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Review

Restructuring of basal ganglia circuitry and associated behaviors triggered by low striatal D2 receptor expression: implications for substance use disorders

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Dopamine D2 receptors (D2Rs) consistently emerge as a critical substrate for the etiology of some major psychiatric disorders. Indeed, a central theory of substance use disorders (SUDs) postulates that a reduction in D2R levels in the striatum is a determining factor that confers vulnerability to abuse substances. A large number of clinical and preclinical studies strongly support this link between SUDs and D2Rs; however, identifying the mechanism by which low D2Rs facilitate SUDs has been hindered by the complexity of circuit connectivity, the heterogeneity of D2R expression and the multifaceted constellation of phenotypes observed in SUD patient. Animal models are well-suited for understanding the mechanisms because they allow access to the circuitry and the genetic tools that enable a dissection of the D2R heterogeneity. This review discusses recent findings on the functional role of D2Rs and highlights the distinctive contributions of D2Rs expressed on specific neuronal subpopulations to the behavioral responses to stimulant drugs. A circuit-wide restructuring of local and long-range inhibitory connectivity within the basal ganglia is observed in response to manipulation of striatal D2R levels and is accompanied by multiple alterations in dopamine-dependent behaviors. Collectively, these new findings provide compelling evidence for a critical role of striatal D2Rs in shaping basal ganglia connectivity; even among neurons that do not express D2Rs. These findings from animal models have deep clinical implications for SUD patients with low levels D2R availability where a similar restructuring of basal ganglia circuitry is expected to take place.

Keywords: Addiction, basal ganglia, cocaine, D2 receptors, dopamine, G-protein coupled receptors, medium spiny neurons, striatum, substance use disorders, synaptic transmission

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Individuals who abuse stimulant drugs, such as cocaine and amphetamine, and those that abuse alcohol display low binding potential for a D2-like agonist in the striatal nucleus of the caudate, putamen and the nucleus accumbens (Volkow *et al.* 1993, 1996, 2001). Obese patients also have low availability of striatal D2Rs when tested using positron emission tomography (PET; Volkow *et al.* 2008). Conversely, high levels of D2Rs in the forebrain have been reported in individuals suffering from schizophrenia (Wong *et al.* 1986). Psychomotor dysfunction and alterations in inhibitory control and motivation are three common symptoms of these disorders. Motivation and motor function are regulated by dopamine through activation of D2Rs and as such, these receptors have surfaced as a likely mediator of these symptoms. Here, we will present evidence that D2Rs mediate the cellular and behavioral response to stimulant drugs and discuss how varying levels of D2Rs in the basal ganglia affect connectivity and circuit function to create vulnerability to stimulant abuse and dependence.

Link between stimulant abuse and D2Rs

An influential theory of addiction is that low levels of striatal dopamine D2Rs predispose individuals to develop SUDs, specifically towards stimulant drugs such as cocaine and amphetamine. This hypothesis originated from PET imaging studies, which showed that cocaine users had lower levels of D2R availability in the striatum compared to healthy controls and also lower glucose metabolism in the orbitofrontal and cingulate cortices, indicative of decreased activity in these cortical areas (Volkow *et al.* 1993, 2001). Furthermore, the ability of a mixed D1/D2 receptor agonist to suppress plasma human growth hormone and prolactin levels is severely blunted in cocaine abusers relative to non-abusing controls, providing additional neuroendocrine evidence for dysregulated dopamine and dopamine receptor functioning in cocaine abusers (Hollander *et al.* 1990). Low D2R availability is found in the striatum of cocaine abusers during early withdrawal and also after detoxification and protracted withdrawal, suggesting that this is not a temporary response to cocaine abstinence but rather a long-lasting alteration relative to healthy controls (Volkow *et al.* 1990, 1993).

Multiple pre-clinical studies have been conducted to address whether low D2R availability is a cause or a consequence of stimulant abuse. The results and conclusions point to evidence that both mechanisms are in play. For example, studies conducted in non-human primates and rodents showed that chronic self-administration of stimulant drugs leads to decreased striatal D2R availability, suggesting a consequential role (Besson *et al.* 2013; Conrad *et al.* 2010; Moore *et al.* 1998; Nader *et al.* 2002). However, other studies in non-human primates found that dominant monkeys display higher levels of striatal D2Rs than subordinate monkeys and that they are more resistant to compulsive cocaine seeking and taking (Morgan *et al.* 2002). Evidence for a causative role of D2Rs also comes from rodent work showing that outbred rats with low levels of D2Rs or mice with a genetic deletion of D2Rs demonstrate increased cocaine self-administration, while virally induced upregulation of D2Rs can lead to decreases in cocaine self-administration in rats (Caine *et al.* 2002; Dalley *et al.* 2007; Edwards *et al.* 2007; Thanos *et al.* 2008). Further, in an elegant study in non-human primates, Nader and colleagues assessed D2R availability before and during cocaine self-administration. They found that initial D2R availability is negatively correlated with the rate of cocaine self-administration, and also that chronic cocaine self-administration further reduced the levels of D2Rs, which remained low even during protracted abstinence (Nader *et al.* 2006). Thus, it appears that striatal D2R availability is both a cause and a consequence of stimulant abuse behaviors.

Impulsivity is a trait linked to poor inhibitory control and is thought to be a root cause of the vulnerability to developing addiction. High impulsivity, assessed in rodents using the five-choice serial reaction time task (5-CSRT), predicted high rates of cocaine self-administration (Dalley *et al.* 2011; Jentsch & Taylor 1999; Verdejo-Garcia *et al.* 2008). Further, high impulsivity was associated with lower D2R availability (Besson *et al.* 2010, 2013; Caprioli *et al.* 2015; Dalley *et al.* 2007). Abnormalities in fronto-striatal circuitry were associated with poor self-control in human stimulant abusers as well as in their non-stimulant abusing biological siblings, supporting the idea that these traits and neuro-circuitry alterations predate the drug taking (Ersche *et al.* 2012).

Taken together, a unified hypothesis is emerging from the literature in which low function of striatal D2Rs causes deficits in inhibitory control and behavioral disinhibition, which contribute to the development of impulsivity and compulsive stimulant use and dependence. The current challenge is determining how low levels of D2R function leads to deficits in behavioral inhibition, to impulsivity, and to stimulant abuse. New findings discussed in this review suggest that decreased function of striatal D2Rs causes a reorganization of striatal connectivity that involves a strengthening of the lateral inhibition between medium spiny neurons (MSNs), which in turn, affects the behavioral response to stimulant drugs. Thus, behavioral or pharmaceutical interventions that enhance D2R availability or activate D2Rs should continue to be considered as novel treatments for stimulant abuse.

