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Review

The Brain on Drugs: From Reward to Addiction

Nora D. Volkow[1,*] and Marisela Morales[1]
[1]National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: nvolkow@nida.nih.gov
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Advances in neuroscience identified addiction as a chronic brain disease with strong genetic, neurodevelopmental, and sociocultural components. We here discuss the circuit- and cell-level mechanisms of this condition and its co-option of pathways regulating reward, self-control, and affect. Drugs of abuse exert their initial reinforcing effects by triggering supraphysiologic surges of dopamine in the nucleus accumbens that activate the direct striatal pathway via D1 receptors and inhibit the indirect striato-cortical pathway via D2 receptors. Repeated drug administration triggers neuroplastic changes in glutamatergic inputs to the striatum and midbrain dopamine neurons, enhancing the brain's reactivity to drug cues, reducing the sensitivity to non-drug rewards, weakening self-regulation, and increasing the sensitivity to stressful stimuli and dysphoria. Drug-induced impairments are long lasting; thus, interventions designed to mitigate or even reverse them would be beneficial for the treatment of addiction.

The nature of addiction is frequently debated as either a personal "lifestyle choice" or a "biological vulnerability." Current evidence shows that most drugs of abuse exert their initial reinforcing effects by activating reward circuits in the brain and that, while initial drug experimentation is largely a voluntary behavior, continued drug use impairs brain function by interfering with the capacity to exert self-control over drug-taking behaviors and rendering the brain more sensitive to stress and negative moods. Indeed, individuals with genetic vulnerabilities, exposed to chronic stress, or suffering from comorbid psychiatric conditions, as well as those who abused drugs during early adolescence, are at greater risk of transitioning into the automatic and compulsive behaviors that characterize addiction.

Drugs modulate the expression of genes involved in neuroplasticity through epigenetic and possibly RNA modifications, ultimately perturbing intracellular signaling cascades and the neuronal circuits whose dysfunction have been implicated in the long-lasting changes associated with addiction. Here, we highlight some of the most significant and recent findings in drug reward and addiction, describing the circuit, behavioral, and synaptic mechanisms underlying this process. Space limitations do not allow us to review the intracellular signaling cascades and epigenetic modifications associated with addiction; thus, we refer readers to recent reviews on these topics (Heller et al., 2014; Nestler, 2012; Pascoli et al., 2014a).

Drug Reward Signaling in Brain

Dopamine (DA) neurons located in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) play a key role in the processing of reward-related stimuli, including those associated with drugs of abuse (Wise, 2008). Drugs of abuse, through their different pharmacological effects, increase the release of DA in the shell subregion of the NAc (Di Chiara, 2002), mimicking the phasic DA neuronal firing that leads to very fast DA increases (Owesson-White et al., 2009) and thus the mechanism through which the brain signals reward (Box 1). The large DA increases triggered by phasic DA cell firing are necessary to stimulate D1 receptors (D1R) in the NAc.

DA neurons in the VTA fire in either a tonic (1–8 Hz) or a transient (<500 ms) high-frequency phasic mode (>15 Hz), with the phasic mode resulting in larger DA increases than the tonic mode. Though it was initially believed that DA signaling in the brain encoded for reward, more recent findings have revealed that it encodes for a reward prediction signal. Specifically, these studies have shown that phasic DA firing is time locked to unexpected or novel reward but is also triggered by cues that predict reward. Moreover, the firing frequency of DA neurons triggered by cues is associated with the expected reward value and its probability of delivery, but if the expected reward does not materialize, DA cell firing is inhibited (Schultz, 2002). Changes in the response patterns of DA cell firing are modulated by more distinct projections for tonic than for phasic firing (Box 1). Changes in phasic DA firing patterns modify the strength of cortico-striatal glutamatergic synapses, thus altering signaling in D1R- and D2R-expressing GABAergic medium spiny neurons (MSNs) (Paladini and Roeper, 2014). This is distinct from DA signaling in the NAc driven by release from tonic DA neuron firing, which results in lower DA increases than from phasic firing but that are sufficient to stimulate D2R signaling and have been mostly associated with motivational drive (Dreyer et al., 2010; Trifilieff et al., 2013). Though most studies link drug-induced neuroplasticity with the fast and large transient DA changes triggered by drugs, the contribution from

Box 1. Modulation of VTA DA Neuronal Firing

Recent pseudorabies virus-based methods for monosynaptic network tracing have shown that neurons from many brain areas synapse on distinct VTA DA neuron subpopulations (Lammel et al., 2014) and that neurons from the dorsal raphe (DR) provide the majority of monosynaptic inputs (Ogawa et al., 2014). Studies of the influence of these projections on DA neurons have been limited to a few brain structures (Paladini and Roeper, 2014). For instance, the control of tonic firing of VTA DA neurons involves the stria terminals and the ventral pallidum (Georges and Aston-Jones, 2001; Mahler et al., 2014), whereas the control of phasic firing of VTA DA neurons involves the pedunculo pontine tegmentum (PPT), the subthalamic nucleus (STN), and the laterodorsal tegmentum (Floresco et al., 2003; Lodge and Grace, 2006). VTA DA neurons receive GABAergic innervation from local GABAegic neurons, the NAc, globus pallidus, and rostromedial tegmental nucleus, among others. These GABAergic projections are implicated in the control of burst timing (Paladini and Roeper, 2014). It is likely that phasic and tonic changes in DA neuronal firing triggered by repeated drug administration, reflect neuroplastic changes in these regions and on inputs that relay to them. For example, the lateral habenula (LHb) indirectly inhibits VTA DA neurons via its inputs to GABA neurons in rostromedial tegmental nucleus (Ji and Shepard, 2007), eliciting aversion (Lammel et al., 2012), and these inputs are modified by repeated cocaine administration (Meye et al., 2015). Thus, future studies will be able to assess their contribution to the dysphoria and enhanced stress reactivity in addiction.

