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INTRODUCTION

Drug addiction can be considered as a chronic and relapsing psychiatric disorder induced by repeated pharmacological manipulation of the so-called mesolimbic reward circuitry by drugs of abuse. It can be viewed as maladaptive neural plasticity that occurs in vulnerable individuals in response to repeated exposure to drugs. Thus, addictive drugs change brain properties that normally permit us to adapt to environmental stimuli (Kelley, 2004; Everitt and Robbins, 2005). By changing motivational circuitry, addictive drugs progressively orient behavior toward drug-seeking and drug-taking strategies that are life-long behavioral changes that built our understanding of the consequences of this synergistic signaling for the actions of drugs of abuse.

Keywords: receptors, addiction, dopamine, crosstalk, signaling, ERK, GPCR, striatum

CONVERGENCE OF DOPEAMINE AND GLUTAMATE SIGNALING ONTO STRIATAL ERK ACTIVATION IN RESPONSE TO DRUGS OF ABUSE

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Despite their distinct targets, all addictive drugs commonly abused by humans evoke increases in dopamine (DA) concentration within the striatum. The main DA Ganine nucleotide binding protein couple receptors (GPCRs) expressed by medium-sized spiny neurons of the striatum are the D1R and D2R, which are positively and negatively coupled to cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling, respectively. These two DA GPCRs are largely segregated into distinct neuronal populations, where they are co-expressed with glutamate receptors in dendritic spines. Direct and indirect interactions between DA GPCRs and glutamate receptors are the molecular basis by which DA modulates glutamate transmission and controls striatal plasticity and behavior induced by drugs of abuse. A major downstream target of striatal D1R is the extracellular signal-regulated kinase (ERK) kinase pathway. ERK activation by drugs of abuse behaves as a key integrator of D1R and glutamate NMDAR signaling. Once activated, ERK can trigger chromatin remodeling and induce gene expression that permits long-term cellular alterations and drug-induced morphological and behavioral changes. Besides the classical cAMP/PKA pathway, downstream of D1R, recent evidence implicates a cAMP-independent crosstalk mechanism by which the D1R potentiates NMDAR-mediated calcium influx and ERK activation. The mounting evidence of reciprocal modulation of DA and glutamate receptors adds further intricacy to striatal synaptic signaling and is liable to prove relevant for addictive drug-induced signaling, plasticity, and behavior. Herein, we review the evidence that built our understanding of the consequences of this synergistic signaling for the actions of drugs of abuse.

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**STRIATAL MSN AT THE JUNCTION OF DA AND GLUTAMATE TRANSMISSION**

**THE DA AND GLUTAMATE HYPOTHESES OF ADDICTION**

Despite their different principal targets of action, common drugs of abuse have a shared secondary consequence on DA transmission (Di Chiara and Imperato, 1988). Dialysis for extracellular levels of DA in the striatum revealed large increases from basal levels by administration of drugs abused by humans including cocaine, amphetamine, and morphine. This increase in DA levels is significantly higher in the ventral region, NAc, than in the dorsal part. Both regions receive dense DA afferents from the ventral tegmental area (VTA). In a physiological context, DA transmission in the striatum influences motor control, decision making, attention and working memory, and learning of reward associated stimuli (Robbins and Everitt, 2002). Therefore, it is believed to be involved in disorders where these processes are perturbed such as Parkinson’s disease (PD), Huntington’s disease, attention deficit hyperactivity disorder (ADHD), and Tourette’s syndrome as well as addiction. The DA hypothesis of addiction has gained significant support but the precise role of DA in reward anticipation and motivation is still under intense investigation (Wise and Roitman, 1987).

DA transmission is not believed to register the context of the drug experience, rather it is an associative learning performed by corticostriatal and subcorticol glutamatergic innervations to the striatum, which supports this (Hyman et al., 2006). While an acute treatment with drugs such as cocaine does not drastically alter the concentration of glutamate liberated in the striatum, the repeated administration or self-administration of drugs results in a potentiated glutamate availability at the synapse (Pierce et al., 1996; Zhang et al., 2001). This loss of control of glutamate transmission has been proposed to underlie the shift in behavioral control that is seen after repeated drug treatment (Kalivas, 2009). Importantly, these two important transmission systems engaged by drugs of abuse, namely DA and glutamate, converge anatomically in the striatum.

