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Biological substrates of addiction

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Abstract

This review is an introduction to addiction, the reward circuitry, and laboratory addiction models. Addiction is a chronic disease hallmarked by a state of compulsive drug seeking that persists despite negative consequences. Most of the advances in addiction research have centered on the canonical and contemporary drugs of abuse, however, addictions to other activities and stimuli also exist. Substances of abuse have the potential to induce long-lasting changes in the brain at the behavioral, circuit and synaptic levels. Addiction-related behavioral changes involve initiation, escalation and obsession to drug seeking and much of the current research is focused on mapping these manifestations to specific neural pathways. Drug abuse is well known to recruit components of the mesolimbic dopamine system, including the nucleus accumbens and ventral tegmental area. In addition, altered function of a wide variety of brain regions is tightly associated with specific manifestations of drug abuse. These regions peripheral to the mesolimbic pathway likely play a role in specific observed comorbidities and endophenotypes that can facilitate, or be caused by, substance abuse. Alterations in synaptic structure, function and connectivity, as well as epigenetic and genetic mechanisms are thought to underlie the pathologies of addiction. In preclinical models, these persistent changes are studied at the levels of molecular pharmacology and biochemistry, *ex vivo* and *in vivo* electrophysiology, radiography and behavior. Coordinating research efforts across these disciplines and examining cell type- and circuit-specific phenomena are crucial components for translating preclinical findings to viable medical interventions that effectively treat addiction and related disorders.

WHAT IS ADDICTION?

Addiction is a devastating disease that imposes a substantial toll on afflicted individuals, friends and relatives, and society as a whole. According to the American Society of Addiction Medicine, addiction is a primary, chronic disease of brain reward, motivation, memory and related circuitry. An important characteristic of addiction is the inability to

consistently abstain from addiction-related behavior despite negative consequences. Each year in the United States, abuse of tobacco, alcohol, and illicit drugs has been estimated to exact over \$130 billion in direct health care costs and over \$600 billion related to dysfunction social and occupational factors¹.

Most often, addiction refers to the abuse of psychoactive substances², that affect neuronal function by altering the chemical balance of the brain. Ingestion of these agents has the potential to alter behavior, consciousness and mood; such agents include alcohol, tobacco, cannabinoids, opioids, stimulants, hallucinogens, club drugs and some prescription drugs. Non-drug addictions have also been suggested including, sex, gambling and other behaviors. Likewise, the concept of overindulging in consumption of highly palatable foods has been recently debated as being an addiction. This should not be surprising since drugs of abuse hijack the natural reward circuitry evolutionarily optimized for survival³.

More specifically, addiction is a chronic disease that is characterized by a cyclical, relapse-laden progression through several phases of maladaptive behavior, particularly evident in substance use⁴. Individuals with addiction repeatedly progress through periods of bingeing (high levels of consumption), withdrawal (abstinence, in the presence of anxiety and/or negative affect) and preoccupation (intense craving and anticipation of next use). Ingestive behavior at disease onset is generally impulsive and is thought to be motivated mainly by positive reinforcement (i.e. euphoria obtained from substance ingestion). As the disease progresses, the motivation underlying drug seeking shifts towards negative reinforcement (i.e. relief of withdrawal symptoms), eventually giving way to compulsive habitual behaviors. While all drugs of abuse act on a final common pathway, the sensitization and intensification of these symptoms and phases is thought to recruit distinct neural circuits and local networks. Thus, to an extent, it is possible to map the phases (symptoms) of addiction-related behaviors to function of specific neural circuits.

NEUROPHARMACOLOGY OF ABUSED SUBSTANCES

To understand how abused substances cause long-lasting changes in the brain, it is first necessary to introduce how they acutely alter brain function (reviewed in⁵). Drugs of abuse elicit their effects through different mechanisms, yet all of them recruit the natural reward pathways of the central nervous system, the mesocorticolimbic dopamine system. A primary action of abused substances is to directly or indirectly facilitate the release of the neurotransmitter dopamine, produced in the ventral tegmental area (VTA) of the midbrain, in the nucleus accumbens (NAc)⁶.

The main receptor targets for drugs of abuse have been defined and for the most part are membrane-bound receptors. However, understanding the complex physiological consequences and downstream signaling cascades activated by drugs of abuse remains a challenge. Here we will briefly describe the targets for the most well studied drugs of abuse (Table 1). Psychostimulants, including cocaine and amphetamines, act directly on the presynaptic dopamine-releasing axon terminals of VTA afferents. Cocaine inhibits the clearance of dopamine from the synaptic cleft by blocking plasma membrane monoamine transporters, whereas amphetamines alter the function of both the vesicular monoamine

transporters and plasma membrane transporters^{7,8}. Nicotine binds to the orthosteric binding site of the nicotinic acetylcholine-gated cation channel (nAChR). Nicotine is thought to act primarily through activation of $\alpha 4\beta 2$ -containing nAChRs on dopamine cell bodies and axon terminals, thereby directly leading to dopamine release in the NAc⁹. However, nicotine-mediated activation of $\alpha 7$ homomers on dopamine axon terminals and presynaptic glutamatergic afferents may also be involved. Opiates, such as heroin and morphine, activate G-protein coupled receptors, specifically $G_{i/o}$ -coupled μ -opioid receptors (μ OR), which, within the VTA, are expressed mainly on γ -aminobutyric acid (GABA)-ergic interneurons. Activation of μ ORs leads to hyperpolarization of the VTA interneurons, disinhibition of the dopamine projection neurons, and enhanced dopamine release in the NAc¹⁰. Similarly, several other classes of abused drugs – barbiturates/benzodiazepines, PCP/ketamine, and cannabinoids – are thought to facilitate dopamine release in the NAc via similar mechanisms¹¹. Like other non-stimulant drugs of abuse, ethanol causes a net disinhibition of VTA dopaminergic neurons¹². However, ethanol has a rich pharmacology involving direct interactions with glutamatergic and GABAergic ion channels.

NEUROCIRCUITRY OF REWARD

Seminal work from Olds and colleagues demonstrated that rodents will work to electrically stimulate relatively discrete areas of the brain¹³. Considering the observation that humans find stimulation of these same areas pleasurable, these regions are taken for granted as being part of the brain-reward circuitry¹⁴. Subsequently, others showed that animal models will work to self-administer drugs of abuse (but not other drugs) and that this self-administration behavior is disrupted by lesioning these brain-reward regions¹⁵. The critical regions in the reward circuitry are now widely accepted to include the mesolimbic dopamine system, more specifically the VTA-NAc pathways (Figure 1)¹⁶. Yet, the VTA-NAc pathways are only part of a series of parallel, integrated circuits, which involve several other key brain regions. These other regions include, but are not limited to, the prefrontal cortex (PFC), hippocampus and basolateral amygdala (BLA), which provide excitatory drive within the reward circuit. Auxiliary regions, including the dorsal striatum and extended amygdala, sustain habitual behaviors and stress responses in addiction.

Dopamine centers

Dopamine plays a central role in motivation and reward processing. However, dopamine-deficient mice still demonstrate a degree of reward learning, suggesting it is not necessary for this process¹⁷. This illustrates that although dopamine is key to reward, the entire rewarding process is much more complex. Within the mesolimbic system, dopamine is produced in midbrain dopamine neurons located in the VTA, the retrorubral field and the substantia nigra (SN). The VTA also contains GABAergic neurons, which regulate VTA dopamine neuron function¹⁸, at least some of which project to and modulate NAc cholinergic interneurons¹⁹. In addition to having widespread projections throughout the brain, retrograde tracing techniques have shown that the VTA receives diverse afferent inputs, which likely differentially influence behavioral output (reviewed in ²⁰).

