Phasic Dopamine Release in Appetitive Behaviors and Drug Addiction

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Abstract: Although dopamine is implicated in the development of addiction, it is unclear how specific dopamine release patterns are involved with drug seeking. Addictive drugs increase tonic dopamine levels on the order of minutes, as well as phasic dopamine release events that occur on a subsecond time scale. Phasic dopamine release is associated with the initiation of goal-directed behaviors, and has been shown to promote drug seeking. Prior experience with addictive drugs modulates the synaptic and intrinsic properties of dopamine neurons, affects the pattern of dopamine neuron firing and release, and alters dopamine-dependent behaviors related to drug addiction. In this review, we synthesize the known drug-dependent changes to the dopamine system along with the established functions of phasic dopamine release in order to provide a framework for conceptualizing the role of phasic dopamine release in drug addiction. Because drug addiction is commonly thought to involve changes in brain circuits important for natural reinforcement, we first present the role of phasic dopamine release in appetitive and goal-directed behaviors in the context of contemporary theories regarding the function of dopamine. Next, we discuss the known drug-induced changes to dopamine neurons and phasic release in both *in vitro* and *in vivo* preparations. Finally, we offer a simple model that chronic drug experience increases the contrast, or 'signal to noise', of phasic dopamine release to basal dopamine levels in response to drug-related stimuli, which could result in aberrant associations between cues and reinforcers that contribute to the development of addiction.

Keywords: Dopamine, drug abuse, addiction, voltammetry.

Numerous theories have been developed to model aspects of drug addiction, both from a psychological [1-4] and a neurochemical perspective [2, 5-10]. In particular, the dopamine system is thought to play an important role in addiction [2, 5-10]. With the development of techniques that can analyze subsecond neurotransmitter release events in behaving rodents, it has become evident that phasic, subsecond dopamine release is involved with promoting drug seeking [11], as well as other appetitive [12, 13] and goal-directed behaviors that do not involve abused substances [14-18]. While others have established theories regarding the role of dopamine in the development of addiction [2, 5-10], there has been far less theoretical discussion on the specific function of phasic dopamine release in regards to drug abuse. In this review, we provide background on the dopamine system, describe methods used to detect dopamine release, and discuss current theories about dopamine's role in behavior. Because addiction is commonly thought to usurp the neural circuitry involved with natural reinforcement [2] and learning and memory [5, 6], we will first discuss the role of phasic dopamine release in appetitive and learned goal-directed behaviors that do not involve abused substances. Finally, we will summarize the changes to the dopamine system after drug exposure in rodents and humans, review studies that explicitly examined drug-dependent effects on phasic dopamine release, and suggest how drug-dependent alterations in the dopamine system could affect phasic dopamine signaling. The goal of this review is to extend upon existing theories of dopamine function in addiction and provide a conceptual framework for the role of phasic dopamine release in drug-related behaviors.

THE DOPAMINE SYSTEM: ANATOMY, RECORDINGS, AND DETECTION

Anatomy

The ventral tegmental area (VTA) and the neighboring substantia nigra pars compacta (SN) contain the primary dopamine-producing neurons in the brain [19]. The VTA is thought to play a particularly important role in drug abuse [7]. The SN has been examined far less in the context of addiction, with many studies focusing on its role of in motor control [20]. A large proportion of the neurons whose cell bodies are in the VTA contain dopamine. For example, in the rat, 2/3 of the approximately 40,000 VTA neurons contain tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, and as such are presumably dopaminergic [19, 21]. The non-dopamine producing cells in the VTA are likely γ-aminotbutyric acid (GABA)- and glutamate-producing; however, there is some debate whether glutamate and dopamine are co-released from the same neurons [22, 23] or if glutamate and dopamine neurons exist in distinct populations [24]. A typical VTA neuron will project to only a single nucleus, though there is considerable variability between VTA-projection targets in the percentage of VTA neurons that are dopamine-producing [19]. Specifically, of the VTA neurons projecting to the nucleus accumbens (NAcc), ~85% are dopamine-producing, while ~50% of the neurons projecting to the amygdala are dopamine-producing, and ~30% of the neurons projecting to the prefrontal cortex (PFC) are dopamine-producing [19]. As with its projections, the VTA receives input from a diverse array of brain regions including the PFC, NAcc, bed nucleus of the stria terminalis, lateral dorsal tegmentum (LDT), pedunculopontine

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tegmental nucleus (PPT), amygdala and areas of the hypothalamus [25-28]. Thus, the VTA is a heterogeneous brain region with extensive afferent input and efferent projections.

Dopamine Neuron Recordings

In extracellular electrophysiological studies, dopamine neurons are identified *in vivo* primarily based on the presence of a triphasic and long-duration action potential waveform [29, 30]. These neurons exist in one of three states: (1) hyperpolarized and quiescent, (2) firing single-spike action potentials in regular or irregular patterns at relatively low rates (2-10 Hz), or (3) firing action potentials in bursts up to 15-30 Hz [29, 31, 32]. Reports suggest that a large portion of dopamine neurons may be electrically coupled [33, 34], which could aid in the synchronicity of dopamine neuron firing patterns. While dopamine neuron firing patterns are often further categorized for subsequent analysis [31, 32, 35, 36], it is important to note that a given dopamine neuron can both fire in a single-spike pattern, as well as in bursts [37]. Furthermore, the amount of bursting can vary considerably across neurons, and as such, the degree of bursting may be more appropriately viewed as a continuum [37]. The burst firing of dopamine neurons requires glutamatergic input, activation of N-methyl-D-aspartate (NMDA) receptors, opening of high-threshold calcium currents, and finally activation of calcium-activated potassium currents to terminate the burst [38]. Furthermore, activation of brainstem nuclei such as the PPT and LDT are involved in dopamine neuron burst generation [38, 39] and increased dopamine levels in the NAcc and striatum [27, 40]. Although robust immunohistochemical methods can identify dopamine, GABA, and glutamate neurons in the VTA [24], electrophysiological identification of neuronal subtypes using only the action potential waveform can be problematic [41]. *In vivo* juxtacellular labeling of recorded VTA neurons in the rat demonstrate that many neurons with a triphasic and long-duration waveform actually are not dopaminergic [42], although it has been suggested that these findings are difficult to interpret because of methodological considerations [30]. Dopamine neuron firing patterns have been examined *in vivo* both in anesthetized and awake, behaving preparations. Although the patterns of dopamine neuron firing are similar (e.g. single-spike and burst firing) whether the recordings are performed in awake or anesthetized rodents, it should be noted that anesthesia can affect certain pharmacological responses [43] and reduce the number of spontaneously active and bursting dopamine neurons when compared to recordings in awake preparations [32, 44, 45].

In contrast to *in vivo* recordings, dopamine neurons in brain slice preparations do not spontaneously fire action potentials in bursts, but rather typically exhibit regular action potential firing [46]. The reported frequency of putative dopamine neuron firing in the slice varies whether one uses extracellular (3-8 Hz) [46], perforated-patch (2-5 Hz) [47], or whole-cell recordings (1-3 Hz) [41, 48]. Regardless of the recording technique, the firing of putative GABA neurons is significantly greater than dopamine neurons and is usually higher than 10 Hz [46]. Although the firing rate can provide a crude segregation of neuronal subtypes in brain slices, a more reliable electrophysiological marker of dopamine neurons was found to be the presence of the hyperpolarization-activated, cyclic nucleotide-regulated cation current (I_h) [46]. However, subsequent work determined that not all cells with the I_h produced dopamine [41, 49], which may be explained by differences between species, as TH is present in 98% of VTA neurons with the I_h in the mouse [48], but only in ~50% in the rat [41]. Recent studies highlight that the electrophysiological properties and pharmacological manipulations can identify dopamine neuron content in brain slices if the projection target of the neuron is known [50, 51]. Although electrophysiological characteristics may not always be an accurate predictor of dopamine neurons, the I_h is commonly used as a marker of dopamine content in many brain slice electrophysiological studies.

