K27ac (enhancer) and H3K4me3 (promoter) of isolated germ cells. Cocaine was further noted to increase the activities of acetyltransferase KAT8/MOF, deacetylase SIRT1, and methyltransferase KMT2C/G9A. Moreover, cocaine has been shown to decrease the activity of HDAC1, 2, and 3 in mice NAc, further causing changes in synaptic plasticity (González et al., 2020). Interestingly, these cocaine-induced epigenetic changes were found to be mediated by the dopamine receptor 1 (DRD1) (Campbell et al., 2021; González et al., 2020).

Acute cocaine administration to mice has been reported to promote H4 acetylation, leading to increased *c-fos* expression (involved in the initial response to psychostimulants) in the mouse striatum. However, following repeated cocaine exposure, H4 acetylation at the *fosB* promoter increased the expression of Δ*fosB* and *fosB*, increasing the animals' sensitivity to cocaine. Moreover, Δ*fosB* accumulation recruits HDAC1 to the *c-fos* promoter, thus decreasing *c-fos* expression and activity. This is also accompanied by an H3 acetylation-mediated upregulation of *bdnf* and *cdk5*. Notably, the long-term accumulation of these effects has been shown to correlate with the increased reward response and locomotor activity that typically occurs after cocaine use (Bastle and Neisewander, 2016).

Acute cocaine exposure has also been shown to promote *dnmt3a* and *dnmt3b* expression in the NAc of mice, which was associated with the hypermethylation of the *pp1* promoter, and subsequent decreased *pp1* gene expression. The resulting decrease in protein Ser/Thr phosphatase activity may then contribute to the increased phosphorylation of MeCP2 protein (at serine 421), preventing its activity as a transcriptional repressor. In rats, MeCP2 phosphorylation in the striatum and NAc has been shown to regulate the response to cocaine (Ausió, 2016).

Opioids

The ability of opioids to promote epigenetic modifications has also been reported. In contrast to cocaine, chronic opioid administration has been shown to decrease H3 acetylation at the *bdnf* promoter in the ventral tegmental area, leading to decreased *bdnf* expression, thus impairing the maintenance of the synaptic structure (Koo et al., 2015).

Epigenetic changes at the *Oprm1* gene (coding for the μ-opioid receptor) seem to play a central role in the response to opioids. For example, increased methylation at the *oprm1* promoter and histone deacetylation have been shown to cause decreased *oprm1* mRNA expression, leading to decreased sensitivity of μ-opioid receptors and subsequent enhanced tolerance to opioids (Jindal et al., 2021). MeCP2 expression is crucial to the silencing of μ-opioid receptors in the dorsal root ganglion, as this epigenetic repressor recruits HDAC1 and binds to the methylation regions of the *oprm1* promoter (Sun et al., 2021).

A recent study examining the genome-wide changes in human midbrain specimens collected from autopsies of opioid abusers showed that these substances promote several transcriptional changes in the midbrain that could be associated within inter-related gene hubs. Genes in two of these hubs, associated with synaptic transmission and other neuronal functions, displayed down-regulated expression in opioid users, whereas genes in a third large drug-responsive gene hub associated with immune modulation and transcription regulation (e.g., *fos, fosl1, fosl2, jun, junb, atf3*) were found to be mostly upregulated. Noteworthy, this same study found strong evidence of downstream gene regulation by long noncoding RNA (lncRNAs). For example, the lncRNA *MIR210HG* was associated with increased levels of *gadd45b* (involved in neuronal gene expression via DNA methylation) and *nfkb1a* (related to the regulation of NF-kB-mediated transcription), which have been implicated in drug abuse (Saad et al., 2019).

Future perspectives

Epigenetics is a fast-evolving field, which in recent years has provided interesting insights towards understanding the mechanisms underlying the (neuro)toxicity of substances of abuse and their correlation with SUDs. Considering the reversibility of most epigenetic changes, it is reasonable to expect that the development and/or repurposing of pharmaceuticals able, for example, to inhibit DNA methylation (e.g., azacytidine) or histone deacetylase activity (e.g., valproic acid) could represent interesting therapeutics to SUDs. Alternatively, DNA methylation can be pharmacologically altered by the administration of methionine, an amino acid present in the diet, whose metabolism yields methyl groups that may act as donors for DNA methylation. Indeed, rats receiving methyl supplementation via daily, systemic administration of methionine showed reduced rewarding and motivating effects in response to cocaine (Wright et al., 2015). Administration of HDAC inhibitors trichostatin A and phenylbutyrate to rats subjected to the cocaine self-administration paradigm, dose-dependently reduced

the rats' motivation for the drug, hence their cocaine self-administration (Romieu et al., 2008). Also, the class I-specific inhibitor MS-275 was shown to reduce the alcohol-drinking motivation of rats trained to self-administer high alcohol levels (Jeanblanc et al., 2015).

Notably, as most epigenetic marks are specific to certain brain regions, SUD-related therapeutics would also have to be targeted to those same regions (e.g., NAc, prefrontal cortex). For example, nanotubes containing such agents have already been tested for their ability to attach to a neuron-specific receptor ligand (e.g., a cocaine-like molecule) and further bind to the same receptor of the ligand (e.g., a dopamine transporter in the case of cocaine), releasing the therapeutic agent in the target neuron. Also, non-replicative viral vectors comprising, for example, a DNA sequence from a gene of interest or a short-interfering RNA (siRNA), could also be used (e.g., using the CRISPR-Cas9 system), to target such gene or siRNA to specific locations (e.g., specific neuron population) to either increase gene expression or prevent gene transcription, respectively.

Moreover, the detection of epigenetic modifications in children could pave the way to identifying potential risks for developing addictions or any developmental disorders, as well as novel therapeutic targets. Interestingly, this strategy has already provided interesting insights regarding the predisposition of children to develop leukemia (Ramos et al., 2018), and has allowed associating specific DNA methylation changes in the blood of newborns with gestational age-related health effects (Merid et al., 2020).

As evidence accumulates on the key role played by non-coding RNAs in the regulation of several distinct intracellular signaling processes, it becomes important to analyze existing databases to ascertain candidate noncoding RNAs that may be involved in SUD-related mechanisms. For example, analysis of enriched targets in the Knowledge-base of addiction-related genes database using bioinformatic tools has led to the identification of miR-495 as a candidate miRNA associated with the regulation of expression of several addiction-related genes (Bastle et al., 2018).

Conclusions

The relevance of epigenetic changes, including DNA methylation or histone modifications, to the modulation of several biological processes has been unveiled in recent years, with specific epigenetic modifications or mutations in key elements of the epigenetic machinery having been correlated with a distinct set of disorders (mostly related to cognitive impairment or childhood brain overgrowth).

In particular, there is increasing evidence demonstrating the association of drugs of abuse with epigenetic changes, namely with the methylation of specific genes (e.g., *fosB*, *drd1, oprm1*), histone modifications (e.g., H3K9me2), and changes in the activity of enzymes responsible for epigenetic modifications (e.g., DNMTs, HDACs). All these changes may modulate the drug users' response to these same substances, by affecting the expression of genes mostly related to reward, tolerance, or neuronal plasticity, which have been suggested to underlie the onset of SUDs.

At the same time, considering the reversibility of most of epigenetic changes, their identification and assessment of their contribution to the onset to distinct disorders, open interesting perspectives towards finding novel therapeutic strategies for such disorders. In this sense, understanding the role of drug-induced epigenetic modifications assumes a critical role to assess the potential risks of developing a given disorder, as well as to improve therapeutical strategies.

Conflict of interests

The authors declare that they have no conflict of interests.

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