

New approach to the neurobiological mechanisms of addiction

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Much progress has been made in the last decade in the understanding the neural substrates of drug addiction, transmitters involved, epigenetic background and their relation to learning and memory but much remains to be elucidated and strong effort is necessary to integrate the rich information at the molecular, cellular systems, and behavioral levels to further clarify the mechanisms and therapy of this complex disease. The aim of this review is to collect and interpret the latest opinions in the development, the underlying mechanisms and therapy of addiction as a disease of central nervous system. The neurocircuitry, the transmitters and the epigenetics of addiction are discussed.

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Drug addiction is a chronically relapsing disorder that has been defined by DSM IV and is characterized by drug-seeking behavior, loss of control of intake, and motivational withdrawal syndrome when access to the drug is prevented, showing psychological dependence (negative emotional and psychic state: e.g. dysphoria, anxiety, irritability), and physical dependence (e.g. cramps, diarrhea, secretions, and abdominal pain etc.).

The most abused substances are (NIDA, 2013):

- Cocaine
- Heroin
- Inhalants
- K2/Spice herbal mixtures (synthetic marijuana)
- LSD (Acid)
- Marijuana
- MDMA (Ecstasy)
- Methamphetamine
- Bath Salts (Synthetic cathinones: mephedrone, methylone)
- Club Drugs (GHB, ketamine and Rohypnol)
- PCP/Phencyclidine
- Prescription Drugs
- Salvia

NEUROCIRCUITRY OF ADDICTIVE DISORDERS

The knowledge of the role of neurocircuitry in the advance of the addiction syndrome might be the scientific basis to understand the molecular, genetic, and

neuropharmacological neuroadaptations that are key factors of vulnerability for developing and maintaining addiction (Fig. 1).

The dopamine projections originate from the ventral tegmental area (VTA) and target the nucleus accumbens, prefrontal cortex, amygdala and hippocampus.

As a general rule, all substances of drug abuse inducing addiction activate a common reward pathway, the mesocorticolimbic dopamine system, although by different mechanisms. However, there are “nonaddictive” drugs never inducing addiction: hallucinogens (e.g. LSD) or ketamine-like dissociative anesthetics. Their target is different from that of addictive drugs: they do not act through the mesolimbic DA system but they target cortical and thalamic circuits (see Lüscher, 2012).

According to a latest hypothesis for addictive disorders mesolimbic dopamine codes for the difference between the expected and actual reward and constitutes a strong learning signal (Lüscher, 2012).

Addictive drugs have at least three specific different cellular mechanisms to activate the mesolimbic system:

- *G-protein coupled receptors* (GPCR) Drugs binding to G_i family of GPCR inhibit neurons through postsynaptic hyperpolarization and presynaptic regulation of transmitter release. In the VTA these drugs act on the local GABA inhibitory interneuron (Lüscher, 2012).

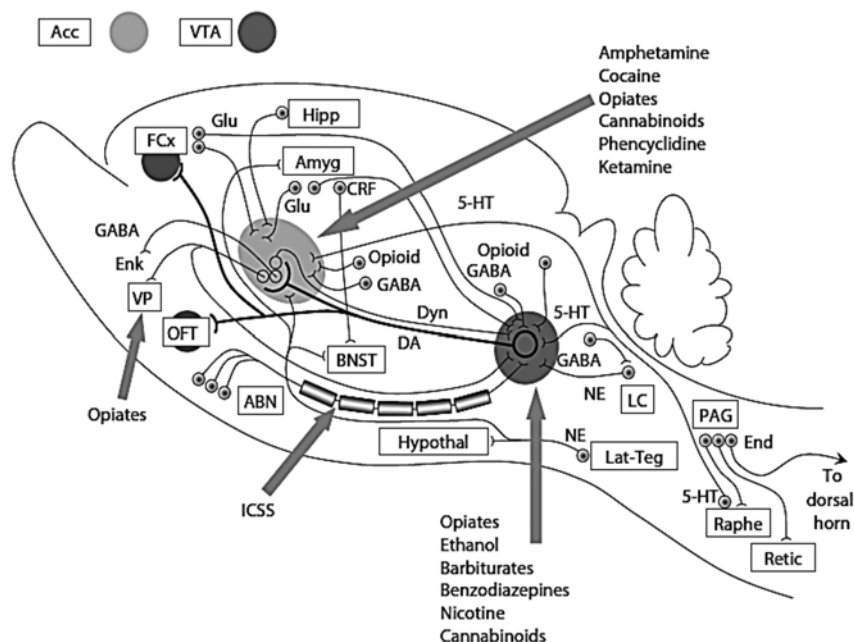


Figure 1 Schematic representation of the brain reward circuitry of the rat brain

Sites at which various addictive drugs act to enhance brain reward mechanisms, produce drug seeking behavior, drug-taking behavior, drug craving and relapse to drug-seeking behavior (Gardner, 2011).

Abbreviations: ABN, Anterior bed nuclei of the medial forebrain bundle; Acc, nucleus accumbens; VP, ventral pallidum; VTA, ventral tegmental area; Amyg, amygdala; BNST, bed nucleus of the stria terminalis; CRF, corticotrophin-releasing factor; DA, mesocorticolimbic dopaminergic system; Dyn, dynorphinergic neurons in Acc; LC, locus ceruleus; Enk, enkephalinergic neurons in Acc; FCx, frontal cortex; GABA, GABAergic inhibitory fiber systems in the LC and Acc; Glu, glutamatergic neural systems from VTA and Acc; 5-HT, serotonergic (5-hydroxytryptamine) fibers, from anterior raphe nuclei and project to the cell body region of VTA and Acc; ICSS, descending, myelinated component of the brain reward circuitry, preferentially activated by electrical intracranial self-stimulation; NE, noradrenergic fibers, from LC; Opioid, endogenous opioid peptide systems VTA, Acc; PAG, periaqueductal gray matter; Raphe, brainstem serotonergic raphe nuclei.

- *Ionotropic receptors or ionchannels* Drugs acting through this mechanism have combined effects on DA and GABA neurons to enhance DA release.
- *Dopamine transporter* targeted drugs either block reuptake or stimulate nonvesicular release of DA.

Thus, all addictive drugs have in common that they enhance (directly or indirectly or even transsynaptically) dopaminergic reward synaptic function in the nucleus accumbens (NAc). Drug self-administration is regulated by NAc DA levels, and keeps NAc DA within a specific elevated range (to maintain a desired hedonic level). Addiction appears to correlate well with a hypodopaminergic dysfunctional state within the reward circuitry of the brain.

Other transmitters. Also, the participation of serotonergic, opioid, endocannabinoid, GABAergic and glutamatergic mechanisms in addiction was repeatedly demonstrated (for rev see Koob and Volkow, 2010).

Most of addictive drugs are voluntarily self-administered by laboratory animals and they enhance the functioning of the reward circuitry of the brain (producing the 'high' that the drug user seeks). Although originally believed to simply encode the set point of hedonic tone, these circuits are now believed to be functionally far more complex, also encoding attention, expectancy of reward, disconfirmation of reward expectancy, and incentive motivation. 'Hedonic dysregulation' within these circuits may lead to addiction. The 'second-stage' dopaminergic component in this reward circuitry is the crucial addictive-drug-sensitive component (Hyman, 2005).

