Structural plasticity in mesencephalic dopaminergic neurons produced by drugs of abuse: critical role of BDNF and dopamine

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INTRODUCTION

Structural plasticity in neurons can be defined as a series of measurable changes in the morphologically defined components of the neuron, i.e., numbers, size, and composition of soma, dendrites, axons, and synapses, occurring over time and in response to changes in the cell environment. Structural plasticity can also be seen as one aspect of neuroadaptation, a general process present in neurons of specific neural circuits when responding to repeated physiologic stimuli, pathologic agents, or effective doses of pharmacologic substance, including addictive drugs (Ikemoto and Bocci, 2014). These stimuli act by engaging molecular mechanisms that are critical for cell growth and survival, their impact on the cell morphology being defined by the stimulus intensity and by genetic and epigenetic predisposing factors that constitute the neuroadaptive potential of the cells.

Since 1990s neuroadaptation and plasticity have been recognized to be relevant in addiction disorders characterized by chronic misuse of neuroactive substances (Koob, 1992; Nestler, 1992; Di Chiara, 1995; Everitt et al., 2001). In mammals, pharmacologic agents characterized by their addictive properties, for example psychostimulants (e.g., cocaine, amphetamines), opioids (e.g., heroin and morphine), nicotine, cannabinoids, and alcohol, were found to engage dopaminergic neurons of the mesocorticolimbic contiguous located in the ventral tegmental area (VTA) (Koob, 1992; Di Chiara, 1995; Koob and Le Moal, 2005; Chen et al., 2010). These neurons produce dopamine (DA) as principal neurotransmitter, project to cortical and limbic brain structures and are involved in regulation of motivation, reward, motor response selection, mood, and arousal. While a large body of experimental findings supports the role for dopaminergic neurotransmission in mediating the addictive properties of these drugs (Koob, 1992; Di Chiara, 1995; Koob and Le Moal, 2005; Kalivas and O’Brien, 2008; Chen et al., 2010), less research was dedicated to the structural changes occurring during exposure to addictive drugs or following their withdrawal. The initial interest on structural plasticity was focused on glutamatergic and GABAergic neurons of nucleus accumbens and prefrontal cortex, i.e., on neurons located in terminal fields of the mesencephalic dopaminergic system (Robinson and Kolb, 1997, 2004; Russo et al., 2010) rather than on their presynaptic side. In dopaminergic neurons structural plasticity was indirectly inferred on the basis of changes in the expression of “marker” proteins though to be involved in structural changes, such as axonal neurofilaments (Nestler, 1992), a phenomenon only later confirmed using morphological techniques (Sklar-Tavron et al., 1996). In fact, by definition, structural plasticity requires morphologic evidence. Dopaminergic neurons are generally identified by immunocytochemistry or immunofluorescence with selective antibodies that recognize tyrosine hydroxylase (TH) or dopamine transporter (DAT; Köhler and Goldstein, 1984). When applied to the post-mortem study in mammalian brains, TH immunocytochemistry allows reliable estimate of soma size and neuron counts in substantia nigra (SN, also identified as A9) and VTA (also identified as A10). Conversely, a proper analysis of the dendrite length and branching is not possible, due to the...
complex overlapping of the dendritic arborizations of adjacent dopaminergic neurons. Visualization of dendrites and dendritic spines of a single neuron requires different approaches, such as the classical Golgi-Cox staining (Juraska et al., 1977) associated with immunohistochemistry (Spiga et al., 2011) intracellular injection with Lucifer Yellow via micropipettes (Sklar-Tavron et al., 1996) or diolistic gene gun delivery of fluorescent dyes (Shen et al., 2008). Dopaminergic neurons can be studied in vitro using primary cell cultures from the ventral mesencephalon of rodent embryos or newborns (Shimoda et al., 1992; Collo et al., 2008). The in vitro approach allows the simultaneous evaluation of soma size, dendritic arborization, dendritic spines, neurochemistry, and intracellular molecular signaling due to their sparse distribution in the culture dish and their standardized control conditions (Collo et al., 2008, 2012).

In this article we summarize the evidence of structural plasticity occurring in mesencephalic dopaminergic neurons following exposure to addictive drugs, focusing on soma, and dendritic arborization rather than synapses and addressing the key molecular intracellular signaling involved.