Heterogeneity of D2Rs throughout the basal ganglia

Expression, isoforms and subcellular localization

D2Rs are expressed by many different cell-types throughout the basal ganglia. The highest levels are found in the dorsal striatum, nucleus accumbens and olfactory cortex and tubercles, but they are also expressed in the cortex, septum, amygdala, hippocampus, hypothalamus, ventral tegmental area/substantia nigra, pituitary and retina (Beaulieu & Gainetdinov 2011). Within the striatum, D2Rs are expressed on at least three different neuronal types: on indirect-pathway medium spiny neurons (iMSNs) which are a subpopulation of striatal GABA projections neurons highly abundant (~48 % of neurons), on cholinergic striatal interneurons that make less than 2% of striatal neurons, and on the afferents to the striatum from midbrain dopamine neurons (Fig. 1; Bello *et al.* 2011; Delle Donne *et al.* 1996, 1997; Li *et al.* 2012; Sesack *et al.* 1994) There are also reports of D2Rs in a subset of GABA interneurons and on glutamate afferents to the striatum from cortical neurons (Bamford *et al.* 2004; Centonze *et al.* 2003; Higley & Sabatini 2010; Maurice *et al.* 2004; Surmeier *et al.* 2011)

There are two isoforms of D2Rs, D2L and D2S for 'long' and 'short', which are generated by alternative splicing. Their selective expression patterns provide an additional layer of complexity to the already heterogeneous pattern of D2R expression. There is an appreciable consensus that the D2S isoform possesses properties more typical of the D2 autoreceptor, the receptors expressed in neurons that release dopamine (Gantz *et al.* 2015b; Khan *et al.* 1998; Lindgren *et al.* 2003; Usiello *et al.* 2000). The D2L isoform, on the other hand, is predominantly found on striatal projection neurons (Centonze *et al.* 2002; Lindgren *et al.* 2003; Usiello *et al.* 2000); however, it should be noted that this isoform is also expressed in dopamine neurons (Jomphe *et al.* 2006; Khan *et al.* 1998; Neve *et al.* 2013). Functionally, different roles have been linked to the D2L and D2S isoforms. For example, the cataleptic effects of the D2-like antagonist haloperidol are absent in mice lacking the D2L isoform (Usiello *et al.* 2000). The extra 29-amino acid domain in the third intracellular loop of the D2L, which is absent in D2S, is thought to serve as an interaction site for G-proteins or confer unique signaling properties (Picetti *et al.* 1997). Other studies suggest this domain grants some resistance to receptor desensitization and internalization (Gantz *et al.* 2015b; Ito *et al.* 1999; Itokawa *et al.* 1996; Liu *et al.* 1992; Thibault *et al.* 2011).

Global deletion of D2Rs as well as systemic or striatal-specific treatment with D2-like agonists and antagonists have revealed an important role for D2Rs in controlling the behavioral response to stimulants (Baker *et al.* 1996; Britton *et al.* 1991; Caine *et al.* 2002; Chausmer & Katz 2001; Chausmer *et al.* 2002; Spealman *et al.* 1999). However, the heterogeneity of D2R expression, be it via localization to different neuronal types or the expression of different isoforms, should be considered when interpreting these data as it might help reconcile the seemingly disparate findings of genetic and pharmacological manipulations (Fig. 2).

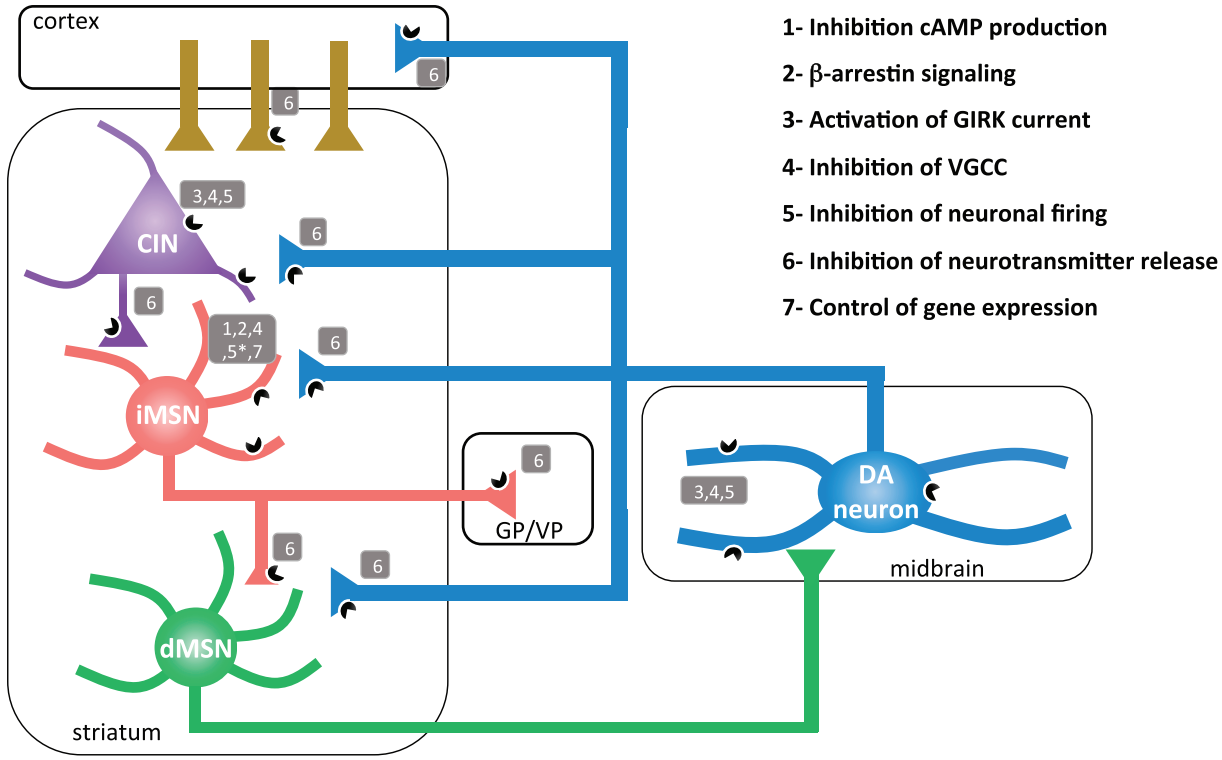

Figure 1: Dopamine D2Rs have heterogeneous cellular localization and functions within the basal ganglia. Schematic diagram depicting D2R localization to indirect-pathway medium spiny neurons (iMSNs, coral), dopamine terminals emanating from the midbrain dopamine neurons (DA, blue), cholinergic interneurons (CIN, purple) and glutamatergic cortical inputs (brown). Grey boxes containing numbers 1-7 correspond to the different cellular effects that have been attributed, thus far, to D2R activation and signaling on that specific cell type. * indicates disparate findings in the literature. dMSN, direct pathway medium spiny neuron; GP, globus pallidus; VP, ventral pallidum.

Molecular effectors and cellular response to D2Rs

D2Rs belong to a family of $G_{\alpha i/o}$-coupled receptors that also includes the D3 and D4 receptors. Multiple effectors and signaling pathways have been identified as mediators of the cellular response to D2R activation. It is important to be aware that the expression of these effectors varies among the different neuronal types expressing D2Rs, and as such, the cellular and behavioral responses to D2R activation are also heterogeneous throughout the brain.