We recently showed abundant glutamatergic projections from the DR to VTA DA neurons that innervate the NAc, whose activation induced DA release in NAc and evoked reward (Qi et al., 2014). The DR is best known as a serotonergic structure that regulates emotional behaviors. However, findings on the role of DR serotonergic neurons in reward have been inconsistent (Cohen et al., 2015; Fonseca et al., 2015; Liu et al., 2014; McDevitt et al., 2014; Miyazaki et al., 2014), which is likely to reflect, in part, the functional diversity of these neurons. In this regard, cellular recordings from DR serotonergic neurons in behaving mice have revealed that they convey reward information through tonic as well as phasic firing and that they signal reward and punishment on multiple timescales (Cohen et al., 2015). The DR also has glutamatergic and GABAergic neurons, some of which co-release serotonin, and thus future studies are necessary to tease apart the specific targets of the diverse serotonergic neurons and of their neighboring GABAergic and glutamatergic neurons (Liu et al., 2014; McDevitt et al., 2014; Qi et al., 2014). In this regard, we recently showed that, within the VTA, DR neurons expressing the vesicular glutamate transport (VGluT3) preferentially establish synapses on DA neurons (Qi et al., 2014). These DR-VGluT3 neurons provide a major glutamatergic input to VTA DA neurons, including those that innervate the NAc. Selective activation of these DR-VGluT3 fibers results in VTA glutamate release, NAc DA release, and reward (Qi et al., 2014). Notably, these DR VGluT3-glutamatergic neurons (some of which may co-release serotonin) are highly interactive with the serotonergic system (Commons, 2009). Thus, a better understanding of the function and connections of the diverse DR neurons will help us determine whether they serve as a link between reward and mood regulation and whether they contribute to the high co-morbidity between drug use and depression.

the longer-lasting stimulation of D2R (also D3R and D4R) has been much less investigated.

VTA DA neurons project predominantly to the NAc, where DA interacts with D1R, D2R, and D3R, which are mainly expressed in MSNs. Stimulatory striatal MSNs that express D1R (D1R-MSNs) signal through the direct striatal pathway, whereas those that express D2R (D2R-MSNs) signal through the striatal indirect pathway and act in an inhibitory manner. D3R mostly co-localize with D1R-MSNs, with which they heteromerize, potentiating their function (Marcellino et al., 2008). The ventral striatal direct and indirect pathways have distinct roles in modulating reward and motivation. The direct pathway is associated with reward, whereas the indirect one is associated with punishment (Hikida et al., 2010; Kravitz et al., 2012). Thus, DA receptor stimulation of the direct pathway directly mediates reward, whereas DA-receptor-mediated inhibition of the indirect pathway opposes aversive responses. This could explain why maximal drug reward is obtained when DA binds to both D1R and D2R. However, in contrast to the situation in the dorsal striatum, where the direct and indirect pathways are fully segregated, in the NAc, both D1R- and D2R-expressing MSNs project into the ventral globus pallidum (Smith et al., 2013b). To be reinforcing, drug-induced DA increases need to be fast and sufficiently large to stimulate low-affinity D1R in addition to D2R, leading to the activation of the direct pathway and the inhibition of the indirect pathway. D1R stimulation in the NAc by itself is sufficient to produce drug reward (Caine et al., 2007), whereas D2R stimulation is not (Caine et al., 2002; Durieux et al., 2009; Norman et al., 2011), and maximal reward occurs when both D1R and D2R are activated (Steinberg et al., 2014; Welter et al., 2007). Indeed, brain imaging studies in humans have documented that fast DA increases triggered by drugs are associated with the "high" associated with drug abuses, whereas stable DA increases are not (Volkow et al., 2008). Specifically, when large DA increases triggered by stimulant drugs were achieved over a short time period (<10 min), they were associated with reward, whereas DA increases achieved over 60 min were not. The rate dependency for a drug's rewarding effects might explain why the time course of the subjective "high" is much shorter than the longer-lasting DA increases triggered by drugs such as cocaine and more notable methylphenidate (Figure 1). Presumably, stimulation of D1 and D2R only occurs when drugs achieve fast peak concentrations, whereas as the concentration of DA starts to decrease, D2R are predominantly stimulated (Luo et al., 2011). This may also explain why routes of administration that achieve faster and higher drug levels in the brain, such as smoking and intravenous injection, are more rewarding and addictive than routes of administration that result in slow brain uptake, like oral administration.

DA increases that are sufficiently large to activate D1R, such as those induced by drugs in the NAc, can induce associative learning, also referred to as conditioning (Zweifel et al., 2009). Stimuli (including contextual or environmental) associated with the drug become conditioned and, with repeated co-exposure, will trigger phasic DA neuronal firing in the VTA, resulting in fast, large, and short-lasting DA increases in the NAc. The DA increases triggered by these conditioned stimuli (CS) are believed to reflect the expectation of receiving a reward. Glutamatergic projections into D1R-expressing MSNs coming from the amygdala (involved in emotional reactivity), hippocampus (involved in memory), and ventral PFC (involved in salience attribution) mediate these conditioned responses. The increased dopaminergic signaling that follows exposure to the CS ensures that

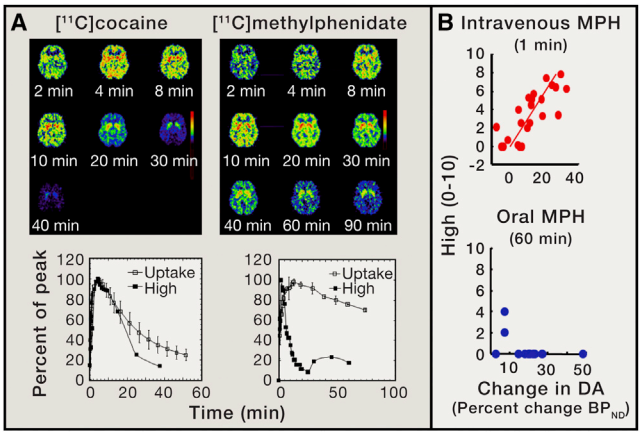

Figure 1. Fast Brain Uptake and Fast DA Increases Triggered by Drugs Are Necessary for Reward

(A) Pharmacokinetics of cocaine and methylphenidate (MPH) in the human brain and relationship to the drug-induced "high." Upper panels show axial brain images of the distribution of [^{11}C]cocaine and [^{11}C]methylphenidate at different time points (in minutes) following their intravenous administration. Lower panels show time activity curves for the concentration of [^{11}C]cocaine and [^{11}C]methylphenidate in the striatum in conjunction with the temporal course of the "high" experienced after intravenous administration of the drug. These findings suggest that the "high" is associated with the initial fast rate of uptake of the drug in brain and presumably the associated fast DA increases they trigger.

