The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons

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The striatum plays a key role in mediating the acute and chronic effects of addictive drugs, with drugs of abuse causing long-lasting molecular and cellular alterations in both dorsal striatum and nucleus accumbens (ventral striatum). Despite the wealth of research on the biological actions of abused drugs in striatum, until recently, the distinct roles of the striatum’s two major subtypes of medium spiny neurons (MSNs) in drug addiction remained elusive. Recent advances in cell-type-specific technologies, including fluorescent reporter mice, transgenic, or knockout mice, and viral-mediated gene transfer, have advanced the field toward a more comprehensive understanding of the two MSN subtypes in the long-term actions of drugs of abuse. Here we review progress in defining the distinct molecular and functional contributions of the two MSN subtypes in mediating addiction.

Keywords: medium spiny neurons, addiction, nucleus accumbens, cell-type-specific, D1+ MSNs, D2+ MSNs, cocaine, dopamine

INTRODUCTION

Drugs of abuse exert potent molecular and cellular alterations in both dorsal striatum (dStr) and ventral striatum (nucleus accumbens, NAc), and many of these changes occur in medium spiny neurons (MSNs), the principal projection neurons in dStr and NAc, which account for 90–95% of all neurons in these regions. However, researchers have until recently been unable to clearly define the differential role of the two MSN subtypes in addiction-related phenomena. The two MSN subtypes are differentiated by their enrichment of dopamine receptor 1 (D1) or dopamine receptor 2 (D2) as well as several other genes (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990, 1991; Bernard et al., 1992; Ince et al., 1997; Lobo et al., 2006, 2007; Heiman et al., 2008; gensat.org) and by their distinct projections through the cortico-basal ganglia pathway (the direct vs. indirect pathways; Gerfen, 1984, 1992). Early work suggested that drugs of abuse exert most influence on the D1+ MSNs, with the use of numerous dopamine receptor agonists and antagonists providing important insight into the functional and molecular roles of each MSN in drug reward behaviors (Self, 2010). However, current cell-type-specific methodologies, including fluorescent reporter mice that express GFP under D1 or D2 bacterial artificial chromosomes (BACs; Gong et al., 2003; Valjent et al., 2009; gensat.org), conditional mouse models such as the use of tetracycline-regulated inducible transgenic mice (Chen et al., 1998; Kelz et al., 1999), and transgenic mice expressing Cre-recombinase using D1 or D2 BACs, yeast artificial chromosomes (YACs), or knock-in mice (Gong et al., 2007; Lemberger et al., 2007; Heusner et al., 2008; Parkintra et al., 2009; Valjent et al., 2009; Bateup et al., 2010; Lobo et al., 2010; gensat.org) as well as cell-type-specific viral-mediated gene transfer (Cardin et al., 2010; Hikida et al., 2010; Lobo et al., 2010; Ferguson et al., 2011), have provided profound new insight into the precise molecular underpinnings of each MSN subtype and their regulation by drugs of abuse (Table 1).

Recent findings support the conclusion of a more predominate role for D1+ MSNs in producing the reinforcing and sensitizing effect of drugs of abuse, with most robust molecular changes occurring in these MSNs. For instance, acute exposure to psychostimulants potently induces numerous signaling molecules including FosB, ERK, c-Fos, and Zif268 in the D1+ MSNs, while repeated cocaine preferentially induces ΔFosB and alters GABA receptor and other ion channel subunits in this cell-type as well (Robertson et al., 1991; Young et al., 1991; Berretta et al., 1992; Cenci et al., 1992; Moratalla et al., 1992; Hope et al., 1994; Bertran-Gonzalez et al., 2008; Heiman et al., 2008). Furthermore, disrupting or over-expressing specific molecules, such as ΔFosB, DARPP-32, or Nr3c1 (the glucocorticoid receptor), in D1+ MSNs typically mimics the drug-related behaviors observed when these alterations are made in a non-cell-type-specific manner, while disrupting such genes in D2+ MSNs often causes an opposite response (Fiendberg et al., 1998; Kelz et al., 1999; Deroche-Gamonet et al., 2003; Zachariou et al., 2006; Ambroggi et al., 2009; Bateup et al., 2010). Nonetheless, we cannot rule out an important contribution of the D2+ MSNs in adaptations to drugs of abuse, because cocaine exposure alters gene expression in both MSN subtypes (Heiman et al., 2008) and D2-receptor agonists and antagonists exert potent effects in behavioral assays (Self, 2010). Indeed, recent findings show that molecular signaling adaptations in D2+ MSNs potently modify an animal’s behavioral response to drugs of abuse (Lobo et al., 2010). The latter findings showed that loss of TrkB (the receptor for BDNF) in D2+ MSNs results in similar behavioral responses to cocaine as total TrkB knockout from the NAc, showing for the first time a selective dominant role for a molecular pathway in D2+ MSNs in mediating the effects of drugs of abuse.
Finally, recent literature reveals that the two MSNs exert antagonistic effects in drug-related behaviors, where activation of D1+ MSNs or inhibition of D2+ MSNs enhances an animal’s sensitivity to a drug of abuse (Hikida et al., 2010; Lobo et al., 2010; Ferguson et al., 2011). These findings are consistent with opposing roles of the two MSNs and their direct vs. indirect pathways in the basal ganglia in motor behaviors (Alexander et al., 1986; Albin et al., 1989; Graybiel, 2000; Kravitz et al., 2010). This recent

