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REVIEW ARTICLE

# Epigenetic Mechanisms Mediate Nicotine-Induced Reward and Behaviour in Zebrafish

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**Abstract:** Nicotine induces long-term changes in the neural activity of the mesocorticolimbic reward pathway structures. The mechanisms involved in this process have not been fully characterized. The hypothesis discussed here proposed that epigenetic regulation participates in the installation of persistent adaptations and long-lasting synaptic plasticity generated by nicotine action on the mesolimbic dopamine neurons of zebrafish. The epigenetic mechanisms induced by nicotine entail histone and DNA chemical modifications, which have been described to lead to changes in gene expression. Among the enzymes that catalyze epigenetic chemical modifications, histone deacetylases (HDACs) remove acetyl groups from histones, thereby facilitating DNA relaxation and making DNA more accessible to gene transcription. DNA methylation, which is dependent on DNA methyltransferase (DNMTs) activity, inhibits gene expression by recruiting several methyl binding proteins that prevent RNA polymerase binding to DNA. In zebrafish, phenylbutyrate (PhB), an HDAC inhibitor, abolishes nicotine rewarding properties together with a series of typical reward-associated behaviors. Furthermore, PhB and nicotine alter long- and short-term object recognition memory in zebrafish, respectively. Regarding DNA methylation effects, a methyl group donor L-methionine (L-met) was found to dramatically reduce nicotine-induced conditioned place preference (CPP) in zebrafish. Simultaneous treatment with DNMT inhibitor 5-aza-2'-deoxycytidine (AZA) was found to reverse the L-met effect on nicotine-induced CPP as well as nicotine reward-specific effects on genetic expression in zebrafish. Therefore, pharmacological interventions that modulate epigenetic regulation of gene expression should be considered as a potential therapeutic method to treat nicotine addiction.

**Keywords:** Epigenesis, nicotine reward, zebrafish, histone acetylation, DNA methylation, conditioning place preference, HDAC inhibitor.

## 1. INTRODUCTION

Tobacco consumption has gradually decreased in developing countries (CDC, 2008; http://www.cdc.gov/ tobacco/data_statistics/tables/trends/cig_smoking). Recent data indicate that about 63% of the international population is protected by at least one tobacco consumption control measure. Nevertheless, up-to-date research suggests approximately 7.2 million people around the world continue to prematurely die annually due to illnesses caused by smoking tobacco cigarettes [1, 2]. Tobacco abusers primarily die from cancer and cardiovascular and pulmonary disease [3]. Another crucial problem with tobacco smoking is related to a well-defined sequence of drug usage and abuse that affects people in western societies. In this sequence, frequent use of non-illegal drugs, such as tobacco and/or alcohol, precedes the use of cocaine and other illicit drugs [4-6]. Nonetheless, cellular and molecular mechanisms underlying substance use shift remain virtually unknown [5].

The principal alkaloid present in tobacco is nicotine, which binds to nicotinic acetylcholine receptors (nAChR) expressed in different tissues of the body of many vertebrate and invertebrate animals. NAChRs are ligand-gated cation channels that are widely distributed throughout the central and peripheral nervous system, skeletal muscles, and immune system [7]. NAChRs are expressed in nearly every region of the brain, both at pre- and postsynaptic sites [8, 9]. NAChRs expressed in neurons of the mesolimbic dopamine circuit and interrelated structures, such as the amygdala, hippocampus, and habenula (Hb), are critical mediators of reinforced behaviour and rewarding properties of nicotine [10]. The rewarding effects of nicotine on the brain are principally mediated by α4, β2, α6 and α7 nAChR subunits [11]. However, more recently, experimental evidence has convincingly demonstrated that α5, α3, and β4 nAChR subunits are also strongly associated with nicotine addiction [12].

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Interactions among the environment, exteroceptive and visceral sensitivity, and gene expression regulate animal behaviour. Slight differences in the interaction among genes, interoception and the environment may be responsible for generating either vulnerability or resilience to the compulsive seeking of drugs [13-15]. At the molecular level, epigenetic mechanisms integrate interoceptive and exteroceptive sensitivity by controlling gene transcription and RNA splicing to maintain homeostasis and regulate the adaptive behaviour of cells and organisms that is why unregulated epigenetic mechanisms can lead to disease and maladaptation, such as neurodegenerative disorders, cancer and drug addiction [16-22]. Regarding addiction, chromatin structure and gene expression are epigenetically reconfigured in neurons of the reward mesolimbic pathway by repeated exposure to drugs [5, 23, 24]. Epigenetic regulation of gene induction or silencing generates persistent alterations in neuronal circuitry functioning that may result in long-lasting drug-seeking compulsive behaviours [25, 26]. Unfortunately, this maladapted plasticity of the reward circuitry occurs in spite of the adverse consequences of drug consumption for homeostasis and the psychophysical well-being of consumers.

Epigenetics is defined as heritable chemical modifications to DNA and histones, which are able to control the transcription of genes without altering the coding sequence of DNA [27-29]. Every epigenetic enduring change could be heritable and reversible. Epigenetic modifications of DNA molecules and histones induce the remodeling/repositioning of nucleosomes and reorganization of the chromatin structure, making DNA more or less accessible to transcriptional machinery components [30].

Different types of chemical modifications are involved in the epigenetic control of gene expression by altering the chromatin condensation status and binding of the transcriptome components (Fig. **1**). Among epigenetic mechanisms, DNA methylation has been associated with long-term chemical modifications that generate silencing of genes. Epigenetic post-translational modifications of histones, depending on the particular chemical group modified in these proteins, can activate or inactivate gene expression. Other important epigenetic mechanisms involve non-coding RNA gene silencing, RNA-editing, non-coding DNA regulatory sequences, prion-based epigenetic inheritance and Polycomb group proteins. The latter are a set of transcription repressors involved in developmental morphogenesis, chromosome X inactivation, and haematopoiesis. Other functions controlled by epigenetic mechanisms include protection against the viral genome and retrotransposon insertion, DNA replication and cell proliferation, DNA repair and maintenance of genomic stability, and aging [22, 31, 32].

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) and occurs in cytosines within CpG islands. It has been more recently demonstrated that guanine and adenine can also be methylated [33-36]. DNA methylation is a reversible epigenetic mechanism because methyl groups can be removed from DNA bases by the coordinated action of demethylases with DNA repair protein complexes [37-40].

Histones are post-translationally modified by several mechanisms, including acetylation, methylation, phosphorylation, ADP ribosylation, ubiquitylation, and sumoylation, among other more recently described chemical modifications of histone tails [41, 42]. Histone tail acetylation is regulated by histone acetyltransferases (HATs), which have been associated with short- and long-term epigenetic modifications [20, 43, 44]. On the other hand, histones are deacetylated by histone deacetylases (HDACs), which are a target of pharmacological treatments for several diseases [45, 46]. Generally, acetylated histones induce an "open" state of the chromatin structure [47], which enables access to transcription factors and RNA pol II to DNA. Histone acetylation and deacetylation must be maintained under homeostatic equilibrium since their deregulation results in different pathological conditions [22, 48]. Furthermore, histones can be methylated by histone methyltransferases (HMTs), and this kind of epigenetic modification can be either repressive or permissive for gene transcription [49]. For instance, methyltransferase activity of Polycomb group proteins catalyzes lysine 27 methylation of histone H3, causing gene repression [50].

It has been suggested that the homeostatic balance of enzymes that catalyze post-translational modifications of histones and DNA methylation, as well as expression of factors that work as acetyl- and methyl-readers, is disrupted in neurons of the reward dopamine circuitry in addicted people and animal models of addiction [13, 51, 52]. For example, histone H3 and H4 acetylation increases in the main structures of the reward pathway as a consequence of acute or chronic exposure to psychostimulant drugs, such as nicotine, opiates and alcohol. Drug-stimulus induces unbalance between HAT and HDAC activity generating a high degree of histone tail acetylation in neurons of the nucleus accumbens (NAcc) [5, 23, 46, 53-55].

Furthermore, epigenetic regulatory interaction between histone and DNA methylation has been described to regulate the integration of environmental and interoceptive stimuli at the transcriptional level [18, 56]. In this regard, DNMT activity is recruited to chromatin by heterochromatin protein 1 (HP1), which binds to histone H3 trimethylated in lysine 9 (H3K9me3) [57]. Moreover, DNMT3a recruitment and maintenance of DNA methylation at intergenic regions require the presence of histone H3 dimethylated in lysine 36 (H3K36me2) in the nucleosome [58, 59].

It has also been suggested that DNA methylation may work in concert with histone tail modifications to dynamically regulate memory formation by remodelling chromatin structure in the adult nervous system [60, 61]. There are, however, some controversial findings regarding cause-effect mechanisms since it has been observed that DNA methylation patterns can guide histone modifications, whereas other studies suggest that histone tail chemical modifications guide DNA methylation status and gene expression silencing [48, 62].

## 2. NICOTINE REWARD

Nicotine modulates synaptic inputs to brain mesolimbic structures in an acute and long-lasting manner [63, 64]. Chronic nicotine exposure modifies the synaptic activity of diverse brain neuronal circuits, including mesolimbic dopamine pathway structures, and induces upregulation of nAChRs causing long-term addictive behaviors [7, 8]. Repeated nicotine exposure induces synaptic circuitry plasticity, such

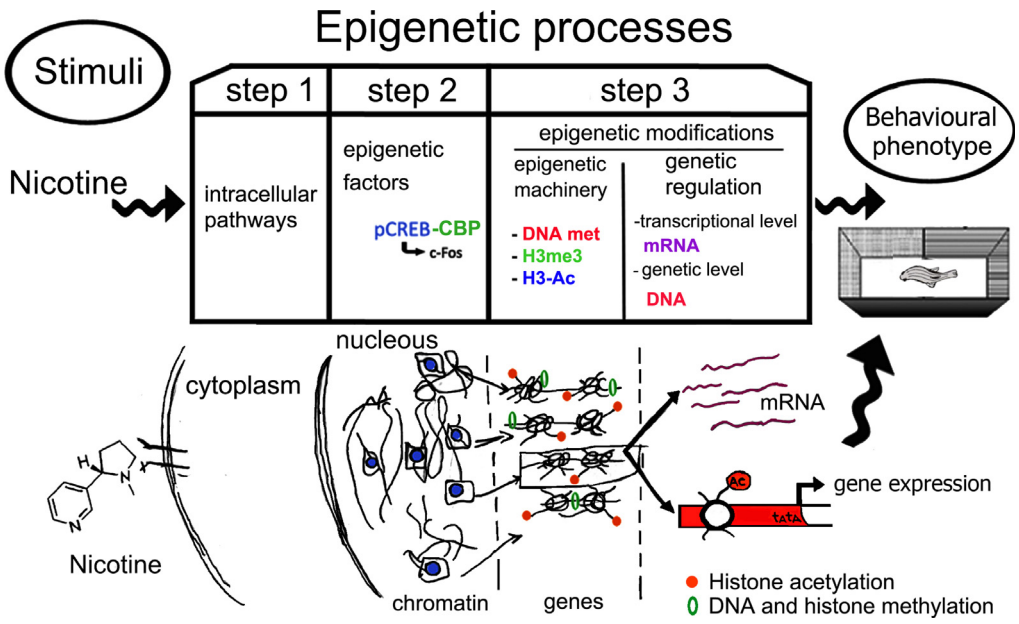


**Fig. (1).** Scheme and drawing depicting some of the epigenetic processes involved in nicotine reward in the zebrafish brain. The epigenetic mechanisms principally studied in our laboratory and described in this review are histone H3 acetylation (H3-Ac) and methylation (H3me3), as well as DNA methylation. Acetylation was assessed by using the HDAC inhibitor phenylbutyrate and immunocytochemistry of some H3-acetylated. Methylation was studied by means of treatments with a methyl donor (L-methionine) to promote and enhance methylation of histones and DNA and a DNA methyltransferase activity inhibitor (5-Aza-2′-deoxycytidine). The permissive state of gene transcription allowed by histone acetylation in specific gene promoters is depicted in the cartoon. In contrast, DNA methylation is generally repressive for transcription. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

as enhancement of glutamatergic input from cortical prefrontal areas onto dopamine terminals, projection neurons coming from the ventral tegmental area (VTA), and intrinsic neurons of the NAcc [65-71]. In nicotine addiction, different nAChR subtypes appear to differentially mediate somatic *vs.* affective components of withdrawal [72, 73]. The Hb, a small bilateral nucleus in the midbrain, is highly conserved among vertebrate species and has been implicated in nicotine addiction and negative reward among other physiological functions, including stress responses, learning and mood disorders [74, 75]. The Hb is highly interconnected with the interpeduncular nucleus (IPN), a singular structure in the midbrain tegmentum, forming a neuronal axis. The Hb-IPN axis is special considering nicotine reward because it densely expresses virtually all neuronal subtypes of nAChR [76, 77].