The three stages of drug addiction:

- 'binge/intoxication',
- 'withdrawal/negative affect', and
- 'preoccupation/anticipation' (craving).

Critically, drug addiction progresses from occasional recreational use to impulsive use to habitual

compulsive use. This correlates with a progression from reward-driven to habit-driven drug-seeking behavior. This behavioral progression correlates with a neuroanatomical progression.

The transition of recreational use to addiction involves neuroplasticity of these structures and a cascade of neuroadaptations through these structures.

Latest advanced imaging studies demonstrated that the key morphological elements for the three stages are:

- ventral tegmental area (VTA) and ventral striatum as a focal point for the *binge/intoxication* stage,
- the extended amygdala in the *withdrawal/negative affect* stage,
- a widely distributed network of the orbitofrontal cortex–dorsal striatum, prefrontal cortex, basolateral amygdala, hippocampus, and insula in the *preoccupation/anticipation* stage (*craving*), and
- the cingulate gyrus, dorsolateral prefrontal, and inferior frontal cortices in *disrupted inhibitory control*.

Withdrawal. The brain circuits mediating the pleasurable (euphoric) effects of addictive drugs are anatomically, neurophysiologically and neurochemically different from those mediating physical dependence, and from those mediating craving and relapse (Koob and Volkow, 2010).

Craving and relapse. The three classical reasons are:

- *Drug-triggered relapse:* involves the nucleus accumbens and the neurotransmitter dopamine.
- *Stress-triggered relapse* involves (i) the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the neurotransmitter corticotrophin-releasing factor, and (ii) the lateral tegmental noradrenergic nuclei of the brain stem and the neurotransmitter norepinephrine.
- *Cue-triggered* (reexposure to environmental cues: people, places, things) previously associated with drug-taking behavior) relapse involves the basolateral nucleus of the amygdala, the hippocampus and the neurotransmitter glutamate.

EPIGENETIC MECHANISMS

Epigenetic mechanisms transduce environmental stimuli to promote stable alterations in chromatin structure to activate or repress gene transcription (Jaenisch and Bird, 2003).

The definition of epigenetics includes not only heritable changes in gene expression but also stable changes in gene expression that do not include

changes in DNA sequence (Bird, 2007; Siegmund et al., 2007). Sobor et al. (2007) worked on offspring of chronically treated mothers to study the vulnerability and transfer of the addiction state to adult rat. There are important genetic variations in vulnerability to drug addiction, yet environmental factors such as stress and social failures also alter brain-reward mechanisms to mediate vulnerability to addiction.

Chronic drug exposure alters gene expression in the brain and produces long-term changes in neural networks that underlie compulsive drug taking. The drug-induced changes in synaptic plasticity and subsequent gene expression are translated into chronic neuroadaptations mediated by highly synchronized and dynamic patterns of gene regulation. It is now widely accepted that epigenetic mechanisms contribute to drug-induced structural, synaptic, and behavioral plasticity by regulating expression of gene networks. The psychostimulants are most studied group of abused substances related their addiction to epigenetic modulation.

Schmiedt et al. (2013) recently reviewed how alterations in histone modifications, chromatin remodeling, DNA methylation, and microRNAs regulate gene expression and contribute to addiction to psychostimulants with a focus on the epigenetic mechanisms that regulate brain-derived neurotrophic factor (BDNF) expression. Coming closer to epigenetic signatures that define psychostimulant addiction may lead to new, efficacious treatments for drug craving and relapse.

Chromatin regulation

Chromatin is a complex structure that consists of negatively charged DNA wrapped around octamers of positively charged histone proteins (Fig. 2).

Histons are simple alkaline proteins usually occurring in cell nuclei, combined ionically with DNA. *Nucleosome* is a unit in which a molecule of a histone is bound to a segment of the DNA chain of genetic material. Changes in these units are associated with changes in the physical state and function of the chromatin during cell division and the transcription of the genetic message. Posttranslational modifications to histones and chromatin remodeling are epigenetic mechanisms that alter access of transcriptional machinery to promoter regions thereby regulating patterns of gene expression (Cheung et al., 2000; Berger, 2007; Strahl and Allis, 2000).

As it was reported from different laboratories chromatin remodeling, including stable enzymatic

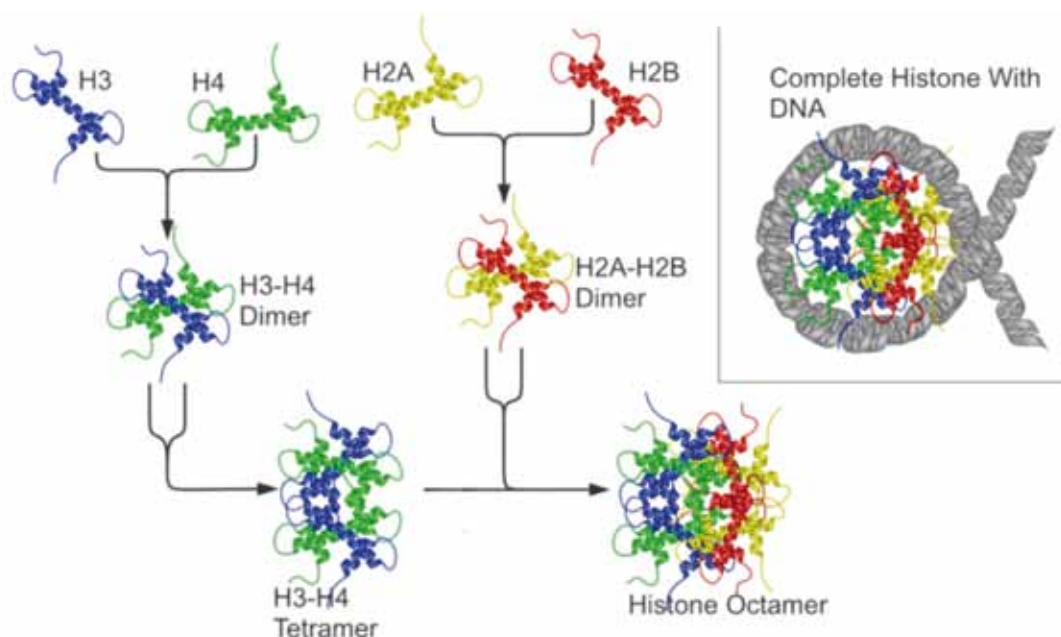


Figure 2 Schematic representation of histone

modifications to DNA and histone proteins, is associated with persistent changes in gene expression that may underlie drug addiction (Renthal and Nestler, 2008; Maze and Nestler, 2011; Schroeder et al., 2008).

The amino-terminal tails of histones contain specific amino acid residues that are sites for several posttranslational modifications such as acetylation, methylation, or phosphorylation.