The canonical molecular and cellular effects downstream of D2R activation have been attributed to the G protein-dependent inhibition of adenylyl cyclase and subsequent reduction of intracellular levels of cyclic adenosine monophosphate (cAMP). D2R-mediated regulation of cAMP production suppresses the activity of protein kinase A (PKA) and of the dopamine- and cAMP-regulated phosphoprotein-32 (Fig. 1, #1; DARPP-32, Beaulieu & Gainetdinov 2011; Kebabian & Calne 1979; Kebabian & Greengard 1971). D2R agonists also activate extracellular signal regulated kinases (ERK) primarily through a G protein-dependent mechanism (Lan *et al.* 2009; Peterson *et al.* 2015b; Fig. 1, #1). Through membrane delimited protein interactions, activation of D2Rs can also inhibit voltage-gated calcium channels (VGCC) to decrease intracellular calcium concentration (Fig. 1, #4), and activate G-protein gated inward rectifying potassium (GIRK) channels to hyperpolarize the membrane potential of neurons (Fig. 1, #3; Kuzhikandathil *et al.* 1998; Lavine *et al.* 2002; Missale *et al.* 1998; Nishi *et al.* 1997). Dissociation of $G_{\beta\gamma}$ subunits activates GIRK channels with a time constant on the order of hundreds of milliseconds (Logothetis *et al.* 1987; Wickman *et al.* 1994). As a result of this signaling, neurons with high expression of GIRK channels, such as in midbrain dopamine neurons, show a marked hyperpolarization and fast inhibition of action potential firing upon D2R activation (Anzalone *et al.* 2012; Beckstead *et al.* 2004; Bello *et al.* 2011; Paladini *et al.* 2003). In contrast, D2R activation in striatal MSNs and cholinergic interneurons (CINs) has been shown to result in an inhibition of L- and N-type voltage-gated calcium channel activity (Hernandez-Lopez *et al.* 2000; Yan *et al.* 1997), as well as in a reduction of the magnitude of calcium transients in the dendrites of striatal MSNs, measured using 2-photon laser microscopy (Day *et al.* 2008; Mizuno *et al.* 2007). This D2R mediated inhibition of calcium influx is thought to mediate the potent D2R-mediated inhibition of neurotransmitter release from dopamine terminals and GABAergic terminals from iMSNs (Adrover *et al.* 2014; Bello *et al.* 2011; Dobbs *et al.* 2016; Jones *et al.* 1999; Fig. 1, #6).

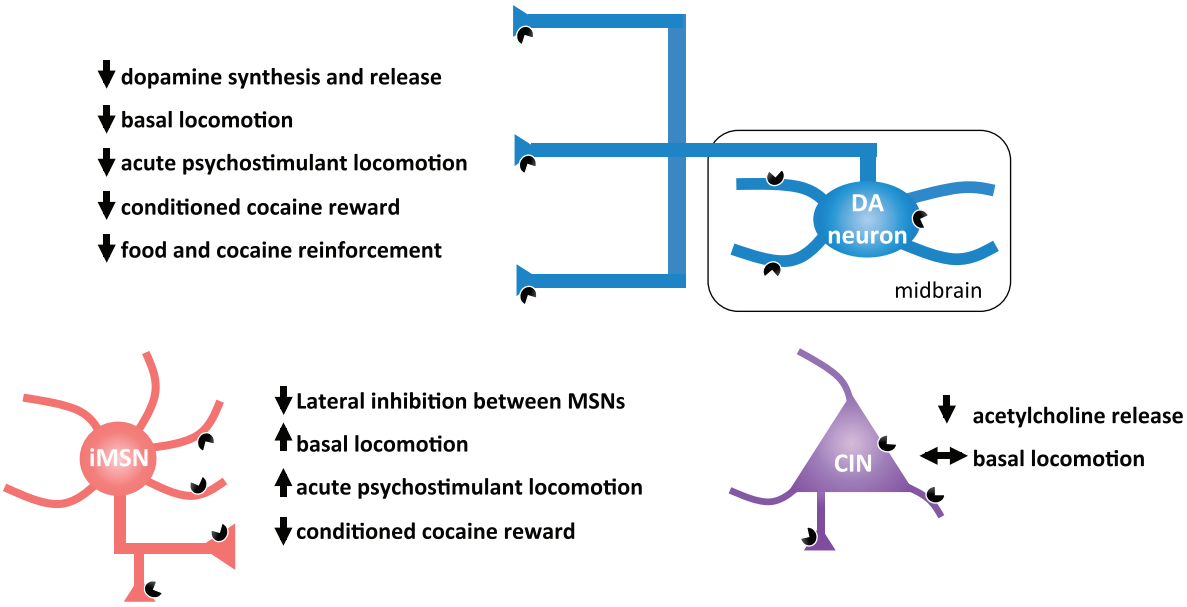

Figure 2: Behavioral and cellular consequences of activation of D2Rs localized to different cell-types. Schematic diagram depicting the different behavioral and cellular functions of D2R activation and signaling at different location and cell-types within the basal ganglia. D2Rs on dopamine terminals are critical for exerting inhibitory control over dopamine synthesis and release as well as the psychomotor, rewarding and reinforcing properties of cocaine. D2Rs on iMSNs are critical for constraining GABAergic transmission to promote basal locomotion and acute psychomotor activation in response to cocaine. D2Rs on cholinergic interneurons regulate acetylcholine release without affecting basal locomotion (double-ended arrow).

A G-protein independent mechanism has also been reported to contribute to the cellular and behavioral effects of D2R activation. Non-selective dopamine receptor agonists and psychostimulants regulate Akt and glycogen synthase kinase-3 (GSK3) signaling pathways via the activation of beta-arrestin signaling molecules (for a full review see (Beaulieu *et al.* 2011; Beaulieu *et al.* 2009). The beta-arrestin pathway has traditionally been associated with receptor desensitization and internalization (Shenoy & Lefkowitz 2011). However, it is now known that beta-arrestin dependent activation of MAP kinases can regulate the trafficking, insertion and conductance of ion channels important for intrinsic excitability and plasticity (Thomas & Huganir 2004). Biased D2R ligands (Allen *et al.* 2011; Free *et al.* 2014), mutant mouse lines (Beaulieu *et al.* 2005), and evolutionary trace analysis (Peterson *et al.* 2015a) have also revealed significant contributions of the beta-arrestin-dependent signaling pathways in mediating the functional consequences of D2R activation (Fig. 1, #2). For example, a recently generated biased D2R ligand devoid of $G_{i/o}$ protein signaling but with partial agonism for the beta-arrestin pathway mitigated schizophrenia-like behaviors in a rodent model, such as hyperlocomotion, impaired pre-pulse inhibition and novel object recognition, and enhanced conditioned avoidance, while inducing a much lower level of catalepsy than haloperidol (Park *et al.* 2016).

Changes in gene expression are reported upon chronic manipulations of D2R activation levels. Low D2R activation causes an enhancement of prepro-enkephalin mRNA and a decrease of Substance P mRNA in the striatum (Fig. 1, #7; Baik *et al.* 1995; Gerfen *et al.* 1990; Romano *et al.* 1987). Further, changes in the excitability of striatal neurons have been reported following chronic overexpression and down-regulation of D2Rs (Cazorla *et al.* 2012; Lemos *et al.* 2016). These excitability changes, as well as the decreases in dendritic branching seen after D2R overexpression (Cazorla *et al.* 2012), likely involve changes in gene expression.

xThe large variety of signaling pathways and effectors engaged by D2Rs provide ample opportunity for cell-type specificity in the cellular response to dopamine, which goes beyond the receptors subtypes. At the same time, the diversity sometimes complicates the interpretation of pharmacological experiments and precludes us from making generalizations and extrapolations based on the D2R effect in one neuronal type to another.

Cell-type specific cellular and synaptic consequences of D2R activation

The next section focuses on the cellular functions of D2Rs expressed on midbrain dopamine neurons, striatal GABAergic MSNs, and to lesser extent, striatal CINs. Dissecting out the role of the striatal D2Rs expressed in the different cells-types within the striatum is critical for ultimately revealing the mechanism by which low D2R levels can facilitate SUDs. Indeed, it is possible that only D2Rs expressed within one cell type are responsible for triggering the vulnerability.