It has become very clear that exposure to psychostimulant drugs in combination with environmental and interoceptive factors affect DNA methylation and covalent histone tail modifications in neurons of brain structures activated by drug reward [54, 78-80]. Nicotine action on the mesocorticolimbic circuitry provokes long-lasting modifications at the molecular level, including epigenetic regulation of gene expression [5, 81]. The rewarding properties of nicotine, as well as other drugs of abuse, are studied in vertebrate animal models using self-administration and the conditioned place preference (CPP) task.

Regarding animal models to study drug reward, zebrafish (*Danio rerio*) are vertebrates of small size that have diurnal habits, including locomotor activity and feeding behaviour. This species of teleost fish is relatively easy to reproduce and maintain in laboratory conditions and possesses diverse advantages to study locomotor and exploratory behaviour as well as many other physiological parameters of a diurnal animal. It has been conclusively demonstrated that zebrafish is an excellent experimental animal model to study addictive properties of drugs of abuse and addiction genetics [82].

Concerning the rewarding properties of nicotine, this species has been used to evaluate biochemical, genetic and epigenetic processes occurring in its brain mesolimbic circuitry (Fig. **2**) after applying different nicotine treatments. Furthermore, a variety of behavioural tests have also been specifically developed for zebrafish to better characterize drug reinforcing properties. Recently, interesting studies have further highlighted zebrafish relevance as a translational model to identify genes involved in nicotine addiction and smoking behaviour in humans. Authors have discovered a crucial role for *slit3* by using a zebrafish mutant defective for this gene that is unable to develop nicotine preference. It has been demonstrated that this gene is also fundamental for nicotine addiction in humans [83]. Importantly, mutational and screening methodologies have been designed for describing new genes that might be involved in drug addiction [82, 83].

CPP remains so far the most studied behavioral task adequate to evaluate drugs of abuse reward in zebrafish. On the contrary, self-administration of drugs, widely used with rodents, has not been extensively developed for studies dealing with drug reward in zebrafish. There is only one compelling study that suggests that zebrafish can be effectively used to investigate drug addiction by self-administration [84]. CPP is a classical conditioning task in which an animal is taught to associate two *a priori* unrelated stimuli, that is, an environment with a rewarding drug. After repeated exposures to a drug in a particular environment, a single exposure to the

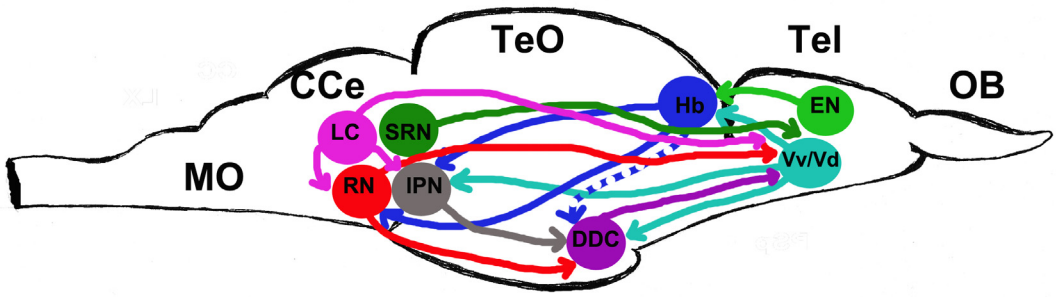


**Fig. (2).** Schematic sagittal view of the zebrafish brain depicting nuclei and pathways of the mesolimbic reward circuitry in zebrafish. The main structures of the zebrafish brain are also labelled. Different neurotransmitter pathways of the reward circuitry are colour-coded; 1) acetylcholine: dark green, 2) dopamine: purple & cyan, 3) glutamate: blue, 4) GABA: gray & light green, 5) norepinephrine: pink, and 6) serotonin: red; Dotted lines represent presumed connectivity.
**Abbreviations:** DDC: dopaminergic diencephalic cluster, EN: entopeduncular nucleus, IPN: interpeduncular nucleus, Hb: habenula, LC: locus coeruleus, RN: raphe nucleus, SRN: superior reticular nucleus, Vd: dorsal nucleus of the ventral telencephalic area, Vv: ventral nucleus of ventral telencephalic area; Zebrafish brain main areas: MO: medulla oblongata, CCe: corpus cerebelli, TeO: tectum opticum, Tel: telencephalon, OB: olfactory bulb; The drawing was based and modified from a study [149]. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

environment without the drug generates an animal´s response of drug-seeking [85-88].

CPP to nicotine has been well characterized in zebrafish, which have demonstrated higher CPP scores than the ones observed in rodents [89-93]. It has been reported that the CPP task can be studied in zebrafish as a translational research model for predicting human behaviour regarding drug abuse [88, 91]. Moreover, zebrafish offer the possibility of dissolving drugs of abuse such as nicotine, caffeine and opiates in the fish tank water. This experimental approach reduces stress and animal manipulation to a minimum by avoiding drug injections. Moreover, in the case of studying nicotine preference, in contrast to rodents, zebrafish can be tested with a broader range of doses without causing aversive effects [90, 92, 94, 95].

## 3. EPIGENETIC CHANGES INDUCED BY NICOTINE EXPOSURE

A growing number of studies suggest that epigenetics, that is, the processes controlling the accessibility of regulatory proteins to DNA, is key to understand how addiction develops and perhaps how it can be treated. In fact, HDAC inhibitors (HDACi) are currently under eager investigation because of their potential applicability in treating cancer and several neurological and psychiatric disorders.

## 4. NICOTINE EFFECTS ON THE BRAIN REWARD CIRCUITRY *VIA* HISTONE POST-TRANSLATIONAL MODIFICATIONS

Nicotine causes long-term changes in the mesolimbic reward circuitry [96, 97]; however, molecular, cellular and circuitry mechanisms that involve nicotine preference remain virtually unknown. Exposure to nicotine induces long-term neuronal changes in gene expression controlled by epigenetic mechanisms, such as histone acetylation regulated by HATs and HDACs [5, 81, 96-99]. It has been reported that nicotine has the capacity of facilitating histone H3 and H4 acetylation status by inhibiting HDAC activity in the striatum of mice [5]. According to these studies, the nicotine effect may lead to a hyperacetylated state of histones that favours gene expression. On the other hand, it has also been reported that long-term nicotine exposure decreases H3K14 acetylation at the *brain-derived neurotrophic factor* (*bdnf*) Exon IV promoter region in rats. This effect was prevented by treatment with the HDACi sodium butyrate suggesting that specific HDAC activity can be induced by nicotine self-administration [99]. These findings suggest that nicotine reward also promotes a repressive chromatin structure silencing specific gene transcription.

Eighteen isoforms of HDACs classified in four classes (I, IIa and b, III, and IV) have been identified in mammals [100]. HDAC1 and HDAC2 proteins share more than 80% identity and act together in repressive complexes [101]. We have found that whereas nicotine induces CPP, the HDACi phenylbutyrate (PhB), selective for class I and IIa HDAC isoforms, abrogates nicotine-CPP consolidation in rats. Nicotine-CPP induces HDAC2 expression in the dorsal striatum and NAcc, the main nucleus of the mesolimbic reward pathway of the rat. This effect of nicotine-CPP correlates with the findings dealing with a nicotine-induced decrease in acetylation of the *bdnf* promoter described above [99]. Moreover, inhibiting HDAC activity with PhB causes a reduction of nicotine-seeking behaviours and HDAC2 expression [98]. The HDACi PhB inhibits preference but not aversion for nicotine. Therefore, HDAC inhibition primarily affects the motivational component of nicotine reward but not learning or mnesic components because it did not affect nicotine aversion examined *via* a classical associative learning task [79]. It has been observed that self-administration of cocaine but not of sucrose is affected by inhibiting HDACs, suggesting that HDAC inhibition does not induce a common effect on the reward pathway in rats [102]. On the other hand, nicotine-conditioned rat brains show significant increases of H3K9 acetylation (H3K9ac), suggesting an effect of nicotine reward favouring transcription of specific genes [98].

We have evaluated for the first time HDACi disruptive effect on nicotine-environmental cue associative learning consolidation by using a five-choice conditioned place preference task in zebrafish. To this end, we have used a fish tank radial maze (FTRM) consisting of five arms of different colours [95]. By using this task, nicotine-CPP was robustly induced and consolidated even after testing nicotine-seeking behaviours during three consecutive days with distracting stimuli, such as the opening of novel arms- during testing sessions. As it was later found with a conventional CPP tank of two compartments [93], treatment with PhB during conditioning causes a disruptive effect on the reinforcing properties of nicotine. Nicotine reward in adult zebrafish, examined *via* CPP tasks, is dependent on HDAC activity in neurons of the mesolimbic pathway structures. Relative levels of HDAC1 mRNA (only one paralogue gene for *hdac1* and *hdac2* exists in zebrafish) are increased in nicotine-place conditioned zebrafish. Class III HDAC SIRT1 transcriptional expression is also increased by intermittent day-night nicotine pre-exposure followed by the establishment of a very robust nicotine-induced CPP. In contrast, reduced levels of SIRT1 mRNA are observed when chronic pre-exposure to nicotine or treatment with PhB prevents nicotine-CPP induction in zebrafish [93].

Furthermore, we have observed that H3K9ac is significantly increased by nicotine-induced CPP in the posterior tuberal nucleus (PTN) and the dorsal and ventral nuclei of the ventral telencephalon or subpallium (Vd/Vv) of the adult zebrafish brain (Fig. **2**). PTN and Vd/Vv are the mesolimbic structures considered homologous to the VTA and NAcc, respectively, in the reward pathway of mammals. Repetitive nicotine exposure also increases H3K9ac in the dorsal Hb (dHb) [92], which interconnects with the interpeduncular nucleus that negatively modulates the VTA and NAcc activity [75]. Therefore, our studies indicate that a widespread and static state of acetylation of histones, promoted by inhibiting class I and IIa HDAC activity, abrogates nicotine-induced place preference and associated nicotine-seeking behaviours in zebrafish and rats. Moreover, nicotine reward established via a classical conditioning task in zebrafish and rats can increase the expression of specific types of HDAC, likely causing gene repression. Therefore, nicotine-induced increase in H3K9ac, which promotes specific gene transcription, must be balanced by HDAC1/2 and SIRT1 activity in key nuclei of the reward pathway to promote nicotine-induced long-term neuronal plasticity in the mesolimbic circuitry.

We have also found in rats and zebrafish that cAMP-responsive element protein phosphorylation (pCREB) increases in structures of the reward pathway immediately after the establishment of nicotine-CPP. Therefore, pCREB could be critical to induce brain changes related to nicotine reward-environmental cue associations [93, 97, 98]. Treatment with PhB during conditioning has no effect on pCREB levels observed in the majority of the structures of the reward pathway, suggesting that the increment in pCREB is independent or upstream of the nicotine reward effect on histone acetylation. However, HDACi treatment reverses the effect of nicotine-CPP on pCREB levels in the IPN and dHb, suggesting that these two areas of the mesolimbic circuitry are relevant for the inhibitory effect of the HDACi PhB on nicotine reward in zebrafish [97].

The observed increase in pCREB induced by nicotine-CPP might suggest the recruitment of CREB binding protein (CBP) to form pCREB-CBP complexes that bind to cAMP response elements (CRE) in promoter regions. CBP is a HAT and acetylates N-terminal ends of nearby histones, such as H3K9, to promote euchromatin formation and transcription [103]. Intermittent day-night nicotine pre-treatment, which potentiates nicotine-CPP consolidation, promotes the transcriptional expression of CBP in the brain portions containing the reward circuit structures of zebrafish [97].

