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Neurotrophic Factors and Structural Plasticity in Addiction

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Abstract

Drugs of abuse produce widespread effects on the structure and function of neurons throughout the brain's reward circuitry, and these changes are believed to underlie the long-lasting behavioral phenotypes that characterize addiction. Although the intracellular mechanisms regulating the structural plasticity of neurons are not fully understood, accumulating evidence suggests an essential role for neurotrophic factor signaling in the neuronal remodeling which occurs after chronic drug administration. Brain-derived neurotrophic factor (BDNF), a growth factor enriched in brain and highly regulated by several drugs of abuse, regulates the phosphatidylinositol 3'-kinase (PI3K), mitogen-activated protein kinase (MAPK), phospholipase C γ (PLC γ) and nuclear factor kappa B (NF κ B) signaling pathways, which influence a range of cellular functions including neuronal survival, growth, differentiation, and structure. This review discusses recent advances in our understanding of how BDNF and its signaling pathways regulate structural and behavioral plasticity in the context of drug addiction.

1. Introduction

An essential feature of drug addiction is that an individual continues to use drug despite the threat of severely adverse physical or psychosocial consequences. Although it is not known with certainty what drives these behavioral patterns, it has been hypothesized that long-term changes that occur within the brain's reward circuitry are important (Figure 1). In particular, adaptations in dopaminergic neurons of the ventral tegmental area (VTA) and in their target neurons in the nucleus accumbens (NAc) are thought to alter an individual's responses to drug and natural rewards, leading to drug tolerance, reward dysfunction, escalation of drug intake, and eventually compulsive use (Everitt et al., 2001; Kalivas and O'Brien, 2008; Koob and Le Moal, 2005; Nestler, 2001; Robinson and Kolb, 2004).

There has been a major effort in recent years to determine the cellular and molecular changes that occur during the transition from initial drug use to compulsive intake. Among many types of drug-induced adaptations, it has been proposed that changes in brain-derived neurotrophic factor (BDNF), or related neurotrophins, and their signaling pathways alter the function of neurons within the VTA-NAc circuit and other reward regions to modulate the motivation to take drugs (Bolanos and Nestler, 2004; Pierce and Bari, 2001). A corollary of this hypothesis is that such growth factor-induced cellular and molecular adaptations are reflected in morphological changes of reward-related neurons. For example, chronic stimulant administration increases branching of dendrites and the number of dendritic spines and

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dynamically increases levels of BDNF in several brain reward regions, whereas chronic opiate administration decreases dendritic branching and spines as well as BDNF levels in some of the same regions (for review see (Robinson and Kolb, 2004; Thomas et al., 2008). Moreover, chronic morphine decreases the size of VTA dopamine neurons, an effect reversed by BDNF (Russo et al., 2007; Sklair-Tavron et al., 1996). However, direct, causal evidence that these structural changes drive addiction remains lacking.

The proposal that BDNF may be related to structural plasticity of the VTA-NAc circuit in addiction models is consistent with a large literature which has implicated this growth factor in regulation of dendritic spines. For instance, studies using conditional deletions of BDNF or the TrkB receptor show that they are required for proliferation and maturation of dendritic spines in developing neurons as well as the maintenance and proliferation of spines on neurons throughout the adult brain (Chakravarthy et al., 2006; Danzer et al., 2008; Horch et al., 1999; Tanaka et al., 2008a; von Bohlen Und Halbach et al., 2007).

Although the exact molecular mechanisms by which BDNF mediates structural plasticity of the brain's reward circuitry remain unknown, recent studies suggest that specific pathways downstream of BDNF are modulated by drugs of abuse, and that these neurotrophic factor-dependent signaling changes correlate with morphological and behavioral end-points in animal models of drug addiction. In this review, we discuss new advances in our understanding of how opiates and stimulants regulate neurotrophic factors signaling and the cellular and behavioral consequences of these effects. We also propose areas for future investigation to address the paradoxically opposite effects of stimulants and opiates on neuronal morphology and certain behavioral phenotypes consistent with addiction.

2. Neurotrophin signaling pathways

Uncovering the signaling pathways that mediate neuronal development and survival has been a long-time goal of neuroscience research. However, neurotrophic factor signaling in the adult central nervous system (CNS) has over the past decade become an important area of interest, as neurotrophic signaling has been shown to modulate neural plasticity and behavior throughout an organism's life (for review see (Chao, 2003)). The first neurotrophic factor identified, nerve growth factor (NGF), was isolated in 1954 (Cohen et al., 1954); cloning of the gene itself did not occur until 1983 (Scott et al., 1983). This discovery was followed closely by the purification and identification of additional NGF-like growth factors that defined a neurotrophin family: BDNF (Barde et al., 1982; Leibrock et al., 1989), neurotrophin-3 (NT3) (Hohn et al., 1990; Maisonpierre et al., 1990), and neurotrophin-4/5 (NT4/5) (Berkemeier et al., 1991). Neurotrophin family members are paralogs and share significant homology (Hallbook et al., 2006); all are polypeptides that homodimerize and are found in both immature and mature forms in the CNS. While it has long been thought that the cleaved ~13 kDa mature form was the active signaling molecule, recent studies indicate that the pro- (immature) forms of the neurotrophins, which retain their N-terminus, are detectable in the brain (Fahnestock et al., 2001) and mediate signaling cascades distinct from the mature peptides. The actions of NGF in the adult CNS are largely localized to cholinergic cells in the basal forebrain, while the distribution of the other neurotrophins is much more widespread.