Histone Acetylation. Acetylation of basic lysine residues in histone tails decreases the electrostatic interactions between histone proteins and negatively charged DNA. Hyperacetylation of promoter regions is associated with *increased* gene expression, whereas hypoacetylation is correlated with *decreased* gene expression (Kurdistani et al., 2004). Recent studies also indicate the role of histone acetylation in *cocaine-taking* behavior. Systemic administration of an histone deacetylase (HDAC) inhibitor before the initiation of daily cocaine self-administration sessions decreased the number of cocaine infusions self-administered, suggesting that histone acetylation decreases the reinforcing efficacy of cocaine (Romieu et al., 2008). Histone acetylation and chromatin remodeling are functionally relevant as both pharmacological inhibition and genetic manipulation of HDACs alter behavioral responses to cocaine. Systemic and intraac-

cumbens administration of HDAC inhibitors significantly enhances cocaine-induced locomotor activity and conditioned place preference (CPP) (Renthal et al., 2007). These results indicate that cocaine-induced behavioral plasticity is mediated, in part, by increased acetylation of gene networks (Schmiedt et al., 2011).

Increased expression of the immediate early genes *Fos* and *Fosb* in the NAc following *acute* cocaine administration is associated with increased histone H4 acetylation at their promoter regions (Kumar et al., 2005). *Fosb* is a unique Fos family member because of its extraordinary stability which is mediated in part by its phosphorylation by casein kinase II (McClung et al., 2004). Sustained induction of *Fosb* is a common consequence of long-term drug exposure which has been documented for cocaine, amphetamine, morphine, nicotine, ethanol, cannabinoids, and phencyclidine. *Fosb* could represent a type of molecular switch that contributes to relatively prolonged aspects of drug addiction.

Chronic cocaine exposure also is associated with increased histone acetylation at distinct promoter regions. Cocaine-induced alterations in histone H3 acetylation and corresponding changes in gene expression are stable during *abstinence* (Freeman et al., 2008) which suggests that cocaine-induced chromatin

remodeling produces persistent changes in gene expression that may underlie drug craving and relapse.

Similarly, the role of histone acetylation in *amphetamine*-induced behavioral responses from behavioral sensitization to the locomotor-activating effects was reported (Shen et al., 2008). Furthermore, repeated methamphetamine administration increases histone H3 acetylation at unique gene promoters in the limbic forebrain (Shibasaki et al., 2011). These results might suggest that amphetamine-induced behavioral plasticity is also regulated, in part, by changes in chromatin structure.

Histon Methylation. Recent evidence indicates that psychostimulant exposure alters gene expression, in part, through changes in histone methylation. Histone H3 methylation is decreased in adult rats exposed to *cocaine* during adolescence and these epigenetic marks run parallel with altered gene expression in adulthood (Black et al., 2006, Schmiedt et al., 2013). These findings suggest also that cocaine exposure during adolescence produces long-lasting changes in gene expression that are mediated by chromatin remodeling. *Amphetamine* abstinence is also associated with changes in histone H3 methylation. Histone H3 methylation is increased at the *Fos* promoter in the striatum following repeated amphetamine exposure and is associated with decreased transcription of this immediate early gene (Renthal et al., 2008). Consistent with these results, expression of the histone H3 methyltransferase is increased in the striatum following chronic amphetamine exposure (Renthal et al., 2008).

Histone Phosphorylation is another posttranslational modification that is associated with increased gene transcription. Acute amphetamine (Rotllant and Armario, 2012) and cocaine (Brami-Cherrier et al., 2009) administration transiently increases histone H3 phosphorylation. Although histone acetylation and phosphorylation are both associated with increased gene transcription, these epigenetic mechanisms can act in concert or independently to regulate gene expression (Brami-Cherrier et al., 2007). The role of histone phosphorylation in psychostimulant-induced behavioral responses remains to be determined.

DNA Methylation. In addition to posttranslational histone alterations, enzymatic modifications to DNA sequences also translate environmental stimuli such as drug exposure into altered patterns of gene expression and enduring behavioral phenotypes. DNA methylation involves the addition of methyl groups to cytosine-guanine dinucleotides (CpG) in the genome by DNA methyltransferases (DNMTs) (Suzuki and

Bird, 2008). DNA methylation is a dynamic process that functions to either promote or repress gene expression (Suzuki and Bird, 2008).

Emerging evidence suggests that psychostimulant-induced changes in gene expression are regulated by DNA methylation: e.g. cocaine self-administration correlates well with increased expression of the methyl-CpG-binding protein. (Host et al., 2011). Further evidence for a role of DNA methylation in cocaine-induced synaptic and behavioral plasticity comes from studies of DNMTs. Acute cocaine administration increases DNA methylation as well as the expression of DNMT3A and DNMT3B in the NAc (Anier et al., 2010).

Genome-Wide Studies. Drug-induced histone modifications can be identified and characterized across the genome using genome-wide (GW) promoter arrays or massively parallel DNA sequencing platforms (ChIP-chip) (Renthal et al., 2009; Zhou et al., 2011). These high-throughput methods characterize complex drug-induced signatures of epigenetic regulation including various histone modifications that regulate transcription of gene networks and may underlie drug-induced behavioral plasticity. *Chronic cocaine* exposure regulates gene transcription by either increasing histone H3 or H4 acetylation (to elevate mRNA levels), or by increasing histone H3 dimethyl-K9/27 (to reduce mRNA expression) (Renthal et al., 2009). Chronic cocaine exposure also decreases repressive histone methylation in the NAc and ChIP-Seq reveals that these histone marks are associated with intergenic genomic regions (Maze et al., 2011). These results suggest that cocaine-induced histone methylation produces heterochromatic *derepression* and increases expression of retrotransposable elements that in turn regulate gene transcription (Maze et al., 2011).

A recent study used whole genome sequencing of mRNA transcripts to characterize histone methylation and gene expression in postmortem hippocampal tissue from cocaine-dependent subjects (Zhou et al., 2011). Most of the GW studies identify dynamic chromatin signatures following chronic cocaine exposure that might play critical roles in drug taking and seeking. Taken together, these studies show that cocaine acts to alter patterns of gene expression in the NAc and hippocampus through epigenetic mechanisms (i.e., histone acetylation and methylation) that promote stable, persistent changes in gene expression. Thus, genome-wide studies identify dynamic chromatin signatures following chronic cocaine exposure and reveal novel gene targets and molecular regulatory

pathways that may play critical roles in drug taking and seeking. It is not clear whether other psychostimulants and drugs of abuse exert their behavioral effects through similar or divergent epigenetic regulation of gene networks.

MicroRNAs have emerged as a new class of epigenetic regulators that are capable of altering synaptic plasticity and behavior. MiRNAs are a class of non-protein coding RNA transcripts (~19–24 nucleotides) that regulate gene expression at the posttranscriptional level (Ambros, 2004; Rodriguez et al., 2004). More than 33% of the mammalian genome is subject to miRNA regulation and each miRNA targets on average 200 mRNA transcripts (Friedman et al., 2009 a,b). A growing literature indicates that miRNAs have diverse effects on gene expression including mRNA degradation, increased mRNA translation, chromatin remodeling, and DNA methylation (for rev see Schmiedt et al., 2013).

It is well established now that molecular regulation of gene expression by miRNAs is a complex phenomenon. In addition to repressing protein synthesis and directing sequence-specific degradation of complementary mRNA, the miRNA/miRISC (miRNA induced silencing complex) complex has been shown to induce gene expression by activating mRNA translation (Steitz and Vasudevan, 2009). miRNAs also remodel chromatin structure and increase DNA methylation thereby altering expression of target genes and, in some cases, inducing gene activation (Place et al., 2008).