Alternatively, it is possible that low levels of D2R expression at each different cell-type makes small contributions that summate or even synergize to confer vulnerability for SUDs.

D2 autoreceptors mediate feedback inhibition over dopamine levels at synapses

D2Rs expressed on dopamine neurons are often referred to as D2 autoreceptors because they are activated by dopamine released from the same neuron, or neighboring neurons, as discussed below. In midbrain dopamine neurons of the ventral tegmental area and the substantia nigra *pars compacta*, D2 autoreceptors suppress both the firing of action potentials and dopamine release from these neurons. Decades of research by the group of JT Williams and others have shown that D2 autoreceptor activation inhibits dopamine neuron excitability by increasing potassium conductances and hyperpolarizing the membrane potential (Fig. 1, #3,5; Beckstead *et al.* 2004; Lacey *et al.* 1987; Luscher & Slesinger 2010; Mercuri *et al.* 1997). Further, local electrical stimulation delivered with a pattern that mimics the burst firing of dopamine neurons (5 pulse, 40 Hz, 0.5 milliseconds duration) can evoke slow inhibitory post-synaptic currents (IPSCs) that are mediated by activation of D2 autoreceptors in midbrain dopamine neurons (Fig. 1,#3; Beckstead *et al.* 2004, 2007; Ford *et al.* 2006). This local stimulation protocol in the midbrain was shown to generate dopamine transients that directly precede the slow D2R-mediated IPSCs in dopamine neurons (Ford *et al.* 2009). Thus, synchronized burst firing of action potentials triggers dopamine release, presumably from somatodendritic compartments of neighboring neurons, that in turns activates D2 autoreceptors to evoke a slow hyperpolarization that prevents further firing for a few seconds. Further confirmation came from experiments using targeted deletion of D2 autoreceptors to dopamine neurons, which resulted in a loss of the evoked D2R-IPSCs and of the D2R-like agonist mediated suppression of firing of dopamine neurons (Fig. 1, #3,5; Anzalone *et al.* 2012; Bello *et al.* 2011). It is indeed thought that D2 autoreceptors mediate critical feedback inhibition of this circuit by limiting dopamine neuron firing and neurotransmitter release (as discussed below).

Bath application of cocaine enhances evoked D2R-IPSCs by blocking the dopamine transporter and increasing the extracellular dopamine concentration upon release (Beckstead *et al.* 2004). A recent study by Gantz et al. also revealed the existence of spontaneous D2R-IPSCs, which were observed in the absence of stimulation and were insensitive to the sodium channel blocker TTX. These miniature D2R-IPSCs are thought to result from the spontaneous release of single vesicles of dopamine from DA neurons (Gantz *et al.* 2015a). Interestingly, a single non-contingent administration of cocaine (20 mg/kg) enhanced both evoked and spontaneous D2R-IPSCs suggesting that the magnitude of the D2 autoreceptor response is plastic and can be regulated by stimulants (Gantz *et al.* 2015a).

D2 autoreceptors are also localized to pre-synaptic terminals in the striatum from dopamine neuron projections. These presynaptic D2 autoreceptors exert a potent and reliable inhibition of dopamine release that can be measured using fast scan cyclic voltammetry (IC_{50} for quinpirole of ~30 nM in rodents; (Fig. 1, #6 Adrover *et al.* 2014; Bello *et al.* 2011; Ding *et al.* 2010; Groves & Wilson 1980; Kennedy *et al.* 1992; Lemos *et al.* 2016; Phillips *et al.* 2003). It remains unclear whether this effect is due to suppression of calcium channel activation or activation of GIRKs on the terminals. Presynaptic D2R-mediated inhibition is engaged by endogenous dopamine during trains of stimulus pulses that mimic the phasic firing pattern of dopamine neurons. Indeed, both sulpiride pre-treatment and selective deletion of D2Rs from dopamine neurons enhance the peak and area of dopamine transients evoked by trains of electrical stimuli (Fig. 1, #6; Bello *et al.* 2011; Lemos *et al.* 2016). Another condition during which D2 autoreceptors are engaged is during exposure to cocaine. By blocking dopamine transporters and enhancing extracellular dopamine levels around the release sites, cocaine engages D2Rs on the presynaptic terminals of dopamine neurons and further inhibits dopamine release (Adrover *et al.* 2014; Bello *et al.* 2011; Holroyd *et al.* 2015). In the absence of D2 autoreceptors, cocaine application leads to an even larger amount of extracellular dopamine (Holroyd *et al.* 2015). Furthermore, the deletion also results in increased TH activation and suggests increased dopamine synthesis (Anzalone *et al.* 2012; Bello *et al.* 2011). Collectively these recent findings generated from cell-specific Drd2 knockout mice demonstrate a larger extracellular concentration of dopamine at synaptic sites in the absence of D2R-mediated inhibitory regulation.

D2R regulation of cholinergic interneuron activity

There is strong evidence that D2Rs are localized to CINs within the striatum (Maurice *et al.* 2004). Less understood is the functional role of D2Rs on CINs and very little known regarding the effect of cocaine on these cells. Microdialysis studies have demonstrated that D2Rs can suppress the release of acetylcholine (Fig. 1, #6; DeBoer & Abercrombie 1996; DeBoer *et al.* 1996). In an *in vitro* slice preparation, agonist for D2/3 receptors can reduce the firing rate by inhibiting NaV channels (Fig. 1, #5; Maurice *et al.* 2004). CINs have both tonic and phasic firing modes. Following thalamocortical stimulation, CINs can shift into a burst-pause mode, in which the pause following the burst is blocked by D2/3 antagonist (Fig. 1, #5; Ding *et al.* 2010). Furthermore, selective optogenetic stimulation of dopamine fibers generates a D2R-dependent pause in CIN firing rate and produces a 'burst-pause' activity mode in CINs located in the NAc shell (Fig. 1, #3,4,5; Chuhma *et al.* 2014). Finally, deletion of D2Rs from CINs did not produce changes in tonic firing rate or intrinsic membrane properties, but significantly reduced the 'pause' component when the cell was stimulated and shifted into a 'burst-pause' mode (Fig. 1, #5; Kharkwal *et al.* 2016a). Collectively, these data suggest that D2Rs in CINs play a more prominent role in regulating phasic activity of these interneurons.

Acute cocaine application appears to prolong the pause in CINs, presumably by increasing D2R activity, although this has not been unequivocally shown (Ding *et al.* 2010). Another study showed that *in vitro* cocaine application increased firing of CINs (Witten *et al.* 2010). Further studies are needed to

clarify the actions of cocaine in CIN activity and the cellular and synaptic mechanisms involved.

In addition to releasing acetylcholine, activity of CINs can evoke dopamine release via activation of nicotinic acetylcholine receptors present on dopamine axons (Cachope *et al.* 2012; Threlfell *et al.* 2012). It was recently demonstrated that selective deletion of D2Rs from CINs leads to a 10% reduction in D2R mediated inhibition of dopamine overflow indicating a small contribution of D2Rs on CINs to the overall D2R-mediated inhibition of dopamine release (Kharkwal *et al.* 2016a) . Further, activation of D2Rs on CINs are thought to be required for striatal long-term depression (LTD) of glutamatergic synapses on MSNs by reducing ACh release from CINs and subsequently limiting M1 muscarinic receptor activation on MSNs (Wang *et al.* 2006). These are two examples by which D2Rs on CINs can indirectly affect the output of striatal neurons and the dopamine modulation. While there has been some progress in understanding the role of D2Rs on CINs, more research is needed to improve our understanding of the role of these D2Rs in shaping striatal connectivity and the cellular and behavioral responses to cocaine.