Mechanisms controlling HDAC activation have not yet been elucidated. Several Class II HDACs are activated *via* kinase phosphorylation, including $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and cyclin-dependent protein kinase 5 (CDK5) [104, 105]. It is tempting to suggest that nicotine repetitive exposure provokes brain effects *via* nAChR that may involve kinase activation and phosphorylation of several targets, such as CREB protein and HDACs, in neurons of the mesolimbic pathway. Moreover, nicotine induction of CREB phosphorylation and CBP expression suggests pCREB-CBP complex formation, which in turn produces H3K9 acetylation promoting specific gene expression in cells of the reward nuclei of rat and zebrafish brains. In this regard, nicotine-activated nAChR induce the expression of transcription factors *via* CREB phosphorylation, such as the immediate early genes *c-fos* and *egr-1* regulating mechanisms of plasticity in neuronal circuits [106, 107].

Intermittent exposure to nicotine followed by short periods of abstinence in zebrafish significantly increases the reinforcing properties of nicotine. The persistence of nicotine effects on the reward circuit is likely mediated by changes in HDAC1 and SIRT1 expression, CREB phosphorylation and CBP transcriptional induction, regulating H3K9 acetylation and α6 and α7 nAChR subunit upregulation [93, 97]. Epigenetic mechanisms induced by nicotine preference also involve changes in the expression of the enzymes that regulate DNA methylation/demethylation and the induction of HDAC1-Sin3A-MeCP2 complex formation, which represses gene expression [108], as it has been observed in the reward circuit of rats [98]. Interestingly, treatment with the HDACi PhB abolishes nicotine-induced CPP and drug-seeking behaviours, whereas it completely abrogates HDAC1 and MeCP2 transcriptional expression in the zebrafish brain [97].

In conclusion, a bias in the acetylated state of various histone isoforms induced by a non-selective HDACi might disrupt a dynamic regulation of histone acetylation/deacetylation by specific signalling systems, such as the one mediated by nAChR in the dopamine mesolimbic circuitry, which is necessary to establishing nicotine-seeking behaviours and nicotine-induced priming for the dependence of other drugs of abuse [81].

## 5. NICOTINE AND HISTONE DEACETYLASE EFFECTS ON OBJECT PREFERENCE IN ZEBRAFISH

As it was previously described, zebrafish can learn to associate unconditioned and conditioned stimuli to produce a conditioned response reinforced by nicotine [90, 92-95]. The HDACi PhB prevents this association between stimuli and the locomotor response induced by nicotine during the CPP task in rats and zebrafish [90, 95, 97]. Therefore, taking into

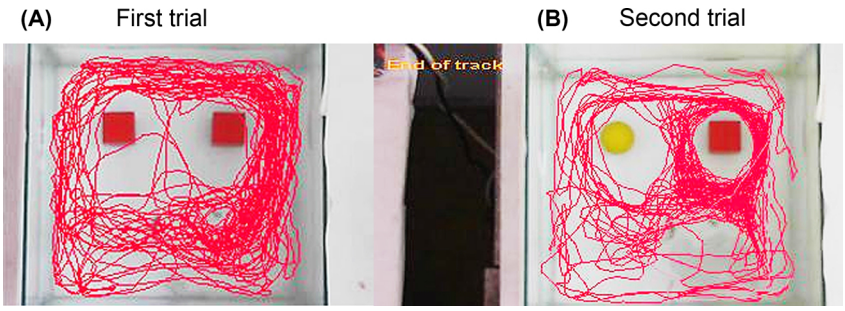


**Fig. (3).** Picture representing the whole swimming trajectory (red tangle) of one zebrafish exploring for 5 minutes two objects submerged in the tank. (A) In the first trial and after three days of habituation to the tank, the zebrafish was placed back in the tank and explored for the first time two identical objects, in this case, red cubes. Before the second trial, one of the familiar objects was replaced by a novel object of different colours and shapes (in this case, a yellow ball was introduced in the tank). (B) After a short (90 min) or long (24 h) interval, the zebrafish was introduced in the tank and the locomotor activity of the zebrafish exploring the familiar and novel object was recorded. Zebrafish in different groups were treated with free-drug water, nicotine or/and the HDAC inhibitor phenylbutyrate (PhB) for 10 min immediately after the first trial was concluded. Zebrafish spent more time exploring the familiar red object, showing neophobia and preference for red over yellow objects of the same or different shapes. Nicotine in the short term and PhB in the long term significantly improved zebrafish awareness and recognition memory of familiar *vs.* novel objects [110]. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

account zebrafish's ability for associative learning, zebrafish exploratory behaviour in an object preference task has been examined to analyze the effect of nicotine and HDACi on recognition memory consolidation (Fig. **3**). Nicotine significantly enhances the exploration of a novel or familiar object by zebrafish when novel object preference was examined [109-111]. Size and colour are object features instrumental for zebrafish recognition memory. It has also been observed that acute administration of nicotine improves short-term but not long-term recognition memory for novel objects in zebrafish [110].

Memory consolidation in rodents is prevented by histone deacetylation regulated by HDAC activity [112, 113]. Taking into account that the HDACi sodium butyrate has been used to modify memory consolidation and potentiates long-term object recognition memory in rodents [114-116], we have assessed the effect of the HDACi PhB on recognition memory for novel objects in zebrafish [110]. Therefore, PhB effect on short- and long-term memory in the adult zebrafish was examined for the first time. It has been observed that the inhibition of HDACs with PhB enhanced long-term novel object recognition memory in zebrafish without affecting this type of memory when it was assessed in the short term. These findings, as in mice, support that epigenetic mechanisms involving a high degree of histone acetylation that promotes transcription are instrumental in consolidating and prolonging recognition memory for objects.

Another important consideration regarding findings dealing with nicotine and the HDACi PhB effects on object preference in zebrafish suggests that acute treatment with nicotine and PhB likely causes short- and long-term changes, respectively, in the activity of neuronal circuits in the brain involved in attention and recognition of visual cues. These neuronal activity changes induced by nicotine and an enhanced degree of histone acetylation might be accountable for improving spatial awareness and perception of colour, form and size of objects by zebrafish. Nicotine and HDACi in the brain significantly modify in the short and long term, respectively, locomotor activity and sensory interactions of adult zebrafish with environmental cues. Therefore, apparently, nicotine and HDACi can stimulate attention and improve discrimination of the visual system helping for object and context recognition memory in zebrafish [110].

## 6. NICOTINE EFFECT ON HISTONE METHYLATION

Drugs of abuse can also directly regulate histone methylation. Histone methylation can induce or repress gene expression and methylation effects are context-dependent [117]. For example, histone H3 methylation at lysine 9 (H3K9me3) was found to be reduced in the NAcc after chronic cocaine administration [118]. This process is mediated by cocaine-induced downregulation of specific histone methyltransferases (HMT), G9a and GLP, which catalyze H3K9me3 formation [119]. Nicotine self-administration in rodents significantly decreases methylation at H3K27me3 and H3K9me2 sites in the promoter regions of *bdnf* and *cyclin-dependent kinase 5* (*cdk-5*) [99, 120]. Acute treatment with nicotine reduces H3K9me2 levels and transcriptional expression of HMTs, and also decreases H3K9me2 specific binding to *bdnf* promoter in primary cultures of mouse cortical neurons and human lymphocytes [121]. Methylation of residues K27 and K9 of the H3 induces heterochromatin formation and represses gene expression [50, 57, 122]. Therefore, nicotine reward likely promotes transcription of these genes in the rodent brain.

L-methionine (L-met), which increases methionine availability, favours methylation of histones and DNA [123]. L-met supplementation was assessed on a nicotine-CPP task in zebrafish [124]. Three exposures to a low dose of L-met orally administered during conditioning are sufficient to abrogate nicotine reward effects on the zebrafish brain as well as several nicotine preference-associated locomotor behaviours. Interestingly, L-met treatment also causes an ansiolytic-like behaviour in zebrafish. DNA methylation effects are discussed in the next section, therefore, we focus herein on the effect of nicotine reward on histone methylation. Nicotine exposure during conditioning significantly decreases

H3K9me3 in the PTN and Vd/Vv (Fig. **2**), which, as aforementioned, are two main structures in the reward pathway of zebrafish [124]. L-met supplementation blocks nicotine-induced CREB phosphorylation and significantly increases H3K9me3 in five key brain regions responsible for drug-seeking and reward in nicotine-place conditioned zebrafish. Moreover, relative expression levels of two HMT (G9a and SuV39H) mRNA are downregulated by nicotine preference in mesolimbic areas of the zebrafish brain. In opposition, treatment with L-met during nicotine conditioning increases the transcriptional expression of the two HMTs. Therefore, a decrease in histone methylation in the brain areas associated with in drug reward associated areas might contribute addictive behaviours and underlie restatement [44].

## 7. NICOTINE AND DNA METHYLATION

DNA methylation simultaneously with post-translational modifications of histones is a key epigenetic mechanism involved in the control of gene expression. However, little is known concerning the regulation of addiction-like behaviors by DNA methylation.

DNMTs catalyze a reaction that adds a methyl group to the carbon-5 position of a cytosine (5mC), which is principally observed at CpG sites [28]. DNMT1 maintains methylated patterns in inherited strands of DNA through cell divisions, whereas DNMT3 catalyzes *de novo* DNA methylation. The presence of 5mC attracts methyl-CpG-binding domain proteins (MBD1-MBD4, MeCP2, and Kaiso among others), which recruit repressor complexes [28, 62, 125] and HDACs [126]. These methylated DNA binding proteins are known to be responsible for repressing gene transcription [127, 128].

Reversion of DNA methylation is regulated by a family of ten-eleven translocation (TET) methylcytosine dioxygenases that oxidize 5mC to 5-hydroxymethylcytosine (5hmC) [38]. In turn, TET enzymes can oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). 5fC and 5caC can be removed through a base excision repair mechanism of damaged DNA to produce plain cytosine [39, 129, 130]. Gadd45a regulates epigenetic gene activation by catalyzing repair-mediated DNA demethylation [131].

Looking for compounds that can reduce the rewarding properties of drugs of abuse, we and others focus attention on DNA methylation, hypothesizing that epigenetic regulation of gene transcription mediated by DNA methylation could be accountable for the neuronal changes induced by addictive drugs.

Cocaine induces global DNA hypomethylation and chronically administered L-met inhibits cocaine-CPP in mice [132]. It has also been observed that chronic exposure to L-met reduces the behavioural sensitization to cocaine and addictive-like behaviours *via* DNA methylation-dependent processes in rodents [133]. At the molecular level, methionine reverses hypomethylation induced by cocaine at CpG islands in the *c-fos* promoter. Repeated administration of nicotine for four days downregulates the expression of DNMT1 and prevents methionine-induced hypermethylation of glutamic acid decarboxylase 67 promoter, increasing its expression in GABAergic interneurons in the frontal cortex and hippocampus of mice [80].

It has been reported that brain levels and tissue distribution of 5mC and 5hmC are comparable between zebrafish and mammals. All DNMTs, as well as the majority of demethylation pathways, are highly conserved [134].

We have first observed, in zebrafish, that relative levels of DNMT1, DNMT3, TET1 and Gadd45 mRNA are significantly modified by chronic pre-exposure to nicotine for 14 days before nicotine-CPP in portions of the zebrafish brain containing the structures of the dopamine reward pathway [97]. The transcriptional expression of DNMT3 is increased, whereas levels of DNMT1 mRNA are reduced by chronic exposure to nicotine, which prevents nicotine-place preference consolidation. In opposition, DNMT1 transcriptional expression is significantly enhanced, whereas levels of DNMT3 mRNA are decreased in zebrafish treated with nicotine during daylight only, which mimics exposure intervals in smokers and induces strong consolidation of nicotine-place preference. Therefore, it seems that *de novo* DNA methylation mechanisms are sinhibited, whereas maintenance methylation is intensified in zebrafish exposed to a day-night nicotine cycle that potentiates the rewarding properties of nicotine. Moreover, DNA demethylating enzymes TET1 and Gadd45 were highly expressed in zebrafish pre-exposed to chronic and intermittent day/night-nicotine. These pieces of evidence point out a dynamic process of DNA methylation maintenance by DNMT1 and demethylation regulated by TET1 and Gadd45. The findings suggest that nicotine reward consolidation also requires hypomethylated states, which are attained by inhibiting *de novo* methylation of DNA and promoting demethylation of specific genes.