Detecting Dopamine Levels

Microdialysis and fast-scan cyclic voltammetry (FSCV) are the two primary analytical techniques commonly used to detect dopamine levels *in vivo*. Microdialysis involves the perfusion of fluid through an implanted probe with an inlet port, outlet port, and a dialysis membrane [52]. Small molecules in close proximity to the outside of the probe can diffuse through the dialysis membrane to be collected in the dialysate fluid on the inside of the probe for subsequent analysis. A more accurate estimate of the concentration of a specific analyte around the probe can be determined by using no net flux microdialysis. This variation of conventional microdialysis involves perfusing known concentrations of the analyte of interest through the probe. If the input analyte concentration is higher relative to the extracellular space, the analyte will diffuse out of the probe leading to a lower output analyte concentration in the dialysate. Conversely, if the input analyte concentration that flows into the probe is lower relative to the extracellular space, the analyte will diffuse into the probe leading to a higher output analyte concentration in the dialysate. The estimated analyte concentration in the brain is found at the point where there is no net flux, or rather diffusion, of the analyte across the microdialysis probe. While microdialysis provides excellent analyte selectivity and sensitivity, there are a few drawbacks associated with this technique. For example, microdialysis probes are relatively large in diameter (200-300 μM), which can disrupt tissue for up to 1.4 mm from the probe location [53]. While it was originally thought that an accurate measurement of absolute analyte levels could be achieved using either no net flux or low perfusion rate microdialysis preparations [52], the damage due to the probe size can lead to lower estimates of the analyte concentration [54]. Another issue with microdialysis is its temporal resolution, which typically is on the order of minutes, though recent attempts have improved the resolution for some analytes to as low as 14 s [52, 55]. Therefore, microdialysis is not well suited to examine analyte changes to behaviorally relevant discrete stimuli, but rather is adept at identifying basal or slow/tonic changes in analyte levels, often on a minute time scale. For the purpose of this article, we will use the expression 'basal dopamine levels' to refer to dopamine that is detected primarily with microdialysis in the absence of any overt behaviorally relevant stimuli or acute experimenter-induced treatment (pharmacological or electrical), and is thought to be around 5 – 20 nM in the NAcc [56]. Importantly, basal

dopamine levels are related to the number of spontaneously active dopamine neurons, as well as to the firing pattern and firing rate of dopamine neurons under these conditions [57].

In contrast to microdialysis, electrochemical techniques offer excellent temporal resolution to isolate phasic neurotransmitter release events but are less selective at differentiating between certain analytes of interest. When used to detect dopamine, electrochemical techniques take advantage of the fact that application of a modest potential (~200 mV *vs* Ag/AgCl) to a suitable electrode is sufficient to drive electrolysis of dopamine to dopamine-*o*-quinone [58]. The current produced by the electrolysis can be measured at the electrode and is proportional to the number of molecules undergoing oxidation (i.e. dopamine to dopamine-*o*-quinone), and therefore the concentration at the electrode surface. Different command waveforms can be used for the application of the potential to the electrode, the simplest being a continuous, constant potential in (constant-potential) amperometry. This variant has microsecond temporal resolution and is often used to study the kinetics of dopamine release and reuptake; however, it offers little chemical selectivity since any oxidized compound will be detected with constant-potential amperometry recordings, which has deterred researchers from using this technique in behaving animals. A more selective electrochemical method is fast-scan cyclic voltammetry (FSCV), which utilizes a triangle input waveform to separate the electrolysis from different analytes into temporally-resolved peaks in the output current. Since the voltage is swept to an oxidizing potential and back, current is generated (in opposite directions) during the oxidation and reduction processes, whereby producing two electrochemical peaks for a given compound making chemical resolution more robust. FSCV can be employed to record dopamine release in awake, behaving rodents [59-61], and is capable of detecting changes in dopamine levels that occur in the range of 0.1 to 100 s [62]. Using FSCV recordings in the NAcc, it was found that brief electrical activation (< 1 s) of the dopamine system transiently elicited a phasic increase in dopamine levels up to 1 μM in awake, behaving rodents, although the increase is smaller in anesthetized preparations [63, 64]. For the purpose of this article, we will use the expression 'phasic' to refer to dopamine that is detected on the order of seconds with electrochemical techniques and 'tonic' to refer to dopamine that is detected on the order of minutes with microdialysis. While electrical stimulations of the midbrain that mimic the burst firing frequency of dopamine neurons elicits a greater summation of dopamine release relative to regularly-spaced lower-frequency stimulations [64], it is important to note that the specific firing pattern responsible for phasic dopamine release is currently unknown. For example, phasic dopamine release could potentially arise from dopamine neurons burst firing, or firing single-spikes in a coordinated fashion, or some combination of both. Regardless, many studies have been able to examine the function of the dopamine system using microdialysis to assess basal or slow/tonic changes in dopamine levels, and electrochemistry to identify phasic dopamine release events.

THE ROLE OF DOPAMINE IN APPETITIVE AND GOAL-DIRECTED BEHAVIORS: PHARMACOLOGY, ELECTROPHYSIOLOGY, AND GENETIC APPROACHES

Since the identification of dopamine over 50 years ago [65], a number of theories have been developed to explain the role of dopamine in appetitive and goal-directed behaviors. We will briefly discuss some of the prominent theories and the supporting evidence to provide a framework for understanding the current views of dopamine function. We would like to preface that these theories are not necessarily mutually exclusive, but rather they provide different perspectives on the role of dopamine in behavior. More in depth discussion on theories of dopamine function can be found elsewhere [66-71].

The most well-established and uncontroversial theory of dopamine function is that dopamine release is involved with sensorimotor behaviors [66, 68]. Dopamine plays a key role in motor tasks, as this is the primary deficit observed with those suffering from Parkinson's Disease (PD), a disease that leads to the selective degeneration of dopamine neurons [72]. However, it should be noted that symptoms of PD do not typically appear until a majority of SN dopamine neurons and terminals have degenerated [20, 73]. Furthermore, mice that are deficient in dopamine production are catatonic and require supplements for survival and normal motor behaviors [74].

In addition to enabling normal motor activity, many lines of evidence support a critical role of dopamine in motivation [66, 68]. For example, manipulations that mildly impair dopamine signaling in the NAcc without altering motor behavior have been found to shift food consumption from a preferred food option that required lever pressing for receipt toward a less palatable food option that was freely available [75-78]. In another behavioral assay that examined effort and motivation, rats were given an option in a T-maze to obtain a lower food reward with no obstacle or a higher food reward that required climbing over a barrier. Systemic dopamine receptor antagonism [79, 80] or local dopamine depletions in the NAcc [81] shifted the response from the high reward side to the low reward side. Importantly, rats still preferred the high reward side when the barrier was removed under conditions when dopamine signaling was impaired, which suggests that these manipulations were not a result of a learning deficit [79-81]. Motivation is also assessed in operant tasks under progressive ratio (PR) reinforcement schedules. Under PR reinforcement paradigms, the operant requirement (often lever pressing) increases on subsequent trials until the 'break-point', which is the number of lever presses for reinforcer delivery on the last completed trial and is a measure motivation [82]. Inhibiting dopamine signaling in the NAcc reduces the break-point for natural reinforcers [82-84]. Conversely, enhancing dopamine signaling in the NAcc by local amphetamine injections [85] or in mice with impaired dopamine transporter function [86] increases the break-point for natural reinforcers. Together, these studies highlight that dopamine, especially within the NAcc, may

function to overcome the motivational costs required for completing tasks requiring a high level of effort [68, 87].

The 'incentive-salience' hypothesis of dopamine builds upon the general motivational hypothesis discussed above [66]. In short, incentive-salience is the neural representation of motivational value generated in response to a reward-related stimulus. This motivational representation is dynamic and can be applied to internally generated or externally experienced reward-related stimuli to give the stimulus incentive value, which can take control of behavior. In this hypothesis, it is thought that dopamine modulates the incentive value of reward-related stimuli [66]. This hypothesis separates 'liking' of rewards, as measured by hedonic responses, from 'wanting' of the reward, as measured by motivational metrics [66]. Specifically, a variety of insults to the function of the dopamine system do not affect taste-reactivity or 'liking' [88-90]. Conversely, enhancing dopamine levels in dopamine transporter knock-down mice increases the 'wanting' for natural reinforcers as evidenced by increased break-points under PR reinforcement schedules [86] and shorter latencies to obtain a reinforcer [91]. These general findings have been mirrored in human studies where dopamine levels correspond to self-reports of 'wanting' and not to 'liking' [92, 93].

Studies employing electrophysiological recordings of dopamine neurons in awake, behaving animals provide evidence that dopamine can encode a 'prediction-error' signal in the brain [70]. In both primates and rats, it was found that dopamine neurons increase firing to the receipt of a reward, but after training dopamine neurons instead fire to cues that predict the availability of the reward [94, 95]. Interestingly, when a predicted reward is omitted, the firing of dopamine neurons is depressed [95]. Together, this evidence suggests that dopamine neuron firing signals the scalar discrepancy between the actual reward obtained and that predicted [95]. In support, dopamine neuron firing correlates with the probability of reward availability [96], the uncertainty of reward delivery [97], as well as the magnitude of the reward [98]. Some argue that the increase in dopamine neuron firing in these reward-related paradigms occurs too fast for any cortical-mediated computation to occur [67, 99], which suggests that dopamine may be important for performing low computational, sub-cortical-dependent decisions [100]. Alternatively, it has been suggested that dopamine release may function as a novelty signal that reinforces efferent copies of recently completed behaviors [67]. Regardless, dopamine neuron activation in these tasks maps extraordinarily well onto a teaching signal proposed in the theoretical learning models in the field of reinforcement learning [71, 95, 101, 102].