Recent studies indicate that *compulsive cocaine consumption* is mediated, in part, by miRNAs. Cocaine self-administration increases expression of miR-212 in the dorsal striatum (Hollander et al. 2010) and it is associated with decreased cocaine self-administration suggesting that up-regulation of striatal miR-212 is a compensatory mechanism decreasing the motivational properties of cocaine (Hollander et al., 2010).

Chronic cocaine exposure also increases expression of miR-181a and decreases expression of miR-124 and let-7d in the mesocorticolimbic dopamine system. While increased expression of miR-124 attenuates cocaine-induced conditioned place preference (CPP), increased expression of miR-181a in the NAc enhances cocaine CPP (Chandrasekar and Dreyer, 2011). Differential behavioral effects of miR-124, let-7d, and miR-181a are associated with distinct changes in gene expression in the NAc (Chandrasekar and Dreyer, 2011; Saini et al., 2007). These and similar results suggest that complex miRNA regulatory path-

ways modulate cocaine-induced behavioral plasticity by directing expression of gene networks.

Thus, miRNAs are epigenetic regulators that play a critical role in translating drug-induced changes in synaptic plasticity into persistent neuroadaptations associated with drug addiction. Although miRNAs may represent promising new targets in the development of novel therapies to treat drug craving and relapse, future studies are needed to determine the precise role of miRNAs and their targets in the molecular mechanisms underlying drug addiction.

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF)

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that includes nerve growth factor, neurotrophin-3, and neurotrophin 4/5 (Thoenen, 1995). Synaptic secretion of BDNF and subsequent TrkB (tropomyosin receptor kinase B) receptor activation are associated with increased glutamatergic activity (Hartmann et al., 2001; Balkowiec and Katz, 2002).

Furthermore, BDNF promotes both early and late-phase long-term potentiation, LTP (Bramham et al., 1996).

BDNF is a key mediator of the activity-dependent processes in the brain that have a major impact on neuronal development, neurogenesis and plasticity.

Impaired control of neuronal activity-induced BDNF expression mediates the pathogenesis of various neurological and psychiatric disorders. Different environmental stimuli, such as the use of pharmacological compounds, physical and learning exercises or stress exposure, lead to activation of specific neuronal networks. These processes induce tight temporal and spatial transcriptional control of numerous BDNF splice variants through epigenetic mechanisms. Recent reviews highlight findings on the dynamic and long-term epigenetic programming of BDNF gene expression by the DNA methylation, histone-modifying and microRNA machineries, and summarize the current knowledge on the activity-dependent BDNF mRNA trafficking critical for rapid local regulation of BDNF levels and synaptic plasticity (Schmiedt, 2013; Karpova, 2013).

BDNF mRNA is expressed abundantly in cortical and midbrain dopamine neurons and also in striatal neurons (Lipska et al., 2001). Cortical pyramidal neurons are thought to supply ~80% and DA neurons ~20% of BDNF protein within the striatum (Altar et al., 1997). Endogenous BDNF mRNA and protein are

differentially regulated in mesolimbic and cortical neurons in response to acute and repeated administration of psychostimulants or during extended periods of drug abstinence (Meredith et al., 2002; Fumagalli et al., 2007; Saylor and McGinty, 2008). In addition, a persistent BDNF protein response develops in mesolimbic, striatal, and cortical structures and lasts for extended durations during cocaine abstinence (Grimm et al., 2003; McGinty et al., 2010). Altered expression of BDNF in this network of reciprocally interconnected structures following cocaine exposure and/or drug abstinence suggests that BDNF may constitute an important component of cocaine-induced plasticity.

Moreover, suppression of endogenous BDNF signaling, by infusing a neutralizing antibody to BDNF in the dorsal striatum, decreases cocaine intake (Im et al., 2010). Thus, exogenous infusion or manipulation of endogenous BDNF levels has a selective functional impact in different target areas that are critical to mediating or preventing cocaine-induced dysfunctional neuroadaptations (Schmiedt et al., 2013).

Epigenetic mechanisms involved in psychostimulant induced expression of BDNF: (i) *Histone acetylation, methylation and phosphorylation* at BDNF promoters. Histone acetylation at lysine, is an epigenetic imprint of transcriptionally active chromatin, whereas histone methylation is mostly associated with repressed chromatin. Phosphorylation at serine, threonine, or tyrosine, is generally linked to gene activation. A growing body of evidence suggests that epigenetic mechanisms of regulation are important for the modulation of drug-induced *bdnf* transcription. Cocaine-induced increases in *bdnf* transcription are associated with increased histone acetylation at several *bdnf* promoters (Kumar et al., 2005; Sadri-Vakili et al., 2010; Schmidt et al., 2011 and 2013). (ii) Different environmental stimuli affect the *DNA methylation* pattern and lead to the formation of the repressive (more condensed) or active (more open) chromatin structure, resulting in the decreased or increased gene transcription. The transfer of a CH₃ group from the methyl donor S-adenosylmethionine to the cytosine in the CpG dinucleotide sequence is catalyzed by DNA methyltransferases, DNMT. Low DNA methylation level is correlated with the increased gene transcription level through the binding of transcription factors, histone acetylases, and RNA polymerase II, to promoter region. Increased amount of methylated CpG sites recruits methylated DNA-binding proteins, such as MeCP2, and a chromatin silencing complex that contains histone

deacetylases (HDAC), and histone methyltransferases, (HMT), resulting in the repressed gene transcription (Karpova, 2013). Reduced DNA methylation of various regions across the *bdnf* locus has been detected following many stimuli that increase BDNF expression (Lubin et al., 2008; Ma et al., 2009). Evidence for the functional importance of DNA methylation in the regulation of BDNF transcription comes largely from studies in which MeCP2 (methyl-CpG-binding protein-2) expression has been disrupted. Expression of MeCP2 is up-regulated following chronic cocaine self-administration in the dorsal striatum of rats where knockdown of MeCP2 expression is associated with impaired cocaine-dependent up-regulation of BDNF protein (Im et al., 2010; Host et al., 2011). (iii) miRNAs represent a third epigenetic mechanism that may contribute to psychostimulant-induced expression of BDNF. Although a number of miRNAs can bind directly to BDNF (Friedman et al., 2009a; Muinos-Gimeno et al., 2011), the relevance of this epigenetic mechanism for the in vivo regulation of BDNF levels remains to be determined. However, miRNAs may impact cocaine-induced BDNF expression indirectly via regulation of MeCP2 expression and CREB activation. Cocaine self-administration is associated with increased expression of miR-132 and miR-212 in the dorsal striatum, both of which can repress the expression of MeCP2 (Klein et al., 2007; Hollander et al., 2010; Im et al., 2010). MeCP2 appears to exert a complementary repression of miR-132 and miR-212, suggesting that these transcriptional regulators are engaged in a homeostatic feedback loop (Klein et al., 2007; Im et al., 2010). Overexpression of miR-212 in the dorsal striatum attenuates cocaine-induced up-regulation of MeCP2 expression and inhibits cocaine-induced up-regulation of BDNF protein (Im et al., 2010). Interestingly, in addition to its effects on MeCP2 expression, miR-212 has been shown to amplify CREB signaling and to increase cocaine-induced expression of CREB-target genes including *Fos* (Hollander et al., 2010; Im et al., 2010). BDNF transcription is also regulated by CREB as well as MeCP2 (Shieh et al., 1998) and it remains to be determined why the effects of miR-212 overexpression on MeCP2 appear to dominate with respect to BDNF regulation over the effects on CREB.