Treatment with the HDACi PhB strongly induces the transcriptional expression of DNMT1, suggesting that a global state of histone acetylation causes DNMT1 gene expression induction. These findings also suggest that upregulation of DNMT1 expression is not sufficient for generating molecular changes in neurons of the mesolimbic circuit that lead to nicotine reward, but likely these molecular changes must be balanced by nicotine-induced regulation of HDAC activity. Treatment with PhB shows no effect on the transcriptional expression of TET1. Therefore, a further increase in DNMT1 transcriptional expression might be responsible for a high degree of gene methylation that, together with a high level of unregulated histone acetylation, prevents nicotine preference consolidation [97].

Furthermore, treatment for three days with a low dose of L-met completely abrogates nicotine-induced CPP and seeking behaviours associated with nicotine reward in zebrafish [124]. Nicotine-CPP inhibition caused by L-met correlates with transcriptional upregulation of DNMT1 and DNMT3. Moreover, L-met supplementation significantly reduces transcriptional expression of TET1 demethylase in the brain portions containing the structures of the reward circuitry of nicotine-conditioned zebrafish. Changes in mRNA levels of demethylases and methylases are accompanied by an increase in the percentage of cells immunopositive for 5mC and H3K9me3 in structures of the reward pathway.

Nicotine-CPP appears to have an opposite effect to treatment with L-met since nicotine-CPP consolidation correlates with a significant reduction of DNMT3 transcriptional expression, which catalyzes *de novo* methylation of DNA

strands, whereas the expression of TET1 demethylase increases. As aforementioned, nicotine-CPP correlates with HMT expression decrease.

Repeated administration of nicotine upregulates nAChR subunits [11, 135]. The upregulation of α6 and α7 but not β2 nAChR subunits is involved in nicotine-induced neuronal adaptations after nicotine-CPP in zebrafish [89, 90, 93, 124]. The HDACi PhB treatment during conditioning abolishes α6 and α7 nAChR subunit upregulation and nicotine-CPP.

Glutamatergic NMDAR1 is also upregulated after nicotine-CPP consolidation. Treatment with L-met during nicotine-place conditioning abrogates transcriptional upregulation of α6 and α7 nAChR subunits and NMDAR1. The disruptive effect on CPP given by L-met administration could be, at least in part, mediated by inhibiting α6 and α7 nAChR subunits and NMDAR1 upregulation.

Treatment with the DNMT inhibitor, decitabine, or 5-Aza-2′-deoxycytidine (AZA), during nicotine-place conditioning causes the opposite effect to L-met by increasing nicotine-CPP scores in zebrafish [124]. Similar findings have been described in rats using cocaine-induced CPP [136]. Moreover, treatment with AZA slightly potentiates the effect of nicotine-CPP on α6 and α7 nAChR subunit and NMDAR1 transcriptional expression. It has also been observed that AZA treatment reduces the transcriptional expression of enzymes related to the methylation of DNA and histones [124].

The observed findings indicate that an increase in global methylation is detrimental to the establishment of the nicotine rewarding properties in the mesolimbic circuitry of zebrafish. In contrast, global DNA hypomethylation by AZA facilitates nicotine reward.

By using the methylation-specific PCR technique (MSP) [137, 138], it has been found that nicotine-CPP in zebrafish induces hypomethylation in promoter regions of specific genes. Nicotine-CPP produces hypomethylation in the promoter region of the nAChR subunit and NMDAR1 genes [124]. This epigenetic mechanism likely underlies the upregulation of these neurotransmitter receptor mRNAs induced by nicotine-CPP in zebrafish reward circuitry nuclei.

In contrast, treatment with L-met increases the number of copies of the methylated (transcriptionally silenced) form of the α7 nAChR subunit and NMDAR1 genes. Treatment with AZA in nicotine-conditioned zebrafish, as it has been observed with nicotine-conditioned animals in the absence of the DNMT inhibitor, increases the number of copies of the hypomethylated (transcriptionally permissive) form of these neurotransmitter receptor subunits, indicating that epigenetic regulation of expression of these genes plays a preponderant role in codifying the reinforcing properties of nicotine. Furthermore, L-met and AZA effects on the nicotine-CPP score are, at least in part, given by their ability to epigenetically regulate the expression of nAChR subunits and NMDAR1.

Methionine exposure reduces glutamate uptake and extracellular ATP catabolism in zebrafish telencephalon, dangerously increasing both neurotransmitters levels in the synaptic cleft [139]. Methionine was chronically administered to reduce cocaine-CPP in mice; however, it has been observed that long-term methionine exposure can provoke toxicity in humans and Alzheimer´s disease-like memory deficiency in mice [140-142]. Although it has been suggested that DNA methylation induces only long-term effects on gene expression [133], we have found that three days of L-met administration twenty minutes before conditioning are sufficient to abolish nicotine-induced CPP consolidation in zebrafish, suggesting that DNA methylation also triggers short-term neuroadaptations in the reward mesolimbic system of vertebrate [124].

## 8. EXPERIMENTAL AND CLINICAL TRIALS CARRIED OUT WITH SODIUM-4-PHENYLBUTYRATE, 5-AZA-2′-DEOXYCYTIDINE, AND L-METHIONINE

Several experimental studies in animal models of human disease as well as clinical trials indicate an important role for histone deacetylase inhibitors in treating human diseases. PhB has been approved by the US Food and Drug Administration (FDA) to treat urea cycle disorders. Clinical trials and experimental studies have indicated the potential benefit of using PhB for treating different types of cancer, cystic fibrosis, hemoglobinopathies, neurodegenerative and motor neuron disorders, such as Huntington´s and Parkinson´s disease and retinal and hepatic ischemia [56, 143]. Clinical trials have demonstrated significant amelioration of amyotrophic lateral sclerosis symptoms by using PhB in combination with other drugs [144]. Translational studies in mice have suggested a potential role of PhB for treating genetic myopathies and stroke [145]. DNA methylation and histone acetylation play a key role in the epigenetics of atherosclerosis [146]. Moreover, clinical trials have examined the combined and sequential action of demethylating agents, AZA and HDACi, for fighting HIV/AIDS [147]. Decitabine or AZA has also been approved by the FDA and is indicated as an antineoplastic agent for the treatment of patients with myelodysplastic syndromes (MDS) [148].

The effects of PhB are not only due to its function as an HDACi. PhB also plays a role as an ammonia sink and chemical chaperone [145]. Therefore, many more basic and translational studies need to be conducted to get insight into molecular mechanisms that mediate PhB, AZA and L-met effects on drug addiction [26, 46, 79, 98, 99, 124, 132, 149]. Regarding epigenetic effects, PhB and L-met likely act by inducing histone highly acetylated states and DNA and histone methylation, respectively, which completely abrogate nicotine-reward. It is likely that these compounds prevent gene expression regulation-driven, in nuclei of the reward circuitry, by drug of abuse repeated intake and drug-environment associations during habit acquisition for drug consumption. PhB is an approved pharmacological agent; it can exert potent metabolic and epigenetic actions on gene expression and stimulatory effects on long-term memory. A short treatment with L-met at low doses, avoiding toxicity issues, has also proved to prevent nicotine reinforcement. All of these features make them very interesting and powerful drugs to potentially treat drug addicts. This ultimate purpose will require conducting further experimental, and translational studies of drug reward and eventually clinical trials.

## CONCLUSION

The study of epigenetics involved in nicotine reward in the zebrafish model gives insight into the molecular mecha-

nisms that participate in establishing stimuli (drug)-environmental cues (exteroceptive sensitivity) associations during a learning experience (CPP). Our studies and studies of others have demonstrated a crucial role of histone acetylation/deacetylation and methylation/demethylation and DNA demethylation/hypomethylation occurring in neurons of the reward circuitry and interrelated structures when an individual becomes addicted to drugs.

Nicotine seeking is modulated by histone acetylation, since a class I and IIa HDAC inhibitor abolishes nicotine reward as well as nicotine-induced nAChR subunit upregulation, and is linked to changes in the expression of HDACs and HATs, together with increased CREB phosphorylation, in addition to other epigenetic factors that bind to DNA, allowing the transcription of specific genes. Nicotine induction of CREB phosphorylation, necessary to establish nicotine reward, is abrogated by global histone acetylation and methylation and DNA methylation. Treatment of zebrafish with HDAC inhibitors improves long-term but not short-term memory, suggesting a complex interaction between memory and generation and maintenance of drug addiction, probably mediated by histone acetylation. Moreover, nicotine effects and nicotine-CPP establishment are also associated to changes in HMT, DNMT and DNA-demethylase transcriptional expression. Nicotine reward is likely associated to demethylation of histone 3, which when highly methylated induces chromatin condensation and transcription blockade. Nicotine-induced changes in the brain structures of the reward pathway of zebrafish involve histone post-translational modifications that modulate specific transcription of genes. Some of these genes, such as nAChR and NMDAR subunits, are directly involved in nicotine effects on brain neural activity. A similar scenario was observed with DNA methylation in which administration of L-met and a DNMT inhibitor, respectively, decreases or increases the effectiveness of nicotine to induce seeking behaviours by modifying the expression of α6 and α7 nAChR and NMDA1R subunits.

Therefore, epigenetic mechanisms involving histone and DNA demethylation and histone deacetylation in the brain areas associated with nicotine reward might contribute to trigger and establish addictive behaviours and restatement. Epigenetic modulation that occurs in the brain during reward establishment can be pharmacologically interfered with to abrogate neuronal plastic changes induced by exteroceptive and interoceptive associations that lead to drug addiction.

## CONSENT FOR PUBLICATION

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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## REFERENCES

[1] Ahluwalia, I.B.; Arrazola, R.A.; Zhao, L.; Shi, J.; Dean, A.; Rainey, E.; Palipudi, K.; Twentyman, E.; Armour, B.S. Tobacco Use and Tobacco-Related Behaviors - 11 Countries, 2008-2017. *MMWR Morb. Mortal. Wkly. Rep.,* **2019**, *68*(41), 928-933. http: //dx.doi.org/10.15585/mmwr.mm6841a1 PMID: 31622286

[2] World Health Organization. WHO report on the global tobacco epidemic, 2017: monitoring tobacco use and prevention policies. Geneva, Switzerland: World Health Organization. **2017**. Available from: https://apps.who.int/iris/bitstream/handle/10665/255874/9789241512824-eng.pdf;jsessionid=5B12F0106C9C5146FD02389C555F41F2?sequence=1external

[3] Benowitz, N.L. Nicotine addiction. *N. Engl. J. Med.,* **2010**, *362*(24), 2295-2303. http: //dx.doi.org/10.1056/NEJMra0809890 PMID: 20554984

[4] Keyes, K.M.; Hamilton, A.; Kandel, D.B. Birth cohorts analysis of adolescent cigarette smoking and subsequent marijuana and cocaine use. *Am. J. Public Health,* **2016**, *106*(6), 1143-1149. http: //dx.doi.org/10.2105/AJPH.2016.303128 PMID: 27077359

[5] Levine, A.; Huang, Y.; Drisaldi, B.; Griffin, E.A., Jr; Pollak, D.D.; Xu, S.; Yin, D.; Schaffran, C.; Kandel, D.B.; Kandel, E.R. Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. *Sci. Transl. Med.,* **2011**, *3*(107), 107ra109. http: //dx.doi.org/10.1126/scitranslmed.3003062 PMID: 22049069

[6] Mehra, V.M.; Keethakumar, A.; Bohr, Y.M.; Abdullah, P.; Tamim, H. The association between alcohol, marijuana, illegal drug use and current use of E-cigarette among youth and young adults in Canada: results from Canadian Tobacco, Alcohol and Drugs Survey 2017. *BMC Public Health,* **2019**, *19*(1), 1208. http: //dx.doi.org/10.1186/s12889-019-7546-y PMID: 31477067

[7] Papke, R.L. Merging old and new perspectives on nicotinic acetylcholine receptors. *Biochem. Pharmacol.,* **2014**, *89*(1), 1-11. http: //dx.doi.org/10.1016/j.bcp.2014.01.029 PMID: 24486571

[8] Henderson, B.J.; Lester, H.A. Inside-out neuropharmacology of nicotinic drugs. *Neuropharmacology,* **2015**, *96*(Pt B), 178-93. http: //dx.doi.org/10.1016/j.neuropharm.2015.01.022