| Gene/function manipulated | Cell-type | Method | Effects mediated by drugs of abuse | References |
|---------------------------|-----------|--------|-----------------------------------|------------|
| NR1 (NMDA) subunit         | D1+       | Mutated NR1 knocked into the D1 locus | Diminished cocaine sensitization and CPP | Heusner and Palmiter (2005) |
| NR1 (NMDA) subunit         | D1+       | D1-Cre knockout | Attenuated amphetamine sensitization | Beutler et al. (2011) |
| mGluR5                     | D1+       | D1-Cre knockout | Attenuated cue-induced cocaine seeking | Novak et al. (2010) |
| Acetylcholine muscarinic receptor 4 | D1+ | D1-Cre knockout | Enhanced behavioral sensitization to cocaine and amphetamine | Jeon et al. (2010) |
| Cannabinoid receptor 1 (CB1) | D1+       | D1-Cre knockout | Blunted response to THC | Monory et al. (2007) |
| Glucocorticoid receptor nuclear receptor 3c1 | D1+ | D1-Cre knockout | Diminished cocaine self-administration | Ambroggi et al. (2009) |
| BDNF receptor (TrkB)       | D1+       | D1-Cre knockout | Enhanced cocaine sensitization and CPP | Lobo et al. (2010) |
| BDNF receptor (TrkB)       | D2+       | D1-Cre knockout | Diminished cocaine sensitization and CPP | Lobo et al. (2010) |
| c-Fos                      | D1+       | D1-Cre knockout | Diminished cocaine sensitization, diminished cocaine extinction | Lobo et al. (2010) |
| AFosB                      | D1+       | Overexpression with tetracycline inducible D1 mouse line | Enhanced morphine CPP, enhanced morphine tolerance, diminished morphine analgesia, enhanced cocaine sensitization and CPP | Zachariou et al. (2006), Kelz et al. (1999) |
| DARPP-32                   | D1+       | D1-Cre knockout | Diminished cocaine sensitization | Bateup et al. (2010) |
| DARPP-32                   | D2+       | D2-Cre knockout | Enhanced cocaine sensitization | Bateup et al. (2010) |
| ChR2, light-activated neuronal activity | D1+ | Conditional AAV viruses + D1-Cre | Enhanced cocaine CPP | Lobo et al. (2010) |
| ChR2, light-activated neuronal activity | D2+ | Conditional AAV viruses + D2-Cre | Diminished cocaine sensitization | Lobo et al. (2010) |
| Tetanus toxin light chain inhibited synaptic transmission | D1+ | Overexpressed with AAV virus with substance P promoter | Diminished cocaine CPP and sensitization | Hikida et al. (2010) |
| Tetanus toxin light chain inhibited synaptic transmission | D2+ | Overexpressed with AAV virus with enkephalin promoter | No change in CPP, slight decrease in sensitization | Hikida et al. (2010) |
| hM4D DREADD inhibit neuron firing | D1+ | Overexpressed with HSV virus with dynorphin promoter | Diminished amphetamine sensitization | Ferguson et al. (2011) |
| hM4D DREADD inhibit neuron firing | D2+ | Overexpressed with HSV virus with enkephalin promoter | Enhanced amphetamine sensitization | Ferguson et al. (2011) |
| Inducible diphtheria toxin receptor – ablates cells | D2+ | Overexpressed with A2A-Cre | Enhanced amphetamine CPP | Durieux et al. (2009) |
As already noted, all drugs of abuse activate dopaminergic input to the NAc and related limbic brain regions (Volkow et al., 2004; Wise, 2004; Nestler, 2005). For instance, psychostimulants such as cocaine or amphetamine act directly on the dopaminergic reward pathway by interfering with the dopamine transporter: cocaine blocks the transporter and amphetamine reverses the transporter, both actions resulting in a build up of dopamine in the synapse which can activate downstream dopamine receptors on target neurons (Figure 1). The two MSNs are most notably differentiated by their enrichment of D1 vs. D2-receptors although single-cell RT-PCR studies reveal that D1+ MSNs express low levels of the D2-like receptor, D3 and D2+ MSNs express low levels of the D1-like receptor, D3 (Surmeier et al., 1996). The two MSNs require glutamatergic innervation to drive neural activity; dopamine oppositely modulates these functional responses via stimulation of distinct dopaminergic innervation to drive neural activity; dopamine oppositely modulates these functional responses via stimulation of distinct molecular signaling exhibited by these two MSN subtypes in relation to their functional roles and responses to drugs of abuse.