BRAIN ANTIREWARD SYSTEMS AND ADDICTION

The opponent brain reward processes are hypothesized to produce inhibition of brain reward, and to show progressive enhancement of strength over time.

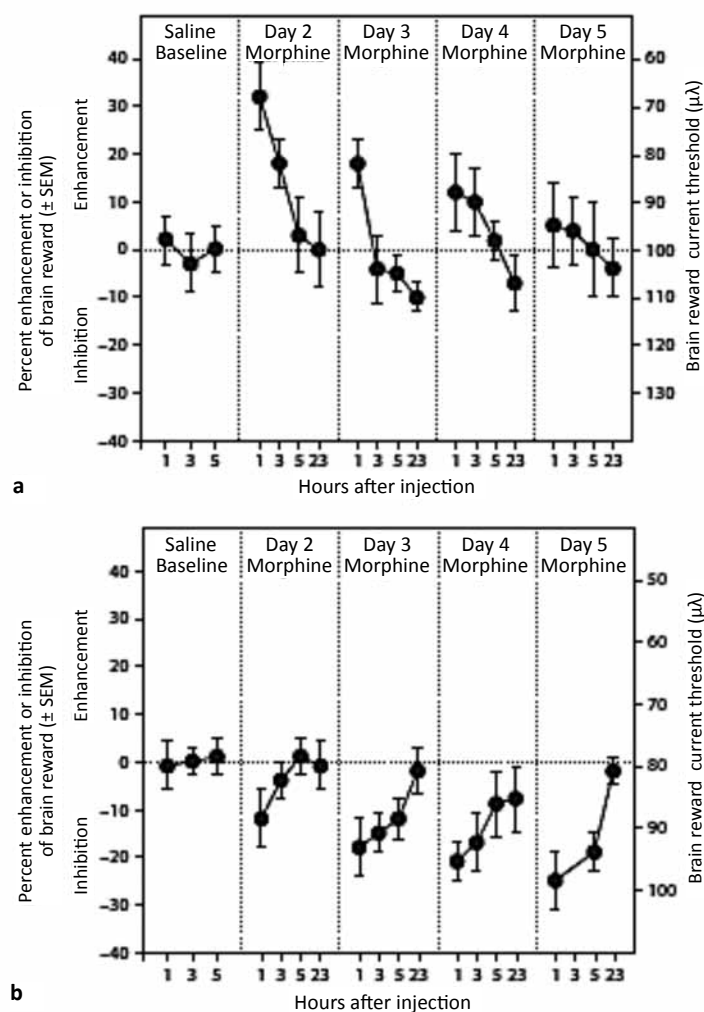


Figure 3 Proreward (a) and antireward (b) brain stimulation reward substrates activated by opiates (Gardner, 2013)

The *proponent* (*proreward*) and *opponent* (*antireward*) processes are distinguished (Koob and le Moal, 2008; Gardner, 2011).

The *proponent* brain reward processes are supposed to occur simultaneously and oppose each other in a mutually inhibitory fashion. Using electrical brain stimulation reward in laboratory rodents, Gardner and Lowinson (1993) reported proreward and antireward processes that correspond well to Koob's hypothesized proponent and opponent brain reward mechanisms. The proreward and antireward mechanisms have important implications for understanding the nature of the overall shift in reward level or hedonic tone produced by addictive drugs. If the reward-enhancing effect is combined with the

reward-inhibiting effect, it appears that administration of an opiate (e.g. morphine) initially produces a strong enhancement of brain reward (the high) that is countered by only a weak simultaneous antireward process. The net effect on brain reward or subjective hedonic tone is significant reward enhancement (high). However, with *repeated* opiate administration, the proreward mechanism gradually diminishes, while the antireward mechanism grows progressively stronger (Fig. 3).

Thus, with repeated opiate administration, the overall net effect on hedonic tone becomes more and more inhibitory since the opponent processes grow stronger and are opposed by progressively weaker proponent processes.

While brain reward is enhanced by acute administration of addictive drugs, conversely, in withdrawal, brain reward is inhibited, sometimes profoundly (Gardner, 2011). It should be well noted that this hedonic withdrawal state is unrelated to the physical withdrawal state experienced concomitantly by addicts experiencing acute withdrawal (e.g. cramps, diarrhea and physical pain during opiate withdrawal).

REWARD DEFICIENCY SYNDROME (RDS)

The term Reward Deficiency Syndrome (RDS) refers to a failure of the system that normally confers satisfaction include drug and alcohol abuse, overeating, heavy cigarette smoking, gambling and hyperactivity (Blum, et al., 1996 a,b and 2012). RDS might result from a dysfunction in the “brain reward cascade,” a complex interaction among neurotransmitters (primarily dopaminergic and opioidergic).

Individuals who have a family history of several addictions may be born with a deficiency in the ability to produce or use these neurotransmitters, due to a genetic defect, especially to hypoactivity of dopamine receptors. Thus, elevated stress levels, together with polymorphisms of dopaminergic genes and other neurotransmitter genetic variants, may have a cumulative effect on vulnerability to addiction. Thus, according to this hypothesis addiction appears to correlate with a hypodopaminergic dysfunctional state, producing an addiction-prone personality. Neuroimaging studies in humans confirm this hypothesis. Credible evidence also implicates serotonergic, opioid, endocannabinoid, GABAergic and glutamatergic mechanisms in addiction as denoted in the brain reward cascade hypothesis.

A “bio-psycho-social model” of etiology holds very well for addiction.

According to the hypothesis treating RDS (e.g., drug addiction such as cocaine dependence) should include, at least in part, DA D2 agonist therapy. Peng et al. (2010) evaluated the slow-onset long-acting monoamine reuptake inhibitor 31,345, a trans-aminotetralin analog, in a variety of addiction related animal models. Their findings suggested that 31,345 is a cocaine-like slow-onset long-acting monoamine transporter inhibitor that may act as an agonist therapy for cocaine addiction. However, its pattern of action appeared to be significantly different from that of methadone used as an agonist opioid therapeutic modality.

Peng et al. (2010) suggested that ideal agonist substitutes for cocaine should be similar to methadone’s

actions, that is, functionally antagonizing cocaine’s action while blocking monoamine transporters to augment synaptic DA.

REWARD-RELATED LEARNING AND MEMORY

Addiction might be considered as a disease of maladaptive learning. Preclinical studies suggest that physiological learning processes are similar to changes observed in addicts at the molecular, neuronal, and structural levels (Kiefer and Dinter, 2013; Martin et al., 2000; Krügel et al., 2013; Pázmány et al., 2013). Based on the importance of classical and instrumental conditioning in the development and maintenance of addictive disorders, many have suggested cue-exposure-based extinction training of conditioned, drug-related responses as a potential new treatment of addiction. It may also be possible to facilitate this extinction training with pharmacological compounds that strengthen memory consolidation during cue exposure. Another potential therapeutic intervention would be based on the so-called reconsolidation theory. According to this hypothesis, already-consolidated memories return to a labile state when reactivated, allowing them to undergo another phase of consolidation–reconsolidation which can be pharmacologically manipulated. These approaches suggest that the extinction of drug-related memories may represent a viable treatment strategy in the future treatment of addiction (Kiefer and Dinter, 2013).