[9] Nashmi, R.; Lester, H.A. CNS localization of neuronal nicotinic receptors. *J. Mol. Neurosci.,* **2006**, *30*(1-2), 181-184. http: //dx.doi.org/10.1385/JMN:30:1:181 PMID: 17192671

[10] Leslie, F.M.; Mojica, C.Y.; Reynaga, D.D. Nicotinic receptors in addiction pathways. *Mol. Pharmacol.,* **2013**, *83*(4), 753-758. http: //dx.doi.org/10.1124/mol.112.083659 PMID: 23247824

[11] Changeux, J.P. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat. Rev. Neurosci.,* **2010**, *11*(6), 389-401. http: //dx.doi.org/10.1038/nrn2849 PMID: 20485364

[12] Bühler, K.M.; Giné, E.; Echeverry-Alzate, V.; Calleja-Conde, J.; de Fonseca, F.R.; López-Moreno, J.A. Common single nucleotide variants underlying drug addiction: more than a decade of research. *Addict. Biol.,* **2015**, *20*(5), 845-871. http: //dx.doi.org/10.1111/adb.12204 PMID: 25603899

[13] Egervari, G.; Ciccocioppo, R.; Jentsch, J.D.; Hurd, Y.L. Shaping vulnerability to addiction - the contribution of behavior, neural circuits and molecular mechanisms. *Neurosci. Biobehav. Rev.,* **2018**, *85*, 117-125. http: //dx.doi.org/10.1016/j.neubiorev.2017.05.019 PMID: 28571877

[14] Morrow, J.D.; Flagel, S.B. Neuroscience of resilience and vulnerability for addiction medicine: From genes to behavior. *Prog. Brain Res.,* **2016**, *223*, 3-18. http: //dx.doi.org/10.1016/bs.pbr.2015.09.004 PMID: 26806768

[15] Ersche, K.D.; Meng, C.; Ziauddeen, H.; Stochl, J.; Williams, G.B.; Bullmore, E.T.; Robbins, T.W. Brain networks underlying vulnerability and resilience to drug addiction. *Proc. Natl. Acad. Sci. USA,* **2020**, *117*(26), 15253-15261. http: //dx.doi.org/10.1073/pnas.2002509117 PMID: 32541059

[16] Amir, R.E.; Van den Veyver, I.B.; Wan, M.; Tran, C.Q.; Francke, U.; Zoghbi, H.Y. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.,* **1999**, *23*(2), 185-188. http: //dx.doi.org/10.1038/13810 PMID: 10508514

[17] Takiguchi, M.; Achanzar, W.E.; Qu, W.; Li, G.; Waalkes, M.P. Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp. Cell Res.,* **2003**, *286*(2), 355-365. http: //dx.doi.org/10.1016/S0014-4827(03)00062-4 PMID: 12749863

[18] Yan, Y.; Kluz, T.; Zhang, P.; Chen, H.B.; Costa, M. Analysis of specific lysine histone H3 and H4 acetylation and methylation status in clones of cells with a gene silenced by nickel exposure. *Toxicol. Appl. Pharmacol.,* **2003**, *190*(3), 272-277. http: //dx.doi.org/10.1016/S0041-008X(03)00169-8 PMID: 12902198

[19] Hande, M.P.; Azizova, T.V.; Geard, C.R.; Burak, L.E.; Mitchell, C.R.; Khokhryakov, V.F.; Vasilenko, E.K.; Brenner, D.J. Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome. *Am. J. Hum. Genet.,* **2003**, *72*(5), 1162-1170. http: //dx.doi.org/10.1086/375041 PMID: 12679897

[20] Levine, A.A.; Guan, Z.; Barco, A.; Xu, S.; Kandel, E.R.; Schwartz, J.H. CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*(52), 19186-19191. http: //dx.doi.org/10.1073/pnas.0509735102 PMID: 16380431

[21] Hwang, J.Y.; Aromolaran, K.A.; Zukin, R.S. The emerging field of epigenetics in neurodegeneration and neuroprotection. *Nat. Rev. Neurosci.,* **2017**, *18*(6), 347-361. http: //dx.doi.org/10.1038/nrn.2017.46 PMID: 28515491

[22] Qureshi, I.A.; Mehler, M.F. Epigenetic mechanisms underlying nervous system diseases. *Handb. Clin. Neurol.,* **2018**, *147*, 43-58. http: //dx.doi.org/10.1016/B978-0-444-63233-3.00005-1 PMID: 29325627

[23] Renthal, W.; Kumar, A.; Xiao, G.; Wilkinson, M.; Covington, H.E., III; Maze, I.; Sikder, D.; Robison, A.J.; LaPlant, Q.; Dietz, D.M.; Russo, S.J.; Vialou, V.; Chakravarty, S.; Kodadek, T.J.; Stack, A.; Kabbaj, M.; Nestler, E.J. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron,* **2009**, *62*(3), 335-348. http: //dx.doi.org/10.1016/j.neuron.2009.03.026 PMID: 19447090

[24] Nestler, E.J.; Lüscher, C. The molecular basis of drug addiction: linking epigenetic to synaptic and circuit mechanisms. *Neuron,* **2019**, *102*(1), 48-59. http: //dx.doi.org/10.1016/j.neuron.2019.01.016 PMID: 30946825

[25] Walker, D.M.; Nestler, E.J. Neuroepigenetics and addiction. *Handb. Clin. Neurol.,* **2018**, *148*, 747-765. http: //dx.doi.org/10.1016/B978-0-444-64076-5.00048-X PMID: 29478612

[26] Hamilton, P.J.; Nestler, E.J. Epigenetics and addiction. *Curr. Opin. Neurobiol.,* **2019**, *59*, 128-136. http: //dx.doi.org/10.1016/j.conb.2019.05.005 PMID: 31255844

[27] Waddington, C. Canalization of development and the inheritance of acquired characters. *Nature,* **1942**, *150*, 563-565. http: //dx.doi.org/10.1038/150563a0

[28] Jaenisch, R.; Bird, A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.,* **2003**, *33*(Suppl.), 245-254. http: //dx.doi.org/10.1038/ng1089 PMID: 12610534

[29] Jiang, Y.; Langley, B.; Lubin, F.D.; Renthal, W.; Wood, M.A.; Yasui, D.H.; Kumar, A.; Nestler, E.J.; Akbarian, S.; Beckel-Mitchener, A.C. Epigenetics in the nervous system. *J. Neurosci.,* **2008**, *28*(46), 11753-11759. http: //dx.doi.org/10.1523/JNEUROSCI.3797-08.2008 PMID: 19005036

[30] Bernstein, B.E.; Meissner, A.; Lander, E.S. The mammalian epigenome. *Cell,* **2007**, *128*(4), 669-681. http: //dx.doi.org/10.1016/j.cell.2007.01.033 PMID: 17320505

[31] Francis, N.J.; Saurin, A.J.; Shao, Z.; Kingston, R.E. Reconstitution of a functional core polycomb repressive complex. *Mol. Cell,* **2001**, *8*(3), 545-556. http: //dx.doi.org/10.1016/S1097-2765(01)00316-1 PMID: 11583617

[32] Di Croce, L.; Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat. Struct. Mol. Biol.,* **2013**, *20*(10), 1147-1155. http: //dx.doi.org/10.1038/nsmb.2669 PMID: 24096405

[33] Christmann, M.; Verbeek, B.; Roos, W.P.; Kaina, B. O(6)-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: enzyme activity, promoter methylation and immunohistochemistry. *Biochim. Biophys. Acta,* **2011**, *1816*(2), 179-190. http: //dx.doi.org/10.1016/j.bbcan.2011.06.002 PMID: 21745538

[34] O'Brown, Z.K.; Greer, E.L. N6-methyladenine: a conserved and dynamic DNA mark. *Adv. Exp. Med. Biol.,* **2016**, *945*, 213-246. http: //dx.doi.org/10.1007/978-3-319-43624-1_10 PMID: 27826841

[35] Beh, L.Y.; Debelouchina, G.T.; Clay, D.M.; Thompson, R.E.; Lindblad, K.A.; Hutton, E.R.; Bracht, J.R.; Sebra, R.P.; Muir, T.W.; Landweber, L.F. Identification of a DNA N6-adenine methyltransferase complex and its impact on chromatin organization. *Cell,* **2019**, *177*(7), 1781-1796.e25. http: //dx.doi.org/10.1016/j.cell.2019.04.028 PMID: 31104845

[36] Yue, Y.; Liu, J.; He, C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. *Genes Dev.,* **2015**, *29*(13), 1343-1355. http: //dx.doi.org/10.1101/gad.262766.115 PMID: 26159994

[37] Lorsbach, R.B.; Moore, J.; Mathew, S.; Raimondi, S.C.; Mukatira, S.T.; Downing, J.R. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). *Leukemia,* **2003**, *17*(3), 637-641. http: //dx.doi.org/10.1038/sj.leu.2402834 PMID: 12646957

[38] Tahiliani, M.; Koh, K.P.; Shen, Y.; Pastor, W.A.; Bandukwala, H.; Brudno, Y.; Agarwal, S.; Iyer, L.M.; Liu, D.R.; Aravind, L.; Rao, A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science,* **2009**, *324*(5929), 930-935. http: //dx.doi.org/10.1126/science.1170116 PMID: 19372391

[39] Ito, S.; Shen, L.; Dai, Q.; Wu, S.C.; Collins, L.B.; Swenberg, J.A.; He, C.; Zhang, Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science,* **2011**, *333*(6047), 1300-1303. http: //dx.doi.org/10.1126/science.1210597 PMID: 21778364

[40] Antunes, C.; Sousa, N.; Pinto, L.; Marques, C.J. TET enzymes in neurophysiology and brain function. *Neurosci. Biobehav. Rev.,* **2019**, *102*, 337-344. http: //dx.doi.org/10.1016/j.neubiorev.2019.05.006 PMID: 31078565

[41] Lawrence, M.; Daujat, S.; Schneider, R. Lateral thinking: how histone modifications regulate gene expression. *Trends Genet.,* **2016**, *32*(1), 42-56. http: //dx.doi.org/10.1016/j.tig.2015.10.007 PMID: 26704082

[42] Tolsma, T.O.; Hansen, J.C. Post-translational modifications and chromatin dynamics. *Essays Biochem.,* **2019**, *63*(1), 89-96. http: //dx.doi.org/10.1042/EBC20180067 PMID: 31015385

[43] Kouzarides, T. Acetylation: a regulatory modification to rival phosphorylation? *EMBO J.,* **2000**, *19*(6), 1176-1179. http: //dx.doi.org/10.1093/emboj/19.6.1176 PMID: 10716917

[44] Kim, H.D.; Call, T.; Magazu, S.; Ferguson, D. Drug addiction and histone code alterations. *Adv. Exp. Med. Biol.,* **2017**, *978*, 127-143. http: //dx.doi.org/10.1007/978-3-319-53889-1_7 PMID: 28523544

[45] Taunton, J.; Hassig, C.A.; Schreiber, S.L. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science,* **1996**, *272*(5260), 408-411. http: //dx.doi.org/10.1126/science.272.5260.408 PMID: 8602529

[46] Schroeder, F.A.; Penta, K.L.; Matevossian, A.; Jones, S.R.; Konradi, C.; Tapper, A.R.; Akbarian, S. Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. *Neuropsychopharmacology,* **2008**, *33*(12), 2981-2992. http: //dx.doi.org/10.1038/npp.2008.15 PMID: 18288092

[47] Shogren-Knaak, M.; Ishii, H.; Sun, J.M.; Pazin, M.J.; Davie, J.R.; Peterson, C.L. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science,* **2006**, *311*(5762), 844-847. http: //dx.doi.org/10.1126/science.1124000 PMID: 16469925

[48] Vaissière, T.; Sawan, C.; Herceg, Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat. Res.,* **2008**, *659*(1-2), 40-48. http: //dx.doi.org/10.1016/j.mrrev.2008.02.004 PMID: 18407786

[49] Wang, H.; Huang, Z.Q.; Xia, L.; Feng, Q.; Erdjument-Bromage, H.; Strahl, B.D.; Briggs, S.D.; Allis, C.D.; Wong, J.; Tempst, P.; Zhang, Y. Methylation of histone H4 at arginine 3 facilitating tran-

scriptional activation by nuclear hormone receptor. *Science,* **2001**, *293*(5531), 853-857.
http: //dx.doi.org/10.1126/science.1060781 PMID: 11387442