**DOPAMINE RECEPTOR SIGNALING IN D1 VS. D2 MSNs**

As already noted, all drugs of abuse activate dopaminergic input to the NAc and related limbic brain regions (Volkow et al., 2004; Wise, 2004; Nestler, 2005). For instance, psychostimulants such as cocaine or amphetamine act directly on the dopaminergic reward pathway by interfering with the dopamine transporter: cocaine blocks the transporter and amphetamine reverses the transporter, both actions resulting in a build up of dopamine in the synapse which can activate downstream dopamine receptors on target neurons (Figure 1). The two MSNs are most notably differentiated by their enrichment of D1 vs. D2-receptors although single-cell RT-PCR studies reveal that D1+ MSNs express low levels of the D2-like receptor, D3 and D2+ MSNs express low levels of the D1-like receptor, D3 (Surmeier et al., 1996). The two MSNs require glutamatergic innervation to drive neural activity; dopamine oppositely modulates these functional responses via stimulation of distinct dopaminergic receptor subtypes: by positively modulating excitatory glutamatergic input through D1 receptor signaling via G_{i/o}, which stimulates adenyl cyclase leading to increased PKA activity, whereas dopamine negatively modulates this input through D1-receptor signaling via G_{i/o} and G_{o}, which inhibit adenyl cyclase causing decreased PKA activity (Surmeier et al., 2007; Gerfen and Surmeier, 2011). In reality, each receptor exerts complex effects on many additional downstream signaling pathways. At rest, the two MSN subtypes are generally inhibited, they are in what researchers have termed the down-state. Excitatory glutamatergic synaptic activity can release the MSNs from this down-state and shift them into a more depolarized state (the up-state). Dopamine oppositely modulates the excitatory glutamatergic shift to the up-state. D1 activation of PKA enhances Cav1 L-type Ca^{2+} channel activity, decreases somatic K^{+} channel activity, and downregulates Cav2 Ca^{2+} channels that control activation of Ca^{2+} dependent, small-conductance K^{+} (SK) channels, resulting in increased spiking in these MSNs (Surmeier et al., 2007; Gerfen and Surmeier, 2011). In contrast, D2 signaling inhibits the up-state transition, thereby preventing increased spiking, via reduction of Cav1 L-type Ca^{2+} channel activity and Nav1 Na^{+} channel activity while increasing K^{+} channel currents (Surmeier et al., 2007; Gerfen and Surmeier, 2011; Figure 1). Such opposite alterations in the two MSNs suggest that increased dopamine signaling elicited by drugs of abuse should enhance glutamatergic activation of D1+ MSNs and reduce glutamatergic activation of D2+ MSNs. In reality, such responses are far more varied and complex for reasons that remain poorly understood. This topic will be addressed further below.