(B) Regression slopes between MPH-induced DA increase (assessed as the reduction in the specific binding of [^{11}C]raclopride to D2R and D3R) and the self-reports of "high" when MPH was administered intravenously, which results in very fast drug delivery in the brain (measures initiated at 1 min post MPH) and when MPH was administered orally, which results in very slow brain uptake (measures initiated at 60 min post MPH). MPH-induced DA increases resulted in a "high" only when it was given intravenously, but not when given orally, consistent with fast DA increases being necessary for drug reward. Figure modified from Volkow et al., 1995, 1999, 2001b.

the individual will have the motivation to engage in the behaviors necessary to procure the reinforcer, be it natural or pharmacological. Because D1R have a lower affinity for DA than D2R, cue exposure or drug intoxication will lead to D1R occupancy only when peak DA levels are present; all the while, DA binding to D2R will be longer-lasting and persist even after peak levels have subsided (Luo et al., 2011). Thus, while DA stimulation of D1R-MSNs in the direct pathway signals the expectation for the reward, DA stimulation of D2R-MSNs in the indirect pathway is more likely to sustain the motivation needed to procure and consume the reinforcer.

For natural reinforcers such as food or sex, the DA signals triggered by the CS drive the motivation to get the reward since with their repeated delivery the DA cells stop firing in response to their consumption (Schultz et al., 1997). This is in sharp contrast to the response to drugs of abuse, which due to their pharmacological properties, continue increasing DA release during their consumption. Thus, DA in the NAc will increase upon exposure to drug cues, which will trigger the desire to take the drug (craving) also during their consumption, which will sustain the motivation to continue consuming them. This may explain why drugs are more likely to result in compulsive patterns of administration than natural reinforcers. However, the DA increases triggered by cocaine, and presumably other drugs, activate D2R auto-receptors inhibiting DA cell firing and DA release (Bello et al., 2011), which is perhaps why the intensity of the cocaine "high" is reduced with subsequent

Box 2. Opioid Regulation of the Mesolimbic DA Pathway

Endogenous opioids modulate DA neuronal firing in midbrain (Margolis et al., 2014) and striatal MSNs (Gianoulakis, 2009), where dynorphin co-localizes in D1R-MSNs and enkephalin in D2R-MSNs (Gerfen et al., 1990). Endogenous opioids are implicated in hedonic responses to natural and drug rewards (Le Merrer et al., 2009) and also in the adaptations that follow repeated drug exposures and drug relapse (Koob et al., 2014).

Microdialysis studies measuring the effects of various drug classes (including stimulants, opiates, THC, and alcohol) have found increases in extracellular levels of endogenous opioids in the NAc and VTA (reviewed in Murphy, 2015). The roles of endogenous opioids in drug reward are particularly well established for alcohol and opiates, whose intake is reduced in mu opioid receptor (*MOR*) knockout mice and decreased by administration of opioid receptor antagonists (reviewed in Tseng et al., 2013). Moreover, naltrexone, an opiate antagonist, is an FDA-approved medication for the treatment of alcoholism and opiate use disorders. In animal models, MOR antagonists interfere with cocaine and nicotine reward (Berrendero et al., 2010; Giuliano et al., 2013); however, clinical trials with naltrexone have failed to show therapeutic benefit for cocaine or nicotine use disorders.

The endogenous opioid system also contributes to the effects of stress on drug consumption. Specifically, dynorphin, through its activation of kappa receptors (KOR), is implicated in the stress-induced potentiation of drug reward (reviewed in Ehrich et al., 2014). Activation of KOR on DA terminals inhibits DA release in the NAc, which is implicated in the dysphoria that follows drug withdrawal (Tejeda et al., 2012). These findings have generated interest in KOR antagonists or partial agonists as medications to prevent relapse in addiction (Al-Hasani et al., 2013; Butelman et al., 2012; Grosshans et al., 2015; Schlosburg et al., 2013; Smith et al., 2013a).

The effects of drugs on the opioid system in the human brain have been investigated with positron emission tomography (PET) using [^{11}C]carfentanil to assess MOR and their occupancy by enkephalins. These studies have shown that acute alcohol, but not intravenous amphetamine, increases enkephalins (Guterstam et al., 2013; Mitchell et al., 2012). Increases in enkephalins after cigarette smoking have been inconsistent, which is likely to reflect, in part, the influence of *MOR* gene variants in these responses. More specifically, increases were observed only in smokers with the AA variant of the *MOR* A118G polymorphism (Domino et al., 2015).

Brain-imaging studies of MOR in alcoholics and cocaine abusers have reported increased [^{11}C]carfentanil binding, which has been interpreted to reflect reduced levels of endogenous enkephalins (hence, decreased competition for ligand binding) though they could also reflect MOR upregulation (Weerts et al., 2011; Zubieta et al., 1996). In cocaine abusers, the increases in [^{11}C]carfentanil binding have been associated with worse clinical outcomes (reviewed in Volkow, 2010). In contrast, studies in smokers have shown no changes in [^{11}C]carfentanil binding (Kuwabara et al., 2014).

Brain-imaging studies of delta opioid receptors in alcoholics (measured with PET and [^{11}C]methylnaltrindole) showed no differences when compared to controls (Weerts et al., 2011). To our knowledge, no PET studies have been done in substance abusers using kappa receptor ligands.

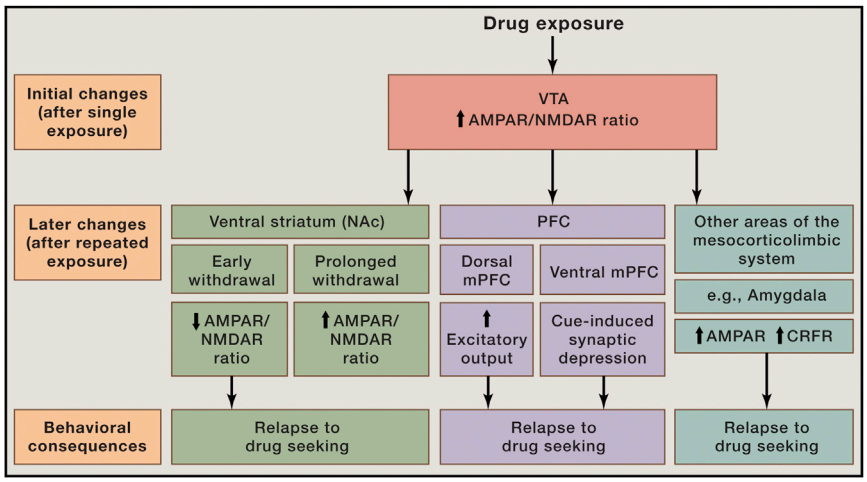

Figure 2. Drug-Induced Synaptic Neuroplasticity in Brain Reward Circuitry

Mesocorticolimbic brain areas where there is evidence of drug-induced neuroplasticity along with the associated synaptic modifications and their behavioral consequences. AMG, amygdala; NAc, nucleus accumbens; (m)PFC, (medial) prefrontal cortex; VTA, ventral tegmental area; CRFR, corticotropin releasing factor. Figure modified from van Huijstee and Mansvelder (2014) and work from Krishnan et al. (2010) and Lee et al. (2013).

administrations, whereas the motivation to continue to take the drug continues unabated.

