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Viral strategies for targeting cortical circuits that control cocaine-taking and cocaine-seeking in rodents

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Abstract

Addiction to cocaine is a chronic disease characterized by persistent drug-taking and drug-seeking behaviors, and a high likelihood of relapse. The prefrontal cortex (PFC) has long been implicated in the development of cocaine addiction, and relapse. However, the PFC is a heterogeneous structure, and understanding the role of PFC subdivisions, cell types and afferent/efferent connections is critical for gaining a comprehensive picture of the contribution of the PFC in addiction-related behaviors. Here we provide an update on the role of the PFC in cocaine addiction from recent work that used viral-mediated optogenetic and chemogenetic tools to study the role of the PFC in drug-taking and drug-seeking behavior in rodents. Following overviews of rodent PFC neuroanatomy and of viral-mediated optogenetic and chemogenetic techniques, we review studies of manipulations within the PFC, followed by a review of work that utilized targeted manipulations to PFC inputs and outputs.

Introduction

Drug addiction is a chronic relapsing disorder characterized by compulsive drug-taking and drug-seeking, loss of control over drug intake, and a persistent craving for the drug. Although only a subset of individuals that try drugs (~15–20% for cocaine) will develop these pathological behaviors, it is a public health epidemic with far reaching economic and social consequences (Anthony et al., 1994; Wagner and Anthony, 2002). The prefrontal cortex (PFC) regulates a wide range of cognitive, perceptual, emotional, and motivational processes, and dysregulation of these processes has been implicated in numerous neuropsychiatric disorders, including addiction. In particular, the PFC appears to play a critical role in the formation and maintenance of addictive behavior to drugs through its regulation of salience attribution, drug-associated memory expression, decision making, and

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inhibitory control, with important differences in function between cortical subdivisions (Goldstein and Volkow, 2011; Hogarth et al., 2013; Kalivas, 2009; Peters et al., 2013; Van den Oever et al., 2010; Volkow and Morales, 2015).

The objective of this review is to provide an update on the role of the PFC in cocaine addiction from work that has used viral strategies. Viral vectors are an essential technology for manipulating activity of neuronal populations with the precision necessary to dissect complex circuits like those of the PFC. In particular, viral-mediated optogenetic and chemogenetic tools allow for a high degree of both spatial and temporal precision in experiments that often cannot be achieved with alternative methods like lesioning or pharmacological manipulation. These tools can be used to target afferent or efferent projections in the brain, as well as specific brain regions and neuronal subpopulations within a given area, and all of these strategies have been widely applied in the study of addiction-related circuitry. Since the vast majority of published work dissecting cortical circuits with viral vectors has been performed in rodent models, this review will predominantly focus on the functional subdivisions of rodent PFC (but see Feil et al. (2010) and Koob and Volkow (2016) for a broader review of cortical circuitry in addiction). First, an overview of rodent PFC neuroanatomy is provided, followed by an overview of the optogenetic and chemogenetic viral tools that were used in the PFC studies. Next, a review of studies of manipulations within the PFC are discussed, followed by a review of work that targeted manipulations to PFC inputs and outputs.

PFC Anatomy

Composition

The PFC is primarily made up of excitatory glutamatergic cells (pyramidal and spiny stellate cells), which project both within and outside of the cortex and are found in all cortical layers except layer I (Jones, 1984; Staiger et al., 2004; Kubota et al., 2016; Tremblay et al., 2016). In addition, there is a small population (10–15%) of GABAergic interneurons (Meyer et al., 2011). Approximately 40% of these interneurons are parvalbumin (PV) positive, 30% are somatostatin (SOM) positive, and 30% express the 5-HT_{3a} serotonin receptor (Tremblay et al., 2016). These interneuron classes can also be subdivided further based on morphology, physiology, and connectivity patterns. Despite their relatively small population numbers, these interneurons are located in all cortical layers and can make substantial connections within cortex, allowing them to play a critical role in modulating cortical output and function (Kubota et al., 2016; Tremblay et al., 2016).

Layers of Cortex

The cortex is made up of six layers. Layer I is largely composed of fibers; however, there are also GABAergic interneurons in this layer, which can modulate the fibers that pass through it (Garcia-Munoz & Arbuthnott, 2015). Layers II/III are primarily made up of pyramidal cells and receive intra-cortical input as well as input from thalamus. These layers provide significant input to the deep layers of cortex (Kuroda et al., 1998; Kubota et al., 2016; Hirai et al. 2012, Kim et al., 2015). Layer IV is composed mostly of corticocortical spiny stellate and pyramidal cells that receive input from the thalamus (Staiger et al., 2004). Layers V and

VI contain the subcortically projecting pyramidal neurons; although many of these neurons also project within cortex. Layer V neurons have strong projections to the striatum and some thalamic projections whereas Layer VI neurons tend to project to thalamus but not striatum (Thomson, 2010; Kubota et al., 2016; Hirai et al. 2012, Kim et al., 2015; Wilson, 1987; Reiner et al., 2010; Shepherd, 2013; Morishima and Kawaguchi, 2006).