**MEDIUM-SIZED SPINY NEURONS INTEGRATE DA AND GLUTAMATE SIGNALS**

The DA and glutamatergic inputs converge onto the dendrites of the major cell type of the striatum, the MSN. The GABAergic MSNs are generally divided into two groups, based in part on their expression of either the DA D1 receptor (the D1R) or the D2 receptor (D2R; Gerfen et al., 1990). Each MSN population controls the striatal inhibitory output to different basal ganglia structures forming the direct, D1 expressing, and indirect, D2 expressing, striatal projection pathways and are both acted on by exposure to drugs of abuse (Smith et al., 2013). The MSN of the direct pathway inhibit the substantia nigra reticulata (SNr) neurons, which are GABA neurons with high basal firing rate. The MSNs of the indirect pathway have an opposite effect because of their inhibitory link formed by projections to the external globus pallidus (GPe; GABAergic), which in turn projects to the subthalamic nucleus (STN; Glutamatergic). Therefore, the indirect pathway leads to the disinhibition of SNr. Activation of the direct striatonigral pathway disinhibits thalamocortical neurons. The indirect pathway has the opposite effect. DA reinforces the direct pathway and inhibits the indirect pathway, leading to harmonious functions of the basal ganglia. Recent evidence, using genetically encoded calcium sensors, has confirmed original assumptions that a balance exists between the direct and indirect pathway and that generation of movements requires the initiation and selection of wanted motor behaviors (Carlezon et al., 1998; Gui et al., 2013). This is supported by studies where either population were artificially activated using light activated Channel rodopsin. Impressively, when the direct pathway was activated in this manner the mice produced more ambulation in an open field and, accordingly, the activation of the indirect pathway lead to an inhibition of movement (Kravitz et al., 2010). Both pathways are acted on by DA, and while a combinatorial effect is undoubtedly central for the actions of drugs of abuse many studies have highlighted the antagonist relationship between them (recently reviewed Lobo and Nestler, 2011). In general terms, the activation of the D1R expressing MSNs promotes initiation of motor behaviors along with drug reward and sensitizing properties, while the D2R seem to exert an inhibitory influence on these behaviors.

**STRATIAL DA RECEPTORS**

Dopamine transmission is detected by Gauanine nucleotide bind- ing protein coupled receptors (GPCRs), also known as seven-transmembrane receptors. These are metabotropic receptors that share features such as the interaction with G-proteins, from which they gain their name, and the seven alpha-helices transmembrane domain structure that are interconnected by alternating intracellular and extracellular loops. The heterotrimeric G-proteins are formed by a combination of an α-subunit and βγ dimer, that can each lead to activation of signaling effectors. Exactly how the DA receptors couple to the G-proteins is not yet fully understood, but two models have been proposed (Lohse et al., 2008).

The striatum contains all the subtypes of DA GPCR to different extents (Missale et al., 1998). Since the late 1970s the DA recep- tors have been subdivided into two families, originally based on the prediction that one family is positively coupled to adenyl cyclase via Gi proteins and cAMP production, and the other to its inhibition via Gi proteins (Kebabian and Calne, 1979). After the identification of D1 and D2 receptors came the molecular cloning and the subsequent discovery of a D3, D4, and D5 (previously known as the D1b in the rat). The D1R, D2R (Le Moine and Bloch, 1993), and D5R (Rivera et al., 2002) are expressed both in the dorsal and ventral striatum, while the D3 is preferentially expressed in the ventral striatum (Levesque et al., 1992). The D4R, relatively speaking, is not as strongly expressed but is detectable in the striatum (Ariano et al., 1997).

**Focus on striatal D1R: regulation by drugs of abuse**

As already stated, there is a segregation of D1R and D2R expression in the two populations of MSNs. These two founding members of the DA receptor family are GPCR that are distinguished on the basis of their coupling to cAMP production and IP3-mediated signaling (Neve et al., 2004). Although a population of MSN exists that co-express D1R and D2R, the exact percentage seems to depend on the method of analysis, however estimates are in the region of 17% in the ventral striatum (Bertran-Gonzalez et al., 2008). Accumulated evidence suggests that from the first
drug exposure, essential signaling, and transcriptional events necessary for drug-induced alterations in behavior are set in motion primarily in D1R + MSN of the “direct” or striato-nigral pathway (Valentino et al., 2005; Bertran-Gonzalez et al., 2008).

The D1R is expressed in the major brain regions of the reinforcement learning circuitry (i.e., the cerebral cortex, limbic system, thalamus) and within the striatum it is strongly expressed in both dorsal and ventral regions. At the cellular level, the D1R localizes principally along the perisynaptic neck regions of dendritic spines (Huang et al., 1992). A similar conclusion was drawn from immunohistochemical analysis of cortical tissue from humans and primates where the D1R signal was more “extrasympathetic” (Smiley et al., 1994). In striatal spines, the D1R come into close proximity with the glutamate receptors at the post synaptic density (PSD), and they are extensively interconnected via scaffold proteins and intracellular signaling proteins.

Pre-treatment with SCH23390, a D1R antagonist, inhibited acute cocaine-induced locomotor activity and prevented the development of locomotor sensitization in a “one-shot” protocol (Fontana et al., 1993; White et al., 1998). Later studies confirmed that SCH23390 but not D2R antagonist raclopride, prevented the development of locomotor sensitization to cocaine, and furthermore a contribution of the NMDAR to this behavior was identified (Valentino et al., 2000).

Two lines of D1R KO mice lacked the acute locomotor and stereotyped behaviors normally induced by cocaine (Xu et al., 1994; Miner et al., 1996). This altered response was specific to D1R homozygous knockout mice since heterozygous mice still responded (Miner et al., 1995). In a further study, the chronic effects of cocaine were also perturbed, as the animals did not show a sensitized locomotor activity after repeated cocaine administration (Xu et al., 1997). However, the D1R KO have hyper basal locomotor activity, which remained elevated in these studies, so the use of a locomotor readout for cocaine’s effects may not be as relevant as other measures. Although D1R KO mice developed a normal conditioned place preference (CPP) to cocaine (Miner et al., 1995), they did not develop self-administration of cocaine, despite having learned sufficiently the operant association for food (Caine et al., 2007). The D1R contribution to self-administration therefore appeared to be function of the reward and whether context or operant reward learning is recruited relied differentially on functional D1R.