Endogenous opioids and cannabinoids have been also implicated in drug reward responses, in part through the opioid regulation of the mesolimbic DA pathway (Box 2) and through studies of the role of cannabinoids in adaptations that occur with repeated drug exposures (for reviews, see Covey et al., 2014; Panagis et al., 2014).

Transition into Addiction

The ability of drugs to increase DA in the NAc and trigger conditioned responses in both naive and addicted individuals indicates that changes in DA levels alone cannot account for the addiction phenotype. Importantly, addiction seems to emerge gradually, although the rate of this transition varies as a function of several factors, including the type of drug (i.e., faster for methamphetamine and slower for cannabinoids), the pattern of exposure (greater for regular than occasional use), and the developmental stage (faster in adolescence than in adulthood) (Robins and Przybeck, 1985; Schramm-Sapyta et al., 2009). The transition from controlled to compulsive drug taking has been associated with a shift in the involvement of striatal subregions (NAc), implicated in the rewarding response to drugs, to the dorsal striatum that is associated with habit formation (Everitt and Robbins, 2013). The speed with which addiction emerges is influenced by genetics, with some individuals transitioning faster than others due to genetic vulnerabilities (Kessler et al., 2007). To properly emulate the addiction phenotype, animal models of human addiction must feature compulsive drug consumption that occurs in spite of adverse consequences (Piazza and Deroche-Gamonet, 2013). This is because a characteristic of addiction is the failure of the individual to control his/her drug consumption despite catastrophic adverse consequences (i.e., incarceration or loss of job or child custody). Interestingly, in such models, only 10% of experimental animals will develop an addiction phenotype, which is similar to the estimated percentage of drug-exposed individuals who become addicted (Seedall and Anthony, 2013).

The pharmacological mechanisms of action of various drugs types influence the nature of the molecular and cellular changes associated with their repeated consumption. For example, alpha 6 and beta 3 nicotinic receptors are upregulated in DA neurons with repeated nicotine exposure (Visanji et al., 2006), dopamine transporters are downregulated in the striatum with repeated methamphetamine exposure (Groman et al., 2012), and cannabinoid receptors 1 are downregulated in striatum with repeated delta9-tetrahydrocannabinol (THC) exposure (Romero et al., 1997). These changes are likely to contribute to the emergence of tolerance to the drug's effects and the need to use increasingly larger doses in an attempt to achieve the same behavioral effect. In turn, exposure to higher drug doses facilitates the neuroplastic changes that ultimately change the reactivity of brain DA pathways to drugs and drug cues.

Drug-Induced Neuroplasticity in DA Pathways

Drug-induced DA increases trigger various forms of synaptic plasticity that can result in strengthening or weakening of synaptic connectivity in various brain reward regions (Figure 2 and Box 3) (Grueter et al., 2012). These drug-induced neuroplastic changes largely hinge on the epigenetic enhancement or silencing of gene expression and on epitranscriptomic (RNA editing) modulation of translation, two mechanisms whose details are beginning to be uncovered (Kenny, 2014; Robison and Nestler, 2011; Satterlee et al., 2014). Among the transcription factors implicated in the long-lasting neuroplastic changes that follow repeated drug exposure is ΔFosB (Maze et al., 2010). Drug-induced neuroplasticity evokes the same types of molecular processes involved in long-term potentiation (LTP) and long-term depression (LTD) that underlie learning and memory. The changes in synaptic strength that occur as a result of LTP are associated with larger synapses and dendritic spines, while those that follow LTD involve smaller synapses and dendritic spines (De Roo et al., 2008). These synaptic modifications generate a long-lasting molecular memory for the drug's rewarding and conditioning effects that will modify subsequent behaviors (Hyman, 2005).

Most of the studies of neuroplasticity have investigated the effects of chronic cocaine in the NAc (Sesack and Grace, 2010). Chronic cocaine increases dendritic spine density in MSNs (Russo et al., 2010), and ΔFosB is implicated in their generation (Maze et al., 2010). The structural changes observed in

Box 3. Synaptic Plasticity in DA Circuits

Many drugs of abuse, including cocaine, but also morphine, nicotine, and ethanol, can evoke synaptic plasticity in VTA DA neurons (Bowers et al., 2010; Lüscher and Malenka, 2011). Because VTA DA neurons are heterogeneous in their synaptic connectivity, molecular composition, and electrophysiological and signaling properties (Lammel et al., 2014), it is conceivable that drugs differentially affect their subpopulations. For instance, VTA DA neurons that lack DAT or D2R (Li et al., 2013) innervate the medial PFC, but not the NAc (Bannon and Roth, 1983; Lammel et al., 2012; Sesack and Grace, 2010). Moreover, some of the VTA DA neurons innervating the NAc have axons with micro-domains for either DA or glutamate signaling, further emphasizing their diversity (Zhang et al., 2015).

DA regulates excitatory synaptic plasticity both by increasing and decreasing synaptic strength through LTP and LTD. Synaptic strength is controlled by the insertion or removal of AMPAR or NMDAR and by changes in the subunit composition of AMPA receptors. Specifically, the insertion of high-calcium permeable AMPAR (GluR2 subunit) contributes to the drug-induced increases in AMPAR-to-NMDAR ratios associated with LTP in models of addiction (Boudreau et al., 2007; Conrad et al., 2008; Kourrich et al., 2007). These AMPAR have higher single-channel conductance than GluA2-containing receptors (Guire et al., 2008; Liu and Cull-Candy, 2000), and their upregulation increases the responsiveness of MSNs in the NAc to glutamate released by cortical and limbic terminals when exposed to drugs or drug cues (Wolf and Ferrario, 2010). Drug-induced neuroplastic changes have been uncovered in glutamatergic inputs to the NAc from PFC, basolateral amygdala, and ventral hippocampus (Figure 2) (Di Forti et al., 2014; Lee and Dong, 2011; MacAskill et al., 2014; Pascoli et al., 2014b). Recently, the use of genetic cellular tagging has enabled researchers to identify the clusters of neurons within the PFC that trigger excitatory signals into NAc with exposure to cocaine cues (Cruz et al., 2014).