Regions of PFC

The medial prefrontal cortex (mPFC) is typically divided into two major divisions, the dorsal medial (dmPFC) and the ventromedial (vmPFC) (Heidbreder and Groenewegen, 2003; Hoover and Vertes, 2007; Moorman et al., 2015; Vertes, 2004). In rodents, the dmPFC consists of the medial agranular, anterior cingulate, and dorsal aspect of prelimbic cortex and the vmPFC includes the ventral portion of prelimbic cortex and the infralimbic cortex (Berendse and Groenewegen, 1991; Heidbreder and Groenewegen, 2003; Vertes, 2006; Hoover and Vertes, 2007). The dmPFC is typically considered to be more involved with motor tasks than the vmPFC, which is believed to play a larger role in emotion and affective behaviors due to its significant connections with limbic areas. However, despite this proposed functional split, it should be noted that modulation of addiction-related behaviors occurs in both regions (Vertes, 2006; Van den Oever et al., 2010 and see below). The orbital frontal cortex (OFC) is located ventrolaterally to the mPFC in rodents. The OFC is thought to be an important association area as it receives significant input from sensory areas and it has been shown to play a critical role in determining outcome expectancies. In addition, its function is significantly altered by drug use (Öngür and Price, 2000; Schoenbaum et al., 2006).

Connectivity by Region

In rats, the mPFC receives cholinergic inputs from the basal forebrain nuclei, noradrenergic inputs from the locus coeruleus (LC), serotonergic inputs from the dorsal and median raphe nuclei, and dopaminergic inputs from the VTA and SNc (Heidbreder and Groenewegen, 2003; Groenewegen and Uylings, 2000; Uylings et al., 2003). The mPFC forms reciprocal projections with all of these areas (Heidbreder and Groenewegen, 2003; Van den Oever et al., 2010; Thierry et al., 2000; Carr and Sesack, 2000; Uylings et al., 2003; Zaborszky et al., 1997; Gaykema et al., 1991; Jodo et al., 1998). Although both the dmPFC and vmPFC receive inputs from LC, dorsal raphe, VTA/SNc, and glutamatergic inputs from several cortical and thalamic sources, there are also key differences in their connectivity patterns with these regions. The dmPFC projects to motor cortex, brainstem, thalamus, tectum, amygdala, dorsal striatum and the nucleus accumbens (NAc) (Vertes, 2006; Hoover and Vertes, 2007) whereas the vmPFC projects to midline thalamic nuclei, brainstem, bed nucleus of stria terminalis, hypothalamus, amygdala, and NAc (Vertes, 2006; Hoover and Vertes, 2007). In addition, the vmPFC is more likely to receive input from midline thalamic and 'limbic' cortices, such as hippocampus, orbital cortex, and PFC compared to the dmPFC (Vertes, 2006; Hoover and Vertes, 2007). The inputs from hippocampus originate in the CA1 and subiculum regions and project throughout the vmPFC (Swanson, 1981, Jay et al., 1989; Jay and Witter, 1991; Thierry et al., 2000; Vertes, 2006). Additionally, projections from the hypothalamus and amygdala are more heavily concentrated in vmPFC compared to dmPFC (Hoover and Vertes, 2007). Within the vmPFC, there is also some separation in connectivity,

as prelimbic cortex projects to the BLA whereas infralimbic cortex projects to the intercalated cells (McDonald et al., 1996; Berretta et al., 2005; Peters et al., 2009). There also seems to be some topographical distinction in the cholinergic innervation of these regions as the lateral portion of the basal forebrain targets dmPFC while the medial portion targets vmPFC (Hoover and Vertes, 2007). Finally, the OFC forms strong reciprocal projections with the thalamus, amygdala, and subiculum as well as provides input to the VTA, substantia nigra, dorsal striatum and NAc (Groenewegen, 1988; Kita and Kitai, 1990; Groenewegen et al., 1991; Öngür and Price, 2000; Schoenbaum et al., 2006; Hoover and Vertes, 2011; Arguello et al., 2016; Gremel et al., 2016). In addition to these regions, the OFC has a number of intracortical projections as well as additional projections to the septum, substantia innominata, raphe, claustrum, and periaqueductal gray (Hoover and Vertes, 2011).

Corticostriatal inputs

The PFC sends a dense glutamatergic projection to the striatum with individual cortical neurons having the capacity to innervate many medium spiny neurons (MSNs). These corticostriatal projections are topographically organized such that the most dorsal aspects (i.e., the anterior cingulate) project more strongly to dorsal striatum and the most ventral aspects (i.e., the infralimbic and ventral prelimbic regions) project primarily to the NAc shell (McGeorge and Faull, 1989; Vertes, 2006; Van den Oever et al., 2010). Prelimbic cortex also projects to the NAc core (Peters et al., 2009).

Each striatal MSN receives roughly 5000 synapses from cortex (Kincaid et al., 1998). These cortical axons can travel up to 900 μm within the striatum, with synaptic boutons spaced about 10 μm apart (Kincaid et al., 1998; DiFiglia et al., 1978). This cortical innervation of striatum arises from layer V of cortex, and these corticostriatal neurons can project both ipsilaterally and contralaterally to striatum, as well as within the cortex itself. In addition to these corticostriatal neurons, layer V also contains corticocortical neurons, which project primarily within cortex, and corticopontine neurons, which can project ipsilaterally to the pontine nuclei, within the cortex itself, or to a number of other subcortical structures, including striatum, thalamus, pons, subthalamic nucleus, substantia nigra pars compacta (SNpc), and spinal cord (Wilson, 1987; Morishima and Kawaguchi, 2006; Hirai et al., 2012; Kim et al., 2015; Reiner et al., 2003; Reiner et al., 2010; Shepherd, 2013; Kubota et al., 2016).