D2Rs in medium spiny neurons suppress lateral inhibition between striatal neurons

The highest level of D2R expression is found in striatal GABAergic MSNs, which project to the globus pallidus (GP) and ventral pallidum (VP) to form the indirect-pathway output of the striatum. These D2Rs are found in the somatodendritic compartments as well as in the synaptic terminals from these indirect-pathway MSNs (iMSNs) in rodents (Delle Donne *et al.* 1996, 1997). Decades of pharmacological studies in combination with the current knowledge of the circuitry support the idea that activation of D2Rs inhibits the output of iMSNs to foster firing in the GP/VP and mediate locomotion. By extrapolating from what it is known of D2 autoreceptors, the most common sense hypothesis is that D2Rs in iMSNs activate K^+ conductances to decrease excitability and inhibit neuronal firing. However, the direct effects of D2R activation in iMSNs have been inconsistent and sometimes hard to detect, in large part because these neurons are intermingled with identically looking MSNs that express D1Rs instead of D2Rs. There are reports that D2R-like agonists induce membrane hyperpolarization in iMSNs in rat brain slice preparations, suggestive of D2R coupling through GIRK (Fig. 1, #5; Ferguson *et al.* 2011; Lalchandani *et al.* 2013; Orefice *et al.* 2013; Surmeier *et al.* 2011). However, these effects are not reproduced in mouse preparations where iMSNs can be visually identified, as D2R-like agonists fail to produce any significant reduction of intrinsic excitability of iMSNs (Fig. 1, #5; Dobbs *et al.* 2016; Lemos *et al.* 2016). Furthermore, expression of GIRK channels has yet to be confirmed in iMSNs.

D2Rs in iMSNs have been shown to decrease calcium currents and subsequent SK channel activity during the up-states of these neurons (Fig. 1, #4; Hernandez-Lopez *et al.* 2000; Tritsch & Sabatini 2012). By inhibiting calcium signals, D2Rs in iMSNs can also affect the phosphorylation and function of other channels and receptors important in regulating excitability under specific conditions and also can affect gene expression, which could lead to long-term changes in excitability that have been reported following D2R activation and D2R overexpression or knockdown (Cazorla *et al.* 2012; Kourrich & Thomas 2009; Lemos *et al.* 2016).

Possibly one of the most reliable and fast consequences of D2R activation in iMSNs is the suppression of inhibitory synaptic transmission from these neurons within the striatum. Axons from iMSNs form collateral projections that extend within the striatum and form inhibitory GABA synapses on neighboring MSNs, in addition to their long-range projections to GP and VP (Smith *et al.* 1998; Wilson & Groves 1980). The D2R-like agonist quinpirole inhibits GABA synaptic transmission by about 50% from iMSNs to neighboring MSNs in the dorsal striatum, shown using paired recordings (Tecuapetla *et al.* 2009), and following synchronized optogenetic stimulation of iMSN axon collaterals in the nucleus accumbens (Dobbs *et al.* 2016; Fig. 1, #6). Quinpirole also reliably inhibits the amplitude of IPSCs recorded from pairs of MSNs in slices and primary cultures of striatal neurons, while it inhibits only a third of the synaptic connections between pairs of fast-spiking interneurons and MSNs (Fig. 1, #6; Cazorla *et al.* 2014; Kohnomi *et al.* 2012). In all cases, the inhibition was blocked by a D2R antagonist and it was absent in mice lacking D2Rs only in iMSNs (Dobbs *et al.* 2016). The fast and potent suppression of synaptic transmission suggests that D2Rs are localized to presynaptic terminals where they most likely inhibit calcium channels and reduce neurotransmitter release (Fig. 1, #6; Salgado *et al.* 2005). Simultaneous stimulation of GABA inputs from multiple iMSNs reduced action potential firing in D1R-expressing direct-pathway MSNs (dMSNs), indicating that this lateral inhibition is indeed potent enough to control the output of the dMSNs. By suppressing the lateral inhibition onto dMSNs, D2Rs in iMSNs disinhibit the firing of action potentials in dMSNs, thus gating basal ganglia output (Dobbs *et al.* 2016). Surprisingly, recent data revealed that D2R-like agonists are much less efficacious at suppressing transmission from iMSN long-range projections to VP neurons (Dobbs *et al.* 2016), suggesting either differential targeting/trafficking or coupling efficacy of D2Rs on local axon collaterals vs. long-range axonal projections. Taking the old and new findings together, a clearer picture begins to emerge, in which activation of D2Rs in iMSNs leads to a reduction of indirect-pathway output. However, the mechanisms involved appear to be less related to changes in intrinsic excitability, but rather downstream of action potential firing, and mediated by a potent inhibition of neurotransmitter release at axon collaterals within the striatum. At the circuit level, the most prominent function of these D2Rs is to suppress the lateral inhibition between striatal neurons and as consequence, D2Rs in iMSNs have a unique and critical role in controlling striatal output of both the indirect and direct pathways.

Expression levels of D2R in MSNs have profound impact on basal ganglia circuit function

Individuals with SUDs appear to have a chronic reduction of D2R function. In animal models, alterations in the levels

of D2R activation not only affect how the striatum acutely responds to dopamine or stimulants, but also triggers a profound re-organization of basal ganglia circuitry, which could be the key for understanding the vulnerability to drug abuse and changes in patients with chronic substance use disorder. Significant cellular changes and restructuring of synaptic strength and connectivity in the basal ganglia were reported following either up- or down-regulation of striatal D2Rs, which seems to be the root cause of hyperactivity and bradykinesia, respectively (Fig. 3). Kellendonk and colleagues reported that generalized D2R overexpression in the striatum results in down-regulation of Kir 2.1/2.3 channels, which are potassium channels that contribute to the passive conductance of MSNs, and result in higher excitability of MSNs (Cazorla *et al.* 2012). In addition, D2R overexpression reduces dendritic arborization in MSNs and causes an overall reduction in striatal volume. In a subsequent study, investigators used Cre-dependent expression of a dominant negative Kir2.1/2.3 channel to reduce Kir function in a cell-specific manner targeted to iMSNs. This manipulation enhanced MSN excitability as well as led to an increase in bridging collaterals from dMSNs to the GPe, (Cazorla *et al.* 2014), an anatomical and functional connection which is normally relatively weak. The enriched density of bridging collaterals resulted in enhanced GABAergic transmission onto GPe neuron's activity upon direct-pathway activation, indicating that the bridging collaterals form functional inhibitory synapses. Both non-specific and iMSN-specific D2R-overexpression caused a restructuring of striatal circuit connections as well as behavioral changes defined as hyper-locomotion and increased water consumption (polydipsia) (Gallo *et al.* 2015). There is also evidence that transient D2R overexpression in the striatum decreases alcohol consumption (Surmeier & Kitai 1997), however, it this has not been confirmed yet with iMSN-specific overexpression of D2Rs (Gallo *et al.* 2015).