Stimulus-reward and stimulus-action learning processes associate specific cues and contexts, with particular responses such as:

- wanting a reward,
- taking action to gain that reward,
- consummation/consumption.

Learning the predictive significance of a specific cue and connecting that information with appropriate responses require the storage of specific patterns of information in the brain (for review see Hyman et al., 2005 and 2006). This stored information must provide internal representations of the reward-related stimulus, its valuation, and a series of action sequences so that the cue can trigger an efficient and successful behavioral response. The same must be true for aversive cues that signal danger. Phasic dopamine release in the NAc, PFC, amygdala, and dorsal striatum appears to mark the motivational significance and value of particular experiences, cues, or action responses.

The firing of VTA dopamine neurons does not, however, encode specific information about specific experiences, cues, or actions. The dopamine innerva-

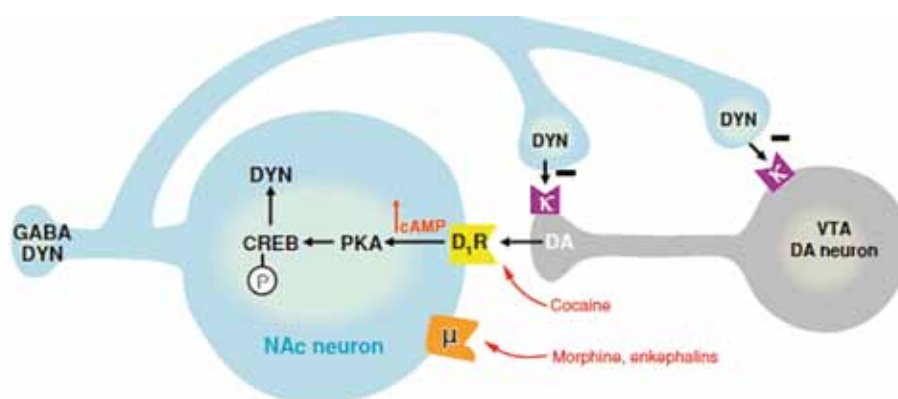


Figure 4 Induction of dynorphin peptides by dopamine

Cocaine and amphetamine activate prodynorphin gene expression in the nucleus accumbens (NAc) and dorsal striatum via D1 dopamine receptors stimulation, the cyclic AMP pathway, and the phosphorylation of CREB (cAMP response element binding protein). Dynorphin peptides are agonists at inhibitory κ (kappa) opiate receptors, resulting in decreased dopamine release. This mechanism may contribute to emotional and motivational aspects of drug withdrawal.

tion of the brain consists of a relatively small number of cell bodies in the midbrain that project widely throughout the neuraxis with single cells innervating multiple targets. LTP and long-term depression (LTD) have been hypothesized to play critical roles in many forms of experience-dependent plasticity, including various forms of learning and memory. Such mechanisms of synaptic plasticity could lead subsequently to the reorganization of neural circuitry by altering gene and protein expression in neurons that receive enhanced or diminished signals as a result of LTP or LTD.

LTP and LTD have thus become important candidate mechanisms for the drug-induced alterations of neural circuit function that are supposed to occur with addiction (Hyman and Malenka, 2001; Malenka and Bear, 2004). There is now good evidence that both mechanisms occur in the VTA, and also in the NAc and other targets of VTA dopamine neurons as a consequence of drug administration. Addictive drugs act on the PFC to produce pathological valuations and to interfere with top-down control of behavior. Although dopamine appears to influence LTP and LTD in the PFC (Hyman et al., 2006; Huang et al., 2004), little is known about the mechanisms by which addictive drugs modify synaptic properties in this region.

The transcription factor most studied in the context of learning and memory is *CREB* (cAMP response element binding protein). CREB is activated upon its phosphorylation by protein kinase A, CaM-kinases (e.g., CaMKIV), or growth factor-

associated kinases, which indicates that CREB is a point of convergence of numerous neurotransmitter intracellular signaling pathways. Gene knockout studies have shown that CREB is required for long-term behavioral memory in diverse animal species (Carlezon et al., 2005). One potentially significant candidate as the target genes and cellular pathways through which CREB might exert effects on memory is the mammalian hippocampus NMDA glutamate receptor signaling pathway (for rev see Hyman et al., 2006). CREB is phosphorylated and activated in several reward-related regions (e.g., VTA, amygdale and frontal cortex) by acute and chronic administration of stimulant and opiate drugs (Konradi et al., 1994; Olson et al., 2005).

The induction of CREB activity appears to become greater and more persistent with repeated drug exposures. The functional significance of this effect is best established within the NAc. Here, the ability of stimulants to induce CREB is mediated via activation of D1 receptor (Konradi et al., 1994). The mechanism underlying opiate induction of CREB could also be dopamine dependent. However, CREB induction in the NAc does not appear to be shared by all addictive drugs; nicotine and ethanol have been reported to decrease CREB activity in this region (Brunzell et al., 2003).

At least some of the CREB-mediated decrease in the rewarding properties of drugs is mediated by the induction of prodynorphin mRNA which encodes the dynorphin peptides (Cole et al., 1995). Dynorphin

acts on kappa opioid receptors on VTA neurons to decrease dopamine release (Fig. 4).

Cocaine and amphetamine have been shown to activate *prodynorphin* gene expression in the NAc and dorsal striatum via D1 dopamine receptors stimulation, the cyclic AMP pathway, and the phosphorylation of CREB (Cole et al., 1995). The resulting dynorphin peptides are transported to presynaptic terminals including terminals found on recurrent collateral axons that feed back on dopaminergic neurons. Dynorphin peptides are agonists at inhibitory kappa opioid receptors, resulting in decreased dopamine release. This mechanism may contribute to emotional and motivational aspects of drug withdrawal (Cole et al., 1995; Kiraly et al., 2006).

Persistent activation of CREB, and the resulting induction of dynorphin, in response to long-term drug exposure would appear to represent a mechanism of *tolerance and possibly dependence* leading to dysphoria during drug withdrawal (Carlezon et al., 2005). The effects of CREB are also mediated via changes in the intrinsic electrical excitability of NAc neurons. The best-established role of CREB in the addiction process is the locus coeruleus, the major noradrenergic nucleus in brain which normally regulates attention and vigilance. Opiate induction of CREB in this brain region is one mechanism underlying opiate physical dependence and withdrawal (see Nestler and Aghajanian, 1997). CREB is also known to be induced by chronic administration of addictive drugs in the VTA, where its effect on drug sensitivity is complex: CREB can either promote or diminish sensitivity to the behavioral effects of cocaine and opiates depending on whether it is induced in more rostral or caudal subregions of this nucleus (Olson et al., 2005).