The role of dopamine receptors in drug abuse is complex and often elusive (Self, 2010). There is an abundance of literature on the role of D1 and D2-receptor agonists and antagonists in modulating rewarding properties and self-administration of drugs of abuse, however, the results differ depending on the type of agonist/antagonist used, the type of delivery (systemic vs. brain region-specific), and the timing of the treatment (Self, 2010). Such results are further confounded by non-striatal specific effects, such as the contribution of pre-synaptic D2-receptors from the VTA or presence of D1 receptors in many other limbic regions, and the lack of specificity of the agonists/antagonists utilized as well as the expression of D1-like and D2-like receptors in both MSN subtypes as noted earlier. In general, it is thought that D1 receptors play a more predominant role in the...
primary rewarding properties of drugs of abuse, whereas D2-receptors play a role in drug seeking mechanisms (Self et al., 1996; Self, 2010). Studies with D1 receptor and D2-receptor knock-out mice provide some insight into the role of these receptors in the two MSNs. D1 knockout mice show a blunted induction of immediate early genes (IEGs) c-Fos and Zif268 in response to cocaine, a diminished response to psychostimulant-induced locomotor activity but with no alterations in cocaine-conditioned place preference (CPP) – an indirect measure of drug reward, and diminished cocaine self-administration and ethanol consumption (Miner et al., 1995; Drago et al., 1996; Crawford et al., 1997; Elmer et al., 2000; Caine et al., 2002; Chausmer et al., 2007; Caine et al., 2007). D2 knockout mice display diminished rewarding effects to opiates and cocaine as well as decreased ethanol consumption but no reduction in cocaine taking (Maldonado et al., 1997; Cunningham et al., 2006; Risinger et al., 2006; Caine et al., 2002; Chausmer et al., 2002; Elmer et al., 2002; Welter et al., 2007). Such data support important roles for D1 and D2-receptors in the two MSNs in multiple aspects of drug abuse, however, the knockouts lack striatal specificity and occur early in development, thus one cannot rule out other brain regions and cell-types and developmental factors in mediating these behaviors. Finally, decreased levels of D2/D1 receptors in striatum, as visualized by brain imaging, has become a common marker of addiction in human patients especially during periods of withdrawal (Volkow et al., 2009). Rodents receiving viral-mediated gene transfer of D2-receptors to the NAc display attenuated cocaine self-administration and ethanol consumption (Thanos et al., 2004, 2008). These studies were not performed in a cell-type-specific manner, so we cannot rule out the possible effect of D2-receptor overexpression influencing D1+ MSNs. This collection of data emphasizes the need to move to more selective approaches, including cell-type-specific, region-specific, and even temporally specific manipulations of the dopamine receptors to better elucidate their functional roles in the two MSN subtypes in drug addiction. Finally, it has been reported recently that D2-GFP homozygote BAC transgenic mice display increased expression levels of the D2-receptor in striatum and enhanced behavioral sensitivity and dopamine signaling to D2 agonists. Moreover, both homozygotes and hemizygotes exhibit blunted behavioral responses to cocaine (Kramer et al., 2011). This study highlights the need to perform thorough characterization of D1 and D2 fluorescent reporter and Cre driver lines. However, the majority of the data collected in this study used homozygotes, which is not the ideal experimental genotype since 5–10% of transgene integrations result in insertional mutations (Meisler, 1992); therefore, the hemizygote genotype is the more reliable experimental genotype. Additionally, this study did not use littermate wildtype controls but used controls on a similar background (Swiss Webster) obtained from Taconic, while their transgenic lines were obtained from GENSAT and MMRRRC. Finally, another group has shown normal cocaine locomotor behavioral responses in D2-GFP hemizygotes (Kim et al., 2011). Thus, future studies using proper controls and proper genotypes must be performed to fully characterize the various cell-type-specific transgenic lines available.

**GLUTAMATE AND GABA SIGNALING IN D1 VS. D2 MSNs**

Medium spiny neurons receive glutamergic input from multiple brain regions including prefrontal cortex, amygdala, and hippocampus, and GABAergic input from local interneurons and perhaps collateral inputs from other MSNs. Net excitatory and inhibitory regulation of MSNs is no doubt crucial in regulating the drug-addicted state, and there is now a growing literature on the complex ways in which drugs of abuse alter glutamatergic neurotransmission in particular in the NAc (Pierce et al., 1996; Thomas et al., 2001; Beurrier and Malenka, 2002; Kourrich et al., 2007; Bachtell and Selig, 2008; Bachtell et al., 2008; Conrad et al., 2008; Kalivas, 2009; Wolf, 2010). Although MSNs are thought to primarily exist in an inhibited down-state under basal conditions with glutamate driving activity of both cell-types, there remains limited information with respect to distinct regulation occurring in D1 vs. D2 MSNs.

ΔFoSB overexpression in D1+ MSNs (see below for more details) enhances the rewarding effects of cocaine and increases levels of the Ca2+-impermeable glutamate receptor subunit, GluR2, in NAc. Furthermore, viral-mediated gene transfer of GluR2 to the NAc similarly enhances the rewarding effects of cocaine (Kelz et al., 1999). However, it is not known whether the induction of GluR2 seen in response to ΔFoSB overexpression in D1+ MSNs is also specific to these neurons, and the viral overexpression of GluR2 is not cell-type-specific, therefore we cannot infer direct conclusions about GluR2 function in these two MSNs in drug reward. Heusner and Palmiter (2005) assessed the role of NMDA glutamatergic conductance in cocaine behaviors by expressing an NR1 subunit, which contains a mutation in the pore that reduces calcium flux, selectively in D1+ MSNs. This group showed that lack of NMDA conductance in D1+ MSNs prevents cocaine-induced CPP and cocaine locomotor sensitization, highlighting the necessity for NMDA signaling in D1+ MSNs for the rewarding and sensitizing effects of cocaine (Heusner and Palmiter, 2005). Furthermore, recently it was found that knocking out the NR1 subunit in D1+ MSNs attenuates amphetamine sensitization and this phenotype was rescued by resupplying the NR1 subunit to D1+ MSNs specifically in the NAc (Beutler et al., 2011). Finally, knockdown of the mGluR5 subunit, using RNA interference, in D1+ MSNs has no effect on the initial rewarding properties of cocaine but diminishes the cue-induced reinstatement of cocaine seeking (Novak et al., 2010). While these data reveal compelling roles for glutamatergic signaling in D1+ MSNs, future work is needed to study glutamatergic systems in D2+ MSNs. Future research should also evaluate how modulation of these glutamate receptor subunits in the two MSN subtypes affects the structural synaptic changes observed in NAc after drugs of abuse (Dietz et al., 2009; Russo et al., 2010), particularly the dendritic alterations observed after cocaine exposure selectively in the D1+ MSNs (Lee et al., 2006; Kim et al., 2011) which may be associated with the increase in miniature excitatory postsynaptic currents observed in D1+ MSNs (Kim et al., 2011). Interestingly, ΔFoSB induction in D1+ MSNs has been related directly to such dendritic adaptations after chronic cocaine (Maze et al., 2010).