In a recent complimentary study from our group, targeted deletion of D2Rs from iMSNs caused a reduction in the *in vivo* firing of MSNs and pallidal neurons in awake, behaving mice. A reduction in the intrinsic excitability was also observed in iMSNs with low expression of D2R. However, the most significant changes were observed in neurons that did not even express D2Rs: a large increase of GABAergic synaptic transmission was observed in D1R-expressing dMSNs and in globus pallidus neurons, the two major synaptic targets of iMSNs (Lemos *et al.* 2016). In dMSNs, there were larger $GABA_A$ receptor mediated tonic currents, measured as gabazine-sensitive holding current, as well as enhanced fast synaptic transmission (higher frequency and amplitude of miniature IPSC). In the GPe, low levels of D2Rs in iMSNs produced a robust increase in the frequency with no change in amplitude of $GABA_A$ receptor mediated mIPSC, suggestive of enhanced release probability following D2R deletion. There are several sources of GABAergic input onto dMSNs and GPe neurons; iMSNs being only one of them. Thus, striatal interneurons (e.g. fast-spiking (FSI) and low-threshold spiking (LTS)), as well as dopamine terminals and GPe feedback connections, can also contribute to the enhanced inhibition observed in the striatum and GP upon D2R deletion

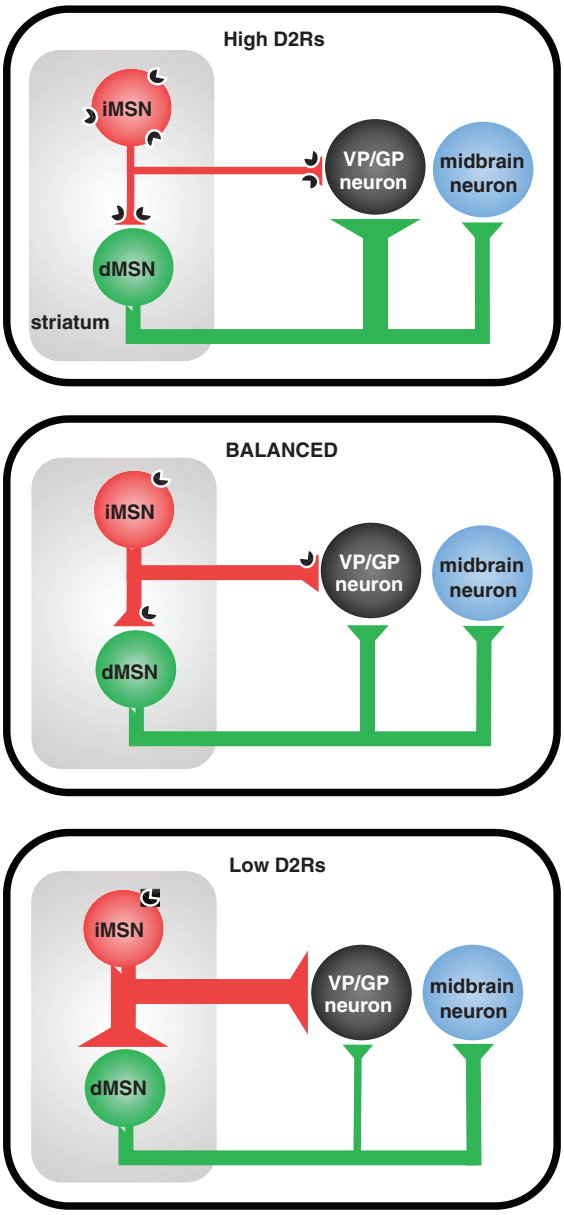

Figure 3: Alterations in D2R levels can lead to aberrant GABA transmission resulting in an imbalance in indirect and direct pathway output. Schematic diagram depicting the hypothesized role of D2R expression levels (top: high, middle: balanced, bottom: low) in iMSNs in regulating the functional balance of iMSN and dMSN output.

(Gittis *et al.* 2011; Glajch *et al.* 2016; Tritsch *et al.* 2012). It has been suggested that MSNs form synaptic connection onto distal dendrites of other MSNs, while FSIs form synaptic connections onto the somas of MSN, based on amplitude and decay time data of evoked IPSC (Tepper *et al.* 2004). Thus, it is might be possible to use mIPSC amplitude information to segregate inputs from different GABAergic sources. However, targeted deletion of D2Rs from iMSNs

produces an enhancement in mIPSC frequency across all amplitudes suggesting a strengthening of iMSN to MSN connectivity as well as FSI to MSN connectivity as is seen in dopamine-depletion models(Gittis *et al.* 2011). Interestingly, acute activation of G_i-signaling using DREADDs reversed the increase in GABA-mediated tonic current and restored levels to normal with no change in mIPSC frequency or amplitude. These data suggested that other sources of GABA transmission, in addition to iMSNs, are also strengthened upon downregulation of D2Rs in iMSNs. Moreover, it indicates that, at least within the striatum, iMSN collateral transmission onto neighboring dMSNs serves to constrain excitability by modulating the size of the GABA tonic current.

Selective deletion of D2Rs in iMSNs produces a robust and reliable decrease in locomotor activity (Anzalone *et al.* 2012; Kharkwal *et al.* 2016b; Lemos *et al.* 2016). To test whether the motor deficit produced by downregulation of D2Rs in iMSNs was due to the increased GABAergic transmission within the striatum, a sub-threshold dose of the GABA-A antagonist picrotoxin was infused into the striatum. While the sub-threshold dose of picrotoxin had no effect on locomotor activity in control mice, it increased locomotion in mice lacking D2Rs in iMSNS (Lemos *et al.* 2016). Further, chemogenetic tools were used to selectively inhibit GABA transmission from iMSNs in the dorsal and ventral striatum, which also led to higher locomotion and confirmed that the enhanced GABA transmission from iMSNs is a main driver of the motor deficit observed in mice lacking D2Rs. In summary, D2Rs localized to iMSNs acutely inhibit GABA and inhibitory synaptic transmission to regulate the output of the indirect-pathway and, at the same time, through the collateral axon connections within the striatum, D2Rs also control the excitability of dMSNs and the strength of connectivity across the entire basal ganglia circuit. Alteration of D2R levels or function results in system-wide restructuring of connectivity that impact the basal ganglia output and behavior and are likely to contribute to the vulnerability to develop SUD.

Regulation of behavioral response to cocaine by D2Rs

Use of conventional pharmacology or global D2R deletion has provided strong evidence for the various roles D2Rs play in behavioral responses to cocaine. However, in some instances these studies have yielded conflicting results. In the following section, it will become clear that in some instances D2R activation at dopamine terminals and D2R activation at iMSN terminals produce opposite behavioral effects, while in other instances they seem to act cooperatively. Being aware of the cell-specific functions of D2Rs can help reconcile some of the controversies that presently exist in the field.