Although anatomical evidence indicates that these two types of corticostriatal projection neurons may preferentially project to different types of striatal MSNs (i.e., striatal neurons that project directly to the SN versus those that project to the SN via the globus pallidus external and subthalamic nucleus), there is electrophysiological evidence to suggest that functionally this separation does not exist (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996; Reiner et al., 2003; Reiner et al., 2010; Kress et al., 2013). Nonetheless, in addition to having different projection patterns, these two types of corticostriatal neuron also have morphological and electrophysiological differences. The corticopontine neurons have a greater hyperpolarization activated current, exhibit a more tonic firing style, are larger, and have a more prominent apical dendrite (Morishima and

Kawaguchi, 2006; Dembrow et al., 2010; Suter et al., 2013; Mason and Larkman, 1990; Hattox and Nelson, 2007; Miller et al., 2008; Gee et al., 2012; Shepherd et al., 2013). In addition, these two neuronal populations express different transcription factors (e.g. corticocortical neurons express SATB2 and p11 whereas corticopontine neurons express CTIP2 and FEZF2) and respond differently to dopamine, serotonin, and acetylcholine (Gaspar et al., 1995; Avesar and Gullledge, 2012; Dembrow et al., 2010; Shepherd, 2013).

Role of the PFC in cocaine addiction

Research exploring the role of the PFC in cocaine addiction has largely focused on the mPFC, which has historically been thought of as a critical region for inhibitory control (Goldstein and Volkow, 2011; Kalivas, 2009). The mPFC becomes hypoactive in both humans (Goldstein and Volkow, 2011) and rodents (Chen et al., 2013) following chronic drug exposure, while also displaying increased activity following exposure to drug-associated cues (Goldstein and Volkow, 2011; Kalivas, 2009; McFarland and Kalivas, 2001). As described, the mPFC can be subdivided into two regions, the dmPFC (i.e., the anterior cingulate and dorsal aspect of prelimbic cortex) and the vmPFC (i.e., the ventral aspect of prelimbic cortex and the the infralimbic cortex) (Heidbreder and Groenewegen, 2003; Moorman et al., 2015; Vertes, 2004), which have different roles in addiction-related behaviors in addition to having distinct patterns of connectivity. Specifically, activation of the dmPFC is generally thought to promote drug seeking, whereas activation of the vmPFC can either promote or inhibit drug seeking depending on experimental parameters, including the type of drug, whether extinction training occurs and targeting of the manipulation (Koya et al., 2009; McLaughlin and See, 2003; Peters et al., 2008; Rogers et al., 2008; Van den Oever et al., 2013; Willcocks and McNally, 2013). Finally, the OFC is thought to mediate salience attribution and valuation of outcomes, such that drug-induced OFC dysfunction may limit behavioral flexibility and contribute to the habitual behavior associated with addiction (Lucantonio et al., 2014; Schoenbaum and Shaham, 2008; Volkow and Morales, 2015). Although these studies provide a general framework for the contribution of each of these regions of the PFC in addiction-related behaviors, it is important to note that there are significant discrepancies in the existing literature with respect to the functional subdivisions that are described here that render comparisons across some studies difficult. In particular, there is large variance regarding whether manipulations extended across most of the mPFC, were restricted to the dorsal or ventral portion, or were isolated to a specific subregion (e.g., prelimbic versus infralimbic cortex).

Viral Tools for Modulating PFC Activity

The continual development of tools for cell-specific targeting of transgenes to selectively alter neuronal activity has dramatically expanded our ability to investigate complex neuronal circuits. Optogenetic and chemogenetic tools, in particular, have been widely adopted for studying the role of the PFC and its subcomponents/projections in behaviors, including those relevant to cocaine addiction. Optogenetics utilizes light-sensitive proteins to transiently modulate neuronal firing on the millisecond time scale, and optical excitation is most frequently achieved using the cation channel channelrhodopsin-2 whereas optical inhibition is typically achieved using the chloride pump halorhodopsin or the proton pump

archaerhodopsin (Allen et al., 2015; Boyden et al., 2005; Deisseroth, 2015; Fenno et al., 2011; Mattis et al., 2011). Many specialized variants of these and other opsins now exist, and though a detailed discussion of these tools is beyond the scope of this review, they have been extensively reviewed by others (Allen et al., 2015; Deisseroth, 2015; Fenno et al., 2011; Mattis et al., 2011). Compared to other approaches, optogenetic tools allow for exceptionally tight temporal control of neuronal activity, which is ideally suited for coupling activity changes to a behavioral action (e.g. lever press, nose poke). However, these methods also have distinct drawbacks, such as the potential for tissue heating/damage, the dependence on high levels of viral expression to achieve neuronal modulation and the need for appropriate stimulation patterns and frequencies (the system can be easily overdriven beyond what is physiologically relevant) (Allen et al., 2015; Deisseroth, 2015). Thus, these issues should be kept in mind when reviewing findings involving their use.

DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) are a widely adopted set of chemogenetic tools that can also transiently modulate neuronal activity. DREADD-induced neuronal activation is achieved using a G_q - or G_s -DREADD (hM₃Dq and rM₃DS) whereas neuronal inhibition is achieved using a $G_{i/o}$ -DREADD (hM₄Di or the newer kappa-opioid receptor derived KORD) (Armbruster et al., 2007; Burnett and Krashes, 2016; Roth, 2016; Smith et al., 2016; Urban and Roth, 2015; Vardy et al., 2015). DREADD activation is accomplished through systemic administration of selective ligands (clozapine-*N*-oxide, DREADD agonist 21, salvinorin B), which results in reversible modulation of the activity of target neurons through the associated G-protein signaling cascades as well as in some cases through modulation of channels (e.g. inward rectifying potassium channels). As a result of the slower onset and offset kinetics, this method has a much slower temporal profile compared to optogenetics, as well as a limited ability to tightly couple neuronal activity modulation with specific behavioral actions. The perturbation of multiple intracellular signaling cascades, including β -arrestin signaling, also raises the possibility of complex cellular effects beyond simple excitation or inhibition. Nonetheless, chemogenetic approaches avoid some complications associated with optogenetics, such as the need to identify appropriate stimulation patterns, as well as being less invasive and often more appropriate for prolonged or repeated manipulations (Burnett and Krashes, 2016; Roth, 2016; Smith et al., 2016; Urban and Roth, 2015). In addition, because DREADDs signal through G-protein cascades, there is downstream amplification of receptor activation, so maximal stimulation or inhibition of cells can be achieved with low levels of viral expression. As well, since modulation of downstream effectors of G-proteins are saturable, over-stimulation of the system cannot occur, so the manipulations are biologically relevant. Although DREADD ligands are highly selective, higher doses of CNO have been reported to have off-target effects on behavior (MacLaren et al., 2016), so use of appropriate control groups when considering DREADD findings is warranted.

Both chemogenetic and optogenetic approaches can be targeted to specific cell types or neuronal projections through the use of selective promoters, Cre recombinase (Cre-)-lox recombination, and/or direct stimulation of axon terminals. With respect to studying cortical regions, a CaM kinase II alpha (CaMKII α) promoter is frequently used to preferentially target excitatory pyramidal neurons. However, it should be noted that this promoter does not exclusively target these cells, as CaMKII α -driven viral markers have been observed in other

neuronal populations, including some parvalbumin-positive cortical neurons (Watakabe et al., 2015). Table 1 provides a summary of the major viral tools that are discussed in this review.

Viral Targeting of Cortical Subdivisions

As noted earlier, the targeting in studies of mPFC function varies considerably, with viral spread in some studies covering a large portion of mPFC and others having restricted expression in dorsal or ventral mPFC or in prelimbic or infralimbic cortex. For the purpose of this review, if viral expression occurred in both dmPFC and vmPFC, then we will refer to these studies as having targeted mPFC. In addition, we will separate studies that targeted dmPFC from those that targeted prelimbic cortex, since the prelimbic studies would also have included some aspects of vmPFC. However, the studies targeting infralimbic cortex will be included in the vmPFC section, but a notation will be made if they were confined to the infralimbic portion of vmPFC.

dmPFC

The role of the dmPFC in addiction-related behaviors has not been studied using viral-mediated gene transfer techniques. However, optical inhibition of dmPFC using halorhodopsin has been shown to attenuate stress-induced reinstatement of food-seeking in rats (Calu et al., 2013), suggesting that the dmPFC promotes reward-seeking.

Prelimbic cortex

Chronic exposure to cocaine can trigger a reduction in baseline mPFC activity in addicted individuals (Goldstein and Volkow, 2011). A similar reduction in neuronal excitability has been observed in rats after prolonged cocaine exposure, and this reduction is especially pronounced in rats that show compulsive drug-taking (e.g., continue to seek cocaine when it is paired with foot shock) (Chen et al., 2013). Optogenetic manipulation of the prelimbic cortex has been used to study the role of this hypoactivity in the loss of inhibitory control. In particular, using AAVs expressing channelrhodopsin and halorhodopsin for neuronal excitation and inhibition, respectively, it was found that optical excitation of prelimbic cortex neurons reduced compulsive (i.e., shock-resistant) cocaine intake whereas optical inhibition of these neurons enhanced compulsive intake (Chen et al., 2013). These results suggest that PFC hypoactivity can, in fact, trigger a loss of inhibitory control over drug consumption. Similarly, photoinhibition of prelimbic cortical neurons with archaerhodopsin was found to enhance cocaine self-administration (Martín-García et al., 2014). However, this manipulation also reduced cocaine-primed reinstatement among rats that had undergone a high-frequency cocaine intake schedule (Martín-García et al., 2014), as did optical inhibition of prelimbic cortex using halorhodopsin (Stefanik et al., 2013). Taken together, these results suggest a more complex view of the PFC, where a reduction in activity of this region can either promote or inhibit drug-taking and drug-seeking behaviors depending on a number of factors including the presence of cocaine during testing, phase of the experimental paradigm (e.g., training, reinstatement), or pattern of drug use.

vmPFC

Similar viral targeting methods have also been used to study the role of vmPFC in drug-taking and drug-seeking behaviors. For example, chemogenetic activation of vmPFC with a G_q -DREADD attenuated cue-induced, but not cocaine-primed, reinstatement of cocaine seeking after extinction, yet had no effect on reinstatement after an equivalent period of drug abstinence without extinction training (Augur et al., 2016). This suggests that extinction-dependent plasticity in the vmPFC is necessary for vmPFC regulation of cue-driven cocaine seeking, possibly through the reconciliation of opposing cue-outcome associations or by preventing neuroadaptations that underlie the incubation of cocaine craving. However, vmPFC control over conditioned cocaine seeking may also be time dependent, as optical stimulation of vmPFC neurons using channelrhodopsin was found to facilitate the extinction of a cocaine conditioned place preference (CPP) after 3 weeks of drug abstinence, but there was no effect of the manipulation when extinction testing was done immediately after conditioning (Van den Oever et al., 2013). Similarly, optical inhibition of vmPFC neurons using halorhodopsin blocked extinction of a CPP memory to cocaine after a 3-week drug withdrawal; however, vmPFC inhibition using Cre-inducible halorhodopsin in transgenic C57BL/6 CaMKII::Cre mice was also able to attenuate the expression of a cocaine CPP 24 h after conditioning (Van den Oever et al., 2013). This work suggests that, during periods of drug abstinence, the circuitry underlying cocaine seeking may reorganize such that the vmPFC transitions from being necessary for the expression of drug-seeking to facilitating the extinction of drug-seeking when drug-associated cues no longer signal drug availability. Furthermore, the infralimbic region of the vmPFC may also play a role in how stressors influence the extinction of drug-seeking. Exposure to inescapable tailshock stress was found to reduce the extinction of cocaine-seeking behavior, whereas exposure to escapable tailshock (i.e., shock was avoided by turning a wheel) accelerated extinction, and this facilitation was blocked by optical silencing of infralimbic neurons using halorhodopsin (Baratta et al., 2015). This work suggests that not only can controllability over a stressor influence how stress effects drug-seeking, but this process is regulated by the infralimbic cortex. Overall, these findings highlight a critical role for the vmPFC in extinction memory of cocaine, but also suggest that the role of the vmPFC is dependent on both the timing and duration of different phases of the experimental paradigm, particularly for drug abstinence and extinction training.