**STRUCTURAL PLASTICITY IN DOPAMINERGIC NEURONS AS CELLULAR NEUROADAPTATION: OPPOSITE EFFECTS OF OPIOIDS AND PSYCHOSTIMULANTS**

Structural plasticity includes hyperplastic and hypoplastic phenomena, i.e., the increase or decrease of number and size of morphologically defined components of the neuron. In the brain reward circuit, drugs of addiction produce both hyperplastic and hypoplastic phenomena, the former generally associated to psychostimulants, the latter to the use of opioids (for review see Russo et al., 2009, 2010). Since chronic exposure to both opioids and psychostimulants produces behavioral sensitization, compulsive drug taking, and relapse after extinction, at the time of their initial discovery these changes appeared somewhat contradictory, casting some doubts about the relevance of structural plasticity in addictive behavior. Recent findings regarding the role of withdrawal state (Spiga et al., 2010; Mazei-Robison et al., 2011), region-specific changes of synaptic spines (Russo et al., 2010) and differential regulation of endogenous neurotrophins, in particular brain derived neurotrophic factors (BDNF) (Russo et al., 2009; Koo et al., 2012), have been advocated as key factors in disentangling this paradox; some possible explanations will be reviewed later in this article.

Chronic exposures to opioids reduce soma size and dendrites of the dopaminergic neurons located in the VTA of adult rodents without reducing the number of neurons (Sklair-Tavron et al., 1996; Spiga et al., 2003; Russo et al., 2007). Opioid-induced hypotrophic effects on soma were observed following either passive dosing or self-administration of heroin or morphine and persist for several weeks during withdrawal. Functionally, chronic exposure to opioids is known to increase VTA neural firing while reduction is observed during withdrawal (Diana et al., 1995; Koo et al., 2012).

Reduced soma size and neural firing were also observed during withdrawal from cannabinoids in rodents (Diana et al., 1998; Spiga et al., 2010). These effects are partially determined by endogenous opioids since acute morphine attenuates the behavioral cannabinoid withdrawal syndrome in mice (Lichtman et al., 2001). Interestingly, chronic exposure to cannabinoids per se does not produce change of soma sizes of VTA dopaminergic neurons. Lack of change in the soma size was also recently showed in rats trained to chronically self-administer cocaine, nicotine, and alcohol when sacrificed in presence of drugs (Mazei-Robison et al., 2011). These data do not rule out the possibility of changes during withdrawal or crash after drug taking “binges,” both conditions associated to a functional hypodopaminergic state (Weiss et al., 1992; Melis et al., 2005; Zhang et al., 2012); so far structural effects were not studied.

Cocaine and amphetamine exposures in vivo increase dendrite arborization and spines in VTA (Mueller et al., 2006; Sarti et al., 2007). In vitro studies on primary cultures of mesencephalic neurons from mouse embryos corroborate this evidence. Dose-dependent increases of soma size, maximal dendrite length, and number of primary dendrites were observed (Collo et al., 2008, 2012). Interestingly, also nicotine was shown to increase structural plasticity of dopaminergic neurons in vitro, effect blocked by mecamylamine and dihydro-β-erythroidine but not methylylcocainethione, suggesting the involvement of α4β2 nicotinic receptor (nAChR; (Collo et al., 2013). These nicotinic hetero-receptors expressed in dopaminergic neurons control DA release and are critical for the reinforcing effects of nicotine in vivo (Picciotto et al., 1998). Consistently, dopaminergic neurons from the mesencephalon of α4 nAChR-subunit knockout (KO) mice did not show nicotine-induced plasticity (Collo et al., 2013).

Prenatal exposure to either cocaine or nicotine during the last gestational phase (E17-21) was associated with significant increase of soma size of dopaminergic neurons in newborns and young mice (Collo et al., 2012, 2013). Prenatal exposures to cocaine and amphetamines produce long-term changes in the behavior and neurochemistry of the mesencephalic dopaminergic system of offspring assessed as adults (Crozier et al., 2003; Lloyd et al., 2013), suggesting a possible association between dopaminergic structural plasticity and liability to develop addiction.