### STRIATAL GLUTAMATE RECEPTORS

The striatum receives vast glutamatergic inputs from cortical and subcortical regions, which converge anatomically with the DA inputs. Early studies used various antagonists of glutamate receptors to show role for glutamate in the development of drug-induced behaviors (Kairis et al., 1989), and lesion studies confirmed this contribution came from the glutamate circuitry coming from hippocampus, the PFC, and amygdala onto the NAc (for review, Wolf, 1996). In MSNs, ionotropic and metabotropic glutamate receptors are expressed in close proximity to the DA GPCR (Hara and Pickel, 2005). This morphological co-localization of DA and glutamate receptors provides a network of possibilities for interactions, including signaling crosstalk and direct interaction in protein complexes.

### Metabotropic glutamate receptors

Glutamate, like DA, can also activate GPCRs. Eight mGluRs are classified into three groups based on sequence homology and G protein interactions: group I (mGluR1, mGluR5), group II (mGluR2, mGluR3), and group III (mGluR4, mGluR6, mGluR7, and mGluR8). Ferraguti and Shigemoto (2006).

mGluR1 and mGluR5, which are G protein-coupled, are mostly located postsynaptically (Mitirro and Smith, 2007). Their activation induces mobilization of intracellular Ca2+ stores and activation of phospholipase C (Schopp and Conn, 1993). mGluR5 KO animals do not self-administer cocaine and do not display locomotor sensitization (Chiamulera et al., 2001). Systems activation of the mGluR5 receptor antagonist, MPEP, decreased cocaine self-administration (Kenny et al., 2003, 2005; Lee et al., 2005; Platt et al., 2008) and attenuated the ability of a priming injection of cocaine (Lee et al., 2005) or cocaine-associated cues (Backstrom and Hyttinen, 2007) to reinstate cocaine seeking. Administration of MPEP into the NAcc shell attenuated cocaine priming-induced reinstatement of drug seeking, an animal model of relapse (Kumaresan et al., 2009). Alongside these data clearly indicated that activation of mGluR5, specifically in the NAcc, may promote the reinstatement of drug seeking.

The activation of the presynaptic Group II mGluRs inhibits cAMP and PKA signaling as they are coupled to Gq protein. The reduction in extrasynaptic glutamate availability after chronic cocaine treatment and withdrawal, due to disruption of the cysteine-glutamate exchanger, removes the toxic activation of group 2 mGluR that would normally inhibit glutamate release. This can explain why during reinstatement, the challenge, or renewed drug experience or cue evokes a potentiated glutamate response, which is not seen with an acute injection of cocaine (Pierie et al., 1996).

The group 3 mGluR contain mGluR4, mGluR6, mGluR7, and mGluR8 and similarly to Group 2 they are negatively coupled to adenyl cyclase activity and found presynaptically in the glutamatergic terminals of the striatum. When an agonist (L-AP4) of the group 3 mGluR was administered into the striatum of cocaine-naïve mice there was no effect on locomotor activity, however, in mice subsequently treated with cocaine the compound was able to prevent the induction of locomotor sensitization (Mao and Wang, 2000).

### Focus on NMDAR: regulation by drugs of abuse

The ionotropic glutamate receptors are classed based on their affinity for synthetic agonists: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate. These are ligand gated ion channels, composed of heteromeric complexes of four integral membrane protein subunits and have long intracellular cytoplasmic tails. Unlike the AMPAR or kainate receptors, the NMDAR channel is blocked by the presence of a magnesium ion in a voltage-dependent manner. In this way they respond to the binding of glutamate only when accompanied by a depolarization of the post synaptic membrane (Mayer et al., 1984). Furthermore their activation requires the binding of a co-agonist (Kleckner and Dingledine, 1988). The NMDAR are permeable to monovalent cations and calcium, and so provide a major entry point for triggering Ca2+ dependent intracellular events.
pathways. The NMDAR subunits are grouped into classes: GluN1, GluN2A-D, and GluN3A-B (formally denoted NR1, etc.). The different subunits contain different agonist binding sites, which confer upon them each with a specific pharmacology. The GluN1 or GluN3 are obligatory to form the ion channel and contain the binding site for the co-agonists glycine (Kleckner and Dingledine, 1988) or L-serine (Moshet et al., 2000) and it is the GluN2 subunits that contain the site for glutamate (Laube et al., 1997).

Context-dependent sensitization induced by single exposure to cocaine is completely prevented in mice pre-treated with the selective NMDAR antagonist, MK801, in a two-injection protocol (Valjent et al., 2010), as well as in the repeated injection protocol (Schenk et al., 1993). Similarly, GluN1-knockdown mice showed an attenuation of sensitization induced by cocaine (Ramsey et al., 2008). The precise location at which NMDA receptors are critical appears not to be limited to DA neurons themselves. Mice with specific inactivation of GluN1 in DA neurons did not show alteration of short-term sensitization, but a decreased long-term sensitization (Engblom et al., 2008; Zwillf et al., 2008).

In contrast, expression of mutant NMDARs in DA-containing MSNs prevented cocaine sensitization (Hesuett and Paimaner, 2005). Altogether, these observations support the hypothesis that NMDARs located in MSNs, in the striatum, and/or on their terminals in the VTA, as indicated by the effects of local infusion of antagonists (Veinya and Queen, 2000), contribute to the development of sensitization.