THE PRINCIPLES OF PHARMACOTHERAPY OF ADDICTIVE DISORDERS

At present, a number of effective pharmacotherapies exist for the treatment of drug addiction (for review see Gardner, 2011; Nutt and Lingford-Hughes, 2008; Funk, 2013).

Opioids: Agonist substitution treatment: the opiate agonist methadone has been shown to be effective in opiate addiction. Even heroin itself has been successfully used as a maintenance pharmacotherapy for opiate addiction. Although the short half-life of heroin makes it a less optimal choice for maintenance therapy, there appears to be a subset of opiate addicts who do better on heroin maintenance than on other opioid agonist therapies. *Antagonists* for highly motivated

individuals with a strong desire to achieve abstinence from the opiate-taking habit, the long-acting oral opiate antagonist naltrexone has proven effective.

Alcohol and marijuana addiction. Antagonists: naltrexone was found to be also effective for some alcoholics wishing to quell alcohol cravings. The reason for the effectiveness of naltrexone in alcoholism is not clearly understood but may relate to the fact that the dopaminergic reward circuitry of the brain is heavily innervated by endogenous opioid peptide (endorphin and enkephalin) circuits, and this opioid peptidergic innervation is known to modulate the reward-enhancing properties of alcohol and other addictive compounds (e.g. Δ^9 -tetrahydrocannabinol, the addictive constituent in marijuana and hashish (Chen et al., 1990; Tanda et al., 1997). A newer mu-opioid receptor antagonist nalmefene which has kappa partial agonist actions, has recently been shown to have efficacy in treating alcohol dependence (Srisurapanont and Jarusuraisin, 2005).

Benzodiazepine abuse is common and there is an effective *antagonist* licensed for human use: flumazenil which is a highly potent, high-affinity antagonist used only by the intravenous route as first-pass metabolism is extensive. Using technology similar to the naltrexone implants, an implantable flumazenil formulation was developed to produce antagonist effect for several months, to treat benzodiazepine dependence. Other benzodiazepine antagonists have been developed and used too (Nutt and Lingford-Hughes, 2008; Funk, 2013).

Cannabis (Δ^9 -tetrahydrocannabinol) dependence is becoming more recognized. It is known that the actions of this substance are mediated via the cannabinoid CB1 receptor for which a number of potent and selective antagonists were developed. They block the effects of cannabis and can precipitate withdrawal, so therapeutic potential as functional antagonists is clearly there. Their target indication is weight loss but they might be used for other indications such as cannabis dependence, too (Nutt, 2005).

Stimulants: One of the great concerns in the addiction field is the poor treatment possibility for stimulant dependence. Cocaine dependence which affects 2.5 million Americans annually, has no US Food and Drug Administration-approved pharmacotherapy, including no cocaine vaccine (Martell et al., 2009). Psychostimulant abuse is a major medical and social problem. *Cocaine, amphetamine (and derivatives), methamphetamine, MDMA (ecstasy: N-methyl-3,4-methylene-dioxy-methamphetamine)* and the new designer synthetic derivatives of *cathinon* are the

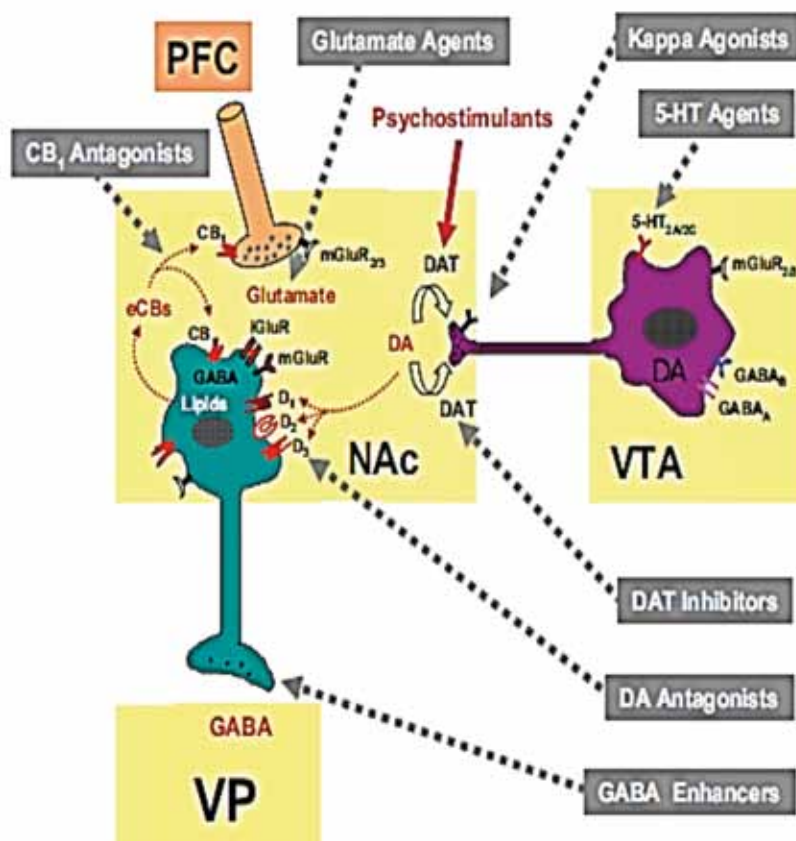


Figure 5 Schematic representation of the VTA-NAC-VP reward pathway
 Actions of psychostimulants. NAC, nucleus accumbens; VTA, ventral tegmental area; VP, ventral pallidum; PFC, prefrontal cortex; DAT, dopamine transporter (Zheng-Xiong and Gardner, 2008).

most commonly abused psychostimulants in humans (Zheng-Xiong and Gardner, 2008). Although many compounds have been tested, few have been found to be even minimally effective.

Similarly to other abused drugs the VTA-NAC-VP pathway and, in particular, functional inhibition of NAC-VP GABAergic projection neurons appears to play a critical role in mediating psychostimulant reward and relapse. The same pathway mediates endocannabinoid reward by activating CB1 receptors located on both GABAergic and glutamatergic neurons and/or terminals that results in an inhibition of medium-spiny GABAergic neurons. This GABAergic hypothesis may also explain why NMDA receptor antagonists have rewarding effects (Fig. 5).

Further, decreased NAC glutamate transmission during cocaine self-administration or early withdrawal/abstinence appears to play a critical role in drug craving and relapse to drug use. Decreased

glutamate tone on mGluR2/3 autoreceptors located on presynaptic glutamate terminals may also provide an explanation for the increased glutamate response upon reexposure to cocaine or cocaine-associated environmental cues (Fig. 5).

Thus, pharmacological strategies that either block NAC DA receptors or increase GABAergic transmission in brain reward circuits could antagonize a given drug's rewarding effects ("antagonist" treatment), while restoration of reduced basal glutamate or DA transmission could be helpful in relieving drug craving or relapse to drug use ("agonist" treatment) (Zheng-Xiong and Gardner, 2008).

As stimulants work indirectly on neurotransmitter pumps to increase release and/or prevent reuptake, the use of *antagonists* is problematic. However, there have been attempts to produce *dopamine uptake blockers* with less abuse potential than cocaine, and animal studies have shown a degree of efficacy, although

human trials to our knowledge have not been reported (Morris et al., 2006). Another, perhaps more promising, use of antagonists in stimulant abuse is the *dopamine D3 receptor*, where in animal studies antagonists have been shown to reduce drug-seeking behaviours. Similarly, dopamine D1 agonism may be stimulant-like then antagonism could attenuate cocaine-seeking behaviour, as it seems to do for novelty-induced behaviours. Perhaps, a drug that combined both actions would have even greater utility (Nutt and Lingford-Hughes, 2008).