The SN is a midbrain dopaminergic region closely related to the VTA. Like the VTA, electrical stimulation of the SN is reinforcing. Evidence suggests that blockade of

glutamatergic or cholinergic signaling into either the SN or VTA alters addiction-related habit formation¹⁷. Also, blockade of dopamine receptor signaling in the terminal beds of either the VTA (NAc) or the SN (dorsal striatum) alters addiction-related behaviors, although to a much greater degree in the NAc. Utilization of optogenetics and genetic engineering in rats and mice has allowed for more detailed analysis of addiction-related behaviors as the light sensitive channels that activate (channel rhodopsins) or inhibit (halorhodopsins) cellular excitability can be expressed in a region- and cell-type specific manner. For instance, selective photostimulation and thus excitation of VTA dopamine neurons supports reward-related behaviors such as conditioned place preference (CPP; described below)^{21,22}.

Like the SN, the VTA also degenerates during the progression of Parkinson's disease and may explain some of the similarities between the PD non-motor symptoms and post-acute-withdrawal syndrome (e.g. depression/irritability, cognitive disruptions). Overall, it is well accepted that activity of midbrain dopamine neurons convey information involved in integration of the rewarding vs. aversive properties of environmental stimuli²⁰.

Dorsal striatum and nucleus accumbens

The striatum is the gate to the basal ganglia, integrating inputs from the cortex, as well as from the thalamic and limbic structures (hippocampus and amygdala). It consists of multiple nuclei differentiated by their anatomical connections and behavioral functions. The striatum is divided into a dorsal region and a ventral region, which includes the NAc. In humans and primates, the dorsal striatum is further divided into the caudate and putamen. The NAc is further divided into two subregions, the core and the shell with the core being more similar to the dorsal striatum and the shell having strong similarities with the extended amygdala (described below). Serial connectivity between the NAc, midbrain, and dorsal striatum may account for the transition from motivated to habitual behaviors observed in the progression of addiction^{23,24}. Thus, drug-evoked changes in synaptic strength and connectivity within the dorsal striatum and the NAc are thought to underlie many behavioral components of addiction²⁵.

The neuronal cell types in the dorsal striatum and NAc include GABAergic projection medium spiny neurons (MSNs), multiple types of GABAergic interneurons and cholinergic interneurons. MSNs comprise approximately 95% of the neurons in the NAc and can be split into two categories (direct (striatonigral) and indirect (striatopallidal) pathway MSNs) based on projection targets, electrophysiology, and expression of neuropeptides and cell surface receptors. For instance, dopamine receptor subtype 1 (D1) and prodynorphin are expressed on one cell type whereas the other cell type expresses dopamine receptor subtype 2 (D2), adenosine 2A receptors, and proenkephalin. D1 receptor expressing MSNs (direct pathway) mainly project to dopaminergic midbrain regions, whereas D2 receptor expressing MSNs (indirect pathway) primarily target the pallidum²⁶. Several studies have demonstrated that these parallel circuits can exert opposing functional effects on behavior^{27–29}, whereas others suggest that the pathways may work in tandem towards the same functional response^{30,31}. Both classes of MSNs generally rest in one-of-two states, an “up state” (~–60mV) or a “down state” (~–90mV)³². Because MSNs generally are quiescent (rest in the down state),

their activity depends heavily on excitatory glutamatergic drive. The dorsal striatum receives excitatory inputs primarily from the associative and sensorimotor cortex and thalamic nuclei and reciprocal dopaminergic innervation from the substantia nigra. In contrast, the NAc receives excitatory inputs from the PFC and limbic regions. Although both the NAc shell and core receive inputs from the VTA, the NAc shell projects back to the VTA while the NAc core projects to the substantia nigra.

The dorsal striatum has been implicated in repetitive behaviors and is thought to mediate stereotypic movements observed during stimulant intoxication. More evidence for the role of dorsal striatal dysfunction in the expression of compulsive behaviors lies in this region's involvement in the pathophysiology of obsessive-compulsive disorder and obsessive-compulsive personality disorder^{33,34}.

NAc lesions, glutamatergic blockade or dopamine receptor blockade disrupt psychostimulant self-administration. Recent work utilizing optogenetics has shown that enhancing firing of D2 or D1 dopamine receptor-specific MSNs in the NAc by photostimulation suppresses and enhances cocaine reward, respectively²⁷. Also using optogenetics, recent studies have attempted to parse out the impact of specific excitatory terminals from the cortical, amygdalar and hippocampal formations in NAc-dependent addiction-related behaviors³⁵. Importantly, stimulation of the axon terminals from each of these regions, within the NAc, can reinforce behavior.

In addition to its well-characterized role in the rewarding and reinforcing properties of drugs of abuse and natural stimuli, the NAc has also been implicated in the placebo effect³⁶ and the processing of pleasant emotions induced by imagery³⁷ and music³⁸. By contrast overactive or otherwise dysfunctional activity in the mesolimbic system is thought to be involved in the manifestation of positive psychotic symptoms (e.g. delusions, hallucinations, grandiosity) shared by acute stimulant-induced psychosis and psychiatric illnesses like schizophrenia and bipolar disorder.

Glutamatergic regions of the reward circuit

The PFC is comprised of the anterior portion of the frontal lobes. Among other functions, the PFC is typically and most consistently associated with executive function, which is an umbrella term for higher-order processes such as planning and forethought, problem solving, and cognitive flexibility. With respect to addiction, the PFC is suggested to regulate the overall motivational significance and determine the intensity of behavioral responding³⁹. The output of the PFC is glutamatergic, and is modulated by dopamine among other neurotransmitters⁴⁰. Accordingly, the activation of the PFC by rewarding stimuli is strongly influenced by the predictability of the reward, which is conveyed in part by release of midbrain dopamine. A hypoactive PFC has been associated with the loss of impulse control that is observed in susceptible individuals⁴¹. Deficits in PFC function have been observed in patients with a variety of psychiatric disorders including substance abuse³⁹, obesity⁴², attention deficit hyperactive disorder⁴³, schizophrenia⁴⁴ and depression⁴⁵.

The hippocampus is a limbic structure that plays a major role in learning and memory and is implicated in addiction-related behaviors. The hippocampus sends glutamatergic projections

to multiple regions within the reward circuitry. In fact, the NAc is innervated by the ventral subiculum of the hippocampus, which is thought to convey contextual and memory-related information and may play a role in relapse to drug seeking behavior⁴⁶. Consistently, direct stimulation of ventral hippocampus axons in the NAc reinforces addiction-related behaviors⁴⁷.

The BLA is a limbic region thought to be necessary for attributing emotional value to cues, thus having an integral role in processing affective (emotional) states⁴⁸. In terms of addiction, the BLA is important for cue- and stress-induced reinstatement of drug seeking behavior. However, lesion studies suggest the BLA is not critical for cocaine self-administration. Similar modulations of BLA output occur during extinction of fear response and drug-seeking⁴⁹ and the BLA, along with the extended amygdala, is a likely neurobiological substrate underlying the comorbidity of addiction and anxiety disorders, especially post-traumatic stress disorder. The BLA therefore acts to integrate the positive or negative value of an environmental stimulus (natural reward, drug of abuse, stress, etc.).