One day after acute cocaine treatment the total expression levels of the NMDAR subunits do not change in the NAcc, however, their subcellular localization is altered, with an increased internalization (Schumann and Yaka, 2009). After a 3-weeks withdrawal from repeated cocaine exposure, global expression levels and surface expression of the NMDAR subunits are increased, specifically in the NAcc. This indicates that during withdrawal some long-term processes are occurring to heighten the glutamatergic receptor levels in the NAcc.

**Signalizing Crosstalk Between D1R and Glutamate Receptors**

D1R-mediated PKA regulation and its influence on glutamate receptors

An acute injection of cocaine does not increase the levels of D1R protein in the dorsal striatum but triggers intracellular signaling cascades that are capable of modulating cell excitability via interactions with ion channels, including the glutamatergic AMPAR and NMDAR. In the striatum, as the D1-like family of DA receptors are coupled to stimulatory Gαs and G-proteins (see Hervé, 2011, for review), the most widely studied consequence of G-protein activation downstream of DA receptors is their influence on PKA-regulated signaling.

After the binding of DA to D1R a change in G-protein association enables the activation of the Ca2+ -insensitive adenyl cyclase 5 (AC5) isoform. The major target of cAMP is the cAMP-dependent PKA that has many targets including the glutamate receptor subunits. The duration of PKA activation is determined by feedback loops due to the activation of phosphodiesterases (PDEs) that are expressed in the striatum and limit cAMP production (Menniti et al., 2006).

Protein kinase A rapidly phosphorylates the NMDAR (Leonard and Hell, 1997) in response to DA, even after just 30 s in presence of a D1E inhibitor (Snyder et al., 1998). This phosphorylation occurs at Ser897 of the GluN1 subunit (Tingley et al., 1997). Phosphorylation of NMDAR subunits is a well-characterized mechanism to control their trafficking to the membrane (Scott et al., 2001; Lav and Zukin, 2007). Ion channels are also targeted by PKA and their phosphorylation can alter the conductance state of the cell. PKA mediated phosphorylation of sodium channels leads to hyperpolarization of MSNs (Schiffmann et al., 1995), and can indirectly diminish N and P/Q-type channel calcium currents that are largely localized to dendrites. On the other hand, L-type currents, at the soma, are potentiated by a PKA mechanism that boosts cellular conductance (Surmeier et al., 1993). Antagonists of the L-type Ca2+ channels can prevent the reinstatement of cocaine seeking, this was linked to the activation of Ca2+-CAM-dependent kinase CaMKII and regulation of AMPAR trafficking (Anderson et al., 2008).

**PKA Regulation of DARPP-32 and Influence on Glutamate Receptors**

A number of the intermediates between PKA and its transmembrane protein targets are kinases and/or phosphatases particularly enriched in the striatum. In the late 80s, the Greengard group characterized many of these, including ARPP-16 (cAMP-regulated phosphoprotein of Mr 16), ARPP-19, ARPP-21 (regulator of calmodulin signaling), ARPP-39, and ARPP-90 (Rap/Gap, Walaas et al., 1983). ARPP-16 and ARPP-19 phosphorylation was confirmed to be strictly dependent on the D1R (Dulubova et al., 2001). DA and cAMP regulated phosphoprotein of Mr 33kDa (DARPP-32) was identified before many of the ARPPs and has received more attention in the context of drugs of abuse as the expression of DARPP-32 is most evident in neurons of the ventral and dorsal striatum (Walaas et al., 1983).

In the context of addiction, the knockout mice for DARPP-32 had diminished hyper-locomotor responses at low doses of acute cocaine (Fenberg et al., 1998; Hiris et al., 1999). Locomotor sensitization to cocaine was absent in the DARPP-32 knockout when a two injection protocol of sensitization (TIPS) was used (Valjent et al., 2005) but not after repeated injections (Hiris et al., 1999). PKA phosphorylates the Thr27 residue of DARPP-32 that permits it to act as an inhibitor of protein phosphatase I (PPI, Hemmings et al., 1984). By this means, DARPP-32 supports PKA driven activity, in particular certain substrates such as the phosphorylation of GluR1 at Ser845 and GluN1 at Ser897. Additional phosphorylation sites regulate DARPP-32 activity including Thr27 by GSK3, Ser97 by GSK3, and Ser130 by CK1 (see Walaas et al., 2011, for review). The Thr27 phosphorylation allows DARPP-32 to regulate its own activity by inhibiting PKA and, in basal conditions this site is phosphorylated. The Ser27 site is located near a nuclear export signal and aids DARPP-32 to remain outside the nucleus when phosphorylated (Stipanovich et al., 2008). The Ser27 residue also allows DARPP-32 to regulate its own activity because it inhibits the dephosphorylation of the Thr27 site by protein phosphatase 2B (PP2B). The gene encoding DARPP-32, pp120, is subject to polymorphisms in humans that gives rise to a truncated form.
When Wittman and colleagues investigated the potentiation of activity plus the D2R-MSN driven hyperactivity. be interpreted as the net result of this D1R-MSN hypo-locomotor inhibition of activity was seen in the D2R conditional knockout. 