In summarizing the experimental support for the sensorimotor, motivational, incentive-salience, and prediction-error theories of dopamine function, it becomes evident that the dopamine system is associated with a diverse array of natural and appetitive behaviors. Furthermore, dopamine likely subserves various functions depending upon the anatomical location, context, and duration of its release [70]. With this theoretical foundation of dopamine's role in behavior, we will now explore the work that has specifically examined phasic dopamine release in the NAcc during appetitive and goal-directed behaviors.

THE ROLE OF PHASIC DOPAMINE RELEASE IN APPETITIVE AND GOAL-DIRECTED BEHAVIORS: ELECTROCHEMICAL APPROACH

The prominent theories of dopamine function developed primarily from the findings of pharmacological, genetic, and electrophysiological experimental techniques. However, these techniques do not provide direct information on dopamine release in forebrain terminal regions during discrete behavioral events on a physiological time scale. Pharmacological and genetic manipulations can produce long-lasting or permanent changes, which prevent using these techniques for isolating behavioral effects related to dopamine changes on a subsecond level. While electrophysiological recordings have excellent temporal resolution, it is not a perfect proxy of dopamine concentration since models of release processes incorporate several non-linear functions [103]. There are currently no reliable electrophysiological criteria for determining the projection target of a given VTA neuron based solely on the action potential waveform, which prevents inferring how dopamine neuron firing is associated with release in a specific brain structure. Therefore, voltammetric approaches, such as FSCV, offer an unparalleled capacity to quantitate phasic changes in dopamine concentration in specific target regions occurring on a physiological time scale. These techniques have provided further insights into dopamine's role in the brain during behavior that are complementary to pharmacological, genetic, and electrophysiological methods. Below we will discuss the findings regarding phasic dopamine release in the NAcc using FSCV in drug-free appetitive and goal-directed behaviors.

Presentation of novel sensory stimuli activates the mesocorticolimbic system. Specifically, electrophysiological recordings in both rats and primates indicate that putative dopamine neurons increase their firing rate in response to tactile stimulation [33], presentation of an auditory stimulus [33], or an unexpected delivery of sucrose [95]. In studies utilizing microdialysis, increases in dopamine overflow are observed after handling [104], and during sexual behaviors [105]. Using FSCV recordings in the NAcc, it was demonstrated that the number of spontaneous transient dopamine release events are enhanced six-fold in response to the presentation of another rat [12, 13]. However, the effect on transient dopamine release events was significantly attenuated with repeated rat presentations, presumably correlating with the reduced novelty and habituation to the other rat [12].

Although the frequency of dopamine transients increased during the presentation of another rat, it is difficult to associate dopamine release to any one specific behavior [12]. Subsequent studies examined phasic dopamine release in response to more easily controlled experimental conditions. Using FSCV, unpredictable intra-oral administration of sucrose was found to increase phasic dopamine release, while administration of an aversive compound, quinine, decreased dopamine release [106]. Interestingly, when rodents self-administered sucrose, dopamine levels increased in response to the presentation of a cue predicting sucrose availability, and the climax of phasically emancipated dopamine in the NAcc coincided with the lever press for sucrose [16]. Control experiments found that unreinforced

cue presentations did not affect dopamine levels in naïve rats, suggesting that the phasic NAcc dopamine release observed in this task was dependent upon a learned association [16]. Further highlighting a role of phasic dopamine release in learned behaviors, FSCV recordings in the NAcc were made from rats undergoing Pavlovian conditioning where a conditioned stimulus (CS+) reliably predicts reinforcer delivery (unconditioned stimulus, US) [14, 17, 18]. Early in training, phasic dopamine responses are observed primarily to the reward delivery (US). After rats learn the CS-US association, dopamine is released to the presentation of the CS, while the response to the US is attenuated. However, a stimulus that did not predict reward availability (CS-) also increased dopamine release to some degree to the onset and offset of the CS-, suggesting some generalization between the conditioned stimuli [14]. Together, these studies using a between-animal design suggest that there is a transfer of the phasic dopamine response from the US to the CS [14, 18] that reflects the electrophysiological recordings in similar paradigms [69, 94]. An important future FSCV experiment would be to utilize a within-animal design so that the time-course of the transfer from the US to the CS could be accurately determined. It should be noted that phasic dopamine release is observed to both the US and CS in rats [14], but dopamine neurons tend to fire only to either the US or the CS in primates [69]. This discrepancy could reflect differences in the species studied, the training paradigm utilized, or functional differences between dopamine neuron firing and release. It should be noted that despite the caveats raised above in relating *in vivo* electrophysiological data to dopamine release, many of these results in rats obtained using FSCV are consistent with the electrophysiological studies performed in behaving monkeys and rats.

Some studies have examined the role of phasic dopamine release during intra-cranial self-stimulation (ICSS) procedures, where learning to lever press for a highly reinforcing electrical stimulation can be assessed within an animal in a single session [15, 71]. A recent report found that the magnitude of dopamine released in the NAcc to cues predicting ICSS availability was correlated with the learning to lever press for electrical stimulation [15]. Specifically, cue-evoked dopamine responses increased in magnitude during acquisition, disappeared during extinction, and reappeared upon reinstatement [15]. These results are exciting since they correlate dopamine responses with learning an operant task and also reflect changes in the contigency of the reinforcement. Somewhat analogous to natural rewards, ICSS is dopamine-dependent [107], although sustained phasic dopamine release to the self-stimulation is not required for operant responding in ICSS paradigms [108]. However, caution should be exercised when extending these results to all aspects of natural reinforcers because ICSS removes the sensory component of reward processing.

To summarize, phasic dopamine release using FSCV has been assessed in many appetitive and goal-directed behaviors, and it is apparent that an increase in the number of phasic dopamine events in the NAcc is associated with novelty and unexpected rewards [12-14]. Experiments employing operant tasks also highlight that cues predicting reinforcer availability elevate dopamine release in the NAcc [15, 16], where the increase in dopamine precedes the operant action [16, 109]. Studies involving Pavlovian conditioning also suggest that phasic dopamine is released primarily to the US early in training and to the CS after extensive training [14, 18]. Therefore, phasic dopamine release is associated with motor output, motivation, the value of reward-related stimuli, and learning. Further experiments with multiple reward magnitudes will be required to determine if phasic dopamine release can also function as a prediction-error signal [69]. In addition to its role in appetitive and goal-directed behaviors, many lines of evidence support a critical role of the dopamine system in addiction-related behaviors [7]. Furthermore, a prevalent theory posits that addiction results in part from pathological changes in brain systems important for reinforcement [2]. Below, we will discuss the effect of addictive drugs on the dopamine system, highlight how drugs alter dopamine neurotransmission, and suggest how these changes, especially to phasic dopamine release, could modulate behavior in regards to drug abuse.

THE EFFECT OF ABUSED SUBSTANCES ON DOPAMINE NEURONS: ELECTROPHYSIOLOGY AND MICRODIALYSIS STUDIES

Acute Effects of Abused Drugs on Midbrain Dopamine Neurons in Drug-Naïve Rodents

In order to understand the role of dopamine in drug abuse, it is important to first discuss how drugs affect the dopamine system acutely, after multiple drug exposures, and after withdrawal from multiple drug exposures. Studies employing microdialysis techniques demonstrate that non-contingent administration of abused drugs such as alcohol, nicotine, opiates, psychostimulants, and cannabinoids increase dopamine levels in the NAcc [110, 111], while drugs with low potential for abuse do not affect dopamine overflow [110]. The cellular mechanism by which addictive drugs increase dopamine levels depends upon the drug studied. Psychostimulants such as amphetamine and cocaine enhance dopamine overflow by affecting dopamine clearance from the extracellular space [112, 113]. Opiates and cannibinoids activate dopamine neurons through inhibition of local GABA input [114-117]. Similar to opiates, ethanol reduces the firing of VTA GABA neurons [118], but also directly modulates the excitability of dopamine neurons [119-121]. Additionally, ethanol affects the excitatory and inhibitory synaptic inputs onto VTA dopamine neurons [122, 123]. Nicotine activates and desensitizes dopamine neurons and inhibitory inputs to dopamine neurons in the VTA [124, 125], but prolonged nicotinic receptor activation is thought to cause a net excitatory effect on the dopamine system that may involve changes in presynaptic glutamate release [124, 126]. Regardless of the cellular mechanism, *in vivo* and *in vitro* recordings of dopamine neurons demonstrate that non-contingent peripheral administration of alcohol [119], nicotine [35, 125], opiates [114, 115], and cannabinoids [127, 128] increase dopamine neuron firing. Furthermore, nicotine [35], opiates [114], and cannabinoids [127] all increase the burst firing of dopamine neurons. Conversely, dopamine neuron firing is attenuated after administration of cocaine [129, 130] and amphetamine [131] in anesthetized animals and brain slices, due to the autoinhibitory effects of

dopamine at high concentrations after psychostimulant exposure [129].