[50] Cao, R.; Wang, L.; Wang, H.; Xia, L.; Erdjument-Bromage, H.; Tempst, P.; Jones, R.S.; Zhang, Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science,* **2002**, *298*(5595), 1039-1043.
http: //dx.doi.org/10.1126/science.1076997 PMID: 12351676

[51] Robison, A.J.; Nestler, E.J. Transcriptional and epigenetic mechanisms of addiction. *Nat. Rev. Neurosci.,* **2011**, *12*(11), 623-637.
http: //dx.doi.org/10.1038/nrn3111 PMID: 21989194

[52] Rogge, G.A.; Wood, M.A. The role of histone acetylation in cocaine-induced neural plasticity and behavior. *Neuropsychopharmacology,* **2013**, *38*(1), 94-110.
http: //dx.doi.org/10.1038/npp.2012.154 PMID: 22910457

[53] Botia, B.; Legastelois, R.; Alaux-Cantin, S.; Naassila, M. Expression of ethanol-induced behavioral sensitization is associated with alteration of chromatin remodeling in mice. *PLoS One,* **2012**, *7*(10), e47527.
http: //dx.doi.org/10.1371/journal.pone.0047527 PMID: 23110077

[54] Kumar, A.; Choi, K.H.; Renthal, W.; Tsankova, N.M.; Theobald, D.E.; Truong, H.T.; Russo, S.J.; Laplant, Q.; Sasaki, T.S.; Whistler, K.N.; Neve, R.L.; Self, D.W.; Nestler, E.J. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron,* **2005**, *48*(2), 303-314.
http: //dx.doi.org/10.1016/j.neuron.2005.09.023 PMID: 16242410

[55] Shen, H.Y.; Kalda, A.; Yu, L.; Ferrara, J.; Zhu, J.; Chen, J.F. Additive effects of histone deacetylase inhibitors and amphetamine on histone H4 acetylation, cAMP responsive element binding protein phosphorylation and DeltaFosB expression in the striatum and locomotor sensitization in mice. *Neuroscience,* **2008**, *157*(3), 644-655.
http: //dx.doi.org/10.1016/j.neuroscience.2008.09.019 PMID: 18848971

[56] Kondo, Y. Epigenetic cross-talk between DNA methylation and histone modifications in human cancers. *Yonsei Med. J.,* **2009**, *50*(4), 455-463.
http: //dx.doi.org/10.3349/ymj.2009.50.4.455 PMID: 19718392

[57] Sales-Gil, R.; Vagnarelli, P. How HP1 post-translational modifications regulate heterochromatin formation and maintenance. *Cells,* **2020**, *9*(6), 1460.
http: //dx.doi.org/10.3390/cells9061460 PMID: 32545538

[58] Weinberg, D.N.; Papillon-Cavanagh, S.; Chen, H.; Yue, Y.; Chen, X.; Rajagopalan, K.N.; Horth, C.; McGuire, J.T.; Xu, X.; Nikbakht, H.; Lemiesz, A.E.; Marchione, D.M.; Marunde, M.R.; Meiners, M.J.; Cheek, M.A.; Keogh, M.C.; Bareke, E.; Djedid, A.; Harutyunyan, A.S.; Jabado, N.; Garcia, B.A.; Li, H.; Allis, C.D.; Majewski, J.; Lu, C. The histone mark H3K36me2 recruits DNMT3A and shapes the intergenic DNA methylation landscape. *Nature,* **2019**, *573*(7773), 281-286.
http: //dx.doi.org/10.1038/s41586-019-1534-3 PMID: 31485078

[59] Lomvardas, S.; Maniatis, T. Histone and DNA modifications as regulators of neuronal development and function. *Cold Spring Harb. Perspect. Biol.,* **2016**, *8*(7), a024208.
http: //dx.doi.org/10.1101/cshperspect.a024208 PMID: 27371659

[60] Miller, C.A.; Campbell, S.L.; Sweatt, J.D. DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. *Neurobiol. Learn. Mem.,* **2008**, *89*(4), 599-603.
http: //dx.doi.org/10.1016/j.nlm.2007.07.016 PMID: 17881251

[61] Barrett, R.M.; Wood, M.A. Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. *Learn. Mem.,* **2008**, *15*(7), 460-467.
http: //dx.doi.org/10.1101/lm.917508 PMID: 18583646

[62] Du, J.; Johnson, L.M.; Jacobsen, S.E.; Patel, D.J. DNA methylation pathways and their crosstalk with histone methylation. *Nat. Rev. Mol. Cell Biol.,* **2015**, *16*(9), 519-532.
http: //dx.doi.org/10.1038/nrm4043 PMID: 26296162

[63] Di Chiara, G. Role of dopamine in the behavioural actions of nicotine related to addiction. *Eur. J. Pharmacol.,* **2000**, *393*(1-3), 295-314.
http: //dx.doi.org/10.1016/S0014-2999(00)00122-9 PMID: 10771025

[64] Fagen, Z.M.; Mansvelder, H.D.; Keath, J.R.; McGehee, D.S. Short- and long-term modulation of synaptic inputs to brain reward areas by nicotine. *Ann. N. Y. Acad. Sci.,* **2003**, *1003*, 185-195.
http: //dx.doi.org/10.1196/annals.1300.011 PMID: 14684446

[65] Mansvelder, H.D.; McGehee, D.S. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron,* **2000**, *27*(2), 349-357.
http: //dx.doi.org/10.1016/S0896-6273(00)00042-8 PMID: 10985354

[66] Saal, D.; Dong, Y.; Bonci, A.; Malenka, R.C. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron,* **2003**, *37*(4), 577-582.
http: //dx.doi.org/10.1016/S0896-6273(03)00021-7 PMID: 12597856

[67] Pidoplichko, V.I.; Noguchi, J.; Areola, O.O.; Liang, Y.; Peterson, J.; Zhang, T.; Dani, J.A. Nicotinic cholinergic synaptic mechanisms in the ventral tegmental area contribute to nicotine addiction. *Learn. Mem. (Cold Spring Harb. N.Y.),* **2004**, *11*(1), 60-69.
http: //dx.doi.org/10.1101/lm.70004

[68] Kenny, P.J.; Chartoff, E.; Roberto, M.; Carlezon, W.A., Jr; Markou, A. NMDA receptors regulate nicotine-enhanced brain reward function and intravenous nicotine self-administration: role of the ventral tegmental area and central nucleus of the amygdala. *Neuropsychopharmacology,* **2009**, *34*(2), 266-281.
http: //dx.doi.org/10.1038/npp.2008.58 PMID: 18418357

[69] Mao, D.; Gallagher, K.; McGehee, D.S. Nicotine potentiation of excitatory inputs to ventral tegmental area dopamine neurons. *J. Neurosci.,* **2011**, *31*(18), 6710-6720.
http: //dx.doi.org/10.1523/JNEUROSCI.5671-10.2011 PMID: 21543600

[70] Pistillo, F.; Clementi, F.; Zoli, M.; Gotti, C. Nicotinic, glutamatergic and dopaminergic synaptic transmission and plasticity in the mesocorticolimbic system: focus on nicotine effects. *Prog. Neurobiol.,* **2015**, *124*, 1-27.
http: //dx.doi.org/10.1016/j.pneurobio.2014.10.002 PMID: 25447802

[71] Ostroumov, A.; Dani, J.A. Inhibitory plasticity of mesocorticolimbic circuits in addiction and mental illness. *Trends Neurosci.,* **2018**, *41*(12), 898-910.
http: //dx.doi.org/10.1016/j.tins.2018.07.014 PMID: 30149979

[72] Jackson, K.J.; Muldoon, P.P.; De Biasi, M.; Damaj, M.I. New mechanisms and perspectives in nicotine withdrawal. *Neuropharmacology,* **2015**, *96*(Pt B), 223-234.
http: //dx.doi.org/10.1016/j.neuropharm.2014.11.009

[73] McLaughlin, I.; Dani, J.A.; De Biasi, M. Nicotine withdrawal. *Curr. Top. Behav. Neurosci.,* **2015**, *24*, 99-123.
http: //dx.doi.org/10.1007/978-3-319-13482-6_4 PMID: 25638335

[74] Dao, D.Q.; Perez, E.E.; Teng, Y.; Dani, J.A.; De Biasi, M. Nicotine enhances excitability of medial habenular neurons *via* facilitation of neurokinin signaling. *J. Neurosci.,* **2014**, *34*(12), 4273-4284.
http: //dx.doi.org/10.1523/JNEUROSCI.2736-13.2014 PMID: 24647947

[75] McLaughlin, I.; Dani, J.A.; De Biasi, M. The medial habenula and interpeduncular nucleus circuitry is critical in addiction, anxiety, and mood regulation. *J. Neurochem.,* **2017**, *142*(Suppl. 2), 130-143.
http: //dx.doi.org/10.1111/jnc.14008 PMID: 28791703

[76] Marks, M.J.; Pauly, J.R.; Gross, S.D.; Deneris, E.S.; Hermans-Borgmeyer, I.; Heinemann, S.F.; Collins, A.C. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J. Neurosci.,* **1992**, *12*(7), 2765-2784.
http: //dx.doi.org/10.1523/JNEUROSCI.12-07-02765.1992 PMID: 1613557

[77] Grady, S.R.; Moretti, M.; Zoli, M.; Marks, M.J.; Zanardi, A.; Pucci, L.; Clementi, F.; Gotti, C. Rodent habenulo-interpeduncular pathway expresses a large variety of uncommon nAChR subtypes, but only the alpha3beta4* and alpha3beta3beta4* subtypes mediate acetylcholine release. *J. Neurosci.,* **2009**, *29*(7), 2272-2282.
http: //dx.doi.org/10.1523/JNEUROSCI.5121-08.2009 PMID: 19228980

[78] Numachi, Y.; Yoshida, S.; Yamashita, M.; Fujiyama, K.; Naka, M.; Matsuoka, H.; Sato, M.; Sora, I. Psychostimulant alters expression of DNA methyltransferase mRNA in the rat brain. *Ann. N. Y. Acad. Sci.,* **2004**, *1025*, 102-109.
http: //dx.doi.org/10.1196/annals.1316.013 PMID: 15542706

[79] Romieu, P.; Host, L.; Gobaille, S.; Sandner, G.; Aunis, D.; Zwiller, J. Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *J. Neurosci.,* **2008**, *28*(38), 9342-9348. http: //dx.doi.org/10.1523/JNEUROSCI.0379-08.2008 PMID: 18799668

[80] Satta, R.; Maloku, E.; Zhubi, A.; Pibiri, F.; Hajos, M.; Costa, E.; Guidotti, A. Nicotine decreases DNA methyltransferase 1 expression and glutamic acid decarboxylase 67 promoter methylation in GABAergic interneurons. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(42), 16356-16361. http: //dx.doi.org/10.1073/pnas.0808699105 PMID: 18852456

[81] Huang, Y.Y.; Kandel, D.B.; Kandel, E.R.; Levine, A. Nicotine primes the effect of cocaine on the induction of LTP in the amygdala. *Neuropharmacology,* **2013**, *74*, 126-134. http: //dx.doi.org/10.1016/j.neuropharm.2013.03.031 PMID: 23597510

[82] Klee, E.W.; Schneider, H.; Clark, K.J.; Cousin, M.A.; Ebbert, J.O.; Hooten, W.M.; Karpyak, V.M.; Warner, D.O.; Ekker, S.C. Zebrafish: a model for the study of addiction genetics. *Hum. Genet.,* **2012**, *131*(6), 977-1008. http: //dx.doi.org/10.1007/s00439-011-1128-0 PMID: 22207143

[83] García-González, J.; Brock, A.J.; Parker, M.O.; Riley, R.J.; Joliffe, D.; Sudwarts, A.; Teh, M.T.; Busch-Nentwich, E.M.; Stemple, D.L.; Martineau, A.R.; Kaprio, J.; Palviainen, T.; Kuan, V.; Walton, R.T.; Brennan, C.H. Identification of *slit3* as a locus affecting nicotine preference in zebrafish and human smoking behaviour. *eLife,* **2020**, *9*, e51295. http: //dx.doi.org/10.7554/eLife.51295 PMID: 32209227

[84] Bossé, G.D.; Peterson, R.T. Development of an opioid self-administration assay to study drug seeking in zebrafish. *Behav. Brain Res.,* **2017**, *335*, 158-166. http: //dx.doi.org/10.1016/j.bbr.2017.08.001 PMID: 28811180