Though not as extensively investigated as the NAc, the dorsal striatum also undergoes neuroplastic changes with repeated cocaine exposure; these are implicated in habit learning and in the automatic cocaine consumption triggered by repeated cocaine exposures (Everitt et al., 2008; Hearing et al., 2011; Lavaur et al., 2009; Parikh et al., 2014).

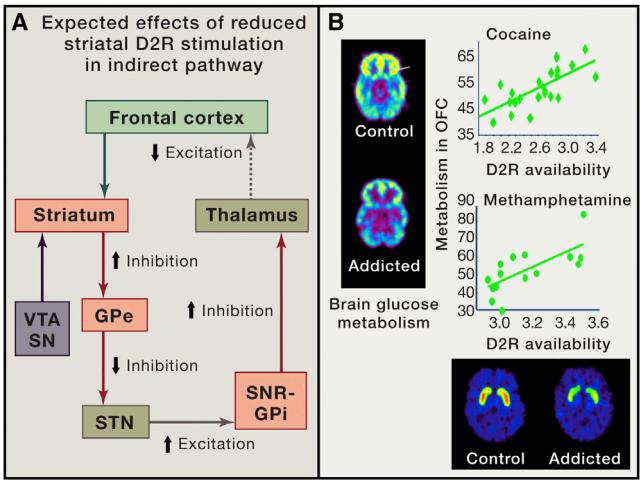

Figure 3. Drug-Induced Reductions in Striatal D2R Are Associated with Decreased Activity in Prefrontal Cortex

(A) Schematic representation of the indirect pathways in which DA neurons from the ventral tegmental area (VTA) and the substantia nigra compacta (SN) provide DA inputs to the striatal GABA neurons expressing D2R (D2R-MSNs). These striatal neurons target GABA cells in the globus pallidum external (GPe), which provide inhibition to glutamatergic neurons within the subthalamic nucleus (STN). The STN glutamatergic neurons provide an excitatory input to GABA neurons present in the substantia nigra reticulata (SNR) and the globus pallidum internal (Gpi), which inhibit glutamate neurons of the thalamus innervating the frontal cortex. Drug-induced reductions in D2R within the striatum impair the inhibition of this indirect pathway by DA, resulting in reduced thalamo-cortical stimulation and consequently reduced activity in the frontal cortex.

(B) Relationship between D2R in striatum and brain glucose metabolism in frontal regions of drug abusers tested both with [^{11}C]raclopride and FDG. Images next to the y axis show axial brain metabolic images at the level of the orbitofrontal cortex, and images below the x axis show axial images of D2R striatal availability for a control and a cocaine abuser. Regression slopes correspond to the association between metabolism in the orbitofrontal cortex (OFC) and D2R availability in striatum in cocaine-addicted and in methamphetamine-addicted subjects.

Figure modified from Volkow et al., 2011.

dendritic spines involve cytoskeletal and actin-myosin rearrangements and other structural proteins that regulate spine morphology and dendritic arborization (Toda et al., 2006). In addition, the formation of new spines is preceded by the generation of "silent" synapses containing NMDARs, but not AMPARs (Huang et al., 2009; Malenka and Nicoll, 1997), which are subsequently unsilenced through the insertion of AMPAR lacking GluA2 (Box 3)(Conrad et al., 2008; Dobi et al., 2011).

Role of Dopamine in Addiction

Repeated exposure to different types of drugs has been associated with downregulation of D2R in striatum (Nader et al., 2006; Thanos et al., 2001; Volkow et al., 2001a). Specifically, studies in rodents and non-human primates have found reduced levels of D2R in the striatum, including in the NAc, upon chronic drug exposures, as well as in animals with a propensity to self-administer drugs (Everitt et al., 2008). In rodents, low levels of D2R in striatum are associated with impulsivity and predict escalating and compulsive administration of cocaine (Everitt et al., 2008). Similarly, human brain-imaging studies of addicted individuals have shown reductions in D2R availability in ventral and dorsal striatum for most of the drugs, except for marijuana (reviewed in Volkow and Baler, 2014). Low levels of D2R in the striatum will result in reduced DA inhibition of the indirect pathway. D2R have high affinity for DA, so they are stimulated by the relatively low DA levels achieved through tonic DA cell firing. Reduced D2R-mediated DA inhibition of the indirect pathway will lead to reduced thalamo-cortical stimulation and consequently reduced activity in PFC brain regions (Figure 3) (Black et al., 2010). Indeed, the reductions in striatal D2R (dorsal and ventral) in drug abusers have been associated with decreased activity in the PFC, including anterior cingulate (ACC) and orbitofrontal (OFC) cortical regions. The ACC and OFC are necessary for self-control and for processing salience attribution, and their disruption is associated with a propensity for impulsive and compulsive behaviors (Volkow and Fowler, 2000). Thus, low levels of D2R in striatum may mediate the risk for compulsive drug taking in part by impairing PFC regions that inhibit prepotent responses and enable flexibility of behavioral choices as a function of changing environments (Volkow et al., 2006a).

Indeed, in rodents, optogenetic stimulation of the PFC prevented cocaine relapse (Chen et al., 2013). In contrast to the findings of low striatal D2R in addicted individuals, which in laboratory animals is associated with vulnerability for compulsive cocaine intake, the chemogenetic stimulation of D2R-MSNs (perhaps somewhat akin to lack of DA inhibition through D2R) has been shown to inhibit cocaine intake in mice (Bock et al., 2013). This seemingly paradoxical finding suggests that the low D2R levels in addicted individuals may reflect reduced postsynaptic and presynaptic receptors and that the firing of D2R-MSNs is not only modulated by D2R, but also by NMDAR, AMPAR, GABAR, $A_{2A}R$, and CB_1R among other receptors. Thus, low D2R levels are likely to unbalance DA's modulation of the indirect pathway, whereas the chemogenetic inhibition of D2R-MSNs interrupts the signaling in this circuit. In this regard, it is interesting to note that D2R agonists tend to decrease cocaine intake rather than increase it, as seen with the chemogenetic inhibition of D2R-MSNs.