OFC

The role of the OFC in behaviors related to addiction has also been studied using viral-mediated optogenetic and chemogenetic tools. Since the OFC is thought to regulate salience attribution and valuation of rewarding outcomes, one behavioral approach used to study this region is to test for Pavlovian over-expectation. Rats trained to self-administer cocaine show a deficit in Pavlovian over-expectation such that distinct conditioned stimuli fail to elicit a summated response when compounded. This deficit can be reversed through optical excitation of OFC neurons using channelrhodopsin (Lucantonio et al., 2014). This finding indicates that OFC dysregulation after cocaine exposure may impair the value representation of instrumental outcomes, or more specifically, impair the ability to flexibly generate value representations of novel outcomes based on prior knowledge or insight into similar outcomes that were previously experienced. Activation of medial OFC neurons with a G_s -

DREADD was shown to enhance sensitivity to devaluation of a food reward, while targeted knockdown of *Bdnf* in the medial OFC of floxed-*Bdnf* mice with Cre-expressing lentivirus blocked sensitivity to devaluation, suggesting that the OFC also plays a role in flexible responding to natural rewards and that this function is dependent on BDNF signaling (Gourley et al., 2016). Finally, the OFC has also been implicated in regulating compulsive (i.e., punishment-resistant) reward-seeking as chemogenetic inhibition of OFC neurons using a $G_{i/o}$ -DREADD decreases VTA self-stimulation when it is paired with foot shock. In addition, neuronal activity and excitability are enhanced in the lateral OFC following VTA self-stimulation paired with foot shock only in punishment-resistant mice (Pascoli et al., 2015). These results suggest that OFC inhibition could impair the updating of reward valuation of the VTA self-stimulation such that the negative value of noxious shock was not fully accounted for once introduced. Together, these studies indicate that OFC hypoactivity in addicted individuals may disrupt the creation of flexible value representations and promote compulsive, punishment-resistant behaviors that are characteristic of addiction.

Viral Targeting of Cortical Inputs and Outputs

Inputs to Striatum

mPFC to NAc: The mPFC has a diverse array of downstream targets; however, the mPFC to NAc projection is by far the most investigated cortical circuit in addiction-related behaviors using viral targeting methods. The striatum is known to play a critical role in cocaine-induced behaviors (Everitt and Robbins, 2005; Berke and Hyman, 2000; Robinson and Berridge, 2000) and the mPFC provides a dense glutamatergic input to the striatum (McGeorge and Faull, 1989; Groenewegen et al., 1991; Van den Oever, 2010; Britt et al., 2012; Wall et al., 2013). Using channelrhodopsin to activate this mPFC input to the NAc core/shell, it was found that stimulation produced both a CPP to the stimulation-paired side and supported self-stimulation. However, these results were also seen when either hippocampal or amygdala inputs to the NAc core/shell were stimulated, indicating that the enhanced reward may be due to increased glutamate irrespective of the source pathway (Britt et al., 2012). As well, another study was unable to achieve self-stimulation of the mPFC to NAc core/shell pathway, although the discrepancies between these studies have been attributed to the differences in optogenetic stimulation parameters (Stuber et al., 2011). The effects of non-contingent cocaine experience on plasticity of mPFC neurons projecting to the NAc core has been examined using optically-evoked excitatory postsynaptic currents (EPSCs) via channelrhodopsin. It was found that cocaine exposure increased N-methyl-D-aspartate receptor (NMDAR) function at mPFC to dopamine D1 receptor (D1R)-expressing MSN synapses in the NAc core, but not at non-D1R-expressing MSN synapses (Joffe & Grueter, 2016). Finally, the role of mPFC projections to the NAc core have also been examined in patterns of alcohol use that are associated with altered NMDAR functioning at mPFC to NAc synapses. Using halorhodopsin-induced optogenetic inactivation of these projections, a significant reduction in aversion-resistant alcohol intake was observed (Seif et al., 2013).

dmPFC to NAc: Cortical inputs from the dmPFC into the NAc core/shell have also been examined in addiction-related behaviors using a combinatorial chemogenetic targeting

approach (i.e., expressing a Cre-recombinase (Cre)-dependent $G_{i/o}$ -DREADD in the dmPFC and a retrograde canine adenovirus expressing Cre (CAV-Cre) in the NAc). It was found that reducing dmPFC activity to the NAc core/shell transiently decreases amphetamine sensitization but has no effect on cocaine self-administration under a progressive ratio schedule of reinforcement (Kerstetter et al., 2015). In addition, prior chemogenetic inhibition of these neurons during cocaine-self-administration lead to an enhancement of drug-seeking on the first days of extinction as well as a paradoxical increase in cocaine-primed reinstatement of drug-seeking. However, chemogenetic inhibition of this dmPFC to NAc core/shell pathway during a reinstatement test attenuated drug-seeking to the cocaine prime (Kerstetter et al., 2015).