D1R MSNs had a diminished basal locomotor activity as measured in D1R-expressing or D2R-expressing MSNs (Bateup et al., 2010). conditional knockout mice for DARPP-32 expression in either the DARPP-32 in locomotor behaviors was further investigated in associative behaviors for reward seeking. The contribution of shuttling of DARPP-32 is particularly important for the learned behavior sequence and found that, in the hippocampus, activation of the mitriary adenylyl cyclase activating peptide (PACAP) receptors stimulated Gi signaling that releases RACK1 from GluN2B and Fyn and increased NMDAR activity. This was associated with PACAP-induced translocation of RACK1 to the nucleus where it induced brain derived neurotrophic factor (BDNF) expression (Yaka et al., 2002). An interaction also exists between Fyn and PSD-95, since the Src homology 2 (SH2) domain of Fyn binds to the third PDZ domain of PSD-95 (Tsunaka et al., 1999). PSD-95 associates with the last four amino acids of the GluN2B subunit, which are in close proximity to the Tyr1472 residues targeted by Fyn. In this way, PSD-95 may bring Fyn nearer to the GluN2B subunit for its phosphorylation and also regulate GluN2B trafficking.

D1R stimulation results in an increase in Fyn colocalization with GluN2B, which is Fyn (and not Src) dependent in the PFC (Hu et al., 2010).

Importantly, it was recently shown that D1R and NMDAR co-stimulation, as well as cocaine in vivo, activated Fyn and phosphorylated GluN2B at the Tyr1472 residue. The activation of Fyn was G-protein dependent, but independent of cAMP production (Pascoli et al., 2011a). The exact mechanisms governing the activation of Fyn in the striatum are, however, not fully understood. The SFK are found in an inactive state when they are phosphorylated on Tyr422 (for mouse Fyn). The dephosphorylation of this site and auto-phosphorylation at Tyr422 leads to a conformational change that opens the activation loop. The inactive conformation involves a binding interaction between the Fyn SH2 and SH3 domains (for review see Saler and Kalia, 2004). Therefore, the above-mentioned interaction of PSD-95 with the SH2 domain of Fyn may favor Fyn activation, but whether it may be a cause or consequence is unclear.

As for the dratvication of Fyn an interesting candidate is striatal enriched protein tyrosine phosphatase (STEP). STEP dephosphorylates GluN2B at Tyr1472 (Snyder et al., 2005). STEP exists in two major catalytic isoforms STEP46 and STEP61, which have distinct N-terminus regions, and each isoform has a specific intracellular location. STEP46 is localized in the cytosolic compartment, while STEP63 is attached to membranes of the ER or PSD. To interact with its substrates, STEPs must bind them using a kinase interacting motif (KIM) domain. The KIM of STEP is phosphorylated by FAK after D1R activation, at Ser160 and Ser221 in STEP61 and Ser49 for STEP46 (Paul et al., 2009). This phosphorylation event prevents STEP binding to its substrates. The Ser160 site is suspected to have a role in the regulation of a proteolytic cleavage sequence (known as PEST sequences) in STEP whereas Ser221 and Ser46 may lose affinity for their substrates after phosphorylation. STEP61 KIM binds to the SH2 and N-terminus domain of (t-DARPP-32), which lacks the Thr75 site and is associated with schizophrenic and bipolar phenotypes (Kumii et al., 2013). These sites are equally subject to dephosphorylation. Thr41 is dephosphorylated by the GSK and thereby allows the activity of PKC. (Nishi et al., 1999). On the other hand, dephosphorylation of Thr34 by PP2A favors the phosphorylation of Thr41 and PKA inhibition. PP2A also dephosphorylates the Ser75 residue. In the striatum, PP2A is activated after cAMP-induced phosphorylation of its B56 subunit.

One extensive study systematically assessed the role of the different residues in DARPP-32 by performing point mutations that replaced the sites that would be normally be phosphorylated by an alanine (Stipanovich et al., 2008). A notable effect was that the Ser75 site in itself controls the subcellular localization of DARPP-32. When Ser75 was mutated to alanine, DARPP-32 was unable to be exported from the nucleus to the cytoplasm and the opposite was observed if a phosphorylation-mimicking mutation, to aspartate, was made. Both these effects were preserved even after activation of the D1R by SKF81297. To investigate the Ser75 mutation to alanine in vivo, mutant mice were also generated and analyzed for alterations in cocaine-induced behaviors (Stipanovich et al., 2008). Firstly, the Ser75Ala-DARPP32 mice had normal acute locomotor responses to cocaine but a reduced locomotor sensitization to a second cocaine injection. Secondly, cocaine-induced CPP was not observed in these mice. Furthermore, the Ser75Ala-DARPP32 mice were also found to have a decreased cocaine-induced CPP response compared to wild-type mice. In addition, the Ser75Ala-DARPP32 mice had a lower breaking point in a food consumption task, which is a measure of cocaine-induced feeding.