**CRITICAL ROLE OF THE BDNF-TrkB SIGNALING IN DETERMINING STRUCTURAL PLASTICITY OF DOPAMINERGIC NEURONS EXPOSED TO ADDICTIVE DRUGS**

Neurotrophic factors that bind to the tropomyosin-related kinase B (TrkB) receptor were shown to be of importance in the development of the central nervous system (CNS) and in shaping neuronal morphology of dopamine neurons and other brain circuits (for a review see Ohira and Hayashi, 2009). In particular, BDNF-TrkB signaling has been extensively studied as critical mediator of the structural changes produced by addictive drugs (Russo et al., 2009; Koo et al., 2012). Mesencephalic dopaminergic neurons significantly express BDNF since prenatal time (Baqi et al., 2005). Still present in adult life, BDNF expression can be transiently increased by psychostimulants in VTA dopaminergic neurons (Graham et al., 2007). These increases consolidate and persist over time during abstinence (Pu et al., 2006) and during extinction of drug self-administration and in craving incubation paradigms (Grimm et al., 2003). Infusion of BDNF in VTA induces long-lasting potentiation of cocaine seeking...
during abstinence (Lu et al., 2004), while BDNF immunoneu- 
ralization attenuates the cocaine addictive behavioral effects 
(Graham et al., 2007). To our knowledge, direct evidence of 
structural changes in dopaminergic neurons during withdrawal, 
abstinence and incubation with psychostimulants is lacking in 
literature. However, in consideration of the well-known BDNF 
neurotrophic properties on dendrites and soma size, it is possible 
to speculate that some structural plasticity could occur. Inter-
estingly, GDNF, another neurotrophic factor, increases in VTA 
during cocaine withdrawal and mediates incubation of cocaine 
craving (Lu et al., 2009), further supporting possible structural 
effects.

Almost opposite effects were observed with opioids: mor-
phine reduces BDNF expression in VTA neurons; low BDNF 
levels were associated with reduced soma size, and local infu-
sion with BDNF normalizes soma size (Sklair-Tayron et al., 1996; 
Russo et al., 2009). Recent studies using conditional KO mice and 
optogenetic technology showed that morphine-induced low 
levels of BDNF in the VTA are associated to hypersensitization 
of VTA dopaminergic neurons to morphine, whose adminis-
tration increases firing and DA release, producing conditioned 
place preference (Koo et al., 2012). Conversely, acute withdrawal 
and abstinence are associated with increased BDNF expression 
and TrkB-mediated plasticity changes that are essentials for neg-
avative reinforcing effects of morphine withdrawal (Vargas-Perez 
et al., 2014). Interestingly, the opioid effects on DA release are 
direct, mediated by GABAergic inhibitory neurons under gluta-
materic control (Bonci and Williams, 1997; Vargas-Perez et al., 
2009; Jalabert et al., 2011), suggesting a role also for these 
networkreceptors.

The main intracellular pathways activated by BDNF-TrkB 
signaling are the MEK-ERK, the PI3K-Akt-mTORC1, the PLCy-
DAG-PCK/Ca2+, and mNKB pathways, all involved in cell survival 
and growth (Kumar et al., 2005; Russo et al., 2009). These path-
ways are not only activated by BDNF but also by G-protein 
coupled receptors (e.g., Girault et al., 2007). Recent evidence 
indicates that cocaine and nicotine activate both MEK-ERK 
and Akt-mTORC1 pathways in primary cultures of dopami-
nergic neurons (Collo et al., 2012, 2013). Phosphorylation in 
these two pathways was found critical for structural plasticity 
since pretreatments with selective inhibitors for ERK, PI3K, 
and mTORC1 block the increase of soma size and dendritic 
assimilation produced by psychostimulants and nicotine (Collo 
et al., 2013). Conversely, morphine exposure is associated with 
reduction in Akt levels and phosphorylation, attenuating mTOR-
dependent phosphorylation (Russo et al., 2007; Mazei-Robison 
et al., 2011). The central role of the PI3K-Akt-mTOR pathway 
in determining soma size of mesencephalic dopaminergic 
nurons is exemplified by the phosphatase and tensin homolog 
(PTEN) KO mice. PTEN is a negative regulator of PI3K whose 
null mutation leads to a constitutive preferential state of activa-
tion of Akt-mTORC1 pathway; the result is a massive increase 
in soma size of dopaminergic neurons already visible in new-
borns, that persists in adult mice (Diaz-Ruiz et al., 2009). Other 
mechanisms affecting dendrite and soma size include the mod-
ulation of Ca2+ levels and the cAMP production, the latter 
not operated by BDNF. A large body of evidence indicates that 
Ca2+-dependent AMPA and NMDA glutamate receptors reg-
ulate dendrite growth in pyramidal neurons and interneurons 
(Hamad et al., 2011). In dopaminergic neurons NMDA-dependent 
anxal growth was described as related to CaMKII phospho-
rylation (Schmitz et al., 2009), while preliminary in vitro data 
indicate a critical role for AMPA receptors. In GABAergic neurons 
located in the VTA, chronic activation of the cAMP-PKA-CREB 
was associated with reduced firing and soma size in dopaminergic 
nurons during morphine withdrawal (Bonci and Williams, 1997; 
Koo et al., 2012), suggesting an indirect involvement in structural 
plasticity.