Similar findings on reinstatement have been reported when optogenetic inhibition by halorhodopsin was performed on prelimbic projections to the NAc core, as cue-induced, cocaine-primed, and cue + cocaine- primed reinstatement of cocaine-seeking were all reduced (Stefanik et al., 2016; Stefanik et al., 2013). This optogenetic inhibition of prelimbic inputs to the NAc core also decreased the spine head diameter, AMPA/NMDA ratio, and spontaneous EPSC amplitude in NAc core MSNs produced by cue-induced reinstatement (Stefanik et al., 2016). Similarly, withdrawal from cocaine self-administration produces maturation of silent synapses in the prelimbic to NAc core pathway, and optogenetically reversing this process using channelrhodopsin and an LTD stimulation protocol inhibited incubation of cocaine craving (Ma et al., 2014).

vmPFC to NAc: The role of the vmPFC projections to the NAc core/shell have been examined more extensively than the dmPFC projections, and there are several optogenetic studies indicating that glutamatergic signaling from this pathway is enhanced following cocaine exposure, and regulates its behavioral effects. For instance, optogenetically-induced LTD on infralimbic terminals in the NAc core/shell (via channelrhodopsin) was sufficient to reverse the cocaine-induced potentiation of glutamatergic neurotransmission on DIR-expressing MSNs caused by non-contingent cocaine administration, as well as to block the expression and persistence of cocaine-induced locomotor sensitization (Pascoli et al., 2012). In addition, optical stimulation of infralimbic terminals within the NAc shell (via channelrhodopsin) revealed that withdrawal from cocaine self-administration increases glutamatergic signaling in DIR-expressing MSNs but not dopamine D2 receptor (D2R)-expressing MSNs (Pascoli et al., 2014). However, although optogenetically-induced LTD of infralimbic terminals on these DIR neurons prior to cue-induced reinstatement was sufficient to restore normal transmission, it increased lever responding non-discriminately (i.e., on both the cocaine-associated and non-associated levers (Pascoli et al., 2014). Optogenetic stimulation via channelrhodopsin has also been used to demonstrate that the presynaptic release probability at vmPFC to NAc shell synapses is enhanced following cocaine withdrawal (Suska et al., 2013). This withdrawal from cocaine produces maturation of silent synapses in the infralimbic to NAc shell pathway, and optogenetically reversing this process (via channelrhodopsin and an LTD stimulation protocol) enhances craving (Ma et al., 2014). Finally, chemogenetic stimulation of the vmPFC to NAc shell pathway (via a Cre-dependent G_q -DREADD combined with CAV-Cre) significantly inhibited cue-induced, but not drug-primed, reinstatement of cocaine-seeking, but only after extinction training (i.e., there was no effect after abstinence) (Augur et al., 2016). Taken together, these studies

suggest that cocaine produces a potentiation in the vmPFC to NAc pathway, and inhibition of this pathway can reduce both cocaine-induced neural plasticity as well as addiction-related behaviors. Nonetheless, as some findings do not fully support this idea, this work also highlights the fact that subregions within both the vmPFC and the NAc appear to play distinct roles in regulating the effects of cocaine.

OFC to dorsal striatum: While most of the viral studies tracing the role of cortical inputs to striatum in addiction behaviors have focused on the role of the mPFC to NAc projection, there has also been some intriguing, related work conducted on the projections from the OFC to dorsal striatum. Using a retrograde HSV-Cre virus in dorsal striatum and a Cre-dependent $G_{i/o}$ -DREADD in OFC, it was found that chemogenetic inhibition of OFC projections to dorsal striatum abolished goal-directed actions and left mice dependent on habitual strategies (Gremel et al., 2016). Further, elimination of the endocannabinoid CB1 receptor from this OFC to dorsal striatum projection (via a retrograde HSV-flippase virus targeted to the dorsal striatum and a Flp-dependent Cre virus targeted to the OFC of Cre-dependent CB1 receptor knockout mice) blocked the transition from goal-directed to habitual strategies (Gremel et al., 2016). Although this study did not directly study drug behaviors, the transition from goal-directed to habitual actions is a critical one in addiction (Everitt and Robbins, 2005), and this work highlights the importance of future research into the role of OFC to dorsal striatum projections in the transition to compulsive drug-taking.

BLA Connections

There has been some recent research examining the role of OFC connections to the BLA in addiction-related behaviors. Using a retrograde AAV-Cre virus and Cre-dependent opsins, it was found that inhibition of the lateral OFC projection to BLA (via a Cre-dependent halorhodopsin and a retrogradely traveling AAV6-Ef1 α -Cre) was sufficient to reduce cue-induced reinstatement of drug-seeking following cocaine self-administration and extinction (Arguello et al., 2016). Interestingly, the BLA and PFC send reciprocal projections to each other, but inhibiting the reciprocal projections (BLA to lateral OFC) in this study had no effect on cue-induced reinstatement of drug-seeking (Arguello et al., 2016). Nonetheless, BLA projections to prefrontal cortex can modulate drug-seeking, as archaerhodopsin-induced optogenetic inhibition of this projection significantly reduced cue-induced reinstatement (Stefanik and Kalivas, 2013). Thus, the research to date on the connections between the PFC and the BLA indicate that this is an important reciprocal projection for modulating drug-seeking, but also emphasizes the functional and anatomical diversity of the different cortical regions and their circuit connections.