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A major consequence of drugs of abuse administration is activation of the MAP kinase/ERK (extracellular-signal regulated kinase) pathway. ERK1 and ERK2 are two closely related mitogen-activated protein kinases (MAP kinases), which are activated by phosphorylation of their activation loop by MAP-kinase and ERK-kinase (MEK 1 and 2). Valjent et al. (2000) demonstrated that acute cocaine elicits a rapid and transient increase in ERK1/2 phosphorylation within the ventral and dorsal striatum that remained after chronic administration. They further demonstrated that ERK1/2 activation was a common feature of most drugs of abuse (Valjent et al., 2001). Even if slight differences could be observed in the kinetic patterns of activation, nicotine, morphine, A9-tetrahydrocannabinol (THC) and psychostimulants, increased ERK1/2 phosphorylation occurred in the reward circuitry, including the prefrontal cortex, the striatum (dorsal part and NAc) as well as the extended amygdala. In all cases, and all structures drug-induced ERK activation was blocked by the D1R antagonist, SCH23390 (Valjent et al., 2004). 3,4-methylenedioxymethamphetamine (MDMA, also known as ecstasy), and ethanol also activate ERK1/2 in the striatum (Salzmann et al., 2003; Ibba et al., 2009). ERK1/2 activation induced by drugs of abuse is functionally relevant since SL327, a pharmacological compound that act on MEK and crosses the blood-brain barrier, prevented the long-term behavioral effects of cocaine, such as CPP (Valjent et al., 2000), or the development of locomotor sensitization (Pascoli et al., 2011b). Furthermore, increased synaptic plasticity LTP was observed in the NAcc, hippocampus, and lateral amygdala slices from ERK1 KO mice, an effect that was specifically reversed by U0126, a selective MEK inhibitor (Mazzucchelli et al., 2002). These findings strongly support that ERK2 is the dominant isoform for neuronal plasticity and behavioral adaptations induced by addictive drugs.

Upstream from MEK, ERK activation induced by cocaine involves the calcium-activated guanine nucleotide exchange factor Ras-GRF1 (Pasano et al., 2009; Pasano and Brambilla, 2011). Knock-out mice for Ras-GRF1 show a significant reduction, but not total inhibition, of cocaine-induced ERK activation, and locomotor sensitization. On the other hand, mice that over-expressed Ras-GRF1 were more sensitive to cocaine treatment than wildtype counterparts (Pasano et al., 2009; Pasano and Brambilla, 2011; Cerovic et al., 2013). Interestingly, Ras-GRF1 is preferentially associated with GluN2B subunits (Krapivinsky et al., 2003). The cross talk between D1R and NMDA receptors implicates a CAMP-independent pathway that increases responsiveness of GluN2B containing NMDA receptors to glutamate (Pascoli et al., 2011a). In striatal neurons, D1R stimulation leads to an increase in Ca\(^{2+}\) influx through NMDARs via SK/Fyn-induced phosphorylation of the GluN2B subunit at Tyr1472. In parallel, PKA-mediated phosphorylation of DARPP-32 promotes ERK activation through an indirect inhibition of STEP. DARPP-32 thus prevents ERK dephosphorylation and contributes to enhanced glutamate-mediated ERK activation (see Figure 1, Valjent et al., 2005).

As stated above, mGluR5 receptors seem to be critically involved in cocaine-induced long term behavioral alterations. A link between amphetamine-induced ERK phosphorylation and mGluR5 was found when using a mGluR5 antagonist, MPEP, which blocked amphetamine induction of ERK in the dorsal striatum (Choe et al., 2002). Surprisingly, this inhibition was not due to mobilization of intracellular Ca\(^{2+}\) stores since dantrolene—a compound that blocks intracellular Ca\(^{2+}\) release from intracellular stores—did not affect amphetamine effects. The Group 1 mGluR can modulate NMDAR signaling onto ERK activation via PKC (Nüsken and Conn, 2010) and also via direct physical interactions (Perroy et al., 2008).

An elegant work from Pascoli et al. (2011b) recently showed that ERK activation was necessary for corticostriatal LTP induced by electrical stimulation in slices of mice treated with cocaine administration in vivo, and that this synaptic plasticity was necessary for locomotor sensitization (Pascoli et al., 2011b). Furthermore, it is now well established that this long-term behavioral adaptation to cocaine relies on gene regulation and new protein synthesis (Valjent et al., 2006a).

Under basal conditions, the unphosphorylated form of ERK1/2 is detected in the cytoplasm of neurons. Upon cocaine administration, the active di-phosphorylated ERKs translocate to the nucleus where they control both epigenetic...
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involves clathrin-dependent endocytosis originating from gluta-
me and dopamine receptors (Brami-Cherrier et al., 2005). In turn, activated MSK1 targets both the cAMP-responsive element-binding (CREB) transcription factor and histone H3 and msk1 knockout mice show a decreased phosphorylation of Ser133-CREB and Ser10-histone H3 induced by cocaine (Brami-Cherrier et al., 2005). This effect on Ser133-CREB phosphorylation was particularly remarkable as this residue is a common substrate for various kinases, including RSK1/2, CaMKIV, and PKA, with PKA being traditionally considered as the major CREB kinase in the striatum (Nestler, 2004). For transcription to occur, DNA decompaction is required to allow the action of the core transcription machinery and transcription factors. Such remodeling of DNA depends on post-translational modifications of histones, including Ser10-histone H3 phosphorylation that positively regulates gene expression (Brami-Cherrier et al., 2009). The default of cocaine-induced Ser10-histone H3 phosphorylation observed in msk1 deficient mice was associated with a striking impact on the induction of immediate early genes, such as c-Fos and dynorphin, which were blocked, whereas the induction of Egr-1/zif268 was spared (Brami-Cherrier et al., 2005). Remarkably, msk1 null mice displayed a decreased psychomotor sensitization to cocaine, whereas CPP was slightly enhanced, in agreement with the role of CREB and dynorphin in the aversive effects of drugs (Carlezon et al., 1998). MSK1-independent genes, such as zif268, thus seem to be more related to the rewarding properties of cocaine, as supported by the lack of cocaine-induced CPP encountered in zif268 knockout mice (Valjent et al., 2006a). Although the role of kinases in histone H3 phosphorylation has been extensively investigated, phosphatases are also implicated in this process. Inhibition of PP1, the phosphatase for Ser10-histone H3, is induced by cocaine via phosphorylation and nuclear translocation of DARPP-32 and is involved in H3 phosphorylation in response to cocaine (Stipanovich et al., 2008).