Other regions in reward circuitry

Although excitatory drive on the NAc-VTA axis is the key final common pathway to addiction, other regions such as the extended amygdala play a key role in distinct addiction-related behaviors¹⁵. The extended amygdala is composed of several basal forebrain regions including the NAc shell, the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CeA) all of which have similar morphology, immunoreactivity, and connectivity. The extended amygdala is the aforementioned area implicated as a key mediator of stress-induced relapse⁵⁰.

Other structures tertiary to the reward circuitry play a role in mediating addiction. For instance, the mesolimbic and nigrostriatal pathways are innervated by a wide variety of brain regions, whose inputs supply information concerning the environment and the animal's motivational and emotional states. One particular region, the hypothalamus, is a highly diverse brain region perhaps best known for its close association with the pituitary gland and the endocrine system. The lateral hypothalamus (LH), which is reciprocally connected to the NAc shell, is a target for self-stimulation⁵¹. Consistent with the critical role of the LH in metabolic homeostasis and reward, drug valence can be modified by metabolic states (for review see⁵²).

ANIMAL MODELS OF ADDICTION-RELATED PHENOMENA

In the interest of space, we will limit our discussion of substance abuse research primarily to preclinical models. Much of our knowledge of addiction and substance abuse comes from preclinical experiments, the vast majority of which are performed in mice or rats. The utility of rodent addiction models are exemplified as follows: there are many variations of rats and mice with strain-specific traits that are particularly useful for modeling aspects of addiction-related behaviors (e.g. high anxiety, alcohol-preferring) and rodents also engage in complex spontaneous and conditioned behaviors that are implicated in substance abuse. Furthermore, mice are highly amenable to genetic manipulation allowing for cell type and region specific manipulation of protein expression. The use of mice or rats also confers pragmatic

advantages in that they are small and relatively inexpensive, easy to house and maintain, and reproduce readily and rapidly.

Behavioral models

Unlike most illnesses and some psychiatric disorders, drug addiction is largely defined by its behavioral components. Effectively reproducing these behaviors in animals is essential to making clinically relevant scientific discoveries. For a complex psychiatric disorder, a comprehensive animal model is likely unattainable. Instead, animal models are designed as a means to examine one or more particular components of a human disorder. When discussing or creating an animal model, there are several types of validity to consider. The first, and arguably most relevant, concept is construct validity, which refers to how meaningfully, interpretably, and powerfully the conclusions drawn from the model can apply to the psychiatric condition⁵³. Construct validity commonly refers to the similarity between the underlying biology of the animals and the patients. Alternatively, construct validity relates to the concept of functional equivalence, that a change in one variable (e.g. stress) should similarly affect the outcome (e.g. drug-seeking) in the model and the clinical population⁵⁴. The concept of functional equivalence is closely related to predictive validity, which more specifically refers to the ability to predict the clinical response to an intervention based on the response in the animal model. Face validity, which refers to how well the animal model resembles components of the psychiatric disorder, should also be considered. Lastly some have proposed considering population validity, an extension of face validity, which dictates that the rate of occurrence of a disease-like behavior should reflect epidemiological data⁵⁵. For example, only approximately 20% of cocaine users transition to clinical cocaine dependence⁵⁶, so a model where only 20% of animals engaged in addiction-like behavior (see ⁵⁷) would be said to have a high degree of population validity. Arguably, this concept is important to ensure that pathological disease-like behavior is being modeled, as opposed to behavior within the normal adaptive range⁵⁸.

Abused substances can be delivered in two ways, contingently (by the subject) or passively (by the experimenter). The distinction is important for two reasons: (1) pathological substance-seeking is a key component of addiction, whereas the prescribed use of medication does not constitute addiction and (2) studies have demonstrated that experimenter-delivered and self-administered abused substances can induce profoundly different neurobiological and behavioral effects^{59,60}. However, not all effects of substances of abuse are dependent upon contingent administration thus both contingent and passive treatment regimens have been utilized to study each phase of addiction pathology.

Modeling the etiology of addiction

Clinical substance dependence is diagnosed when at least 3-of-7 Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria have been met within one year. These criteria include tolerance, withdrawal, escalation, persistence, excessive motivation to obtain the substance, giving up other activities, and perseverance despite self-harm. In addition to these established criteria, the upcoming edition of the DSM (DSM-V) is expected to add a criterion that encompasses drug craving. Considering these criteria is important when discussing an animal model of addiction since, as mentioned, behavioral animal models are

designed to mimic only a particular facet of the disorder. Animal models of addiction typically replicate aspects of less than three of the DSM criteria, and typically measure only one property of drug action.

Stages of addiction—The binge/intoxication phase of addiction is studied by examining the initial effects of substance administration to naïve animals. Acute and short subchronic treatments with abused substances have been shown to induce transient and/or long lasting perturbations in physiology (depending on molecular target) and behavior. Additionally, in contingent administration paradigms, animals tend to escalate their substance intake similarly to humans at the onset of the disorder, especially under long-access conditions. Negative affect/withdrawal is modeled by continuing the administration of vehicle in lieu of the abused substance (i.e. extinction) or, in some cases, by completely removing access. Like in human addicts, drug-seeking behavior in animals often intensifies during the first drug-free session – this phenomenon is referred to as the “extinction burst”. The dwindling of drug-seeking during the extinction phase is not due to a loss, or forgetting, of previous drug-related memories, but instead is an active learning process⁶¹. Acceleration or enhancement of this extinction learning is considered to be a potential avenue for improving clinical outcomes. Arguably the most clinically and therefore experimentally relevant stage of addiction is relapse, or reinstatement of drug seeking behaviors in model systems. Reinstatement is a phase of administration in animal models that occurs after an extinction phase. In animals that have undergone extinction (i.e. those that no longer engage in drug-seeking), drug-seeking behavior can be elicited by several manipulations relevant to the preoccupation/relapse phase of addiction. These reinstatement treatments are similar to events known to precipitate relapse in humans and include: a stressor, a small “priming” drug dose, and a cue or context previously paired with substance delivery. It is also believed that, in patients, cue-induced cravings for cocaine or other drugs increases during the first weeks of abstinence. A similar phenomenon, termed incubation of craving, has been reported in rodents, and several specific circuit, cellular, and molecular players have been implicated⁶².

Reinforcement—Reinforcement is the ability of a stimulus to modify a measurable dimension of instrumental behavior, typically rate, duration, magnitude, or latency. Positive reinforcement occurs when a behavior results in the presentation of an absent stimulus (e.g. receiving a foot massage), whereas negative reinforcement occurs when a behavior results in the removal of a present stimulus (e.g. scratching an itch). Positive and negative reinforcement are not mutually exclusive properties; this is perhaps best evidenced by the development of addiction where behavior is initially driven by intoxication and is eventually driven by alleviation of withdrawal. The most flexible and robust model of a substance’s reinforcing properties is self-administration.