VTA Connections

Although the PFC projections to VTA have not been directly examined in drug-related behaviors, the available evidence on the anatomy of this circuit and the functional importance of both structures in addiction makes this pathway an appealing avenue of future research. Nonetheless, some viral targeting work has been done to examine connections between the mPFC and the VTA in motivated behaviors relevant to addiction. Specifically, removing NMDA receptors from the VTA and its inputs (using transgenic mice in conjunction with CAV-Cre) blocked Pavlovian learning, while restoring NMDA receptors to

mPFC neurons projecting to the VTA (via an NR1 subunit-encoding virus) was sufficient to restore Pavlovian learning (Parker et al., 2011). The VTA provides an important reciprocal pathway back to the PFC and salient environmental stimuli (e.g., a reward-associated cue) can induce burst firing of dopamine neurons in the VTA (Schultz et al., 2002; Fiorillo et al., 2003). Following optical stimulation of VTA dopamine terminals in the prelimbic cortex (using a Cre-dependent channelrhodopsin and transgenic rats that express Cre under the tyrosine hydroxylase (TH) promoter), it was found that mimicking this burst pattern of firing decreased intrinsic inhibition in pyramidal neurons in layer V of prelimbic cortex (Buchta et al., 2017). Similar results were obtained following more sustained chemogenetic activation, using either a Cre-dependent G_q -DREADD or G_s -DREADD (Buchta et al., 2017). These results are consistent with idea that increased dopamine from the VTA enhances mPFC neuronal activity to drive cocaine-seeking behavior (Goldstein and Volkow, 2011; Kalivas, 2009; McFarland and Kalivas, 2001). In contrast, Kabanova et al. (2015) found that channelrhodopsin-mediated activation of VTA neurons projecting to the mPFC produces excitatory postsynaptic potentials in cortical interneurons but inhibitory postsynaptic potentials in layer V pyramidal projection neurons. These effects were blocked by glutamate receptor antagonists, suggesting that they are mediated by glutamatergic drive from the VTA (Kabanova et al., 2015). The seemingly disparate results of these two studies could be due to variance in a number of experimental parameters, including the species used, the region of mPFC targeted, the subtype of VTA neurons expressing the transgenes, and the optical stimulation protocol. These last two variables, in particular, are likely to result in very different levels of released dopamine and/or glutamate from the VTA following stimulation, which could have distinct effects on mPFC neuronal activity. Finally, these studies did not identify which subtype of pyramidal neurons (i.e., corticocortical or corticopontine) they were recording from. Given that these two populations respond differentially to dopamine (Gaspar et al., 1995; Shepherd, 2013), it is possible that the results were reflective of only one of the populations. This raises the intriguing possibility that cue-induced dopamine release could have differential effects on corticocortical and corticopontine neurons, and perhaps it is the relative balance in activity of these two neuronal populations that drive drug-seeking behavior.

Concluding Remarks

This review has focused on the use of viral-mediated based optogenetic and chemogenetic targeting strategies for improving our understanding of the role of the PFC, and its afferent and efferent connections, in behaviors related to cocaine addiction. This work (which is summarized in Table 2) highlights findings that changes in neural activity within the PFC itself contribute to drug-taking and drug-seeking behaviors. In addition, this research underscores that PFC inputs, particularly those terminating in the NAc, regulate downstream neuroadaptations that mediate drug-seeking. Although these studies have largely confirmed what has previously been reported with more traditional methods, such as pharmacological manipulation and lesions, they also reveal that the involvement of the PFC in addiction behaviors is more complex and nuanced than previously appreciated. Given the heterogeneity of cell types and connections across regions of the PFC, it will be especially important moving forward to expand research efforts into some of the understudied portions

of the circuit, including the anterior cingulate and OFC, as well as afferent inputs from the thalamus and VTA. In addition, further circuit dissection at the cellular level is warranted (e.g. parsing effects of corticocortical pyramidal cells from those of corticopontine pyramidal cells and interneuron populations), and is now feasible with promoter based and/or combinatorial viral-mediated gene transfer approaches.

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Highlights

- Prefrontal cortex regulation of drug-taking and drug-seeking is complex and dynamic.
- Optogenetic and chemogenetic viral tools allow for precision dissection of circuit function.
- Novel techniques are providing new insights into the PFC regulation of cocaine addiction.

Table 1

Summary of major viral tools discussed in this review.

Viral Tool	Function
Channelrhodopsin (ChR2)	Optical activation produces influx of sodium ions leading to increased neuronal firing
Archaeorhodopsin (ArchT)	Optical activation results in protons being pumped out of the cell leading to reduced neuronal firing
Halorhodopsin (eNpHR)	Optical activation increases the intracellular chloride concentration leading to reduced neuronal firing
G _q -DREADD (hM ₃ Dq)	Ligand-driven activation increases G _q signaling pathways leading to increased neuronal firing
G _s -DREADD (hM ₃ Ds)	Ligand-driven activation increases G _s signaling pathways leading to increased neuronal firing
G _{i/o} -DREADD (hM ₄ Di, KORD)	Ligand-driven activation increases G _{i/o} signaling pathways leading to reduced neuronal firing
CAV-Cre	A canine adenovirus vector that allows for retrograde transport of Cre recombinase (Cre-)
HSV-Cre/HSV-Flp	A herpes simplex virus 1 vector that allows for retrograde transport of Cre- (or Flippase)
hSyn	Strong promoter that is specific to neurons
CAG/CMV	General promoters that drive strong expression
CaMKIIa	When used in cortex, this promoter provides preferential targeting of pyramidal neurons.