Ternary complex factors (TCFs) are direct ERK targets that control serum responsive element (SRE)-driven transcription. Among TCFs, Elk1 is phosphorylated by ERK in MSN in response to acute cocaine or amphetamine (Valjent et al., 2000; Choe et al., 2002). The role of Elk1 in mediating cocaine responses was difficult to determine because of the redundancy within TCFs (Cesari et al., 2004) and because Elk-1 is found in dendrites and nuclei of neurons (Gambato et al., 1998) where it governs opposite biological outcomes (Benuard et al., 2011b). In MSN, phosphorylation on Ser133/135/137-Elk-1 by ERK triggers its translocation from the cytoplasm to the nucleus (Lavur et al., 2007). The selective inhibition of Elk-1 phosphorylation, downstream ERK, using a cell penetrating peptide, the TAT-DEF-Elk-1 (TDE; Lavur et al., 2007), significantly impaired cocaine-induced IEG expression (cFos, zif268, and Arc) along with dentritic spine formation onto MSN (Benuard et al., 2011a). An unexpected effect of TDE was found on chromatin remodeling since histone H3 phosphorylation in response to cocaine (Stipanovich et al., 2008).

and genetic responses. This cyto-nuclear shuttling of ERK involves clathrin-dependent endocytosis originating from glu-

tamate AMPA receptor (AMPA) endocytosis (Trifoni et al., 2009). Within the nucleus ERK2 can phosphorylate several nuclear kinases, including p90 ribosomal protein S6 kinases (RSK1/2) and the mitogen and stress-activated protein kinases MSK1 and 2. MSK1, but not RSK1/2 nor MSK2, is rapidly phos-
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They performed site-directed mutagenesis experiments and found that GluN1 was increased by NMDA, the authors proposed that the direct interaction of D1R with GluN1 and GluN2A subunits takes place after just 3 mins from exposure to NMDA, MSN spines contained these sites, yet their findings established a model, the “diffusion trap” concept. Lee et al. (2002) demonstrated that D1R interact with the GluN1 and GluN2A subunits, using co-immunoprecipitation studies from rat hippocampal cultures and transfection of Cos-7 cells and HEK293T lines (Pei et al., 2004). Three GST-peptides were constructed based on the sequence of the D1R c-terminal tail (D1-t sequences) and interactions were revealed between the D1-t2 sequence and the GluN1 subunit and D1-t3 sequence and the GluN2A subunit. Subsequent biochemical studies using synthesized peptides were able to determine, by mass spectrometry, that the interaction was likely due to electrostatic forces (Wood et al., 2005). The GluN1 C3 cassette contains the Arg-rich epitope and the D1R contains a corresponding acidic epitope. Mass spectrometry showed that these two regions interacted and interestingly, they found that Ser397 in the D2 acidic epitope could possibly be phosphorylated by CKI and a phosphorylated version of the peptide bound five times more to the GluN1 Arg rich epitope. It remains to be shown in more native receptor environments if indeed this residue favors the association. The stability of the D1-t2/GluN1 interaction after exposure to ligand remains unclear. In hippocampal neurons and in Cos-7 cells co-transfected with D1R and GluN2A, Lee et al. (2002) saw a significant decrease in the co-immunoprecipitation of D1R or GluN2A after treatment with SKF-81297. However, no such changes were seen for the D1/GluN2A interaction.

The D1R/GluN1 interaction was also found in PSD enriched fractions of striatal tissue (Fiorentini et al., 2003). The authors showed that co-expression of the D1R/GluN1/GluN2B subunits allowed their trafficking to the membrane. It had previously been established that GluN1 alone is restricted to the ER and when GluN1 was co-transfected with D1R, the D1R signal also became restricted to the cytosol. It is thought their oligomerization may hide ER retention signals allowing the passage of the receptor to the export machinery. Despite DA not affecting the BRET signal for association, it was shown that SKF-81297 treatment prevented the internalization of D1R but only when co-expressed with GluN1/GluN2B. This may suggest that once at the membrane in a formed complex, stimulation of the D1R stabilized its presence or association. The association of D1R with NMDAR not only alters their trafficking from the ER to the plasma membrane but also along its surface. Scott and colleagues were able to observe that after just 3 mins from exposure to NMDA, MSN spines contained more D1R (Scott et al., 2006). To explain how D1R signal in spines was increased by NMDA, the authors proposed that the direct interaction with NMDAR would “trap” the D1R to the synapse. They performed site-directed mutagenesis experiments and found that if Ser397 or Ser398 in the D2 domain were mutated to alanine the NMDA effect was lost and the D1R no longer bound GluN1 GST-peptides. The authors were unable to confirm a kinase for these sites, yet their findings established a model, the “diffusion trap,” whereby NMDAR activation would cause some change in

The receptor to enhance the binding with D1R and “trap” them to the spine. The ultimate result of the above mentioned diffusion trap would be to potentiate mutually the receptors.