[85] Ninkovic, J.; Bally-Cuif, L. The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. *Methods,* **2006**, *39*(3), 262-274. http: //dx.doi.org/10.1016/j.ymeth.2005.12.007 PMID: 16809048

[86] Darland, T.; Dowling, J.E. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(20), 11691-11696. http: //dx.doi.org/10.1073/pnas.191380698 PMID: 11553778

[87] Mathur, P.; Lau, B.; Guo, S. Conditioned place preference behavior in zebrafish. *Nat. Protoc.,* **2011**, *6*(3), 338-345. http: //dx.doi.org/10.1038/nprot.2010.201 PMID: 21372814

[88] Collier, A.D.; Khan, K.M.; Caramillo, E.M.; Mohn, R.S.; Echevarria, D.J. Zebrafish and conditioned place preference: a translational model of drug reward. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2014**, *55*, 16-25. http: //dx.doi.org/10.1016/j.pnpbp.2014.05.014 PMID: 24887295

[89] Kily, L.J.; Cowe, Y.C.; Hussain, O.; Patel, S.; McElwaine, S.; Cotter, F.E.; Brennan, C.H. Gene expression changes in a zebrafish model of drug dependency suggest conservation of neuro-adaptation pathways. *J. Exp. Biol.,* **2008**, *211*(Pt 10), 1623-1634. http: //dx.doi.org/10.1242/jeb.014399 PMID: 18456890

[90] Kedikian, X.; Faillace, M.P.; Bernabeu, R. Behavioral and molecular analysis of nicotine-conditioned place preference in zebrafish. *PLoS One,* **2013**, *8*(7), e69453. http: //dx.doi.org/10.1371/journal.pone.0069453 PMID: 23894483

[91] Brock, A.J.; Goody, S.M.G.; Mead, A.N.; Sudwarts, A.; Parker, M.O.; Brennan, C.H. Assessing the value of the zebrafish conditioned place preference model for predicting human abuse potential. *J. Pharmacol. Exp. Ther.,* **2017**, *363*(1), 66-79. http: //dx.doi.org/10.1124/jpet.117.242628 PMID: 28790193

[92] Faillace, M.P.; Bernabeu, R.O. Conditioned place preference and behavioral analysis to evaluate nicotine reinforcement properties in zebrafish. In: *Nicotinic Acetylcholine Receptor Technologies*; Ming D., Li.; Bernabeu, R.O., Eds.; Humana Press and Springer Science, **2016**; pp. 51-64. http: //dx.doi.org/10.1007/978-1-4939-3768-4_3

[93] Pisera-Fuster, A.; Rocco, L.; Faillace, M.P.; Bernabeu, R. Sensitization-dependent nicotine place preference in the adult zebrafish. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2019**, *92*, 457-469. http: //dx.doi.org/10.1016/j.pnpbp.2019.02.018 PMID: 30826460

[94] Faillace, M.P.; Bernabeu, R.O. Effects of nicotine and histone deacetylase inhibitors on the brain. In: *Neuroscience of Nicotine 1st edition, Mechanisms and Treatment*; Preedy, V.R., Ed.; Academic Press, Elsevier, **2019**; pp. 365-373. http: //dx.doi.org/10.1016/B978-0-12-813035-3.00045-9

[95] Faillace, M.P.; Pisera-Fuster, A.; Bernabeu, R. Evaluation of the rewarding properties of nicotine and caffeine by implementation of a five-choice conditioned place preference task in zebrafish. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2018**, *84*(Pt A), 160-172. http: //dx.doi.org/10.1016/j.pnpbp.2018.02.001

[96] Jung, Y.; Hsieh, L.S.; Lee, A.M.; Zhou, Z.; Coman, D.; Heath, C.J.; Hyder, F.; Mineur, Y.S.; Yuan, Q.; Goldman, D.; Bordey, A.; Picciotto, M.R. An epigenetic mechanism mediates developmental nicotine effects on neuronal structure and behavior. *Nat. Neurosci.,* **2016**, *19*(7), 905-914. http: //dx.doi.org/10.1038/nn.4315 PMID: 27239938

[97] Pisera-Fuster, A.; Faillace, M.P.; Bernabeu, R. Pre-exposure to nicotine with nocturnal abstinence induces epigenetic changes that potentiate nicotine preference. *Mol. Neurobiol.,* **2020**, *57*(4), 1828-1846. http: //dx.doi.org/10.1007/s12035-019-01843-y PMID: 31848934

[98] Pastor, V.; Host, L.; Zwiller, J.; Bernabeu, R. Histone deacetylase inhibition decreases preference without affecting aversion for nicotine. *J. Neurochem.,* **2011**, *116*(4), 636-645. http: //dx.doi.org/10.1111/j.1471-4159.2010.07149.x PMID: 21166804

[99] Castino, M.R.; Cornish, J.L.; Clemens, K.J. Inhibition of histone deacetylases facilitates extinction and attenuates reinstatement of nicotine self-administration in rats. *PLoS One,* **2015**, *10*(4), e0124796. http: //dx.doi.org/10.1371/journal.pone.0124796 PMID: 25880762

[100] Seto, E.; Yoshida, M. Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb. Perspect. Biol.,* **2014**, *6*(4), a018713. http: //dx.doi.org/10.1101/cshperspect.a018713 PMID: 24691964

[101] Brunmeir, R.; Lagger, S.; Seiser, C. Histone deacetylase HDAC1/HDAC2-controlled embryonic development and cell differentiation. *Int. J. Dev. Biol.,* **2009**, *53*(2-3), 275-289. http: //dx.doi.org/10.1387/ijdb.082649rb PMID: 19412887

[102] Bobadilla, A.C.; Dereschewitz, E.; Vaccaro, L.; Heinsbroek, J.A.; Scofield, M.D.; Kalivas, P.W. Cocaine and sucrose rewards recruit different seeking ensembles in the nucleus accumbens core. *Mol. Psychiatry,* **2020**, *25*(12), 3150-3163. http: //dx.doi.org/10.1038/s41380-020-00888-z PMID: 32985600

[103] Mayr, B.; Montminy, M. Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell Biol.,* **2001**, *2*(8), 599-609. http: //dx.doi.org/10.1038/35085068 PMID: 11483993

[104] Chandramohan, Y.; Droste, S.K.; Reul, J.M. Novelty stress induces phospho-acetylation of histone H3 in rat dentate gyrus granule neurons through coincident signalling *via* the N-methyl-D-aspartate receptor and the glucocorticoid receptor: relevance for c-fos induction. *J. Neurochem.,* **2007**, *101*(3), 815-828. http: //dx.doi.org/10.1111/j.1471-4159.2006.04396.x PMID: 17250652

[105] Yeh, S.H.; Lin, C.H.; Gean, P.W. Acetylation of nuclear factor-kappaB in rat amygdala improves long-term but not short-term retention of fear memory. *Mol. Pharmacol.,* **2004**, *65*(5), 1286-1292. http: //dx.doi.org/10.1124/mol.65.5.1286 PMID: 15102957

[106] Hu, M.; Liu, Q.S.; Chang, K.T.; Berg, D.K. Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways. *Mol. Cell. Neurosci.,* **2002**, *21*(4), 616-625. http: //dx.doi.org/10.1006/mcne.2002.1202 PMID: 12504594

[107] Gueorguiev, V.D.; Cheng, S.Y.; Sabban, E.L. Prolonged activation of cAMP-response element-binding protein and ATF-2 needed for nicotine-triggered elevation of tyrosine hydroxylase gene transcription in PC12 cells. *J. Biol. Chem.,* **2006**, *281*(15), 10188-10195. http: //dx.doi.org/10.1074/jbc.M513806200 PMID: 16452470

[108] Yang, X.J.; Seto, E. Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol. Cell,* **2008**, *31*(4), 449-461. http: //dx.doi.org/10.1016/j.molcel.2008.07.002 PMID: 18722172

[109] May, Z.; Morrill, A.; Holcombe, A.; Johnston, T.; Gallup, J.; Fouad, K.; Schalomon, M.; Hamilton, T.J. Object recognition memory in zebrafish. *Behav. Brain Res.,* **2016**, *296*, 199-210.

http: //dx.doi.org/10.1016/j.bbr.2015.09.016 PMID: 26376244

[110] Faillace, M.P.; Pisera-Fuster, A.; Medrano, M.P.; Bejarano, A.C.; Bernabeu, R.O. Short- and long-term effects of nicotine and the histone deacetylase inhibitor phenylbutyrate on novel object recognition in zebrafish. *Psychopharmacology (Berl.),* **2017**, *234*(6), 943-955.
http: //dx.doi.org/10.1007/s00213-017-4532-x PMID: 28130648

[111] Dean, R.; Duperreault, E.; Newton, D.; Krook, J.; Ingraham, E.; Gallup, J.; Franczak, B.C.; Hamilton, T.J. Opposing effects of acute and repeated nicotine exposure on boldness in zebrafish. *Sci. Rep.,* **2020**, *10*(1), 8570.
http: //dx.doi.org/10.1038/s41598-020-65382-6 PMID: 32444782

[112] Korzus, E.; Rosenfeld, M.G.; Mayford, M. CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron,* **2004**, *42*(6), 961-972.
http: //dx.doi.org/10.1016/j.neuron.2004.06.002 PMID: 15207240

[113] Peleg, S.; Sananbenesi, F.; Zovoilis, A.; Burkhardt, S.; Bahari-Javan, S.; Agis-Balboa, R.C.; Cota, P.; Wittnam, J.L.; Gogol-Doering, A.; Opitz, L.; Salinas-Riester, G.; Dettenhofer, M.; Kang, H.; Farinelli, L.; Chen, W.; Fischer, A. Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science,* **2010**, *328*(5979), 753-756.
http: //dx.doi.org/10.1126/science.1186088 PMID: 20448184

[114] Hawk, J.D.; Florian, C.; Abel, T. Post-training intrahippocampal inhibition of class I histone deacetylases enhances long-term object-location memory. *Learn. Mem.,* **2011**, *18*(6), 367-370.
http: //dx.doi.org/10.1101/lm.2097411 PMID: 21576516

[115] Stefanko, D.P.; Barrett, R.M.; Ly, A.R.; Reolon, G.K.; Wood, M.A. Modulation of long-term memory for object recognition *via* HDAC inhibition. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(23), 9447-9452.
http: //dx.doi.org/10.1073/pnas.0903964106 PMID: 19470462

[116] Haettig, J.; Stefanko, D.P.; Multani, M.L.; Figueroa, D.X.; McQuown, S.C.; Wood, M.A. HDAC inhibition modulates hippocampus-dependent long-term memory for object location in a CBP-dependent manner. *Learn. Mem.,* **2011**, *18*(2), 71-79.
http: //dx.doi.org/10.1101/lm.1986911 PMID: 21224411

[117] Jenuwein, T.; Allis, C.D. Translating the histone code. *Science,* **2001**, *293*(5532), 1074-1080.
http: //dx.doi.org/10.1126/science.1063127 PMID: 11498575

[118] Maze, I.; Feng, J.; Wilkinson, M.B.; Sun, H.; Shen, L.; Nestler, E.J. Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. *Proc. Natl. Acad. Sci. USA,* **2011**, *108*(7), 3035-3040.
http: //dx.doi.org/10.1073/pnas.1015483108 PMID: 21300862

[119] Maze, I.; Covington, H.E., III; Dietz, D.M.; LaPlant, Q.; Renthal, W.; Russo, S.J.; Mechanic, M.; Mouzon, E.; Neve, R.L.; Haggarty, S.J.; Ren, Y.; Sampath, S.C.; Hurd, Y.L.; Greengard, P.; Tarakhovsky, A.; Schaefer, A.; Nestler, E.J. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science,* **2010**, *327*(5962), 213-216.
http: //dx.doi.org/10.1126/science.1179438 PMID: 20056891

[120] Castino, M.R.; Baker-Andresen, D.; Ratnu, V.S.; Shevchenko, G.; Morris, K.V.; Bredy, T.W.; Youngson, N.A.; Clemens, K.J. Persistent histone modifications at the BDNF and Cdk-5 promoters following extinction of nicotine-seeking in rats. *Genes Brain Behav.,* **2018**, *17*(2), 98-106.
http: //dx.doi.org/10.1111/gbb.12421 PMID: 28857504