Interestingly, structural changes of soma size and dendritic 
assimilation of dopaminergic neurons are not specific of addictive 
Drugs. In a recent article a reduction of soma size in the 
VTA was observed in male rats after single and repeated mat-
ing episodes (Pitchers et al., 2014). Naloxone treatment reversed 
soma size reduction and attenuated the longer-term expression 
of experience-induced facilitation of sexual behavior without 
affecting its rewarding properties. In another study, an increase 
of the number, size, and dendritic spines of mesencephalic dopaminergic neurons was associated to exercise and intense 
motor behavior in rats exposed to moderate dose of dopami-
nergic neurotoxins (Real et al., 2013), supporting a role for neu-
rotrophic BDNF-TrkB signaling in behaviorally induced structural 
plasticity.

**DOPAMINE AS NEUROTROPHIC FACTOR: ROLE OF D3 
RECEPTOR SIGNALING IN STRUCTURAL PLASTICITY OF 
DOPAMINERGIC NEURONS**

In addition to its role as a neurotransmitter, DA can act as a 
neurotrophic factor. When released in the extracellular space, 
DA binds to postsynaptic receptors, producing structural plas-
ticity: for example DA increases TrkB phosphorylation via D1 
receptor (Iwakura et al., 2008). DA also binds to presynaptic 
D3, D2s, and D5 receptors located on dopaminergic neurons 
(Zhang and Sulzer, 2012). Functional studies in mutant mice indicate that D2 and D3 receptors are complementary in reg-
ulating phasic and tonic dopamine release from dopaminergic 
nerves terminals in caudate and nucleus accumbens (Le Foll et al., 
2005b; Maina and Mathews, 2010). The intracellular pathways 
activated by the presynaptic DA receptors and related to struc-
tural plasticity are only partially understood, being the majority 
of studies performed in non-dopaminergic cells. Converging find-
ings indicated a primary role for D3 receptors in dopaminergic 
structural plasticity via phosphorylation of MEK-ERK1/2 and 
PI3K-Akt-mTORC1 pathways (Cussac et al., 1999; Beom et al., 
2004; Collo et al., 2012, 2013). Conversely, D2s receptors inhibit 
MEK-ERK1/2 pathway and are negatively coupled with adeny-
late-cyclase (Van-Ham et al., 2007). PLCy activation and β-arrestin 
non-canonical pathways were described for the D2L splice vari-
ant present on postsynaptic neurons (Delguidice et al., 2011). 
Finally, less data are available on D5 receptor, whose role has been 
related to functional plasticity (Schilström et al., 2006; Argilli et al., 
2008).

Direct evidence linking D3 receptors and structural plasticity 
was recently obtained in primary cultures of dopaminergic neu-
rons from mouse embryos. Repeated exposure with low doses
of D3-preferential agonists, such as quinpirole or 7OH-DPAT, increased soma size and the number and length of primary dendrites (Collo et al., 2008). These effects were also produced by drugs of addiction such as cocaine, amphetamine, nicotine, and ketamine, all known to increase extracellular levels of DA in the VTA. Pretreatments with DA D3 selective antagonist SB277011-A and the non-selective D2/D3 antagonist sulpiride resulted in a blockade of dendrite outgrowth and soma size (Collo et al., 2008, 2012). No structural plasticity was observed when treatments with psychostimulants or nicotine were performed in cell cultures from the mesencephalon of D3 KO mice (Collo et al., 2008, 2012, 2013). When nicotine was repeatedly administered to pregnant D3 KO mice during the last gestational phase, no effect was observed on the soma size of VTA neurons of newborns. Recent evidence suggests that D3 receptors work in concert with BDNF-TrkB signaling. In vivo experiment showed that D3 receptor expression depends on the levels of BDNF (Guillin et al., 2003) and that cocaine exposure increases the synthesis of both BDNF and D3 receptors (Le Foll et al., 2005a), while morphine increases the expression of D3 receptors only (Spangler et al., 2003), marking a difference between the two addictive drugs (Figure 1).