The D1R/GluN1 oligomer has been demonstrated to have consequences on cellular plasticity and behavior in the hippocampus (Nai et al., 2010). The authors described that the D1R mediated enhancement of plasticity was via direct interactions with GluN1 that was also necessary for spatial working memory. Studies have also addressed whether these D1R/NMDAR oligomers may be implicated in pathologies. DA and glutamate transmission are frequently considered in studies of PD, and Fiorentini et al. (2008) found a significant decrease in the D1R/NMDAR interaction in both the 6-OHDA lesioned mice and in the L-DOPA induced dyskinetic (LID) mice relative to controls.

As with regards to drugs of abuse, one study did examine the D1R/GluN1 interaction after exposure to cocaine (Sun et al., 2009). By Western-blotting, the authors did not detect any change in total expression of the D1R or GluN1 in the dorsal striatum over a 60-min period following an acute (30 mg/kg) injection of cocaine. The authors reported that at 30 min post-acute cocaine there was a significant decrease in the GluN1 immunoprecipitated by the D1R antibody. The authors speculated that at the time point where they saw their effect corresponds to the point at which ERK activity returns to basal activity, the two may be linked and that the disruption of the D1R/GluR1 interaction may limit ERK activity. These possibilities were not tested and so remain to be investigated. A D2R/GluN2B oligomer was also found to be regulated by cocaine (Liu et al., 2006). In the striatum the association between the third intracellular loop of the D2R and the carboxyl terminal tail of GluN2B was enhanced by cocaine. The binding of D2R to the GluN2B displaces CaMKII and reduced GluN2B phosphorylation at Ser118 and calcium currents. Furthermore this interaction was shown to have functional consequences as a TAT-coupled peptide that mimicked the D2R region of interaction prevented acute horizontal activity and stereotyped behavior normally induced by cocaine. These studies suggest that direct interactions between DA-GPCR and NMDAR will provide another level for interaction, and perhaps possible interventions, with regards to addiction-drug induced signaling.

CONCLUSION PERSPECTIVES

Despite the usefulness of animal models for studies of addiction treatment the majority of therapeutic strategies for cocaine addiction still consist mostly of psychiatric therapy. For psychostimulants in general, there is a marked absence of effective treatments despite considerable neurobiological knowledge. Unlike some other “addictions” substitution regimes do not exist for cocaine. Most strategies are based on targeting the neurotransmitter receptors implicated in the reward circuitry. Dissimilar to alcohol or opiates, the withdrawal syndrome from cocaine is not as severe and thus, therapies can focus more on preventing relapse and craving rather than treating withdrawal symptoms. As DA plays a critical role in motivation, reward, and locomotion, studies have focused on modifying its functions in addicted humans. To counter the DA increase elicited by cocaine, a DA depletor reserpine was tested in humans but found to be ineffective (Winhusen et al., 2007). A D1R antagonist Ecopipam was tested...
A vaccine is currently under phase II multi-site clinical trials, but Anderson, S. M., Famous, K. R., Sadri-Vakili, G., Kumaresan, V., Schmidt, H. (2014). Some success was had with GABAa receptor agonist), proved ineffective for human cocaine studies and successful for human alcohol dependence, NMDAR) did not aid addicts to abstain from cocaine (Kampman et al., 2011). Balance of glutamate and DA transmission. Unfortunately, few studies have shown any promise in humans. The non-competitive glutamate receptor antagonists Amanatide and Memantine (acting on the NMDAR) did not aid addicts to abstain from cocaine (Kampman et al., 2006; Collins et al., 2007). Although promising in rodent cocaine studies and successful for human alcohol dependence, the drug Acamprosate (NMDA type Glu receptor antagonist and GABAa receptor agonist), proved ineffective for human cocaine addicts (Kampman et al., 2011). Some success was had with NAcetylcysteine, which promotes the replacement of intracellular glutamate for cysteine via anti-porters and so reduced glutamate transmission. In a pilot study, it was found to diminish the taking of cocaine by the majority of patients after treatment in the study (Mardikian et al., 2007). This may encourage future studies aiming to tone down Glu transmission in cocaine addiction.

Furthermore, in the early 1990s, a cocaine vaccine was developed. It was believed that the production of antibodies against the cocaine molecules could block its effects and thus help maintain abstinence. Unfortunately, getting patients to produce efficient antibodies proved to be a great limit to this approach. A vaccine is currently under phase II multi-site clinical trials, and some reservations have been voiced regarding ethics of vaccines against addiction being used as an prevention or as a treatment approach. In summary, a considerable number of human and pre-clinical animal studies focus on restoring perturbations in the balance of DA and Glu transmission as a therapy for cocaine addiction, but from animal studies we now know that ERK signaling is a key co-ordinator in this system. We propose that D1R/GluN1 oligomers could participate also in this synergy. While blocking ERK activation by drugs is validated in animal models, its potential for human treatment is limited as it is a kinase implicated in a wide range of cellular processes. Instead, we content that targeting ERK signaling and its downstream partners (MSK-1 and histone phosphorylation, Elk-1 phosphorylation) within D1R-MSN specifically may be a promising strategy. This strategy could include the signaling events that are at the crossroad of D1R and NMDAR synergism in response to cocaine (see Figure 1).

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