Before summarizing the changes to the dopamine system brought about by multiple drug exposures, we would like to highlight that a majority of the studies examined the effect of psychostimulants. Furthermore, it is difficult to parsimoniously reconcile all of the findings in the literature. While an acute exposure to abused drugs elicits clearly identified effects on dopamine neuron firing and dopamine levels, the effect of multiple drug exposures on the dopamine system is far more complicated in part because of differences arising from the drug studied, how the drug is administered (dose, frequency, and route), and the duration after drug experience. Few consistent trends are found in the literature regarding the effect of multiple drug exposures on the dopamine system, but as we will discuss, there are often difficulties with the interpretation of the data due to methodological limitations.

Acute Effects of Abused Drugs on the Dopamine System in Drug-Experienced Rodents

To our knowledge, only a couple of studies have examined dopamine neuron firing patterns during drug self-administration. In rats that self-administered heroin, dopamine neurons increased their firing only after the first heroin infusion in a session [132, 133]. All subsequent heroin infusions were characterized by an increase in dopamine neuron firing before the infusion, which was immediately followed by a decrease in dopamine neuron firing rate after the infusion [132, 133]. While the electrophysiological data is lacking, more is known regarding how dopamine levels are affected by a drug treatment in drug-experienced rodents. Cocaine administered chronically *via* the experimenter [134-136] or through rodent self-administration [135, 137, 138] augments dopamine levels in response to a subsequent cocaine administration. Furthermore, microdialysis studies demonstrate that rodents titrate their drug intake to maintain a stable elevation of dopamine levels whether the drug administered is cocaine [137, 139], amphetamine [140], or heroin [141].

The Effect of Withdrawal After Chronic Drug Treatment on Dopamine Neuron Firing

The effect of withdrawal after chronic drug treatment has been extensively studied in addiction research, though unfortunately a number of administration paradigms have been used so that it is often difficult to compare the findings between studies. For the simplicity and clarity of this discussion, 'chronic' drug treatment refers to any treatment that involved more than one prior drug exposure and 'withdrawal' refers to the period of time after the drug treatment, which does not necessarily signify the presence of overt aversive symptoms or a physiological syndrome. Dopamine neuron firing rate is reduced by chronic treatment of nicotine after 1-day withdrawal [142], ethanol after acute (up to 24 hrs) withdrawal [143, 144], and morphine up to a week of withdrawal [145, 146], but see [147]. Additionally, acute withdrawal from chronic ethanol treatment reduces the number of spontaneously active dopamine neurons [148], as well as the burst firing of dopamine neurons [144], while chronic morphine treatment reduces dopamine burst firing for up a week of withdrawal treatment [145, 146], but see [147]. Interestingly, a 1-day withdrawal after chronic cannabinoid treatment reduced burst firing in SN dopamine neurons, but was without effect on dopamine burst firing in the VTA, or on basal dopamine neuron firing rate in either the SN or VTA [149]. It should be noted that some of the changes in dopamine neuron firing patterns discussed above return to baseline levels within two weeks after cessation of the drug treatment [142, 145].

In contrast to the effects with ethanol, nicotine, morphine, and cannabinoids, chronic treatment with psychostimulants can increase the activity of dopamine neurons during early withdrawal. Specifically, withdrawal (1 – 3 days) after multiple injections of amphetamine reduced the autoinhibitory effects of dopamine receptor activation (subsensitivity) [150-153], which can even lead to an increase in dopamine neuron firing rate with a subsequent amphetamine challenge [151], even though amphetamine acutely reduces dopamine neuron firing in naïve subjects [131]. However, the effects on the dopamine system depend upon the dose of amphetamine administered [150]. Similar to amphetamine, 1-day withdrawal after chronic non-contingent cocaine administration reduced the autoinhibitory effects of dopamine receptor activation, and increased the number of spontaneously active dopamine neurons, as well as the firing rate and burst firing of dopamine neurons [154]. These changes in dopamine neuron properties were found to last for up to 3 days of withdrawal in rats that had self-administered cocaine [155]. Interestingly, the number of spontaneously active dopamine neurons in the VTA was reduced compared to control-treated rats following 2 weeks of withdrawal after chronic non-contingent cocaine treatment [156]. Another study found that 1-week withdrawal from chronic non-contingent intraperitoneal cocaine injections did not affect SN dopamine neurons, but increased the number of spontaneously active dopamine neurons in the VTA [157]. However, 1-week withdrawal after continuous cocaine infusions reduced the number of active SN dopamine neurons and reduced the bursting of VTA dopamine neurons [157]. Therefore, the changes on the dopamine system elicited by drugs are highly dependent upon the route of administration [157].

To summarize, relatively short withdrawal periods (less than 2 weeks) after chronic drug treatment can elicit a variety of effects on dopamine neuron activity that depends upon the drug studied, the dose administered, the route of administration, and the location of the recorded dopamine neurons. However, a few trends have emerged from these studies. First, a transient decrease in dopamine neuron activity is observed within 1 week of withdrawal after chronic treatment of ethanol, nicotine, opiates, and cannabinoids. Second, chronic psychostimulant treatment increases dopamine neuron activity for at least up to 3 days of withdrawal, which may involve subsensitivity of dopamine autoreceptor function. Third, chronic psychostimulant treatment can decrease dopamine neuron activity after longer periods of withdrawal.

A critical aspect of drug addiction is the high prevalence of relapse and the difficulty of abstaining from drug use over extended periods of time [158, 159]. It has been suggested that the intense craving for drugs contributes to relapse [160]. Interestingly, drug-craving is progressively enhanced

after longer periods of withdrawal in rodent models of addiction [161]. A recent report found that longer periods of withdrawal (3 – 6 weeks) after chronic non-contingent treatment of nicotine, amphetamine, cocaine or ethanol all reduced the number of spontaneously active dopamine neurons, which perhaps is important in the development of drug craving [162]. Regardless, more studies are needed in order to adequately examine this hypothesis.

The Effect of Withdrawal After Chronic Drug Treatment on Tonic Dopamine Levels

A large number of studies have examined how withdrawal after drug treatment affects the mesocorticolimbic system. As discussed in the previous section, some trends are evident in the effect of withdrawal on dopamine neuron activity; however, few trends are apparent when examining the literature that has studied the effect of withdrawal on dopamine overflow. Using conventional microdialysis, no net flux microdialysis, or examining tritiated dopamine levels, many have reported that withdrawal from chronic drug treatment decreases basal concentration of dopamine [144, 163-169], while others report no change in basal dopamine levels [134, 135, 143, 163, 170-176], and yet others found an increase in basal dopamine levels [164, 173, 177, 178]. While some studies reported that basal dopamine levels changed depending upon the duration of withdrawal [164, 173], these observed changes were not consistent across studies. For example, some report that withdrawal after chronic cocaine exposure decreases basal dopamine levels in as early as a few hours [169] to as long as 10 days [165], while others found increases in basal dopamine levels during 1 – 4 days of withdrawal [164, 173, 178], and it was reported to have no effect on basal dopamine levels between 24 hours and 2 weeks of withdrawal [134, 135]. Thus, at least for cocaine, no clear temporal effect of withdrawal on basal dopamine levels can be inferred from these studies.

While basal dopamine levels after withdrawal from chronic drug treatment have been assessed with both conventional and no net flux microdialysis, the no net flux method can be more accurate at determining exact dopamine levels immediately surrounding the microdialysis probe [56]; however, it often is not used in behaving rodents because the temporal resolution is relatively poor compared to conventional microdialysis strategies. Some studies employing no net flux microdialysis did not observe any changes in dopamine levels after withdrawal from drug treatment [134, 170, 172, 173], while another found an increase [165], though it was suggested that differences in the buffer composition and flow rate could account for this discrepancy [134]. Interestingly, in a study using no net flux microdialysis, the lack of an effect of withdrawal from cocaine treatment on dopamine levels was accompanied by lower dopamine uptake, which was hypothesized to denote a reduction in basal dopamine levels [170]. Furthermore, some studies have utilized both conventional and no net flux microdialysis and came to different conclusions depending upon the technique used. Withdrawal from drug treatment increased basal dopamine levels when studied with conventional microdialysis, but was without effect when using no net flux microdialysis [134, 173]. Because of the divergent results between conventional and no net flux microdialysis techniques, it is difficult to state with resolve whether withdrawal from chronic drug exposure has a conclusive effect on basal dopamine levels.