In contrast to glutamate, there is a lack of research on GABA function in the two MSNs in addiction models, which is surprising
considering both ethanol and benzodiazepines enhance the effects of GABA and the two MSNs receive dense GABAergic inputs as stated above. There is also considerable evidence pointing to enhanced inhibition in the NAc at least after chronic cocaine exposure (White et al., 1995; Peoples et al., 1998; Zhang et al., 1998; Thomas et al., 2001; Beurrier and Malenka, 2002). Heiman et al. (2008) performed high throughput genetic screening in the two MSNs after chronic cocaine exposure and, interestingly, the most altered biological process in the D1+ MSNs was GABA signaling. In particular, there was potent upregulation of GABA A receptor subunits Gabra1 and Gabra4 as well as the GABA B receptor subunit Gabb3, and this group found that chronic cocaine increases the frequency of small-amplitude GABAergic miniature inhibitory postsynaptic currents (mIPSCs) in D1+ MSNs (Heiman et al., 2008). On the other hand, another group recently showed that chronic cocaine results in an opposite response with decreased frequency and amplitude of mIPSCs in the D1+ MSNs (Kim et al., 2011). However, the latter group did show diminished membrane excitability in the D1+ MSNs after chronic cocaine, which could be a reflection of enhanced GABA tone and is consistent with the field’s assessment of enhanced inhibition in the NAc after exposure to chronic cocaine. Furthermore, such differences between the two groups could simply be due to the timing of cocaine exposure and withdrawal. In general, there is a need to study glutamatergic and GABAergic function in the two MSNs in response to drugs of abuse and the field is now equipped with the resources that make such a cell-type- and region-specific study possible.

**OTHER RECEPTOR SIGNALING IN D1 VS. D2 MSN SUBTYPES**

The two MSNs are differentially enriched in other G-protein-coupled receptors in addition to dopamine receptors. D1+ MSNs express higher levels of the acetylcholine muscarinic receptor 4 (M4; Bernard et al., 1992; Ince et al., 1997) and D2+ MSNs are enriched in both adenosine receptor 2A (A2A; Schiffmann et al., 1991; Schiffmann and Vanderhaeghen, 1993) and G-protein-coupled receptor 6 (Gpr6; Lobo et al., 2007; gensat.org). M4 is coupled to Gi/Go, which would produce an opposite response, compared to D1 receptors, in D1+ MSNs by inhibiting cAMP/PKA activity. Indeed, a D1+ MSN selective M4 knockout displayed enhanced behavioral sensitization to cocaine and amphetamine (Jeon et al., 2010). Furthermore, recent studies using a designer receptor exclusively activated by a synthetic drug (DREADDs) showed that activation of the DREADD Gi/o-coupled human M4 receptor (hM4D) in D1+ MSNs diminished behavioral sensitization to amphetamine, with the opposite response seen in D2+ MSNs (Ferguson et al., 2011). Such data reveal the antagonizing role of M4 receptors in D1+ MSNs in drug abuse. As well, since the hM4D receptor potently inhibits these MSNs, the data provide insight into the effect of altered activity of these two MSNs in drug abuse, which will be discussed further below.

Both A2A and Gpr6 are positively coupled to G i/o/Golf proteins, implicating their role in antagonizing the D2-receptor in D2+ MSNs. Indeed, stimulation of A2A receptors has been shown to reduce both the development and expression of cocaine sensitization (Filip et al., 2006), impair the initiation of cocaine self-administration (Knapp et al., 2001), and antagonize the reinstatement of cocaine seeking elicited by cocaine, D2-receptor stimulation, or cocaine-conditioned cues (Bachtell and Self, 2009). As Gpr6 is also enriched in D2+ MSNs (Lobo et al., 2007), its role in behavioral functions of the striatum should be evaluated. To date, it has been shown to influence instrumental learning (Lobo et al., 2007) but its role in drug abuse models is yet unknown.

The cannabinoid receptor 1 (CB1) is expressed ubiquitously throughout the central nervous system (Mackie, 2008), hence it is difficult to dissect the precise role of specific brain regions and cell-types in mediating Δ9-tetrahydrocannabinol (THC) addiction. Recently, deletion of CB1 from D1+ MSNs was found to modestly affect behavioral responses to THC, including blunted effects in THC-induced hypolocomotion, hypothermia, and analgesia (Monory et al., 2007). It would be interesting to evaluate cannabinoid receptor function in D2+ MSNs since these MSNs express endocannabinoid-mediated long-term depression (eCB-ITD), which requires dopamine D2-receptor activation (Kreitzer and Malenka, 2007).