[121] Chase, K.A.; Sharma, R.P. Nicotine induces chromatin remodelling through decreases in the methyltransferases GLP, G9a, Setdb1 and levels of H3K9me2. *Int. J. Neuropsychopharmacol.,* **2013**, *16*(5), 1129-1138.
http: //dx.doi.org/10.1017/S1461145712001101 PMID: 23067581

[122] Peters, A.H.; Kubicek, S.; Mechtler, K.; O'Sullivan, R.J.; Derijck, A.A.; Perez-Burgos, L.; Kohlmaier, A.; Opravil, S.; Tachibana, M.; Shinkai, Y.; Martens, J.H.; Jenuwein, T. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell,* **2003**, *12*(6), 1577-1589.
http: //dx.doi.org/10.1016/S1097-2765(03)00477-5 PMID: 14690609

[123] Meng, J.; Wang, L.; Wang, J.; Zhao, X.; Cheng, J.; Yu, W.; Jin, D.; Li, Q.; Gong, Z. Methionine adenosyltransferase4 mediates DNA and Histone methylation. *Plant Physiol.,* **2018**, *177*(2), 652-670.
http: //dx.doi.org/10.1104/pp.18.00183 PMID: 29572390

[124] Pisera-Fuster, A.; Zwiller, J.; Bernabeu, R. Methionine supplementation abolishes nicotine-induced place preference in zebrafish: a behavioral and molecular analysis. *Mol. Neurobiol.,* **2021**, *58*(6), 2590-2607.
http: //dx.doi.org/10.1007/s12035-020-02260-2 PMID: 33475949

[125] Shimbo, T.; Wade, P.A. Proteins that read DNA methylation. *Adv. Exp. Med. Biol.,* **2016**, *945*, 303-320.
http: //dx.doi.org/10.1007/978-3-319-43624-1_13 PMID: 27826844

[126] Ng, H.H.; Bird, A. Histone deacetylases: silencers for hire. *Trends Biochem. Sci.,* **2000**, *25*(3), 121-126.
http: //dx.doi.org/10.1016/S0968-0004(00)01551-6 PMID: 10694882

[127] Lang, Z.; Lei, M.; Wang, X.; Tang, K.; Miki, D.; Zhang, H.; Mangrauthia, S.K.; Liu, W.; Nie, W.; Ma, G.; Yan, J.; Duan, C.G.; Hsu, C.C.; Wang, C.; Tao, W.A.; Gong, Z.; Zhu, J.K. The methyl-CpG-binding protein MBD7 facilitates active DNA demethylation to limit DNA hyper-methylation and transcriptional gene silencing. *Mol. Cell,* **2015**, *57*(6), 971-983.
http: //dx.doi.org/10.1016/j.molcel.2015.01.009 PMID: 25684209

[128] Zhu, H.; Wang, G.; Qian, J. Transcription factors as readers and effectors of DNA methylation. *Nat. Rev. Genet.,* **2016**, *17*(9), 551-565.
http: //dx.doi.org/10.1038/nrg.2016.83 PMID: 27479905

[129] Wu, H.; Zhang, Y. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. *Genes Dev.,* **2011**, *25*(23), 2436-2452.
http: //dx.doi.org/10.1101/gad.179184.111 PMID: 22156206

[130] Koivunen, P.; Laukka, T. The TET enzymes. *Cell. Mol. Life Sci.: CMLS,* **2018**, *75*(8), 1339-1348.
http: //dx.doi.org/10.1007/s00018-017-2721-8 PMID: 29184981

[131] Barreto, G.; Schäfer, A.; Marhold, J.; Stach, D.; Swaminathan, S.K.; Handa, V.; Döderlein, G.; Maltry, N.; Wu, W.; Lyko, F.; Niehrs, C. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature,* **2007**, *445*(7128), 671-675.
http: //dx.doi.org/10.1038/nature05515 PMID: 17268471

[132] Tian, W.; Zhao, M.; Li, M.; Song, T.; Zhang, M.; Quan, L.; Li, S.; Sun, Z.S. Reversal of cocaine-conditioned place preference through methyl supplementation in mice: altering global DNA methylation in the prefrontal cortex. *PLoS One,* **2012**, *7*(3), e33435.
http: //dx.doi.org/10.1371/journal.pone.0033435 PMID: 22438930

[133] Wright, K.N.; Hollis, F.; Duclot, F.; Dossat, A.M.; Strong, C.E.; Francis, T.C.; Mercer, R.; Feng, J.; Dietz, D.M.; Lobo, M.K.; Nestler, E.J.; Kabbaj, M. Methyl supplementation attenuates cocaine-seeking behaviors and cocaine-induced c-Fos activation in a DNA methylation-dependent manner. *J. Neurosci.,* **2015**, *35*(23), 8948-8958.
http: //dx.doi.org/10.1523/JNEUROSCI.5227-14.2015 PMID: 26063926

[134] Kamstra, J.H.; Aleström, P.; Kooter, J.M.; Legler, J. Zebrafish as a model to study the role of DNA methylation in environmental toxicology. *Environ. Sci. Pollut. Res. Int.,* **2015**, *22*(21), 16262-16276.
http: //dx.doi.org/10.1007/s11356-014-3466-7 PMID: 25172464

[135] Wonnacott, S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. *Trends Pharmacol. Sci.,* **1990**, *11*(6), 216-219.
http: //dx.doi.org/10.1016/0165-6147(90)90242-Z PMID: 2200178

[136] Fonteneau, M.; Filliol, D.; Anglard, P.; Befort, K.; Romieu, P.; Zwiller, J. Inhibition of DNA methyltransferases regulates cocaine self-administration by rats: a genome-wide DNA methylation study. *Genes Brain Behav.,* **2017**, *16*(3), 313-327.
http: //dx.doi.org/10.1111/gbb.12354 PMID: 27762100

[137] Ohashi, H. Methylation-specific PCR. In: *PCR Cloning Protocols. Methods in Molecular Biology™*; Chen, BY.; Janes, H.W., Eds.; Humana Press, **2002**; 192, pp. 91-97.
http: //dx.doi.org/10.1385/1-59259-177-9:091

[138] Hernández, H.G.; Tse, M.Y.; Pang, S.C.; Arboleda, H.; Forero, D.A. Optimizing methodologies for PCR-based DNA methylation analysis. *Biotechniques,* **2013**, *55*(4), 181-197.
http: //dx.doi.org/10.2144/000114087 PMID: 24107250

[139] Vuaden, F.C.; Savio, L.E.B.; Rico, E.P.; Mussulini, B.H.M.; Rosemberg, D.B.; de Oliveira, D.L.; Bogo, M.R.; Bonan, C.D.; Wyse, A.T.S. Methionine exposure alters glutamate uptake and adenine nucleotide hydrolysis in the zebrafish brain. *Mol. Neurobiol.,* **2016**, *53*(1), 200-209.

http: //dx.doi.org/10.1007/s12035-014-8983-3 PMID: 25421208

[140] Garlick, P.J. Toxicity of methionine in humans. *J. Nutr.,* **2006**, *136*(6)(Suppl.), 1722S-1725S. http: //dx.doi.org/10.1093/jn/136.6.1722S PMID: 16702346

[141] Shorter, K.R.; Felder, M.R.; Vrana, P.B. Consequences of dietary methyl donor supplements: Is more always better? *Prog. Biophys. Mol. Biol.,* **2015**, *118*(1-2), 14-20. http: //dx.doi.org/10.1016/j.pbiomolbio.2015.03.007 PMID: 25841986

[142] Tapia-Rojas, C.; Lindsay, C.B.; Montecinos-Oliva, C.; Arrazola, M.S.; Retamales, R.M.; Bunout, D.; Hirsch, S.; Inestrosa, N.C. Is L-methionine a trigger factor for Alzheimer's-like neurodegeneration?: Changes in Aβ oligomers, tau phosphorylation, synaptic proteins, Wnt signaling and behavioral impairment in wild-type mice. *Mol. Neurodegener.,* **2015**, *10*, 62. http: //dx.doi.org/10.1186/s13024-015-0057-0 PMID: 26590557

[143] Iannitti, T.; Palmieri, B. Clinical and experimental applications of sodium phenylbutyrate. *Drugs RD.,* **2011**, *11*(3), 227-249. http: //dx.doi.org/10.2165/11591280-000000000-00000 PMID: 21902286

[144] Paganoni, S.; Hendrix, S.; Dickson, S.P.; Knowlton, N.; Macklin, E.A.; Berry, J.D.; Elliott, M.A.; Maiser, S.; Karam, C.; Caress, J.B.; Owegi, M.A.; Quick, A.; Wymer, J.; Goutman, S.A.; Heitzman, D.; Heiman-Patterson, T.D.; Jackson, C.E.; Quinn, C.; Rothstein, J.D.; Kasarskis, E.J.; Katz, J.; Jenkins, L.; Ladha, S.; Miller, T.M.; Scelsa, S.N.; Vu, T.H.; Fournier, C.N.; Glass, J.D.; Johnson, K.M.; Swenson, A.; Goyal, N.A.; Pattee, G.L.; Andres, P.L.; Babu, S.; Chase, M.; Dagostino, D.; Hall, M.; Kittle, G.; Eydinov, M.; McGovern, M.; Ostrow, J.; Pothier, L.; Randall, R.; Shefner, J.M.; Sherman, A.V.; St Pierre, M.E.; Tustison, E.; Vigneswaran, P.; Walker, J.; Yu, H.; Chan, J.; Wittes, J.; Yu, Z.F.; Cohen, J.; Klee, J.; Leslie, K.; Tanzi, R.E.; Gilbert, W.; Yeramian, P.D.; Schoenfeld, D.; Cudkowicz, M.E. Long-term survival of participants in the CENTAUR trial of sodium phenylbutyrate-taurursodiol in amyotrophic lateral sclerosis. *Muscle Nerve,* **2021**, *63*(1), 31-39. http: //dx.doi.org/10.1002/mus.27091 PMID: 33063909

[145] Hayashi, G.; Labelle-Dumais, C.; Gould, D.B. Use of sodium 4-phenylbutyrate to define therapeutic parameters for reducing intracerebral hemorrhage and myopathy in *Col4a1* mutant mice. *Dis. Model. Mech.,* **2018**, *11*(7), dmm034157. http: //dx.doi.org/10.1242/dmm.034157 PMID: 29895609

[146] Lee, H.T.; Oh, S.; Ro, D.H.; Yoo, H.; Kwon, Y.W. The Key Role of DNA Methylation and Histone Acetylation in Epigenetics of Atherosclerosis. *J. Lipid Atheroscler.,* **2020**, *9*(3), 419-434. http: //dx.doi.org/10.12997/jla.2020.9.3.419 PMID: 33024734

[147] Bouchat, S.; Delacourt, N.; Kula, A.; Darcis, G.; Van Driessche, B.; Corazza, F.; Gatot, J.S.; Melard, A.; Vanhulle, C.; Kabeya, K.; Pardons, M.; Avettand-Fenoel, V.; Clumeck, N.; De Wit, S.; Rohr, O.; Rouzioux, C.; Van Lint, C. Sequential treatment with 5-aza-2′-deoxycytidine and deacetylase inhibitors reactivates HIV-1. *EMBO Mol. Med.,* **2016**, *8*(2), 117-138. http: //dx.doi.org/10.15252/emmm.201505557 PMID: 26681773

[148] Wishart, D.S.; Feunang, Y.D.; Guo, A.C.; Lo, E.J.; Marcu, A.; Grant, J.R.; Sajed, T.; Johnson, D.; Li, C.; Sayeeda, Z.; Assempour, N.; Iynkkaran, I.; Liu, Y.; Maciejewski, A.; Gale, N.; Wilson, A.; Chin, L.; Cummings, R.; Le, D.; Pon, A.; Knox, C.; Wilson, M. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.,* **2018**, *46*(D1), D1074-D1082. http: //dx.doi.org/10.1093/nar/gkx1037 PMID: 29126136

[149] Scaplen, K.M.; Kaun, K.R. Reward from bugs to bipeds: a comparative approach to understanding how reward circuits function. *J. Neurogenet.,* **2016**, *30*(2), 133-148. http: //dx.doi.org/10.1080/01677063.2016.1180385 PMID: 27328845