Though, theoretically, enhanced signaling through D1R and its activation of the direct pathway would be consistent with facilitation of drug reward (Gore and Zweifel, 2013), the findings related to the consequences of repeated drug exposure on D1R have not been consistent. Thus, whereas some studies have shown that chronic cocaine potentiated D1R signaling (Pascoli et al., 2012), others have reported decreased D1R excitability (Kim et al., 2011), and while in nonhuman primates repeated cocaine exposure was associated with reductions in D1R in striatum (Moore et al., 1998), human studies failed to observe this effect (Martinez et al., 2009).

D1R-MSNs and D2R-MSNs do not fire independently of each other, and during drug exposure, enhanced DA signaling will stimulate one population but inhibit the other. Thus, it is likely that the balance between striatal signaling through the direct (D1R-mediated) and the indirect (D2R-mediated) pathways underlies drug responses and that an imbalance between these pathways may ultimately underlie the behavioral changes observed in addiction. In fact, we have recently shown that, during acute cocaine intoxication, signaling through both D1R and D2R in the striatum was markedly reduced in mice previously exposed to chronic cocaine, although the attenuation was greater for D2R than for D1R (Park et al., 2013). As a result, DA signaling though D1R versus D2R was biased in favor of DA-mediated D1R signaling during the state of intoxication (Park et al., 2013). Since DA stimulation of D1R is associated with enhanced sensitivity to drug reward, a higher D1R-to-D2R signaling ratio during drug intoxication could contribute to compulsive drug taking. The D3Rs, which are highly expressed in the mesolimbic DA system, have also been implicated in the transition to addiction, and D3R antagonists have been proposed as promising targets for the development of addiction treatments (Heidbreder and Newman, 2010). D3R in the NAc are upregulated by chronic cocaine (Conrad et al., 2010), whereas D3R blockade interferes with cocaine reward (Heidbreder and Newman, 2010). In humans, imaging and postmortem studies have found an upregulation of D3R in the NAc of cocaine abusers (Payer et al., 2014; Staley and Mash, 1996). However, findings in mice expressing no D3R (D3R KO mice) have been equivocal, with one study showing that D3R KO animals display enhanced motivation for cocaine-seeking behavior (Song et al., 2012) and another showing no effect on motivation (Caine et al., 2012).

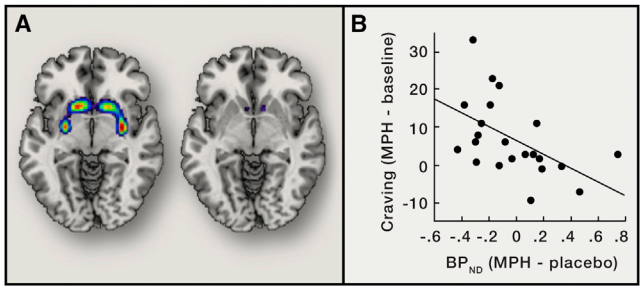

Figure 4. Stimulant-Induced Dopamine Increases Are Blunted in Active Cocaine Users

(A) Brain maps of normal controls (left) and active cocaine abusers (right) showing DA increases in the striatum after methylphenidate (MPH) administration reveal a robust response in controls, but not in cocaine abusers.

(B) Correlation between MPH-induced DA increases (measured as changes in non-displaceable binding potentials or BP_{ND}) in ventral striatum (where NAc is located) and the changes in craving scores (cocaine craving questionnaire [CCS]).

Figure modified from Volkow et al., 2014a.

A powerful approach for investigating changes in DA signaling in addiction is to compare the DA responses triggered by drugs in addicted versus non-addicted individuals. PET studies have made it possible to measure drug-induced DA increases in humans (Volkow et al., 1994)—for instance, in studies that compared the effects of drug intoxication between cocaine abusers and controls, where stimulant drugs such as methylphenidate and amphetamine were used as pharmacological challenges. These studies have consistently shown that the DA increases triggered by stimulants are markedly attenuated in cocaine abusers, both in dorsal and ventral striatal regions (reviewed in Volkow et al., 2014a). These blunted responses are consistent with preclinical findings showing reduced DA signaling during cocaine intoxication in mice chronically exposed to cocaine (Park et al., 2013). Despite these markedly blunted DA responses, the DA increases in NAc were associated with drug-induced craving (Volkow et al., 2014a) (Figure 4). In cocaine abusers (Martinez et al., 2011) and in methamphetamine abusers undergoing substance abuse treatment (Wang et al., 2012), the blunted DA responses to stimulant drugs have been also associated with worse clinical outcomes. Studies in alcoholics have further documented blunted DA increases upon challenge with a stimulant drug, which are consistent with reduced DA cell activity (Martinez et al., 2005; Volkow et al., 2007), but have also uncovered increased brain reactivity to the DA increases, which suggests impaired downstream modulation (Martinez et al., 2005; Volkow et al., 2007, 2013). In contrast, in marijuana abusers, while stimulant-induced DA increases in striatum did not differ from controls (Urban et al., 2012; Volkow et al., 2014b), the brain reactivity to DA stimulation was blunted, an effect that was associated with negative emotionality (Volkow et al., 2014b). Similarly, a PET study using 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine ([^{18}F]-DOPA) reported that the reduced striatal DA synthesis capacity

Box 4. The "Dark Side of Addiction"

The "dark side of addiction" involves allostatic changes that lead to drug use as a means to counteract the dysphoria and distress associated with drug withdrawal and discontinuation. Studies implicate the extended amygdala (central nucleus of the amygdala, bed nucleus of the stria terminalis, and a transition area in the NAc shell), corticotropin-releasing factor (CRF), and norepinephrine in these allostatic responses (Koob, 2013). Upregulation of the dynorphin/kappa opioid receptor system has also been associated with the dysphoria and the increased sensitivity to stress during drug discontinuation (see also Box 2). In parallel, downregulation of signaling though neurotransmitters associated with positive rewards, including DA, enkephalins (Zubieta et al., 1996), endocannabinoids (Serrano and Parsons, 2011), and the reduced DA inhibition through D2R of the indirect pathway, which signals aversive responses (Danjo et al., 2014), might also contribute to dysphoria in addiction.

Recently, studies have identified the lateral habenula (LHb) as a brain region disrupted by drugs that might also contribute to the dark side of addiction (Velasquez et al., 2014). The LHb, through its projection to the rostromedial tegmental nucleus, can inhibit DA cell firing (Ji and Shepard, 2007). The LHb is activated upon exposure to unrequited expectation and aversive stimuli, which could therefore also contribute to the enhanced sensitivity to stress in addiction.