The glucocorticoid receptor, Nr3c1, is also broadly expressed in the CNS and periphery. Stress-induced glucocorticoid secretion can potentiate maladaptive behaviors including drug addiction (Frank et al., 2011). In particular, disrupting glucocorticoid signaling in D1+ MSNs by deleting Nr3c1 diminished the motivation these mice display to self-administer cocaine, and this is consistent with previous data where Nr3c1 was deleted from the entire brain (Ambroggi et al., 2009). These data are consistent with other findings described in this review, showing a predominant role for D1+ MSNs in mediating many of the effects of drugs of abuse.

Finally, we recently disrupted BDNF signaling in the two MSNs by deleting its TrkB receptor selectively from each MSN subtype. We observed opposite effects on cocaine-elicited behaviors: cocaine-induced locomotor activity and the induction of cocaine CPP were enhanced after TrkB deletion from D1+ MSNs, but attenuated after deletion from D2+ MSNs (Lobo et al., 2010). Interestingly, the deletion of TrkB from D2+ MSNs mimics the effects of total deletion of TrkB from the NAc as well as disruption of BDNF signaling from the VTA (Horger et al., 1999; Graham et al., 2007, 2009; Bahi et al., 2008; Crooks et al., 2010). These findings thus show for the first time a predominant role of a signaling cascade in D2+ MSNs in mediating the effects of a drug of abuse. The predominant role of D2+ MSNs in mediating BDNF’s effects on cocaine-elicited behaviors is not surprising considering both TrkB mRNA and protein are enriched in D2+ MSNs (Lobo et al., 2010; Baydys et al., 2011). The behavioral changes observed in these mice were accompanied by enhanced neuronal activity in the D2+ MSNs upon a selective knockout of TrkB. These findings prompted us to use optogenetic technology to selectively manipulate MSN activity in cocaine reward (see below).

**TRANSCRIPTION FACTORS IN D1 VS. D2 MSNs**

The most compelling evidence for the more robust role of D1+ MSNs in drug abuse comes from literature evaluating induction of intracellular signaling molecules. As stated above, acute doses of psychostimulants induce IEG expression, including c-Fos, Zif268 (Egr1), and FosB primarily in D1+ MSNs in NAc...
Another IEG that has been extensively studied in the two MSN subtypes is FosB. Acute exposure to cocaine induces FosB in D1+ MSNs (Berretta et al., 1992), whereas chronic exposure induces ΔFosB, a stable product of the FosB gene generated by alternative splicing (Hope et al., 1994; Nestler et al., 2001; Nestler, 2008), in D1+ MSNs (Nye et al., 1995; Moratalla et al., 1996; Lee et al., 2006). Similar findings are observed with many other drugs of abuse as well as with natural rewards such as food, sex, and wheel running. For example, chronic wheel running, which is a natural reward (Iversen, 1993; Belke, 1997; Lett et al., 2000), induces ΔFosB in D1+ MSNs but not D2+ MSNs (Werme et al., 2002). To gain functional insight into the role of ΔFosB in the two MSNs, our group generated NSE-tTa lines, termed 11A and 11B, which direct transgene expression to either D1+ or D2+ MSNs, respectively (Chen et al., 1998; Kelz et al., 1999; Werme et al., 2002). Line 11A mice crossed with a Tet-Op ΔFosB line show increased responses to the rewarding and locomotor effects of cocaine (Kelz et al., 1999), which is consistent with ΔFosB induction in D1+ MSNs (Nye et al., 1995; Moratalla et al., 1996). Furthermore, these same mice display increased morphine reward (evaluated by CPP) as well as diminished morphine analgesia and enhanced morphine tolerance, while the 11B Tet-Op ΔFosB mice show no change in morphine reward. Overexpression of a dominant negative antagonist of ΔFosB exerts effects opposite to those seen with ΔFosB, although this mouse model does not distinguish D1 vs. D2 MSNs (Peakman et al., 2003). Together, these data further support the role of ΔFosB induction in D1+ MSNs as an important molecular player in the rewarding properties of drugs of abuse (Zachariou et al., 2006). This phenomenon is also observed in other reward behaviors, in particular, wheel running: 11A Tet-Op ΔFosB mice display increased wheel running behavior, whereas 11B Tet-Op ΔFosB mice display diminished wheel running (Werme et al., 2002). The finding that ΔFosB induction in D1 MSNs promotes reward is consistent with recent findings that such cell-type-selective induction also promotes resilience responses to chronic stress (Vialou et al., 2010). Finally, chronic cocaine induction of ΔFosB in D1+ MSNs was shown to be accompanied by robust long-lasting increases in dendritic spine densities (Lee et al., 2006) and recently ΔFosB in the NAc was shown to be both necessary and sufficient in mediating the increased density of dendritic spines in this brain region (Maze et al., 2010). Such data support a role for ΔFosB in D1+ MSNs in mediating the rewarding aspects of drugs of abuse and natural rewards as well as the accompanying structural plasticity changes. The data also suggest that induction of ΔFosB in D2+ MSNs confers negative consequences to rewarding stimuli. Since ΔFosB induction in D2+ MSNs is seen in response to chronic stress and antipsychotic drug exposure (Hiroi and Graybiel, 1996; Perrotti et al., 2004), further studies of the latter actions are needed.