D2 autoreceptors limit the behavioral responses to cocaine

Given the acute *in vitro* effects of cocaine on D2 autoreceptor function, both at somatodendritic and synaptic bouton compartments, it stands to reason that downregulation of D2 autoreceptors would have an effect on behavioral responses to cocaine. Mice lacking D2 autoreceptors (autoDrd2KO) have increased basal locomotion and are more sensitive to the acute locomotor stimulatory effect of cocaine (Anzalone *et al.* 2012; Bello *et al.* 2011; Kharkwal *et al.* 2016b). In addition, these mice showed conditioned place preference for a low dose of cocaine that did not produce preference in littermate controls, indicating a heightened sensitivity to the rewarding properties of cocaine (Bello *et al.* 2011). The reinforcing properties of cocaine and the incentive salience of cocaine predictive cues were also enhanced when D2 autoreceptor mediated feedback inhibition was absent (Holroyd *et al.* 2015). Additionally, a higher percentage of autoDrd2KO mice met the acquisition criteria for cue-induced cocaine self-administration and showed impaired extinction of cocaine seeking, specifically when drug-paired cues were present (Holroyd *et al.* 2015). These findings are particularly relevant to patient studies, in which individuals suffering from stimulant addiction have a difficult time suppressing drug-seeking behavior in the presence of drug-associated cues (Childress *et al.* 1993, 1994). Acute down-regulation of the Drd2 gene in adult rats using shRNAs caused a similar increase in the locomotor response to cocaine and increase in the progressive ratio for cocaine (de Jong *et al.* 2015). Neither acute nor long-term decrease in D2 autoreceptors affected the rate of operant responding for cocaine, daily cocaine intake, nor changed the ability to extinguish cocaine seeking behavior when drug-associated cues were not present (Holroyd *et al.* 2015). Thus, the behavioral alterations induced by low levels of D2 autoreceptor activation are very specific and sometimes subtle. However, they point to a critical role of D2 autoreceptors in titrating the dopamine concentration at synapses, a mechanism that is especially important when cocaine is on board and the feedback inhibition is engaged. Deficiency in the feedback inhibition then causes larger dopamine transients indicative of a higher concentration of extracellular dopamine, which in turn strengthens learning and the association between cues and drugs.

Motivation for food was also increased under high effort conditions (FR100) following prolonged and acute down-regulation of D2 autoreceptors (Bello *et al.* 2011; de Jong *et al.* 2015). However, when trained under low effort (FR1), no significant changes in progressive ratios for food reward were detected (Holroyd *et al.* 2015).

Thus, reduced D2 autoreceptor signaling on dopamine neurons contributes to a context-dependent increase in the rewarding and reinforcing properties of cocaine in rodents. This could lead to higher vulnerability to engage in cocaine taking behavior and stronger salience of drug paired cues, which could enhance craving and increase the likelihood of relapse. Most PET studies in human and non-human primate have focused on quantifying D2R levels in the caudate/putamen (striatum) because it has the highest density of D2Rs. The assumption has been that decreases in D2R availability seen in abusers is due to low D2Rs in MSNs. However, D2 autoreceptors localized to presynaptic dopamine terminals are likely to contribute to the total D2R availability and also to the vulnerability to abuse and dependence.

Indeed, two recent studies in humans found that low levels of midbrain D2R availability are associated with increased craving for amphetamine as well as increased impulsivity and novelty seeking traits (Buckholtz *et al.* 2010; Zald *et al.* 2008).

D2Rs in MSNs contribute to the acute stimulant response to cocaine

New evidence indicates that cocaine relies on D2Rs in iMSNs, likely localized to axon collaterals within the nucleus accumbens, to elicit its canonical acute increase in locomotion. In the *in vitro* slice preparation, cocaine, like a D2/3 agonist, suppresses the GABAergic synaptic transmission from iMSNs to dMSNs by activation of D2Rs expressed in iMSNs. Mice lacking these receptors lose the suppression of collateral transmission and show a severely depressed cocaine-induced locomotion, demonstrating that D2Rs in iMSNs are required for the acute locomotor response to cocaine. At the same time, chemogenetic rescue experiments indicated that G_i-signaling in iMSNs, which normally occurs following D2R activation, restores the suppression of collateral transmission between MSNs and is sufficient to induce a mild locomotor response that can be further enhanced when paired with cocaine. Thus, a parsimonious interpretation of these results is that activation of D2Rs in iMSNs acts to suppress the inhibition onto dMSNs to allow for direct activation of these neurons by dopamine (likely via D1Rs). Thus, D2Rs in iMSNs act more as a gate that permits cocaine-induced locomotion rather than mediating the psychomotor response *per se* (Dobbs *et al.* 2016). Another interesting finding is that, despite the blunted locomotor response to cocaine, mice with targeted deletion of D2Rs to iMSNs show locomotor sensitization upon repeated cocaine exposure and also cocaine conditioned place preference (see section below). These new findings are in agreement with previous data showing that administration of the D2R-like antagonist sulpiride into the nucleus accumbens attenuates cocaine-induced locomotion (Baker *et al.* 1996; Neisewander *et al.* 1995). Similarly, global D2R deletion abolishes the acute locomotor response to cocaine (Chausmer & Katz 2001; Chausmer *et al.* 2002; De Mei *et al.* 2009; Sim *et al.* 2013; Welter *et al.* 2007).

Thus, cocaine-induced locomotion is mediated by an increase in striatal dopamine acting on two synergistic mechanisms: (1) via direct activation of D1Rs in dMSNs, and (2) via indirect activation of D2Rs in iMSNs, which effectively 'lifts the break' onto neighboring dMSNs. Furthermore, the attenuated acute cocaine locomotor response in mice lacking D2Rs in iMSNs does not appear to arise from developmental alterations from early life deletion of D2Rs since mice with a D2R deletion introduced in adulthood and restricted to the nucleus accumbens also exhibit a blunted locomotor response to acute cocaine.

D2Rs regulate cocaine induced behavioral plasticity and reinforcement

The role of striatal D2Rs in reward learning, behavioral plasticity and self-administration has been controversial in that there have been several conflicting findings within the literature. Locomotor sensitization, defined as enhanced locomotor response to repeated stimulant challenge, has been proposed as a behavioral read-out of cocaine-induced plasticity at the circuit level. Interestingly, in contrast to the effect on the acute locomotor response to cocaine, targeted deletion of D2Rs in iMSNs does not impair locomotor sensitization to cocaine (Anzalone *et al.* 2012; Dobbs *et al.* 2016), demonstrating a dissociation of the role of D2Rs in iMSNs between the acute locomotor response to cocaine and the long-term plasticity that occurs following repeated cocaine exposures. Sensitizat ion is also observed when D2R deletion is restricted to the ventral striatum and induced in adulthood, suggesting that developmental compensation following early-life D2R deletion is not driving this phenotype (Dobbs *et al.* 2016). These findings are consistent with the global D2R knockout mice, which similarly show no effect of D2R deletion on cocaine locomotor sensitization (Sim *et al.* 2013). More transient manipulations of D2R-like Gi-signaling using chemogenetic and optogenetic tools have lead to opposite results and suggest that D2Rs can regulate sensitization. For example, selective activation of hM4Di in iMSNs, the G_i-coupled Designer Receptor Exclusively activated by Designer Drugs (DREADDs), facilitated the development of amphetamine locomotor sensitization in rats (Ferguson *et al.* 2011). Conversely, optogenetic activation of D2R-containing iMSNs suppressed the development and expression of cocaine locomotor sensitization (Lobo *et al.* 2010). There are several factors that could possibly account for the difference in the results, such as the stimulant drug used (amphetamine vs. cocaine), the species (mouse vs. rat), and the different signaling and cellular effects of activating D2Rs, hM4Di, and optogenetic stimulation. However, the most relevant distinction between these studies is likely the transient vs. long-term nature of these perturbations. It is becoming apparent that long-term perturbations (weeks) of D2R levels can produce different phenotypes, and sometimes even opposite results. Unpublished work indicates that mice lacking D2Rs in iMSNs also display a hypersensitive behavioral response to D1R-like agonists and upregulation of D1R signaling, which might prime these mice for cocaine locomotor sensitization.