In the self-administration paradigm, animals are trained in a specific environment such that an instrumental response (e.g. lever-press or hole-poke) results in substance delivery. Food, drink, or alcohol are typically delivered by a hopper or retractable lever, whereas for drugs of abuse, animals are implanted with an intravenous catheter used for systemic drug infusion. A variety of manipulations of the required behavior can be made to gain insight

into specific aspects of addiction. For example a progressive ratio schedule has been used to examine motivation, addition of coincidental punishment can be used to study persistence, responding in the absence of drug is used to measure compulsive drug-seeking, and the reinstatement of extinguished responding is a model of relapse⁵⁴. Additionally some researchers have correlated specific behavioral traits (e.g. persistent or compulsive responding) with changes in receptor expression⁶³, neuron excitability⁴¹, and synaptic plasticity⁶⁴. Patterns of intake during acquisition of cocaine self-administration are also strong predictors of developed behaviors⁶⁵. The versatility of the paradigm, in tandem with its validity, perpetuates the view that self-administration is the “gold standard” of behavioral addiction research.

Reward—Models of reward specifically capture information related to the hedonic valence of the drug or stimulus, whether positive or negative. That is, these models can readily detect aversive properties of a stimulus since reward and aversion exist on one spectrum. The most substantial distinction between models of reward and reinforcement is that models of reward typically involve the passive, experimenter-delivered administration of a stimulus and therefore do not address motivation. Intracranial self-stimulation (ICSS) can be used to study reward by pretreating an animal with a drug prior to a training session: drugs of abuse act synergistically with deep-brain electrical stimulation to facilitate instrumental behavior in the ICSS paradigm¹⁴. It is worth noting that, through the advent of optogenetics, stimulation of distinct, specific neuronal pathways has been demonstrated to be sufficient to reinforce^{28,47,66,67} or punish^{28,67,68} instrumental behavior and such preparations are an ongoing area of research.

Perhaps the most commonly used paradigm to study reward is conditioned place preference (CPP). CPP takes advantage of Pavlovian conditioning in an apparatus with at least two, contextually distinct compartments. Animals are conditioned during several training sessions such that a paired association is formed between the stimulus of interest (unconditioned stimulus, US) and a particular chamber in the apparatus (conditioned stimulus, CS). After the completion of the training sessions, the animal is given unrestricted access to all chambers of the apparatus and the preference or avoidance of the conditioned side is taken as a measure of reward or aversion. CPP has been used to study not just drugs of abuse, but also the rewarding properties of palatable food and drink, novel objects, voluntary exercise, social interaction, copulation, and direct neuronal stimulation (for a comprehensive review, see ⁶⁹). On the other hand, CPP has also been used to study the aversive properties of acute drug treatment, drug withdrawal, and painful stimuli. Like the self-administration paradigm, CPP can be used to study abstinence and relapse by observing extinction learning and reinstatement induced by cues, stress, or drug priming. One important caveat to CPP is that the expression of a place preference necessitates the learned formation of a paired US-CS association, and the effects of amnesic drugs must be interpreted carefully⁷⁰.

Certain anticipatory behaviors have also been used as a measure of the rewarding properties of drugs and natural rewards. Measuring these behaviors requires conditioning an animal such that the conditions of drug delivery or food presentation are held constant and occur at the same manner each time. Like Pavlov’s iconic salivating dogs, rodents will engage in

specific behaviors in the time period immediately before and after reward delivery. These behaviors, including such quantifiable events as digging and rearing⁷¹, and bouts of high-frequency ultrasonic vocalizations (USVs)^{72,73}, are often measured during performance of another task, especially self-administration or CPP. Importantly USVs are a measure of reward, not reinforcement, as evidenced by the fact that control rats given yoked cocaine infusions develop the behavior equally as compared to the contingent group⁷⁴. Analogous behaviors can also be used to study the aversive properties of drugs⁷⁵ or drug withdrawal⁷⁶.

Novelty-seeking and sensation-seeking are personality traits that have been associated with a propensity to use drugs of abuse. There are a few reports of modeling these traits in rodents. For example, rats that strongly prefer environmental novelty are more likely to develop addiction-like cocaine self-administration⁷⁷. A newly developed rodent model is Operant Sensation Seeking (OSS), in which an animal actively responds for the presentation of dynamic sensory stimuli. OSS performance appears to be dependent on similar molecular substrates as psychostimulant self-administration^{78,79}.

Interoceptive state and drug action—The drug discrimination assay is used to model the subjective effects of a drug, also referred to as the interoceptive state. In a human drug user or test subject, the interoceptive state does not necessarily carry emotional valence (as in feeling ‘good’, ‘bad’, or ‘high’), as drugs that are neither rewarding nor aversive can still be detected. There are many different ways to implement a drug discrimination experiment, but two-choice operant paradigms are most commonly used. An animal is trained in a two-choice box where one choice (e.g. left lever press) results in food delivery on days when the animal has been administered drug, while the other choice (e.g. right lever press) is reinforced when the animal has been treated with vehicle. This paradigm is very sensitive and relatively specific for particular pharmacological mechanisms. Substitution studies and antagonism studies have been used to gain insight into the abuse liability and mechanism of action of psychoactive drugs. Similarly in animal models, a drug need not have rewarding or locomotive properties to be used as a training or probe drug in drug discrimination; animals have been successfully trained on rewarding (cocaine, morphine), aversive (atropine, naloxone), and neutral (buspirone, clozapine, desipramine) agents⁸⁰.

Changes in locomotor activity (i.e. either hyperactivity or sedation) are a commonly used measure of drug action as the NAc plays a role in regulating locomotor output. These data must be interpreted carefully since high doses of particular drugs, especially psychostimulants, produce repetitive stereotypical behaviors that interfere with locomotion. In some cases these stereotypies can be very informative, as some have been linked to a specific molecular mechanism of action (e.g. yawning/D3 dopamine receptor⁸¹, head-bobbing/5-HT_{2A} serotonin receptor⁸²). One important applied paradigm of locomotor activity is sensitization. Like in human addicts⁸³, rodents will become more sensitive to particular properties of certain drugs during the initial exposure period. For example locomotion and grooming behavior will continue to increase over the first several injections in a chronic stimulant period. Behavioral sensitization has been demonstrated for up to one year after the treatment, and may in part underlie the long-term neuroadaptations associated with addiction.

Cognition—A wide range of deficits in cognition have been observed in addicted patients. Although there are several potential explanations, current evidence suggests that drug abuse may cause certain cognitive deficits, and conversely certain cognitive deficits may cause a predisposition to abuse drugs and/or become addicted to them. The brain structure most commonly associated with these particular cognitive deficits is the PFC, and altered cortical function has been observed in both human drug abusers as well as chronically-treated animals. The major cognitive deficits generally associated with addiction are related to attention and problem solving. Although most clinical studies have been correlational, it is generally thought that various cognitive traits confer vulnerability to addiction and that substance abuse itself can alter certain aspects of cognition.

One such aspect is impulsivity, which is a personality trait that is characterized by the tendency to make quick, rash decisions as well as an inability to cease inappropriate behavior. Impulsivity can be broadly split into two major components: quick and/or rash decision-making (impulsive choice) and disinhibition of motor responses (impulsive action)⁸⁴. Impulsive choice is modeled in animals primarily via delay discounting, a paradigm that measures the preference for smaller, immediate rewards vs. larger, delayed rewards. On the other hand, impulsive action is modeled in several paradigms, one of which is the Go/No-Go task, where the animal must cease responding when a tone is presented during interspersed “no-go” trials⁸⁵.

Another cognitive domain believed to be involved in addiction is executive function. Executive function refers to a group of “higher order” tasks including problem solving and cognitive flexibility, and is thought to be mediated by the PFC. One commonly-used clinical assay to detect deficits in executive function is the Wisconsin Card Sorting Task, while such deficits are assessed in animals in reversal learning paradigms and in the attentional set-shift task⁸⁶. PFC-mediated “top-down” control of subcortical structures is thought to suppress drug-seeking behavior following extinction training⁴⁹.