In addition to basal dopamine levels, many studies have examined how withdrawal after chronic drug treatment affects drug-stimulated dopamine overflow. Some reported a decrease in drug-stimulated dopamine levels that was evident between 1 – 9 days of withdrawal [134, 135, 168, 173, 176, 179-181], although these changes may be transient [173, 179]. Others have found that early withdrawal (3 - 7 days) after amphetamine treatment does not affect drug-stimulated dopamine overflow [153, 182, 183]. While many report increases in drug-induced dopamine levels after withdrawal [134, 153, 163, 173, 174, 177-179, 182-184], though the timing of these effects varies considerably. One consistent trend is that chronic amphetamine treatment does not affect drug-stimulated dopamine levels early in withdrawal (1 – 4 days), but augments amphetamine-stimulated dopamine levels reliably after 2 – 4 weeks of withdrawal [153, 182-184]. However, there is less agreement between studies in regards to chronic cocaine treatment. Specifically, some reports suggest that chronic cocaine treatment has little effect on drug-induced dopamine levels after 1 day of withdrawal, but robustly increases drug-stimulated dopamine overflow after 3 weeks of withdrawal [174, 185], while others report the opposite [178]. Additionally, some have found that chronic cocaine treatment augments drug-stimulated dopamine levels immediately and for up to 3 weeks [134, 179].

While we have discussed the effects of withdrawal on basal and drug-stimulated dopamine levels separately, it is important to note that these two measures are intertwined. Chronic cocaine treatment was found to increase both basal and cocaine-stimulated dopamine levels after 1-day withdrawal compared to saline-injected controls, but the percentage increase in dopamine levels was significantly less in the cocaine-treated rats [178]. Conversely, a study using a binge cocaine administration protocol reported a decrease in basal dopamine levels, and a lower concentration of dopamine in response to an acute cocaine injection [169]. However, when the effect of a challenge cocaine exposure was presented as a percentage of basal dopamine levels, the cocaine-stimulated dopamine levels were significantly enhanced [169]. Although these two studies found different effects of previous cocaine exposure on basal and drug-stimulated dopamine levels presumably due to variations in drug administration protocols [169, 178], they both highlight that it is exceedingly difficult to interpret the findings from many microdialysis studies that present findings only as percent changes because it is unknown how the respective drug treatments may affect basal dopamine levels.

While some of the discrepancies between studies could be explained by the method of presenting the data, it is important to note that the effects on dopamine levels after withdrawal from chronic drug treatment depends upon the drug studied, as clear differences exist even between psychostimulants [134, 153, 173, 174, 178, 179, 182-184]. Additionally, the dose of the drug administered during the chronic treatment can differentially alter dopamine neuron firing [150] and levels [134]. Another potential source of variability in the reported findings could arise from different

drug-dependent effects in distinct striatal subregions [163, 169, 171, 177, 179], which was not explicitly examined in many studies.

Yet another explanation for the lack of cohesion amongst many of these studies can be due to the fact that detecting dopamine levels depends upon three major components: (i) the basal level of dopamine, which was discussed above, (ii) the quantity of dopamine released, and (iii) the uptake of dopamine. Although this section has focused on changes in dopamine levels determined by microdialysis, this technique is not capable of examining changes in the quantal size of dopamine levels. Therefore, we will briefly mention that in a study using FSCV, it was inferred that prior cocaine experience and 2 weeks of withdrawal led to an increased size of the releasable pool of dopamine [186]. A number of studies have observed changes in dopamine transporter number and function in the NAcc during withdrawal after chronic drug treatment. Specifically, the number of dopamine transporters increase while cocaine is present [187] and return to baseline levels for up to a week of withdrawal [163, 188]. However, dopamine transporter numbers are lower after 10 – 21 days of withdrawal [187, 189-191]. Some studies suggest that there is a functional impairment in dopamine uptake during 1- 7 days of withdrawal [163, 192, 193], although the time course of this effect does not match with the fluctuations in dopamine transporter numbers.

To summarize, many studies have examined the effect of withdrawal after chronic drug treatment on dopamine levels; however, few clear trends are evident. Many of the differences between seemingly similar studies may result from different treatment schedules, locations of the microdialysis probe, as well as the specific microdialysis technique employed. Additionally, caution must be exercised when comparing between treatment groups when microdialysis data is presented only as a percentage change from baseline dopamine levels, as the percentage change in dopamine overflow is directly related to basal dopamine levels [169, 178]. Furthermore, dopamine release is also regulated by quantal size and the function and number of dopamine transporters. Therefore, many of the discrepancies between the studies discussed above may reflect complex interactions between the specific drug-treatment, where dopamine is released, and the dynamics of dopamine clearance.

THE EFFECT OF ABUSED SUBSTANCES ON THE SYNAPTIC INPUTS TO DOPAMINE NEURONS

As discussed above, dopamine burst firing requires glutamatergic input, NMDA receptor activation, opening of high-threshold calcium currents, and finally activation of calcium-activated potassium currents to terminate the burst [38]. Thus, it follows that changes in synaptic inputs, calcium currents, or calcium-activated potassium currents could alter burst patterns of firing in dopamine neurons. Although incomplete, a growing body of evidence suggests that abused drugs can modify synaptic inputs onto dopamine neurons, as well as the currents found in dopamine neurons important for burst generation. For example, many studies have now shown that a single non-contingent injection of cocaine increases the ratio of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor currents to NMDA receptor currents (AMPA/NMDA) on VTA dopamine neurons [8, 194-199]. This effect is observed 24 hours after the cocaine injection, persists for up to 5 days, and is observed in rodents that received a single [198] or chronic non-contingent cocaine treatment [195]. Furthermore, The AMPA/NMDA ratio is thought to be a reliable measure of excitatory synaptic strength, where increases have been associated with enhanced AMPA receptor function [194, 198, 200]. In agreement with enhanced excitatory synaptic function on dopamine neurons, a single cocaine injection also increased the frequency and amplitude of miniature excitatory post-synaptic currents [198]. Furthermore, increases in the AMPA/NMDA ratio are associated with an impaired ability to generate long-term potentiation (LTP), a cellular mechanism that strengthens excitatory synapses and is thought to be important in learning and memory [6, 198]. Thus, an enhanced AMPA/NMDA ratio is likely occluding the ability to elicit LTP because the excitatory synapse cannot be further strengthened [6, 198]. However, for the purpose of this discussion, we highlight that such augmentations in the AMPA/NMDA ratio will promote the efficacy of excitatory inputs to dopamine neurons.

While much of the work examining synaptic alterations on dopamine neurons has focused on the effects of cocaine, similar increases in the AMPA/NMDA ratio on VTA dopamine neurons have been observed 24 hrs after a single injection of amphetamine, nicotine, or morphine [197]. Subsequent work demonstrated that these synaptic changes are rapid, as an enhanced AMPA/NMDA ratio is observed 2-3 hrs after injection of cocaine and amphetamine [201, 202]. Interestingly, *in vitro* exposure to cocaine transiently increases NMDA receptor currents [203] and increases the AMPA/NMDA ratio after 3-5 hrs [201], although this may not extend to all psychostimulants [202].

Many studies examining drug-mediated changes on VTA dopamine neuron synaptic plasticity utilized non-contingent drug-administration, though recent studies have now examined these synaptic changes under conditions where rodents self-administer the drug. For example, an increase in VTA AMPA receptor levels is observed in rats that have self-administered nicotine [204]. Interestingly, food, sucrose, and cocaine self-administration increase the AMPA/NMDA ratio immediately after training, but rats that self-administered cocaine (and not yoked controls) exhibited a persistent increase in the AMPA/NMDA ratio [205]. This study highlights how the changes on VTA dopamine neurons will depend upon the method of drug administration, as chronic peripheral injection of cocaine transiently (< 10 days) affects the AMPA/NMDA ratio [195], though these changes are longer lasting (> 21 days) in self-administering rodents [205]. Originally, it was hypothesized that these changes in glutamate receptor function are responsible for behavioral sensitization, which is an enhanced motor response to a subsequent drug exposure [198]. Instead, further studies found that the AMPA/NMDA ratio is not associated with the development of behavioral sensitization [195, 199], but rather may be important for initiating long-lasting changes, which promote addiction-like behaviors [205, 206].

Recently, addictive drugs have been shown to affect inhibitory synaptic inputs on VTA dopamine neurons. Multiple injections of cocaine [196] and a single injection of morphine [207] reduce the inhibitory input on VTA dopamine neurons. These effects are not unitary across drugs, as a single injection of ethanol was found to increase inhibitory input on VTA dopamine neurons [208, 209]. However, withdrawal after chronic ethanol exposure promotes burst firing of dopamine neurons by inhibiting the function of calcium-activated potassium currents [210]. These studies suggest that exposure to addictive substances can strengthen excitatory synaptic input, reduce inhibitory synaptic input, and alter the function of ion currents in VTA dopamine neurons. While these drug-induced adaptations have not been characterized or identified for all addictive substances, we suggest that these changes will increase efficacy of excitatory inputs on VTA dopamine neurons, which could increase the coordinated activity of VTA dopamine neurons and/or the burst firing of VTA dopamine neurons [211], since this firing pattern is dependent upon glutamatergic input, NMDA receptor activation, and calcium-activated potassium currents [38]. We therefore would expect that behaviorally relevant and drug-associated stimuli would enhance phasic dopamine release to a greater level after experience with drugs. Empirical data of dopamine neuron firing patterns in awake, behaving rodents after drug exposure is lacking, though recent studies have begun to examine these questions by examining phasic dopamine release with FSCV in rodents during drug-related behaviors.