Humans display activation in both the VTA and the habenula in response to aversive events (Hennigan et al., 2015). We had found that diverse VTA cellular phenotypes, which may be involved in different behaviors, synapse on LHb neurons (Root et al., 2014b). This synaptic complexity is reflected in behavioral studies showing that LHb photo-activation of some fibers from the VTA evokes reward (Stamatakis et al., 2013), while activation of others evokes aversion (Hennigan et al., 2015; Root et al., 2014a). The LHb synaptic complexity provided by VTA is not surprising, as multiple streams of information may be encoded and decoded by the different types of VTA cells and their corresponding efferent to downstream brain targets.

observed in marijuana abusers was associated with apathy and amotivation (Bloomfield et al., 2014).

The regional brain activation responses to a stimulant drug also differ between controls and cocaine abusers in ventral prefrontal regions. In control subjects, intravenous stimulant administration decreased the activity of ventral medial frontal regions (OFC and ventral ACC), whereas in cocaine abusers, it activated these regions, which are involved in salience attribution and conditioning (Dosenbach et al., 2006; O'Doherty et al., 2001; Shackman et al., 2011). Activation of the OFC in cocaine abusers was associated with craving (Volkow et al., 2005). In contrast, activity in the right inferior frontal region Ba 44, a key brain region involved in inhibitory control (Aron et al., 2004), was associated with the deactivation of the NAc and ventral PFC upon successful control of cocaine craving (Volkow et al., 2010). This pattern of responses uncovers distinct contributions of PFC regions to addiction on the basis of their striatal projections: dlPFC and inferior frontal regions that project to the dorsal caudate facilitate self-control, whereas ventral PFC regions projecting to NAc facilitate drug taking (Goldstein and Volkow, 2011). This is also consistent with preclinical findings that identified distinct contributions of prelimbic mPFC (PL) and infralimbic mPFC (IL) to cocaine seeking in rats (review in Bossert et al., 2013). Studies using the reinstatement model of relapse found that, after extinction of cocaine self-administration, PL activity promoted cocaine seeking while IL activity inhibited it (Peters et al., 2008). Importantly, in the incubation of the cocaine-craving model (response to cocaine cues progressively increases with time after withdrawal), reversible inactivation of IL, but not PL, decreased "incubated" cue-induced cocaine seeking after prolonged withdrawal, while pharmacological activation of IL, but not PL, increased cocaine seeking during early withdrawal (Koya et al., 2009). However, in the same animal model, optogenetic inhibition of PL neurons (projecting to NAc core) that previously underwent a specific form of cocaine-induced synaptic plasticity (recruitment of silent synapse) decreased incubation of craving, while the opposite effect was observed following inhibition of the IL projection to NAc shell (Di Forti et al., 2014). In clear contrast, in a punishment-induced suppression model of "compulsive" cocaine seeking, in which most rats suppressed cocaine self-administration by shock punishment while a few did not (punishment-resistant "compulsive" rats), optogenetic stimulation of PL inhibited cocaine seeking in punishment-resistant rats while optogenetic inhibition increased it (Chen et al., 2013). However, in the same model, excitotoxic lesions of the PL or IL had no detectable effect on cocaine seeking in punishment-resistant rats (Pelloux et al., 2013). Taken together, the PL and IL appear to play different and complex roles in cocaine-seeking behaviors in rat addiction models, which are highly dependent on the particular behavior being assessed and the experimental procedure used to manipulate local neuronal activity. The results underscore the complexity of the neuroplasticity within the mPFC circuitry, a multimodal brain structure involved in the orchestration of diverse behaviors.

The regional brain responses to drug-associated cues have also been investigated with neuroimaging. These studies have shown that, in cocaine abusers, exposure to cocaine cues triggers DA release in the dorsal striatum and that this effect was associated with craving (Volkow et al., 2006b; Wong et al., 2006). Interestingly, similar increases were not observed in tobacco smokers upon exposure to nicotine cues (Chiuccariello et al., 2013), whereas exposure to alcohol cues in healthy controls resulted in DA decreases (Yoder et al., 2009). Studies with fMRI have shown, more or less consistently, that exposure to cues in substance abusers is associated with increased activation of NAc and VTA (Goudriaan et al., 2013), which most likely reflects not only cue-induced DA increases, but also excitatory stimulation stemming from glutamatergic terminals into NAc and midbrain. These studies have also identified several other regions that are co-activated during cue exposure, including the PFC, cerebellum, limbic regions, and insula (Jasinska et al., 2014). Activation of the insula is noteworthy since this region is involved in interoceptive awareness, contributes to the conscious awareness of drug craving, and is part of the default mode network (DMN) that enables internal mind wandering, perhaps facilitating rumination about drug use in addicted subjects (Naqvi and Bechara, 2010).

Neuronal Circuitry in Addiction

The use of imaging tools to study changes in the brains of individuals suffering from addictions has helped to identify the brain regions and associated circuits that are disrupted and

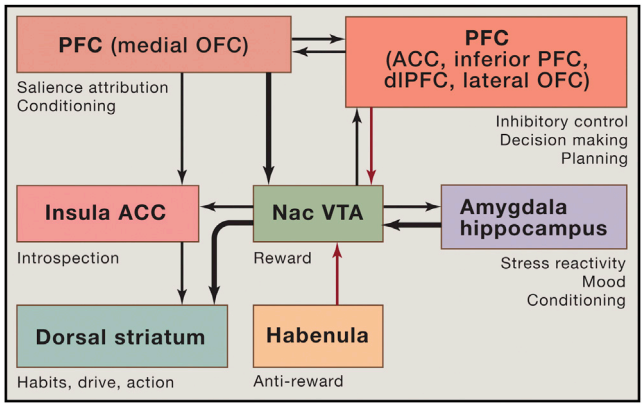

Figure 5. Neuronal Circuitry of Addiction

Proposed neuronal network of interacting brain regions and associated circuits that are disrupted in addicted individuals. Changes occur in reward (NAc and VTA), conditioning/memory (amygdala and medial OFC for emotional and salience attribution; hippocampus and dorsal striatum for memories and habits), executive control (ACC, inferior PFC, dlPFC, and lateral OFC), motivation/drive (medial OFC for attribution of salience, ventral ACC, VTA, dorsal striatum, NAc), interoception (insula and ACC), and aversion/avoidance (habenula). This model proposes that, during addiction, the enhanced expectation value of the drug in the reward, motivation, and memory circuits overrides the control circuits, favoring a positive-feedback loop initiated by consumption of the drug and perpetuated by the enhanced activation of the motivation/drive and memory circuits. These circuits also interact with those involved in mood regulation, including stress reactivity (which includes participation of the extended amygdala, hypothalamus, and habenula) and interoception (which includes participation of the insula, ACC, and the default mode network [DMN] and contributes to a heightened awareness of craving). NAc, nucleus accumbens; VTA, ventral tegmental area; PFC, prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; dlPFC, dorsolateral PFC.

understand how these changes influence behaviors associated with the addiction phenotype. These studies have revealed changes in reward and motivation, resulting in increased motivation toward drugs and drug cues and in decreased motivation for non-drug reward and cues, executive control, resulting in reduced ability to control the urge to take the drug, triggered by cues, emotional states, or an impaired ability to delay gratification, as well as mood and interoceptive circuits, resulting in an enhanced sensitivity to stressors and dysphoria or the so-called "dark side of addiction" (Box 4 and Figure 5; Koob, 2013).