Anxiety and anhedonia—A complex bidirectional relationship exists between addiction and stress/anxiety⁸⁷. At disease onset, drugs can be sought as a means of relieving stress. Eventually drug withdrawal becomes a stressful experience, and, in rehabilitated individuals, stress can trigger relapse to drug seeking behavior. While many anxiety models exist, the canonical model of anxiety in rodents is the elevated plus maze, an arena that consists of one open, vulnerable arm and one closed, sheltered arm. When placed in an elevated maze, rodents exhibit an approach-avoidance conflict between attraction to novel environments and aversion to heightened and/or open spaces. The proportion of time spent in the closed arms, taken as a measure of anxiety, is reliably increased and decreased by anxiogenic and anxiolytic manipulations, respectively⁸⁵.

Negative affective states, along with anhedonia, have been documented to elicit craving and relapse in recovering addicts⁸⁸. Anhedonia is an inability to enjoy pleasurable activities, and often occurs during acute or protracted withdrawal from drugs of abuse. Some of the addiction-related animal models, like ICSS and CPP, have even been used to model anhedonia⁸⁹. Additionally, sucrose preference is often used to study anhedonia in rodents. Animals are given equal access to standard drinking water and an otherwise identical

solution sweetened with sucrose or saccharin. Stressful conditions and drug withdrawal can decrease the preference for sucrose, and chronic antidepressant treatment can reverse that effect^{90,91}.

Comorbidities

Following acute and/or chronic treatment, abused substances can directly cause or exacerbate medical problems in several organ systems throughout the body. To varying degrees, drugs of abuse are known to affect the liver and kidney, the cardiovascular, respiratory and gastrointestinal systems, and of course the brain⁹². Several methods of drug delivery are also known to convey immediate risks to the user, including severe pulmonary episodes, HIV/hepatitis infection, endocarditis, and acute cardiovascular events. The effects of certain abused substances can also mimic symptoms of certain mental illnesses in otherwise healthy individuals.

Comorbidities among addiction and a wide variety of psychiatric disorders have been well documented^{93,94}. These psychiatric disorders include mood/affective disorders, anxiety disorders and obsessive-compulsive disorder, schizophrenia and bipolar disorder, and attention deficit hyperactive disorder. Psychotropic medications used to treat these disorders can also precipitate side effects that elicit or exacerbate substance abuse. Aside from acute drug effects, disease comorbidities can generally be explained by two scenarios⁹⁵: (1) drug use may begin as an alleviation of disease symptoms and/or (2) drug use and another illness brought about by a common genetic and/or environmental cause. Many of these diseases/disorders have been shown to be due to dysfunction of specific brain regions (Figure 2). The occurrence and recurrence of either situation can be caused by the presence of one or more endophenotypes.

An endophenotype is a heritable, symptom-like trait that is present in clinical populations and healthy individuals, especially those with affected first-degree relatives. With respect to addiction and other psychiatric disorders, endophenotypes are essentially behavioral biomarkers that, in a healthy individual, would often be described as a strong personality trait. Endophenotypes related to addiction include impulsivity-compulsivity traits, high levels of anxiety, and executive function deficits⁹⁶⁻⁹⁸. Some of these relationships have also been demonstrated in rodent⁹⁹ and primate¹⁰⁰ models. Moreover, comorbidities among addiction and other disorders may be related to structural or functional abnormalities in specific brain regions that have been correlated to endophenotypes¹⁰¹.

Like in humans, feeding behavior and metabolism can be affected by chronic drug treatment, leading to changes in body weight. Drugs of abuse may also affect circadian rhythm, as measured by electroencephalogram and other measures during polysomnography studies¹⁰². Some evidence also suggests that certain sleep parameters may deteriorate further during abstinence. Not surprisingly, a number of genes regulating circadian rhythm have been implicated in drug addiction¹⁰³.

WHAT ARE THE MOLECULAR MEDIATORS OF ADDICTION PATHOLOGY?

Addiction is the likely result of the long lasting or even permanent reorganization of neurocircuits within the reward circuitry through strengthening/weakening of synaptic connections or neuronal loss due to toxicity. This process recruits signaling mechanisms originating at the site of drug action through second messenger systems, synaptic plasticity mechanisms and transcriptional effects to ultimately change signal propagation through the circuit (Figure 3). We briefly highlight some well documented addiction-related mediators.

Synaptic plasticity and ionotropic glutamate receptors

Addiction is conceptualized as a learning disorder, whereby drugs of abuse hijack the same glutamate-dependent cellular mechanisms that enable learning and memory. Synaptic plasticity is a collective term for when patterns of neural activity alter the strength of the connection between two neurons. Synaptic plasticity, occurring at both excitatory and inhibitory synapses, may be mediated by altering the number of synapses, the quantal size or probability of neurotransmitter release or the functional state or expression of ionotropic glutamate receptors (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-d-aspartate (NMDA) receptors) and GABA_A receptors respectively. AMPA receptors are ligand-gated ion channels that, upon activation by glutamate, flux sodium and other cations resulting in depolarization of the synaptic membrane and are the “workhorse” of the excitatory synapse responsible for basal transmission. Sufficient AMPA receptor dependent depolarization of the neuron can lead to action potential firing and thus propagation of the electrochemical signal through the circuit. Additionally, AMPA receptor dependent depolarization can relieve the magnesium block (conferring voltage dependence) of the ionotropic glutamate NMDA receptor. These channels play a key role synaptic plasticity by fluxing calcium, resulting in activation of calcium-dependent signaling cascades. NMDA receptor activity, as well as other neuromodulatory systems, can induce transient or long lasting changes in synaptic strength. The long lasting increases or decreases in synaptic strength can be classified as either long-term potentiation (LTP) or long-term depression (LTD), respectively. A wide variety of studies have demonstrated that substances of abuse bidirectionally modulate synaptic transmission within several brain regions, including the VTA and NAc, strengthening the concept that synaptic plasticity is a key molecular constituent of experience-dependent behavioral plasticity.

Synaptic plasticity in the VTA—VTA neurons integrate reward and aversion related information from distinct excitatory inputs⁶⁷. Since Ungless et al.¹⁰⁴ first demonstrated drug-induced synaptic plasticity by showing that a single dose of cocaine could precipitate LTP-like changes at VTA dopaminergic neurons, many iterations of this phenomena have been uncovered¹⁰⁵. Similar results have since been obtained following administration of other drugs of abuse including amphetamine, morphine, nicotine and ethanol¹⁰⁶. Importantly, such changes in VTA synaptic transmission were not observed following similar treatment with non-addictive, psychoactive drugs such as the antidepressant fluoxetine¹⁰⁶. In addition to glutamatergic signaling, exposure to drugs of abuse has been shown to modulate inhibitory neurotransmission in the VTA. For example, acute treatment with morphine, cocaine, nicotine, or a stressor, impaired GABAergic LTP (iLTP) onto VTA

dopamine neurons¹⁰⁷. Similarly, methamphetamine treatment was documented to decrease GABA_B autoreceptor function in the VTA¹⁰⁸. Although less explored than excitatory neurotransmission, these studies indicate that changes in drug-induced alterations in GABAergic signaling may play a substantial role in the pathophysiology of addiction. Alongside these noted changes in fast ionotropic signaling are changes in neuron structure. Termed structural plasticity, these experience-dependent changes in cellular morphology have been reported following drug administration paradigms similar to those used in the aforementioned experiments. Increases in VTA DA neuron spine density that parallel the noted electrophysiological changes have been observed following treatment with psychostimulants¹⁰⁹. In addition to the VTA, structural and synaptic plasticity have been observed in various forebrain regions following drug administration regimens.