THE EFFECT OF ABUSED SUBSTANCES ON PHASIC DOPAMINE RELEASE

FSCV has been utilized to examine phasic dopamine release in a variety of model systems, though it is important to note that drug-mediated alterations in dopamine release can result from direct effects on dopamine neuron excitability or from changes in dopamine uptake. Numerous studies have examined the effect of ethanol on dopamine release in striatal brain slices from drug-naïve rats. Specifically, moderate doses of ethanol are without effect on dopamine uptake [212-214]. In contrast, chronic ethanol vapor exposed rats exhibited enhanced dopamine uptake *in vitro*, which was thought to be a compensatory mechanism resulting from the elevated dopamine levels due to the prolonged ethanol treatment [215]. In awake and behaving rodents, acute peripheral injections of ethanol at doses that increase tonic dopamine neuron firing were found to attenuate electrically stimulated dopamine release [216]. This ethanol-mediated reduction of phasic dopamine release was thought to result from enhanced tonic dopamine levels that impaired phasic dopamine release due to the depletion of releasable dopamine and activation of release-regulating autoreceptors [216]. However, intravenous ethanol infusions sometimes increased the frequency of spontaneous phasic dopamine transients in awake, behaving rats [217]. Similar effects have also been observed with cannabinoid receptor activation, where intravenous infusions of cannabinoids reduced electrically stimulated dopamine release, but increased the frequency and amplitude of spontaneous phasic dopamine release events [218]. These findings with ethanol and cannabinoid administration highlight that it can be difficult to parsimoniously use the findings from *in vitro* preparations and artificial electrical stimulations to predict the net effect in awake, behaving rodents. Reduced preparations and electrical stimulations are better suited to examine specific aspects of dopamine transmission, such as the involvement of specific ion channels, changes in release kinetics and the quantity of dopamine release, which are more difficult to accurately ascertain using *in vivo* preparations. Regardless, these findings highlight that ethanol and cannabinoids produce changes in phasic dopamine release.

A number of studies have examined the effect of nicotine on phasic dopamine release in both *in vitro* and *in vivo* preparations. In contrast to ethanol, acute *in vivo* nicotine exposure enhances dopamine uptake in the striatum [219]. Nicotine exerts frequency-dependent effects on phasically stimulated dopamine release *in vitro*, where at low firing rates dopamine release is attenuated, but at high firing bursts nicotine enhances dopamine release [220, 221]. Intravenous infusions of nicotine were also found to increase the frequency and amplitude of spontaneous phasic dopamine release events [217]. In agreement with the findings from other abused substances, intravenous infusions of cocaine also increase spontaneous phasic dopamine release events in the NAcc [62, 217, 222-224]. Interestingly, endogenous cannabinoids modulate the cocaine-, nicotine-, and ethanol-mediated increases in phasic dopamine release, as the effects of drugs on phasic dopamine release are attenuated by systemic cannabinoid receptor antagonism [217]. While the locus of this effect is yet to be determined, it is speculated that it lies within the VTA, where cannabinoid receptor activation reduces GABA release onto VTA dopamine neurons [225]. Together, these findings suggest that abused drugs may exert similar effects on phasic dopamine release even though their respective cellular targets are quite distinct. Future studies are required to systematically examine drug-specific effects on phasic dopamine release. Below we discuss the effects of cocaine on phasic dopamine release, as this has been the most thoroughly studied addictive substance.

Using microdialysis, it was found that an acute administration of abused drugs increase dopamine levels to a greater extent in a limbic structure, such as the NAcc, relative to a motor brain structure, such as the dorsal caudate nucleus [110], although this may not be valid for amphetamine [226]. Furthermore, a meta-analysis of microdialysis studies indicate that cocaine augments dopamine overflow preferentially within the NAcc shell relative to the NAcc core [227]. Consistent with microdialysis findings, a recent report using FSCV identified larger cocaine-mediated effects on phasic dopamine release in the NAcc shell compared to the NAcc core [222]. It was suggested that the preferential effect on dopamine release in the NAcc shell by cocaine could be critically important for the primary reinforcing effects of drug [222]. Similar to differential effects of contingent and non-contingent drug administration on synaptic plasticity on VTA dopamine neurons [205], the effect of cocaine infusions on phasic dopamine release can depend upon the contingency of the administration [223]. No changes in dopamine levels are observed within 10 s of a non-contingent cocaine administration to awake, drug-naïve rats. However, phasic

dopamine events are increased during this time frame with contingent cocaine administration, highlighting that these early dopamine release events (< 10 s after drug delivery) may be important for learned associations, and are not a result of the pharmacological actions of cocaine [223]. Identical to natural reinforcers [15, 16], cues that predict cocaine availability are able to elicit phasic dopamine release that persist even when the drug is not administered [11, 224]. Interestingly, the rise in dopamine levels is associated with the initiation of approach to lever press for cocaine [11, 223, 224], which is thought to be causal since stimulation of dopamine neurons was found to promote this behavior [11]. To summarize, cues associated with drug reinforcers elicits phasic dopamine release [11, 223, 224], similar to what was observed with natural reinforcers [14, 16-18]. In contrast to natural reinforcers, acute administration of addictive substances promotes spontaneous phasic dopamine release events [217], which could be involved with establishing and strengthening associations between environmental cues and the drug.

The field of FSCV recordings in behaving rodents during drug-related behaviors is nascent, and many questions regarding the prolonged effects of drugs on phasic dopamine release remain unanswered. One limitation present in many behavioral studies using FSCV arises from the usage of acute glass-insulated microelectrodes, which need to be physically inserted on each recording day. Due to this approach with acute recordings, FSCV recordings are likely in different locations across days, and successfully inserting electrodes becomes more difficult after multiple recording sessions [61]. We have developed chronically implanted microelectrodes that permit stable FSCV recordings over multiple days and are well suited to address changes in phasic dopamine release over long-lasting behavioral paradigms [228]. Future studies employing chronically implanted FSCV electrodes will be able to test for changes in the pattern of phasic dopamine release during the transition to compulsive drug taking in rodent models of addiction.

CHANGES IN DOPAMINE NEUROTRANSMISSION IN HUMAN ADDICTS

The development of human imaging techniques, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have provided many insights into functional changes within the brain that occur in human addicts [229]. Specifically, fMRI identifies changes in blood oxygen levels, thought to represent changes in neural activity, with a time resolution of a few seconds. In contrast, PET can be used to make specific measurements of neurotransmission by monitoring radiotracers that selectively bind to proteins. For example, radiolabeled dopamine-receptor ligands can be monitored in the brain, and their concentration decreases as they are displaced from receptors following release of endogenous dopamine. Alterations in radioligand binding is often indicative of changes in receptor levels, though it should be noted that changes in the receptor affinity for a radioligand could confound these results. The temporal resolution for PET is in minutes and only increases, not decreases, in dopamine concentration can be detected [10]. As we will describe below, human addicts and rodents exhibit similar alterations in dopamine neurotransmission, though it is important to remember that the temporal resolution of the identified changes are much lower in human studies (seconds to minutes) compared to those using FSCV in rodent models of addiction (subsecond).

While it is well established that the delivery of natural positive reinforcers increases dopamine neuron firing [94, 95] and subsequent release in the striatum [14, 16-18, 106], this was only recently demonstrated in humans using fMRI and PET [230, 231]. Furthermore, exposure to psychostimulants increased dopamine levels in the human striatum that was associated with the reinforcing effects of the drug [232-234], which mirrors the findings from microdialysis and FSCV studies performed in rodents [110, 217].

In addition, the changes to the dopamine system after withdrawal from chronic drug experience are quite similar between rodents and humans. Specifically, stimulated dopamine release was reduced in detoxified cocaine abusers [235], consistent with the attenuated drug-stimulated dopamine overflow in rodents after chronic cocaine exposure found in some studies [135, 178, 180]; however, as discussed above, there is little consensus between many of the studies in the rodent literature. The level of radiolabeled dopamine-receptor ligands under basal conditions has also proved useful in assessing difference in neural function between individuals. Human abusers of alcohol [236], cocaine [237], heroin [238], and methamphetamine [239] have lower levels of dopamine receptor binding compared to non-abusers. In contrast, individuals that are resistant to drug use (close relatives of addicts that do not abuse drugs) exhibit increased dopamine receptor binding, suggesting that higher levels of dopamine receptors in the striatum could protect against the development of addiction [240]. In agreement with human studies, the amount of drug consumed is inversely related to the amount of dopamine receptor binding in the striatum in both rodent [241] and non-human primate addiction studies [242]. These findings have led to the hypothesis that low numbers of dopamine receptors, especially within the striatum, whether due to genetics or previous drug exposure, can make an individual more susceptible to drug abuse [9, 10, 229, 242, 243]. Furthermore, the attenuated activity of dopamine neurons [156, 162] and reduced basal dopamine levels [164-167] found in rodents in withdrawal from chronic drug treatment is consistent with the hypofunctional dopamine system that is observed in human addicts [10].