**OTHER INTRACELLULAR SIGNALING MOLECULES IN D1 VS. D2 MSNs**

One signaling molecule that has been well studied in the two MSNs in the context of drug abuse is the protein kinase, ERK (extracellular signal related kinase). Acute or chronic exposure to cocaine induces phosphorylated ERK (pERK), the activated form of the protein, in the NAc and dStr in D1+ MSNs using D1-GFP and
D2-GFP BAC transgenic reporter mice (Bertran-Gonzalez et al., 2008) and this response is mediated through D1 receptors (Valjent et al., 2000; Lu et al., 2006). This group also showed that pMSK-1 (phospho-MAP and stress activated kinase-1) and histone H3, both targets of pERK signaling, are robustly induced in pERK containing D1+ MSNs after acute cocaine exposure and modestly increased after chronic cocaine (Bertran-Gonzalez et al., 2008). pERK is also induced is response to chronic morphine, in particular, pERK is robustly induced in D1+ MSNs and modestly induced in D2+ MSNs in the NAc shell after withdrawal in response to the context-specific association with morphine (Borgkvist et al., 2008). The precise functional role of pERK in drug addiction remains to be determined. Pharmacological treatment with ERK inhibitors has been shown to decrease cocaine reward, however, a knockout of ERK1 potentiates cocaine reward, suggesting that ERK inhibitors may preferentially be affecting ERK2.

Recently, we showed that optogenetic activation of D1+ MSNs in the NAc, which increases an animal’s rewarding responses to cocaine, potently reduces both pERK1 and pERK2. Future studies manipulating ERK expression in a cell-type-specific manner are necessary to fully address the functional role of ERK signaling in the two MSNs in drug abuse.

DARPP-32 is another signaling molecule that has been extensively studied in response to drugs of abuse. It is well known that acute psychostimulants lead to PKA phosphorylation of DARPP-32 at threonine 34 (T34), causing it to become a potent inhibitor of protein phosphatase 1 (PP-1), which regulates the phosphorylation state of many effector proteins, including transcription factors, ionotropic receptors, and ion channels (Greengard et al., 1999). However, until recently, it was unclear which MSN subtype mediates this biochemical change. Greengard et al. (1999) generated BAC transgenic mouse models that enable the evaluation of DARPP-32 phosphorylation in D1+ or D2+ MSNs by expressing tagged versions of DARPP-32 using D1 or D2 BACs allowing for immunoprecipitation of DARPP-32 from each MSN subtype. These studies demonstrated that acute cocaine treatment increases T34 phosphorylation in D1+ MSNs and induces phosphorylation of threonine 75 (T75) by Cdk5, which inhibits PKA signaling, selectively in D2+ MSNs (Bateup et al., 2008). Finally this group showed that deletion of DARPP-32 from each MSN subtype using D1-Cre and D2-Cre BAC transgenic mice results in opposite regulation of cocaine-induced locomotor activity (Bateup et al., 2010). Loss of DARPP-32 from D1+ MSNs diminished the locomotor effects of cocaine, which mimics previous data evaluating a total DARPP-32 knockout (Fienberg et al., 1998), whereas loss of DARPP-32 from D2+ MSNs enhanced cocaine locomotor responses. Such data provide concrete evidence for differential roles of DARPP-32 in the two MSNs in response to drugs of abuse and illustrate the importance of cell-type-specific methods to fully understand the contribution of these two neuronal types in drug addiction.