In this model of addiction, the motivation to take the drug is not only driven by conditioned responses to cues, but also by negative emotional states. The exposure to drug cues results in the activation of glutamatergic projections from the ventral PFC, the ventral hippocampus, and the amygdala (and presumably medial thalamus) to striatal projections that increase DA signaling and release in the NAc and dorsal striatum. The enhanced craving and desire for drug taking will eventually lead to drug consumption, and although the drug-induced DA increases are markedly attenuated in the NAc, in particular of cocaine abusers and alcoholics, they are sufficient to enhance the craving and to sustain the drive to continue taking the drug (Figure 4), perhaps by stimulation of upregulated D3R that facilitate transmission through the D1R direct pathway (Fiorentini et al., 2010). The ventromedial PFC (including OFC and ventral ACC) in drug-addicted individuals, which in the absence of drug or drug cues is hypofunctional, becomes hyperactive when exposed to drugs or cues, enhancing reward salience calculation through its involvement in the processing of the outcome value of that reward (Volkow et al., 1996).

The control circuit, which relies on the PFC, including ACC, lateral OFC, dlPFC, and the inferior frontal cortex (BA 44), is disrupted in addicted individuals. The reduction in D2R signaling in the striatum leads to reduced activity in these PFC regions (Figure 3), which is necessary for proper control, planning, and flexibility of behaviors and for delaying gratification (Volkow and Baler, 2015). Specifically, the OFC is critically involved in salience attribution; its main contribution is to offer predictive information about alternative options and outcomes, which is essential for shifting behaviors when the reward is no longer reinforcing. Meanwhile, the ACC enables inhibitory learning by keeping tabs on conflicts between predicted and actual outcomes and by conveying that information to inhibitory circuits orchestrated by the dlPFC, the inferior frontal regions (BA 44), and the dorsal caudate. This inhibitory arm of the decision-making process is facilitated, in turn, by tonic DA signaling sustaining activity in ACC, dlPFC, and inferior PFC. Together with the OFC, the insular cortex and the ACC also allow the circuit to estimate the level of uncertainty involved in choosing among alternative options. Finally, the extended amygdala, insula, and lateral habenula, which provide information about emotional salience, interoceptive awareness, and pertinent reward omission events, respectively, contribute to dysphoria, anhedonia, and the enhanced stress reactivity that follows drug withdrawal (Koob and Le Moal, 2005).

In consequence, the addicted individual experiences enhanced reactivity to drug cues and to stressful stimuli, the reactivity to natural reward is decreased, and there is loss of flexibility to adjust the saliency value of reward as a function of their context. Although the neuronal adaptations that follow repeated

Box 5. Translational Opportunities

- Enhance tonic dopaminergic D2R signaling through the indirect pathway to improve control. Pharmacologically, this is challenging because currently available D2R agonists (or partial agonists) also bind to D3R, and stimulation of D3R has been associated with impaired impulse-control disorders (Seeman, 2015).
- Enhance function of prefrontal regions involved in executive function, including self-control via transcranial magnetic or electrical stimulation, mindfulness, or other behavioral interventions, and through medications that increase DA signaling in prefrontal regions (i.e., tomoxetine, oral stimulants, modafinil).
- Decrease the reactivity of stress-associated circuits (extended amygdala, habenula) through the use of biofeedback or medications (CRF or kappa antagonists)
- Decrease the motivation value of conditioned responses to drug cues (by targeting PFC, amygdala, hippocampus) through the use of behavioral extinction interventions, including coupling interventions with medications (i.e., d-cycloserine).
- Reduce dysphoria and enhance hedonic responses to non-drug rewards during withdrawal and drug discontinuation though the use of cognitive behavioral interventions or medications.

Box 6. Perspectives in Reward Circuitry and Addiction

Identification of DA neuron subtypes in VTA and SN and characterization of their projections, inputs, and function.

Investigation of interactions between the nuclei and circuits mediating reward and those involved with mood regulation, including those with the dorsal raphe.

Investigation of the dynamic interactions between coordinated pathways (for instance, the direct and indirect MSNs pathways) in drug reward and in compulsive drug taking.

Investigation of the neurocircuitry that drives "antireward" and the dark side of addiction (Koob et al., 2014), including the role of the habenula and kappa/dynorphin signaling.

Investigation of how genes influence the molecular biology and neurocircuitry that underlies individual heterogeneity in vulnerability and resilience to addiction.

Identification of biomarkers that can be used to personalize prevention and therapeutic interventions in substance use disorders.

drug exposures are not fully understood, the picture that is emerging of the drug-induced neuronal impairments is already helping us to think about interventions that could remediate them through the development of medications/immunotherapies, magnetic or electrical stimulation strategies, and/or behavioral approaches (Box 5). The development of such interventions will benefit from an understanding of the specific circuitry or functional processes being targeted, rather than using abstinence as the only beneficial outcome in addiction treatment. For example, interventions designed to counteract dysphoria or strengthen executive control, even if not resulting in complete abstinence, may improve long-term success and recovery from addiction. In parallel, these neurobiological advances have begun to reveal the molecular and neuronal bases underlying the heterogeneity of the clinical presentation and the outcomes of addicted individuals (Box 6). In the near future, these advances might enable the development of tailored therapeutic interventions on the basis of the specific molecular targets and/or circuits disrupted in a given individual.

In conclusion, uncovering the neurobiology underlying drug abuse has led to the recognition of addiction as a chronic disease of the brain. At the same time, these advances have revealed potential targets for interventions that could usher in a new era of more effective and personalized addiction treatments.

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