Recent human imaging studies have provided insights into the neurochemical events responsible for drug craving, which is thought to be a key contributor to relapse. Specifically, exposure to drug-related cues is thought to promote drug craving in human addicts [244-247], as well as in rodents [7, 161]. Presentation of conditioned cues associated with alcohol [245, 246], nicotine [248], amphetamine [249], and cocaine [247] were all found to increase dopamine transmission in the striatum of humans, consistent with rodent studies using FSCV [11, 223, 224]. In summary, human imaging studies have provided evidence that drugs and drug-associated cues increase dopamine levels and that chronic drug exposure is associated with an impaired function of the dopamine system, which together recapitulates many of the conclusions from the rodent addiction studies discussed above.

THE 'SIGNAL TO NOISE' MODEL OF PHASIC DOPAMINE SIGNALING IN DRUG ADDICTION

Briefly, we will summarize what we have discussed in this review and offer our model regarding the role of phasic dopamine release in the development of drug abuse, which we have also schematically presented in Fig. (**1**). In the absence of behaviorally relevant stimuli in a drug-naïve condition, dopamine neurons will fire action potentials in either single-spike or burst patterns [29, 31, 32], which will contribute to the basal dopamine levels in brain regions receiving dense dopamine input, such as the NAcc. Many lines of evidence suggest that behaviorally relevant and salient stimuli can increase the firing rate of dopamine neurons [31, 33, 94, 95], which may or may not include an increase in burst firing. For clarity, we do not discriminate between transient increases in single-spike firing rate and burst firing, and represent both as a cluster of action potentials in Fig. (**1**). Furthermore, exposure to salient stimuli also enhances phasic dopamine release [12, 14, 17, 18], which is likely due to synchronous firing of dopamine neurons [33, 34] in single-spikes, bursts, or a combination of both (Fig. **1**). However, in a drug-naïve state, the presentation of stimuli associated with drug intake, such as drug paraphernalia or cues predicting drug availability, will not affect dopamine neuron firing or release [11].

In contrast to the drug-naïve state, a number of changes occur within the dopamine system whereby an arbitrary stimulus (drug paraphernalia or cues predicting drug availability) becomes behaviorally-relevant and elicits phasic dopamine release after the association is learned between a cue predicting drug availability and the drug itself [11]. It is important to note that this phenomenon is also observed with learned cue-reinforcer associations with natural and non-drug reinforcers [14-16]; however, cues predicting drug availability appear to be more resistant to extinction than cues predicting non-drug reinforcer availability [15, 224], which suggests that additional changes occur in the dopamine system due to the drug experience. In support, learning an association between cues and natural reinforcers transiently affects the synaptic properties of dopamine neurons [17, 205], while drug experience promotes long-lasting changes (days to weeks) to the intrinsic and synaptic properties of dopamine neurons [198, 205, 210]. Furthermore, dopamine release is thought to modulate synaptic inputs to striatal neurons by inhibiting weak glutamatergic signals and strengthening strong glutamatergic inputs [250, 251]. Therefore, we posit that these prolonged cellular adaptations in dopamine neurons after withdrawal from chronic drug experience will function to (i) increase the efficacy of excitatory glutamatergic inputs onto dopamine neurons that can increase dopamine neuron firing and subsequent release in response to previously weak or neutral stimuli, which will (ii) strengthen previously learned associations, and (iii) develop and strengthen new associations between environmental cues and drugs. Together, these neural changes will enhance the phasic dopamine 'signal' elicited by drug-related cues after withdrawal from chronic drug experience (Fig. **1**).

Furthermore, changes to the 'noise', or rather basal dopamine levels, may in turn amplify the effect of withdrawal on the enhanced dopamine 'signals', leading to a greater 'signal to noise' of phasic dopamine release. Before discussing the experimental support for this analogy, we want to clarify that references to dopamine 'noise' are not meant to imply that basal dopamine levels are without behavioral importance, but rather are only used to conceptualize the contrast of phasic dopamine release events to background dopamine levels. Recently, it was reported that dopamine neuron population activity is attenuated 3 - 6 weeks after the cessation of chronic drug treatment in rodents (represented by Neuron 3 in Fig. **1**), which may suggest a reduction in the dopamine system 'noise' [162]. Although there is debate regarding the role of chronic drug treatment on dopamine levels throughout the striatum in many rodent studies (see above, and dashed lines in Fig. **1**), both human and rodent studies have observed a reduction in stimulated dopamine release [135, 178, 180, 235] and dopamine receptor levels [236-239, 241] after withdrawal from chronic drug treatment, which is consistent with an attenuation of dopamine 'noise'. Together, these findings provide evidence that withdrawal from drugs enhances the contrast, or 'signal to noise' of phasic dopamine release to basal dopamine levels.

We predict that this augmented 'signal to noise' of phasic dopamine release will have important behavioral consequences, especially in regards to drug abuse. Because phasic dopamine release is associated with initiating goal-directed behaviors [11, 16, 223], it follows that the enhanced 'signal to noise' of phasic dopamine signaling will promote drug seeking in response to drug-related stimuli, which is important in the development of addiction. In agreement, human imaging studies find that the presentation of drug-related cues promotes drug craving [244-247] and increases dopamine transmission [245-249].

A corollary of our hypothesis is that chronic drug exposure will also affect the processing of cue-related associations that do not involve drugs. As discussed above, drug experience promotes long-lasting synaptic changes on dopamine neurons, which we have posited will increase the efficacy of excitatory glutamatergic inputs that can increase dopamine neuron firing and subsequent release to previously weak stimuli. However, the studies that determined the presence of drug-dependent synaptic changes on dopamine neurons were performed in brain slices [198, 205]; therefore, it is difficult to ascertain whether the synaptic changes are global (as schematically presented in Fig. **1**), or if the alterations occur on just a subset of dopamine neurons that are preferentially activated by drug-related stimuli. In support of global changes in cue-related associations after drug exposure, amphetamine pre-treatment promotes habit formation after reinforcer devaluation [252], and enhances both inhibitory and excitatory Pavlovian associations [185, 253]. Furthermore, human addicts and healthy subjects with reduced dopaminergic function exhibit impaired decision-making abilities [254-256], highlighting that dysregulation of the dopamine system can alter cognition and behavior. Therefore, addiction can be debilitating for individuals because chronic drug experience not only promotes drug seeking, but also affects proper decision-making.

Because drug exposure produces gross synaptic changes on dopamine neurons [198, 205], we suggest that this would elicit a global enhancement in establishing cue-reinforcer