The conditioned rewarding properties of stimulants have been classically assessed using the conditioned place preference procedure, which involves pairing non-contingent stimulant administration with distinct contexts and cues (Mucha *et al.* 1982). The activity and expression of D2Rs have been implicated in regulating the conditioned rewarding and reinforcing effects of psychostimulants. For example, global D2R knockout mice have normal cocaine place preference (Sim *et al.* 2013; Welter *et al.* 2007). Selective deletion of D2 autoreceptors enhances the acquisition of cocaine conditioned place preference and increases striatal dopamine release (Bello *et al.* 2011). Additionally, mice with a selective D2R deletion from striatal iMSNs acquire cocaine conditioned place preference faster as littermate controls (Dobbs *et al.* 2016).

Studies utilizing pharmacological and global genetic manipulations also suggest a complex role for D2Rs in mediating the reinforcing effects of cocaine. The effect of D2R-like

antagonists on self-administration varies depending on the dose of D2R antagonist and cocaine used. Generally, D2R-like antagonists dose-dependently facilitate self-administration of cocaine, particularly at high doses of cocaine. However, high-doses of D2R-like antagonist produce catalepsy and therefore, inhibit cocaine self-administration as well as many other behavioral outputs (Britton *et al.* 1991; Caine & Koob 1994; Caine *et al.* 2002; Hubner & Moreton 1991; Woolverton 1986). Similarly, global D2R deletion enhances self-administration of high-dose cocaine (Caine *et al.* 2002). It should be noted that at low doses of cocaine, D2 antagonism or deletion produces the opposite or no effect of self-administration. In light of what it is now known about the function of D2Rs localized to different cell-types in the striatum, it is reasonable to interpret these biphasic effects of D2R antagonist/deletion as the result of targeting D2 autoreceptors (shown to be engaged when cocaine is on board) at low doses and D2Rs in iMSNs and CINs causing reduced movement (bradykinesia) and catalepsy at higher doses.

The role of D2Rs in reinstatement to cocaine seeking

Relapse to stimulant abuse has been modeled in animals using reinstatement paradigms, which involves acutely exposing an animal to a drug prime, stressor or drug-paired cue following drug conditioning and extinction (Epstein *et al.* 2006). Multiple acute manipulations result in the reinstatement to drug-seeking behavior and it is possible the pathways underlying each manipulation's effects on reinstatement are different. In the case of D2R regulation of reinstatement behavior, it has been shown that global D2R knockout mice have normal drug-induced reinstatement of cocaine seeking, but do not exhibit stress-induced cocaine seeking (Sim *et al.* 2013). This is in contrast to the findings that D2R-like antagonists classically attenuate drug-primed reinstatement of cocaine seeking (Khroyan *et al.* 2000; Spealman *et al.* 1999). Both D2R-like agonists and the chemogenetic activation of G_i-signaling specifically in iMSNs enhanced motivation for cocaine seeking as measured using drug-primed reinstatement and progressive ratio, respectively (Bock *et al.* 2013; Fuchs *et al.* 2002; Self *et al.* 1996). Moreover, the administration of D2R-like agonists alone reinstate cocaine-seeking behavior (De Vries *et al.* 1999; Fuchs *et al.* 2002; Khroyan *et al.* 2000; Self *et al.* 1996; Spealman *et al.* 1999). These disparate findings again highlight potential differences between chronic reduction/ablation of D2Rs compared to acute pharmacological manipulations of D2R activity.

The general picture that emerges is that low D2R activity facilitates cocaine self-administration but inhibits reinstatement; however, in order to fully parse out the effect of D2R in regulating cocaine taking, seeking and relapse, manipulations that can be targeted specifically to each cell-type expressing D2Rs in the striatum are required. In particular, an important next step is to assess the roles of D2Rs in iMSNs on the self-administration of psychostimulants. For example, studies using mice with targeted deletion of D2Rs to iMSNs could directly test the current theory linking low D2Rs to stimulant abuse. In this case, the use heterozygote mice will be the most relevant since the partial reduction of D2R levels better resemble the down-regulation levels of striatal D2R availability observed in human abusers (Volkow *et al.* 1993, 2001).

Summary and final conclusions

The heterogeneity of D2R expression and function throughout the CNS and within striatum is profound and likely to reflect the involvement of D2Rs in regulating multiple diverse brain functions. At the same time, experimentally these factors have lead to paradoxical results and limited the interpretation of findings from studies using pharmacological manipulations or global genetic D2R deletion. Novel transgenic approaches targeting D2R deletion or overexpression to specific cell-types have greatly aided our understanding of how D2Rs regulate striatal function and behavioral output. These more precise manipulations have also revealed a remarkable complexity of the circuit connectivity and new biological sources of variability, such as the difference between acute and long-term perturbations of D2R level function that seem to trigger different behavioral outcomes.

It is important to highlight a gap of knowledge with regards to the behavioral function of D2Rs localized to CIN and to glutamatergic inputs to the striatum. More studies are also needed in regards to their role in the response to stimulant drugs. A recent study in which D2Rs were selectively deleted from CINs showed no effect on basal locomotion, yet selective disruption of catalepsy induced by D2R-like antagonists and impaired locomotor response to D1R-like agonist (Kharkwal *et al.* 2016a).

At this point, more is known about the physiological and behavioral functions of D2 autoreceptors localized to dopamine neurons and terminals than D2Rs in iMSNs (heteroreceptors). Both from a physiological and a behavioral perspective, there are clear similarities and differences in the function of D2Rs localized to these two disparate cell-types. On the one hand, both the autoreceptors and the heteroreceptors act to inhibit synaptic transmission by reducing neurotransmitter release (dopamine and GABA, respectively) from presynaptic terminals. On the other hand, there are differences in the ability of D2Rs to acutely modulate the intrinsic excitability of these neurons: potent inhibition in midbrain dopamine neurons vs. weak/unreliable effect in GABAergic MSNs. Also, D2R mediated changes in gene expression are reported in iMSNs but less in dopamine neurons so far. Interestingly, D2 autoreceptors and D2Rs localized to iMSNs have opposite roles in regulating the locomotor response to acute dopamine elevation or cocaine exposure, with D2 autoreceptors suppressing and D2Rs on iMSNs facilitating both behaviors.

When assessing behavioral responses to cocaine that require more chronic cocaine exposure, D2 autoreceptors and D2Rs in iMSNs appear to have a more synergistic role in constraining the rewarding and reinforcing properties of cocaine. Interestingly, D2 autoreceptors appear to have an important and newly discovered role in limiting cocaine reinforcement and seeking that has been previously underappreciated.

Long-term reduction of D2R expression levels, that likely occurs in the SUD patient population, not only impacts subsequent responses to stimulant drugs and the cues associated with them, but also cause fundamental changes in the connectivity and synaptic strength of the striatal circuit in ways that impact basic brain function and behavior. We then propose that the long-term cellular changes and the synaptic reorganization generate a vulnerability to acquire stimulant use as well as abuse and relapse. These finding are likely to have clinical implications and further our understanding of the factors and mechanisms that drive the vulnerability to develop stimulant drug use and abuse in humans.

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