MODULATING ACTIVITY OF D1 OR D2 MSNs

Directly modulating the activity of the two MSN subtypes has recently provided novel insight into the molecular and functional role of D1 and D2 MSNs in addiction. We used optogenetic tools combined with a conditional (i.e., Cre-dependent) adeno-associated viral (AAV) vector expressing the blue light-activated cation channel, channelrhodopsin-2 (ChR2). We injected the vector, or a control, into the NAc of D1-Cre or D2-Cre BAC transgenic mice and then stimulated the injected region with blue light to selectively activate D1+ vs. D2+ MSNs in the context of cocaine CPP. We found that activation of D1+ MSNs potentiates induction of cocaine CPP, whereas activation of D2+ MSNs inhibits this induction (Lobo and Nestler, 2010). As noted previously, we observed the same behavioral effects when TrkB was deleted selectively from these MSN subtypes: enhanced cocaine CPP and locomotor activity after TrkB deletion from D1+ MSNs, and reduced cocaine CPP and locomotor activity after TrkB deletion from D2+ MSNs. The likely common action of TrkB knockout and optogenetic stimulation in D2+ MSNs is their increased activity, since deletion of TrkB from these cells increases their electrical excitability. As mentioned earlier, we also found a robust reduction of pERK after TrkB deletion from D1+ MSNs. pERK is a known downstream target of BDNF signaling, therefore, the shared behavioral effects observed after TrkB deletion from D1+ MSNs and from optogenetic activation of these cells might be due to converging effects on pERK activity. However, future work is needed to determine the precise, shared molecular underpinnings that govern the behavioral effects seen after disruption of BDNF signaling and optogenetic control of these two neuronal subtypes.

Other groups have used different tools to modulate activity of the two MSNs in drug abuse models. Hikida et al. (2010) used AAV vectors to express tetracycline-repressive transcription factor (Tet) using the substance P (a D1+ MSN gene) or enkephalin (a D2+ MSN gene) promoters. These vectors were injected into the NAc of mice, in which tetanus toxin light chain (TN) – a bacterial toxin that cleaves the synaptic vesicle-associated protein, VAMP2 – was controlled by the tetracycline-responsive element, to selectively abolish synaptic transmission in each MSN subtype. Consistent with our optogenetic approach, these data showed a role of D1+ MSN activity in enhancing cocaine CPP as well as cocaine-induced locomotor activity, since abolishing synaptic transmission in D1+ MSNs diminished both behavioral effects. In contrast to the optogenetic studies, the authors found no alterations in cocaine CPP after abolishing synaptic transmission in D2+ MSNs, but did observe reduced cocaine-induced locomotor activity in response to the first two cocaine exposures. Interestingly, this group showed that inactivation of the D2+ MSNs played a more profound role in mediating aversive behaviors.

As stated earlier, Ferguson et al. (2011) used herpes simplex virus (HSV) vectors to express an engineered GPCR (a Gδ4-coupled human muscarinic M4 designer receptor exclusively activated by a designer drug, hM4D) that is activated by an otherwise pharmacologically inert ligand using enkephalin and dynorphin promoters to selectively silence D1+ or D2+ MSNs in the dStr. The authors showed that transiently disrupting D2+ MSN activity in dStr facilitated amphetamine sensitization, whereas decreasing excitability of D1+ MSNs impaired the persistence of amphetamine-induced sensitization. Finally, abolishing D2+ MSNs in the NAc at adult ages using diphtheria toxin receptor enhances the rewarding effect of amphetamine (Durieux et al., 2009). Such data are in accordance with our optogenetic findings,
and together implicate opposite roles of D1+ vs. D2+ MSNs in drug addiction, with D1+ MSNs promoting both reward and sensitization responses to psychostimulants and D2+ MSNs dampening these behaviors.

**FUTURE DIRECTIONS**

The field has made tremendous advances toward understanding the selective role of the D1+ and D2+ MSN subtypes in NAc and dStr in mediating the effects of drugs of abuse. In particular, recently developed tools that enable the selective manipulation of these cell-types have played a predominant role in obtaining the majority of this information. What are the next steps? Since the underlying molecular adaptations in drug addiction models are not static, but very dynamic, it is crucial to develop the capability to selectively manipulate signaling molecules of interest in D1+ vs. D2+ MSNs in a temporally precise way. DREADDs and optogenetic tools can help with this time scale manipulation. DREADDs ligands can be administered at different time courses throughout drug behavioral paradigms to parcel out the selective role of signaling receptors in the two MSNs in drug models. Optogenetic tools in particular provide an extremely powerful means to temporally regulate not only neuronal activity but G-protein-coupled receptor signaling using OptoXRs (Airan et al., 2009), glutamatergic signaling (Volgraf et al., 2006; Numano et al., 2009), GABAergic signaling, and even certain intracellular signaling molecules (Wu et al., 2009; Hahn and Kuhlman, 2010). Ultimately, it may be possible to extend these capabilities to optogenetic regulation of transcriptional activity. Likewise, optogenetic tools are making it possible for the first time to study the influence of specific inputs to striatum and to determine whether such inputs impinge in selective ways on D1+ vs. D2+ MSNs (Higley and Sabatini, 2010). The ability to control such signaling and molecular properties with great temporal resolution will allow major steps to be made toward a more comprehensive understanding of the two MSN subtypes, and other cell subtypes in NAc and dStr, in mediating the time course and different phases of drug addiction.

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