Trial information on the use of *sertraline, bupropion, mirtazapine, modafinil, dextroamphetamine, ondansetron, risperidone, aripiprazole, baclofen, and gabapentin, trazodone* was reviewed. Aripiprazole trials appeared in the reviewed literature more frequently than the other medications. Based on the findings of several reviews, no single medication demonstrated consistent efficacy and each trial contained a variety of methodological limitations (Brackins et al., 2011; Funk, 2013).

In a promising clinical study, cocaine vaccine was investigated in cocaine dependence (Martell et al., 2011). Attaining high (≥ 43 $\mu\text{g/mL}$) IgG anticocaine antibody levels was associated with significantly reduced cocaine use but only 38% of the vaccinated subjects attained these IgG levels and they had only 2 months of adequate cocaine blockade. Thus, improved vaccines and boosters are needed for clinical use.

Partial agonist substitution treatment

Opioids: The partial opiate agonist *buprenorphine* is used with success in clinical practice in opioid addiction. **Nicotine:** Partial agonists have now been proven to be effective in nicotine addiction. **Varenicline:** (an $\alpha_4\beta_2$ partial nicotinic agonist) is licensed on the basis of efficacy that exceeds that of the other licensed medications—bupropion and nicotine replacement therapy (Rollema et al., 2007). The principles are the same as with buprenorphine for opioid dependence—substitution treatment to prevent the need to smoke. Interestingly, there is emerging evidence that varenicline may also reduce alcohol consumption suggesting a common nicotinic pathway in several addictions—as animal evidence suggests some effects of related compounds in opioid dependence (for rev. see Nutt and Lingford-Hughes, 2008). **Benzodiazepines:** In an attempt to make benzodiazepine anxiolytics less abused (that would also be less sedating), several partial agonists were made and tested in humans (for rev see Nutt and Lingford-Hughes, 2008).

Suggested future research directions to treat addictions (Nutt and Lingford-Hughes, 2008; Zheng-Xiong and Gardner, 2008):

- For the DA receptor *antagonist* strategy: more selective D3 receptor antagonists and more D3-preferring mixed D3/D2 receptor antagonists might be developed.
- For the DA *agonist* substitution strategy: any compound that “competitively” inhibits psychostimulant binding to DAT or monoamine transporters should be pharmacologically an agonist. This is based on evidence that the therapeutic effects of methadone on heroin addiction appear to be mediated by functional antagonism of heroin’s actions, not a simple “agonist substitution”.
- For the *GABA-based* medication strategy: development of GABA transaminase inhibitors with GABAC receptor antagonist properties may represent a new research direction in medication development for the treatment of psychostimulant addiction.
- For the *cannabinoid-based* medication strategy: development of more potent and selective neutral CB1 receptor antagonists may be more promising than the existing CB1 receptor antagonists e.g., which have significant inverse agonist properties.
- For the *glutamate-based* medication strategy: any compound that modestly elevates extracellular NAc glutamate but with fewer unwanted side-effects, should be developed to treat drug craving and relapse.
- For the *opioid-based* medication strategy: mixed kappa-preferring opioid receptor agonists with partial mu receptor agonist properties may be more promising in attenuating psychostimulant addiction.
- For the *serotonin-based* medication strategy: development of more effective and systemically active 5-HT_{2C} receptor agonists may represent another medication development direction.

CONCLUDING REMARKS

Addiction is a complex disease. There are several known and unknown factors influencing its development. This review summarizes some of the important novel directions to discover the underlying mechanisms and the new promising therapeutic targets for treatment of this neuropsychiatric disorder.

Neural substrates and transmitters. Given its widespread projections within the forebrain, dopamine is a key factor in the progression from pleasurable

experimentation with drugs to long-lived compulsion as persistent associative memories are formed in circuits involving the NAc, PFC, amygdala, and dorsal striatum. Neurotransmitters other than dopamine (GABA, endogenous opioids, serotonerg-, endocannabinoid, purinerg etc. systems), also play important roles in regulating hedonic states and even in reward-related learning.

Epigenetics. Increasing evidence suggests that epigenetic mechanisms including histone modifications, DNA methylation, and miRNAs regulate psychostimulant-induced gene expression profiles in discrete brain regions e.g. NAc and hippocampus. Many changes in chromatin regulation following chronic psychostimulant exposure correlate in time with the expression of maladaptive behaviors including drug taking and seeking. Also, molecular genetic studies have implicated some of the transcriptional regulatory factors in the induction of adaptive forms of neuroplasticity that appear to repress or inhibit drug self-administration. Further characterizing the molecular substrates that regulate chromatin remodeling and gene transcription following chronic drug exposure may identify novel drug targets for drug craving and relapse. miRNAs are epigenetic regulators that play a critical role in translating drug-induced changes in synaptic plasticity into persistent neuroadaptations associated with drug addiction. Although miRNAs may represent promising new targets in the development of novel therapies to treat drug craving and relapse, future studies are needed to determine the precise role of miRNAs and their targets in the molecular mechanisms underlying drug addiction.

Brain-derived neurotrophic factor (BDNF). BDNF is important in regulating synaptic plasticity in the brain areas that process reward information. BDNF was reported in the nucleus accumbens, a brain area critical for the rewarding effects of cocaine, promoting persistent cocaine-seeking behaviors and heightening relapse vulnerability.

Brain Antireward Systems and Reward Deficiency Syndrome (RDS) were also reported to play role in the development of drug addiction.

Although much progress has been made in understanding the neural substrates of drug addiction, transmitters, epigenetics and its relation to learning and memory but much remains to be elucidated and strong effort is necessary to integrate the rich information at the molecular, cellular, systems, and behavioral levels to further clarify this complex disease.

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Az addikció kialakulásának új neurobiológiai mechanizmusai

Bár az utóbbi évtizedben jelentősen megnöttek az addikcióval kapcsolatos ismereteink, annak neurológiai/morfológiai szubsztrátjairól, az érintett transzmitter rendszerekről, az epigenetikai háttérről, a tanulás/memóriatárolási folyamatokkal analóg mechanizmusokról jelentős ismeretanyag gyűlt össze, mégis nagyon sok láncszem hiányzik a teljes megértéshez. Nagy szerepe lesz a közeljövőben a számtalan molekuláris, celluláris, viselkedésfarmakológiai ismeret koherens értelmezésének abban, hogy a megfelelő, hatásos ellenszerek, terápiás eszközök kifejlesztésének tudományos alapjait kidolgozhatjuk. Elsősorban az érintett idegrendszeri hálózat, az addikció kifejlődésének mechanizmusai és a terápiás lehetőségek áttekintése a tanulmány célja az utóbbi évek szakirodalmára támaszkodva.

Kulcsszavak: addikció, drog abúzus, epigenetika, tanulás és memória, brain derived neurotropic factor (BDNF), az addiktív kórképek terápiája