HUMAN EVIDENCE OF ADDICTIVE DRUG INDUCED STRUCTURAL PLASTICITY

No direct human evidence of structural plasticity induced by addictive drugs in dopaminergic neurons is currently available. Post-mortem studies in cocaine users revealed a 16% reduction of melanized dopaminergic neurons with no reported change of soma size (Little et al., 2009), while a reduction of TH levels in dopaminergic terminals of the striatum was found in heroin addicts (Kish et al., 2001).

In vivo neuroimaging studies, which lack cellular resolution, showed reduced 6-FDOPA uptake in the dopaminergic terminals of the striatum of cocaine addicts during 10–30 days of abstinence (Wu et al., 1997). Interestingly, another marker of dopaminergic terminals in striatum, i.e., DAT levels, was found reduced in methamphetamine addicts (Chang et al., 2007) and in tobacco and marijuana smokers (Leroy et al., 2012). Extracellular DA release estimated using the 11C-raclopride displacement techniques indicated a lower DA tone in ventral striatum of cocaine (Martinez et al., 2009) and marijuana users (Volkow et al., 2014), the latter correlated with enhanced stress reactivity and irritability, confirming a hypodopaminergic state. Structural MRI showed a volumetric increase in the left nucleus accumbens in marijuana users (Gilman et al., 2014), enlarged striatum in methamphetamine users (Chang et al., 2007) and reduction in nucleus accumbens, anterior cingulated and orbitofrontal cortex in children exposed in utero to opioids (Walhovd et al., 2007), all findings in line with preclinical observations.

CONCLUSION AND FUTURE RESEARCH

Addictive drugs induce structural plasticity in dopaminergic neurons. While a complete picture of structural changes associated to the different stages of the addiction cycle and its translational value in human is still lacking, differences among the main addictive drugs in producing either hypotrophic or hypertrophic response stand out, driven by the respective down or up regulations of BDNF and extracellular dopamine levels. These effects can be more

FIGURE 1 | Schematic representation of relevant intracellular pathways of BDNF and dopamine dependent structural plasticity in dopaminergic neurons. TrkB, tropomyosin-related kinase B; Src, proto-oncogene tyrosine protein kinase; MEK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase; D3R, dopamine D3 receptor; Giφγ, G protein; PI3K, phosphatidylinositol 3-kinase; Akt, serine threonine kinase or protein kinase B; mTORC1, mammalian target of rapamycin complex 1; p70S6K, p70 ribosomal S6 protein kinase; PD98059, MEK inhibitor; LY294002, PI3K inhibitor; rapamycin, mTORC1 inhibitor; SB277011-A, selective D3R inhibitor.
conspicuous during neural development, as shown in offspring following in utero exposure or in vitro using embryonic-derived cell cultures. Overall, structural changes appear to be related to some differences in targeting of reward and stress circuits that work in parallel to control motivation (Koob, 2013; Ikemoto and Bonci, 2014). These long term structural changes can be seen as substrates of “memory” traces (Nestler, 2013) that would eventually constitute a liability for drug taking relapse.

ACKNOWLEDGMENTS

This manuscript was supported by the grant from ex 60%, University of Brescia to Ginetta Collo. The authors thank Emilio M. Pich for helpful discussion regarding the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 19 October 2014; paper pending published: 29 October 2014; accepted: 06 November 2014; published online: 25 November 2014.

Citation: Collo G, Cavalleri L and Spano P (2014) Structural plasticity in mesencephalic dopaminergic neurons produced by drugs of abuse: critical role of BDNF and dopamine. *Front. Pharmacol.* 5:259. doi: 10.3389/fphar.2014.00259

This article was submitted to Neuropharmacology, a section of the journal *Frontiers in Pharmacology*.

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