Synaptic plasticity in the NAc—In the NAc, changes in excitatory synaptic strength have proven to be more complex as increasing evidence advocates dynamic changes in synaptic function in a synapse-specific and experience-dependent manner²⁵. Nonetheless, current work is built upon seminal studies that suggest a decrease in synaptic strength at excitatory synapses onto NAc shell MSNs following re-exposure to cocaine¹¹⁰. Importantly, this effect is dependent upon the state of cocaine exposure (*in vivo* drug experience), since excitatory signaling in the NAc is potentiated following extended withdrawal but depressed following drug re-exposure¹¹¹. Similar differences in synaptic transmission have been obtained following morphine treatment¹¹², and gross changes in synapse morphology that mirror the electrophysiology have also been observed¹¹³. Prior to, and perhaps facilitating these biphasic synaptic changes that occur following extended withdrawal and re-exposure is the generation of silent synapses that occurs acutely following repeated cocaine exposure¹¹⁴. Silent synapses, synapses having measurable NMDA, but not AMPA, receptor responses, are thought to be substrates for increased neuronal connectivity and may be important for drug-induced behavioral changes. Furthermore, increased number of silent synapses corresponds to an increase in the expression of the NMDA receptor subunit, GluN2B. GluN2B-containing NMDA receptors have also been implicated in the pathophysiology of relapse. For example, heroin-seeking behavior can be blocked by intra-NAc treatment with selective GluN2B antagonists or siRNA¹¹⁵. Similarly, in the NAc, rapid internalization of GluA2-containing AMPA receptors has been shown to be necessary for some of the behavioral effects of acute cocaine¹¹⁶.

Synaptic plasticity in other brain regions has also been demonstrated to be necessary for relapse to drug-seeking behavior. For instance, endocytosis of GluA2-containing AMPA receptors in the medial PFC, is required for cue-induced reinstatement of heroin-seeking¹¹⁷. Both passive and contingent chronic cocaine administration as well as ethanol exposure/withdrawal transiently alters synaptic efficacy in the BNST^{118,119}. Understanding these plastic changes and their molecular substrates is essential to discovering novel treatment options. Regulating and mediating this plasticity are changes in the expression of receptors and channels, the localization of cytoskeletal and scaffolding proteins, and the activation state of kinases, phosphatases and transcription factors¹⁰⁹.

Cell surface receptors, ion channels, and transporters

In addition to signaling through ligand-gated ion channels, glutamate also signals through metabotropic receptors (mGluRs). While activation of Group II mGluRs by LY379268 has been shown to attenuate reinstatement of seeking of cocaine¹²⁰ and heroin¹²¹, the Gq-coupled Group I mGluRs have been the most thoroughly studied with respect to drug abuse, particularly mGluR5¹²². Experiments utilizing selective antagonists or knockout mice have implicated mGluR5 in the rewarding, reinforcing and motivational properties of several classes of abused substances^{123,124} as well as operant sensation seeking⁷⁹. Furthermore, acute cocaine exposure has been shown to lead to intracellular sequestration of mGluR5, mediated in a Homer-dependent manner (see below)¹²⁵, while chronic cocaine administration has been shown to upregulate mGluR5 in the NAc¹²⁶ and hippocampus¹²⁷. Additionally, in some regions, activation of Group I mGluRs have been linked to endocannabinoid (eCB) production.

In addition to its well-known role in stress and anxiety¹²⁸, the eCB system has been implicated in addiction-related processes and behaviors¹²⁹. In fact, drugs of abuse and natural rewards are known to alter brain eCB content¹³⁰. Endocannabinoids attenuate neurotransmitter release through at least two targets, the Gi/o-coupled cannabinoid 1 (CB1) receptor and the transient receptor potential vanilloid 1 (TRPV1). In addition to being activated by tetrahydrocannabinol, the psychoactive ingredient in marijuana, antagonism or genetic deletion of CB1 receptor has been shown to suppress reinstatement of several class of drugs¹³¹. Additionally, CB1 receptor activity is required for reinstatement of sucrose¹³² and corn oil¹³³ seeking, suggesting that CB1 receptor mediates a non-selective motivational component of conditioned reinforcement. Much less is known about TRPV1 and reward processes, but studies utilizing TRPV1 knockout mice suggest that the channel plays a role in the behavioral effects of ethanol¹³⁴ and cocaine¹³⁵.

In clinical populations, few targets have been studied as much as the plasma membrane dopamine transporter (DAT) and dopamine receptors. PET studies have revealed decreased DAT and D2-like dopamine receptor availability in patients who abuse several classes of drugs¹³⁶ as well as in patients with obesity¹³⁷. It should be noted that the majority of these studies were performed using a radiotracer (¹¹C]-raclopride) with high affinity for both D2 and D3 dopamine receptors. The D3 receptor is structurally and functionally homologous to D2 receptors, but is more strictly localized to the mesolimbic circuit, specifically the NAc shell and the islands of Calleja¹³⁸. Through the use of a D3 receptor-preferring ligand, one recent PET imaging study has observed specific upregulation of D3 receptors in methamphetamine abusers¹³⁹. Additionally, evidence from several preclinical models implicate D3 receptor activation in the behavioral effects of psychostimulants¹⁴⁰. In rodent models, antagonists of dopamine receptors decrease the reinforcing effects of cocaine^{141,142}. More specifically, D1-like and D2-like dopamine receptor antagonism in the NAc reduces the reinforcing effects of cocaine^{143,144}. In addition to affecting dopamine receptors and its plasma membrane transporter, psychostimulant abuse is thought to perturb the packaging of dopamine into synaptic vesicles. Studies have linked both cocaine¹⁴⁵ and methamphetamine¹⁴⁶ abuse in human patients with decreased vesicular monoamine transporter availability in the striatum. Interestingly, this phenomenon may be specific to

contingent abuse of the drugs, since drug-induced dopamine release can vary based upon whether the drug is self-administered or passively delivered. For example, extracellular dopamine in the NAc increased to a significantly greater extent in rats that self-administered cocaine than in the yoked controls¹⁴⁷. In addition to dopamine release, the contingency of drug administration can differentially modulate the transcription and expression of a variety of targets.

Structural and regulatory proteins

Structural proteins are critical for the development, maintenance and plasticity of excitatory synapses. As exposure to drugs of abuse leads to remodeling of excitatory synapses, it is not surprising that synaptic scaffolding proteins are important proteins implicated in drug-related behaviors. To date, postsynaptic structural proteins including (but not limited to) postsynaptic density 95 (PSD-95)¹⁴⁸, Kalirin¹⁴⁹, activity-regulated cytoskeleton-associated protein (Arc)¹⁵⁰, A-kinase anchor protein (AKAP)¹⁵¹, integrins¹⁵², spinophilin and neurabin¹⁵³ and Homers¹²⁵ have been implicated in drug related behaviors. For the most part, with the exception of Homers discussed below, these structural proteins act to stabilize postsynaptic densities including ionotropic glutamate receptor trafficking and function as well as synapse formation.