Table 2

Overview of key findings covered in this review.

Target	Methodology	Viral Strategy	Results	Citation
mPFC → NAc	<i>Optogenetic excitation of axon terminals</i>	ChR2	Produces CPP Supports self-stimulation Ø on self-stimulation	Britt et al., 2012 Stuber et al., 2011
mPFC → NAc core	<i>Optogenetic inhibition of axon terminals</i>	CaMKIIα-eNpHR	↓ aversion-resistant alcohol intake	Seif et al., 2013
	<i>Optogenetically-invoked EPSCs</i>	CaMKIIα-ChR2	↑ NMDAR function at D1R- but not D2R-MSN synapses following cocaine	Joffe & Grueter, 2016
dmPFC → NAc	<i>Chemogenetic inhibition</i>	DIO-hM ₄ Di + CAV-Cre in NAc	↓ amphetamine sensitization Ø on cocaine self-administration ↓ cocaine-primed reinstatement prior inhibition ↑ cocaine-seeking during extinction prior inhibition ↑ cocaine-primed reinstatement	Kerstetter et al., 2015
Prelimbic	<i>Optogenetic excitation</i>	CaMKIIα-ChR2	↓ compulsive cocaine intake	Chen et al., 2013
	<i>Optogenetic inhibition</i>	CaMKIIα-eNpHR hSyn-eNpHR CaMKIIα-ArchT	↑ compulsive cocaine intake ↓ cocaine-primed reinstatement ↑ cocaine self-administration ↓ cocaine-primed reinstatement	Chen et al., 2013 Stefanik et al., 2013 Martín-García et al., 2014
Prelimbic → NAc core	<i>Optogenetically-induced LTD with axon terminal excitation</i>	ChR2	↓ incubation of craving Reverses silent synapse maturation after cocaine withdrawal	Ma et al., 2014
	<i>Optogenetic inhibition of axon terminals</i>	CAG-ArchT	↓ cocaine-primed reinstatement ↓ cue+cocaine-primed reinstatement ↓ cue-induced reinstatement ↓ spine head diameter in NAc ↓ AMPA/NMDAR ratio in NAc ↓ sEPSC amplitude in NAc	Stefanik et al., 2013 Stefanik et al., 2016
BLA → Prelimbic	<i>Optogenetic inhibition of axon terminals</i>	CAG-ArchT	↓ cue-induced reinstatement	Stefanik and Kalivas, 2013
vmPFC	<i>Chemogenetic excitation</i>	hSyn-hM ₃ Dq	↓ cue-induced reinstatement after extinction training Ø on cue-induced reinstatement after withdrawal Ø on cocaine-primed reinstatement	Augur et al., 2016
	<i>Optogenetic excitation</i>	CaMKIIα-ChR2	↑ extinction of cocaine CPP after 3-week withdrawal Ø on CPP without withdrawal	Van den Oever et al., 2013
	<i>Optogenetic inhibition</i>	CaMKIIα-eNpHR + DIO-eNpHR in CaMKII::Cre mice	↓ expression of CPP 24 h after conditioning ↓ extinction of cocaine CPP after 3-week withdrawal	Van den Oever et al., 2013
vmPFC → NAc shell	<i>Optogenetic excitation of axon terminals</i>	ChR2	↑ presynaptic release probability during withdrawal	Suska et al., 2013
Infralimbic	<i>Optogenetic inhibition</i>	CaMKIIα-eNpHR	↓ escapable-stress induced facilitation of extinction	Barratta et al., 2015
Infralimbic → NAc	<i>Optogenetically-induced LTD by axon terminal excitation</i>	ChR2	↓ expression of sensitization ↓ persistence of sensitization Reverses cocaine-induced potentiation of glutamatergic signaling on D1 R-MSNs	Pascoli et al., 2012

Target	Methodology	Viral Strategy	Results	Citation
Infralimbic → NAc shell	<i>Chemogenetic excitation</i>	hSyn-DIO-hM ₃ Dq + CAV-Cre in NAc shell	↓ cue-induced reinstatement after extinction Ø on cue-induced reinstatement after withdrawal Ø on cocaine-primed reinstatement	Augur et al., 2016
	<i>Optogenetic excitation of axon terminals</i>	ChR2	↑ glutamatergic signaling in D1R- but not D2R-MSNs during withdrawal	Pascoli et al., 2014
	<i>Optogenetically-induced LTD by axon terminal excitation</i>	ChR2	↑ lever pressing during cue-induced reinstatement non-discriminately Restores glutamatergic signaling in D1R-MSNs after withdrawal ↑ cocaine craving Reverses silent synapse maturation after cocaine withdrawal	Pascoli et al., 2014 Ma et al., 2014
OFC	<i>Optogenetic excitation</i>	CaMKIIα-ChR2	Restores Pavlovian over-expectation after cocaine self-administration	Lucantonio et al., 2014
	<i>Chemogenetic inhibition</i>	CaMKIIα-hM ₄ Di	↓ compulsive (punishment- resistant) VTA self-stimulation	Pascoli et al., 2015
OFC → BLA	<i>Optogenetic inhibition</i>	DIO-eNpHR + AAV-Cre in BLA	↓ cue-induced reinstatement	Arguello et al., 2016
BLA → OFC	<i>Optogenetic inhibition</i>	AAV-Cre + DIO-eNpHR in BLA	Ø on cue-induced reinstatement	Arguello et al., 2016