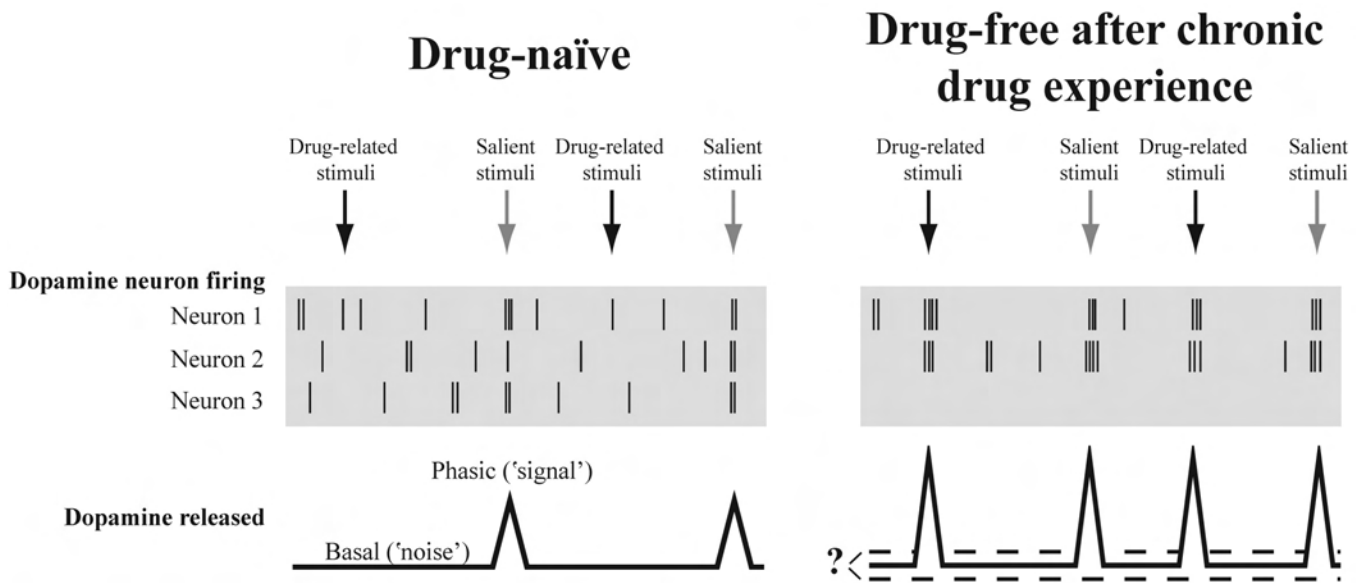

Fig. (1). Schematic representation of changes to the dopamine system after chronic drug experience. (Left) Illustration of the firing patterns of three hypothetical dopamine neurons from a drug-naïve individual where each vertical line represents an action potential. For clarity, there is no distinction between transient increases in firing rate and burst firing, as both are represented by a cluster of action potentials. The specific firing patterns responsible for phasic dopamine release are unknown, but phasic release likely results from coordinated activity of dopamine neurons firing in single-spikes and/or bursts. Notice that behaviorally relevant salient stimuli elicits coordinated activity of dopamine neurons that translates into a phasic increase in dopamine release that could occur in brain regions receiving VTA input, such as the NAcc. However, presentation of drug-related stimuli are without effect on dopamine neuron firing and release in the drug-naïve condition. (Right) After withdrawal from chronic drug treatment, dopamine neuron firing rate and population activity can be reduced, which is represented by fewer spontaneous action potentials and the lack of activity in Neuron 3. While the effect on basal dopamine levels remains controversial, many studies demonstrate instrinsic and synaptic changes on dopamine neurons that could promote the efficacy of glutamatergic inputs on to dopamine neurons. We hypothesize that these intrinsic and synaptic changes will increase dopamine neuron firing and phasic release in response to drug-related stimuli. We propose that this increased 'signal to noise' of phasic dopamine release to basal dopamine levels contributes to the aberrent processing of drug-related stimuli, which in turn can promote drug seeking.

associations (Fig. **1**). It could be argued that a non-discriminate enhancement of cue-reinforcer associations would increase the *magnitude* of all cue-elicited behaviors to similar levels, independent of whether the cue was drug-related [161] or not [185, 252, 253]. Although it is plausible, there is no evidence to our knowledge of drugs altering the synaptic inputs of dopamine neurons that are preferentially activated by drug-related cues. A global increase in the *magnitude* of all cue-elicted behaviors initially seems to conflict with the theory that drug-related cues gain increased salience over non-drug cues [66]. However, addictive substances elicit spontaneous phasic dopamine release events [217], which we suggest could promote the *quantity* of associations between environmental cues and the drug. While the *magnitude* of all cue-reinforcer associations would be elevated during withdrawal from chronic drug treatment, there would be a greater *quantity* of associations between drug-related cues and drugs compared to non-drug-related cue-reinforcer associations, which is consistent with an increased salience of drug-related over non-drug-related cues [66]. Furthermore, if phasic dopamine release acts as a teaching signal, the sustained pharmacological response to abused drugs should continue to update the weight of drug-related cues, whereas phasic dopamine released under normal circumstances will attenuate naturally and does not promote sustained learning [257].

We have sought to propose a model that provides evidence at the cellular and system's levels to explain how drug-related cues promote drug seeking through an increase in the 'signal to noise' of phasic dopamine release after withdrawal. Breaking the link between drug-related cues and drug seeking is an attractive target for therapeutic interventions to reduce the incidence of relapse in human addicts. However, there is also considerable interest in determining the neurochemical changes associated with the development or initiation of addiction-related behaviors. Acute drug administration increases both basal dopamine levels and phasic dopamine release [11, 110, 217, 222-224], which at first pass seems incompatible with our 'signal to noise' model since both the 'signal' and the 'noise' are elevated. However, voltammetric studies measure phasic changes in analyte levels relative to basal levels; hence, voltammetry techniques specifically measure an enhancement of a phasic 'signal' relative to basal 'noise'. Dopamine is phasically released to cues predicting reinforcer availability in a number of reinforcement learning paradigms [14, 15, 17, 223]; therefore, it is possible that the drug-dependent elevations of phasic dopamine release events could promote the association of cues and drug reinforcement [257]. However, a critical future experimental question will be to determine if phasic dopamine release is indeed necessary and sufficient for reinforcement learning.

Throughout this review, we have focused primarily on the effects of abused drugs on dopamine neuron firing, as well as dopamine levels and release. However, it is important to note that dopamine exerts a number of postsynaptic effects throughout the mesocorticolimbic system, which been described in detail elsewhere [23, 250, 251]. Furthermore, the time course of dopamine's effects can range from milliseconds to minutes [23, 70]. Dopamine release in the striatum is suggested to strengthen strong glutamatergic inputs and inhibit weak inputs [250, 251], which provides a plausible general postsynaptic effect of dopamine that is complementary to our 'signal to noise' model of phasic dopamine release after chronic drug treatment. While we have discussed basal dopamine levels and phasic dopamine release separately, it is important to note that basal levels and phasic release are intertwined. Increasing basal dopamine levels activates dopamine autoreceptors, which will open potassium channels that will hyperpolarize dopamine neurons [258], and inhibit phasic dopamine release [259]. Although we have presented a simple model of increased 'signal to noise' of phasic dopamine release, the postsynaptic effects of dopamine are likely complex and will depend upon the amount of dopamine released as well as the region studied.

CONCLUSIONS

Our model posits that after withdrawal from chronic drug treatment, the 'signal to noise' of phasic dopamine release is enhanced, which promotes aberrant stimulus-reinforcer associations that are important in the development of addiction. Our model fits within the framework of many contemporary theories of dopamine function. Specifically, the increase in the 'signal to noise' of phasic dopamine signaling could be interpreted as an enhanced motivation to pursue drugs [68], or may reflect a potentiation in the incentive-value of drug-related stimuli [66], or could provide a 'prediction-error' teaching signal that reinforces certain behaviors [69]. Our model is also consistent with theories regarding the role of dopamine in learning [67, 71]. Specifically, the increased 'signal to noise' of phasic dopamine release may be important for learning cue-reinforcer associations with an acute drug administration, and may also strengthen previously learned cue-reinforcer associations after withdrawal from chronic drug treatment. Regardless, our model proposes that an enhanced 'signal to noise' in phasic dopamine release after chronic drug experience will promote drug seeking and aberrant behaviors related to cue-stimuli associations.

Many theories have been developed to model aspects of drug addiction, both from a psychological and a neurochemical level. Recent psychologically-based reviews suggest that addiction results from changes in the decision making process [3], or that drug craving and substance use are related to an attentional bias toward drug-related cues [1, 4], or involves a decrease in brain reward function with an increase in antireward systems [2]. Neurochemically-based reviews have highlighted important roles of synaptic plasticity [8], learning and memory [6], or changes in neurotransmitter function [2, 9, 10] in the development of addiction-related behaviors. Furthermore, many have highlighted important roles of the dopamine system in the development of addiction [2, 5-10], although it is clear that other neurotransmitter systems and brain regions are involved [7]. Our model complements and extends upon many of established theories regarding the role of dopamine in addiction. Synaptic changes on dopamine neurons and an altered function of the dopamine system have been suggested to be critical in drug addiction [8-10], but we synthesize components of these theories with the extensive literature to make specific hypotheses regarding the function of phasic dopamine release in regards to addiction-related behaviors. Regardless, future studies will be needed to test the predictions in our 'signal to noise' model. The study of phasic dopamine release during behavior is nascent; however, recent improvements in FSCV recording strategies from our lab now permit voltammetric recordings over months [228], which will be an invaluable experimental technique to specifically examine the role of phasic dopamine release in the development of addiction-related behaviors.

Learning Objectives

- The basic anatomy of the VTA dopamine system and common methods to detect dopamine neurons and dopamine release.
- The evidence supporting contemporary theories of dopamine function.
- The role of phasic dopamine release in appetitive and goal-directed behaviors.
- The acute and prolonged effect of abused drugs on dopamine neuron properties, dopamine neuron firing and dopamine release.
- The effect of abused drugs on phasic dopamine release.

Future Research Questions:

- Do dopamine neuron firing patterns change in awake, behaving rodents after withdrawal from chronic drug exposure?
- Do the patterns and amplitude of phasic dopamine release change after withdrawal from chronic drug exposure?
- Is learning associations between cues and non-drug reinforcers affected in a phasic dopamine-dependent manner after chronic drug exposure?
- Is the association between phasic dopamine release and reinforcement learning a causal relationship?

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