One of the most thoroughly studied scaffolding proteins in regards to addiction-related phenomena are the Homer proteins. Homers are scaffolding proteins that regulate cell signaling by regulating Group I mGluR trafficking and extracellular glutamate concentrations¹⁵⁴. Homers are also involved in dendritic spine enlargement and postsynaptic density maturation. Evidence has pointed towards Homer proteins as being crucial for the long-lasting synaptic and behavioral plasticity following drug administration. Along with Arc, the isoform Homer1a is an immediate early gene expressed in response to drug exposure. Other isoforms of Homer are involved in drug response as well. For example, genetic deletion and viral-mediated rescue demonstrated that Homer2 is essential for the neuroplastic effects and rewarding properties of alcohol¹⁵⁵. By contrast, deletion of either Homer1 or Homer2, has been shown to elicit a pre-sensitized state to neurochemical and behavioral changes induced by cocaine¹⁵⁶.

Many drugs of abuse, including cocaine, amphetamines, and opioids, directly bind or functionally interact with the ER-associated intracellular chaperone known as the σ 1 receptor¹⁵⁷. The σ 1 receptor is thought to co-localize with inositol triphosphate receptors and modulate intracellular calcium release. σ 1 activity also regulates transporters and ligand- and voltage-gated ion channels and has been implicated in the reinforcing and addictive properties of psychostimulants¹⁵⁸.

Kinases

One of the most pronounced signaling pathways in addiction includes elements of the adenylate cyclase, cAMP, and protein kinase A (PKA) signaling pathway. PKA is thought to be activated by D1 receptor signaling in response to drug exposure. Among other events, PKA activation can lead to phosphorylation of AMPA receptors and convergence with the protein kinase C pathways onto the extracellular-related kinase (ERK) pathway. Similarly,

Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) is known to phosphorylate AMPA receptors and regulate the activity of striatal excitatory synapses¹⁵⁹. Additionally, CaMKII enhancement of certain K⁺ currents, independent of AMPA receptors, has been demonstrated to promote cocaine reward¹⁶⁰. Like PKA and PKC, CaMKII is also involved in activation of the ERK pathway by drugs of abuse.

Activation of the ERK pathway has been reported following acute and repeated treatment with cocaine, amphetamine, tetrahydrocannabinol, nicotine and morphine in VTA, NAc, extended amygdala and PFC¹⁶¹. Additionally, the ERK pathway is suggested to be dependent on the phosphatase inhibitor dopamine- and cAMP-regulated phosphoprotein-32 (DARPP-32)^{162,163}. Cyclin-dependent kinase 5 (Cdk5), another key component in the pathway, is also implicated in addiction-related behaviors since chronic cocaine exposure has been shown to increase Cdk5 levels in the striatum¹⁶⁴. This pathway plays a variety of diverse roles in different brain regions and disease stages. In the NAc for example, the ERK pathway mediates some of the initial effects of cocaine¹⁶⁵, while in the central amygdala it plays a role in the incubation of cocaine craving¹⁶⁶. ERK activation has been shown to interact with the epigenetic machinery, including the expression of immediate early genes and transcription factors.

Transcription machinery

A variety of epigenetic mechanisms are thought to contribute to the persistent plastic changes related to addiction. Histone modification, DNA methylation, and the production of non-coding RNA and transcription factors have all been implicated in the pathophysiology of substance abuse¹⁶⁷. For instance in the NAc, histone 3 lysine 9 dimethylation by lysine dimethyltransferase G9a has been demonstrated to be required for neuronal structural plasticity and cocaine reward¹⁶⁸. Additionally, cocaine-induced acetylation of histone 3 in the NAc, and subsequent transcriptional activation of CaMKII α , are important events underlying drug seeking motivation¹⁶⁹. Other regulatory proteins have also been implicated in the effects of psychostimulants. Viral manipulations of MeCP2, a methyl-DNA binding protein, established its role in the behavioral effects of psychostimulants, and hypomorphic *Mecp2* mice have deficient amphetamine-induced structural plasticity and immediate early gene activation¹⁷⁰. Accompanying – and sometimes responsible for – these changes in epigenetic machinery is the activation and regulation of transcription factors. The two transcription factors most extensively studied with respect to addiction are FosB and cAMP response element binding protein. These transcription factors are upregulated following exposure to drugs of abuse and likely lead to upregulation of mRNA of the before mentioned Cdk5¹⁶⁴. Overexpression of either FosB¹⁷¹ or cAMP response element binding protein¹⁷² within the striatum has been shown to potentiate the behavioral effects of cocaine, likely through similar mechanisms of gene expression regulation¹⁷³. Interestingly, a more recent study has demonstrated that selective overexpression of FosB within D1-, but not D2-, expressing neurons in the NAc potentiates behavioral responses to cocaine²⁹. Others have shown that FosB is necessary and sufficient for the cocaine-mediated synaptic remodeling and CaMKII induction in D1-expressing neurons¹⁷⁴. Taken together these data highlight the need for the continued need for cell-type specific experimental designs in future research efforts.

CONCLUSION

Addiction is clearly a complicated disease recruiting many neural circuits and intracellular signaling pathways. While we have provided a broad overview of addiction and current research strategies to study addiction-related phenomena, the complexities of the addicted brain are likely to depend on the temporal interactions between brain regions and signalling cascades not addressed in this current review. Nonetheless, it can be argued that addiction research has provided one of the most advanced understandings of experience-dependent plasticity. This is because animal models of addiction can be simplified as the effects of a substance (drugs of abuse) on a biological substrate (the brain). As such, models of addiction are some of the most powerful constructs neuroscientists have to study learning and memory under controlled circumstances. As opposed to developmental or aging related diseases, exposure to drugs of abuse is an inducible model under the temporal control of the experimenter.

The incorporation of cell-type and circuit-specific approaches to study neuroscience allow for rapid advancements in our understanding of addiction. These tools, transgenic mice and optogenetics have been reviewed by Stamatakis and Stuber¹⁷⁵). Optogenetic techniques have made it possible to dissect the function of different inputs to the NAc^{66,165} and VTA^{20,67}, an approach that should illuminate how synapses formed by these various inputs may be differentially modulated by drugs of abuse. The application of these tools simultaneously with *in vivo* electrophysiological techniques has the potential to greatly advance the field of addiction research and our understanding of brain function. As preclinical models of therapeutic relevance emerge, further work could unveil targets to treat addiction-related maladaptive processes with limited negative consequences in human drug addicts. Several labs have demonstrated that acute opto-/chemogenetic manipulation of the reward circuits is a promising avenue for the development of treatments. However the use of these technologies in a long-lasting approach, i.e. to modulate synaptic plasticity *in vivo*, has been less well-documented, and is an exciting direction of future preclinical research.

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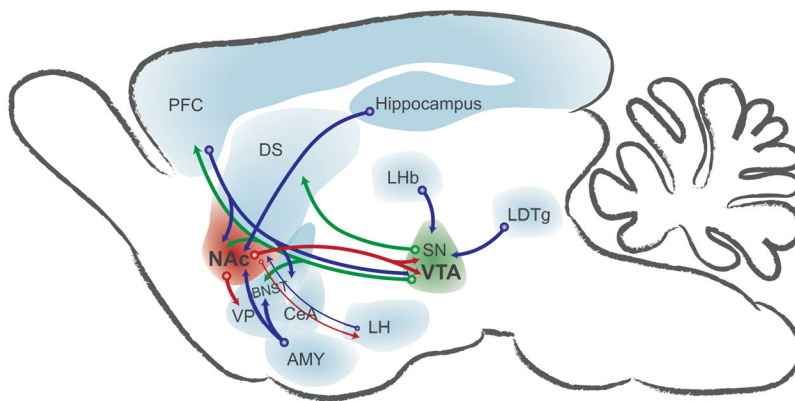


Figure 1. Simplified schematic of the reward circuitry in the rodent brain emphasizing signaling to and from the nucleus accumbens (NAc) and ventral tegmental area (VTA). Glutamatergic transmission drives information through the reward and reward-related circuitry (blue arrows). GABAergic transmission from NAc and other regions dampens target neuronal activity (red arrows). Dopamine release from the VTA and substantia nigra (SN) modulates synaptic transmission in target regions (green arrows). These regions are recruited and undergo synaptic, circuit and genetic adaptations in response to drug experience. AMY, amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; DS, dorsal striatum; LDTg, laterodorsal tegmentum; LHb, lateral habenula; LH, lateral hypothalamus; PFC, prefrontal cortex; SN, substantia nigra; VP, ventral pallidum.

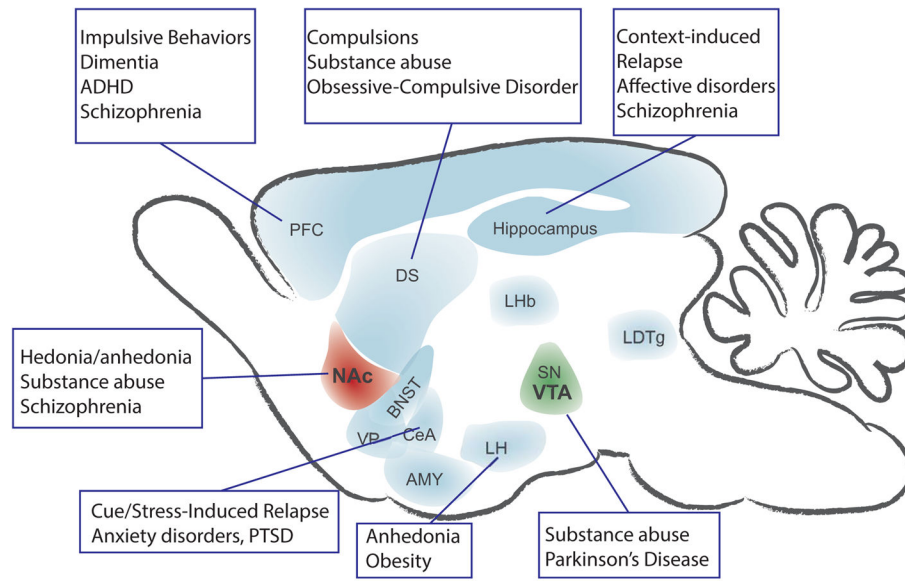


Figure 2. Simplified schematic depicting proposed brain regions involved in disease states exhibiting comorbidities with addiction.

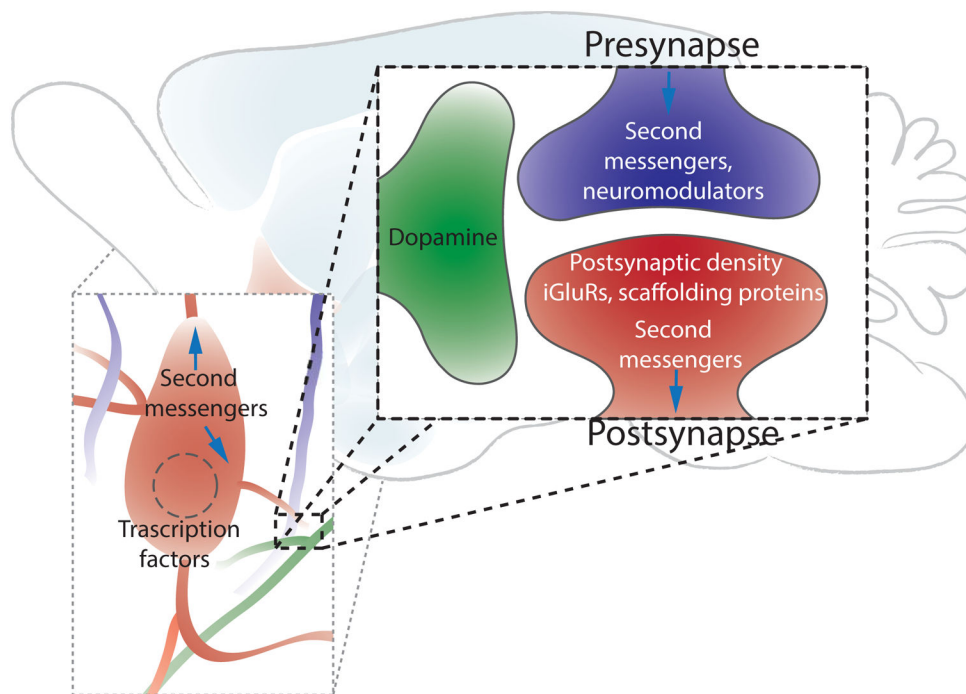


Figure 3. From circuits to synapses, addiction alters the brain. This schematic suggests cellular changes in second messenger signalling and transcription in response to drugs of abuse. Simplified tripartite synapse illustrating molecules known to be affected at different synapses within the reward circuits. Note: addiction-related changes are most often region, cell-type and synapse specific. iGluR, ionotropic glutamate receptors (AMPA and NMDA receptors).

Table 1

Drug(s) of Abuse	Primary Action	Physiological Effects
Nicotine	Nicotinic receptor agonist	Increased blood pressure, heart rate, and alertness
Cocaine, methylphenidate, MDPV derivatives	Plasma membrane dopamine transporter (DAT) inhibitor	Increased blood pressure and heart rate, increased energy and alertness, reduced appetite, anxiety, paranoia, psychosis
Derivatives of amphetamine and cathinone	Vesicular monoamine transporter (VMAT) inhibitor	
Heroin, morphine and semisynthetic analogs, fentanyl	μ -opioid receptor agonist	Euphoria, drowsiness/sedation, nausea, respiratory depression
<i>Salvia divinorum</i> preparations/salvinorin A	κ -opioid receptor agonist	Dissociation, hallucinations, impaired motor function
Phencyclidine (PCP), ketamine	N-methyl-D-aspartate (NMDA) receptor antagonist	Dissociation, delirium, analgesia, impaired motor function
Barbiturates, benzodiazepines, Z-drugs, muscimol	GABA _A receptor agonist/ positive allosteric modulator	Sedation, anxiolysis, amnesia, impaired coordination, muscle relaxation
<i>Cannabis</i> preparations and hashish, tetrahydrocannabinol and synthetic analogues	CB ₁ receptor agonist	Altered perception, impaired learning and memory, increased heart rate and appetite
Tryptamine and phenethylamine derivatives e.g. psilocin, mescaline, LSD, DOM, DMT	5-HT _{2A} receptor agonist	Altered perception, hallucinations, emotional changes, insomnia
Alcohol/ethanol	pleiotropic effects including NMDA receptor, GABA _A receptor action	Relaxation, loss of inhibition, drowsiness, impaired coordination, amnesia
Solvents and inhalants	pleiotropic effects	Stimulation, loss of inhibition, slurred speech, impaired coordination