Further specificity of the neurotrophin signal is produced through the differential expression of neurotrophin receptors, which can be separated into two categories, the tropomyosin-related kinase (Trk) and p75 neurotrophin (p75NTR) receptors. The p75NTR was first identified as a receptor for NGF (Johnson et al., 1986), but actually binds both the immature and mature forms of all four neurotrophins (Lee et al., 2001; Rodriguez-Tebar et al., 1990; Rodriguez-Tebar et al., 1992). As opposed to p75NTR, the Trk family of receptors exhibits specificity for its ligands. The TrkA receptor preferentially binds NGF (Kaplan et al., 1991; Klein et al., 1991),

the TrkB receptor binds BDNF (Klein et al., 1991) and NT4/5 (Berkemeier et al., 1991), and the TrkC receptor binds NT3 (Lamballe et al., 1991). While the mature neurotrophins have an increased affinity for Trk receptors compared to the propeptides, both the immature and mature forms can bind p75NTR with high affinity. Additionally, p75NTR has been shown to form complexes with Trk receptors, and these receptor complexes exhibit an increased affinity for the respective Trk ligands compared to homodimeric Trk.

Trk receptors are single transmembrane spanning proteins composed of an extracellular ligand binding domain and an intracellular region containing a tyrosine kinase domain. Similar to other receptor tyrosine kinases, Trk receptors homodimerize in response to ligand binding, which allows for trans-phosphorylation within the activation loop to increase catalytic activity of the receptor kinase. Trans-phosphorylation at tyrosine residues in the juxtamembrane domain and in the C-terminus generates attachment sites for SH2 (Src homology 2)-type “linker” proteins, such as Src homology domain-containing protein (Shc), and phospholipase C γ (PLC γ), respectively. Shc binding initiates downstream signaling cascades leading ultimately to the activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3'-kinase (PI3K) pathways. Stimulation of the MAPK pathway includes activation of extracellular signal-regulated kinase (ERK), while binding of insulin receptor substrate (IRS) leads to recruitment and activation of PI3K and to the activation of downstream kinases such as thymoma viral proto-oncogene (Akt), also known as protein kinase B (PKB). Phosphorylation and activation of PLC γ leads to the formation of inositol(1,4,5)triphosphate (IP $_3$) and diacylglycerol (DAG) and to the stimulation of protein kinase C (PKC) and cellular Ca $^{2+}$ pathways. These three main signaling pathways—PI3K, PLC γ , and MAPK/ERK—induced by Trk receptor activation are illustrated in Figure 2. Interestingly, there is evidence of differential activation of these three cascades depending on the neurotrophin, receptor type, and signal strength and duration involved (see (Segal, 2003)). The differential activation of these downstream pathways seems particularly relevant to drug-induced changes in neuronal morphology and behavior, as will be detailed in later sections of this review.

Compared to the extensive knowledge of the consequences of Trk receptor activation, much less is known about the role of p75NTR signaling in neurotrophin function. Activation of the Trk effectors generally leads to pro-survival and differentiation signals, whereas the activation of p75NTR initiates both pro-survival and pro-death signaling cascades. Survival signaling through the p75NTR requires downstream Nuclear Factor kappa B (NF κ B) which is thought to be activated indirectly through TNF (tumor necrosis factor) receptor-associated factor 4/6 (TRAF4/6) or receptor interacting protein 2 (RIP2) (for review see (Chao, 2003)). Although neurotrophin signaling allows for a complex variety of signals that depend on the expression pattern of neurotrophins and receptors and the processing of the neurotrophin peptides, this review focuses on drug-induced changes in neurotrophin signaling pathways downstream of BDNF.

3. Drug-induced changes in BDNF in brain reward regions

Changes in levels of BDNF protein and mRNA have been examined in multiple brain regions following administration of many classes of addictive substances. Stimulants produce a widespread, but transient, induction of BDNF protein in the NAc, prefrontal cortex (PFC), VTA, and the central (CeA) and basolateral (BLA) nuclei of the amygdala (Graham et al., 2007; Grimm et al., 2003; Le Foll et al., 2005). Both contingent and non-contingent (i.e., animals yoked to self-administering animals) cocaine administration causes elevated levels of BDNF protein in the NAc (Graham et al., 2007; Liu et al., 2006; Zhang et al., 2002). Likewise, long-term withdrawal of up to 90 days after cocaine self-administration is correlated with increased BDNF protein in the NAc, VTA, and amygdala (Grimm et al., 2003; Pu et al.,

2006), and there is early evidence that epigenetic regulation at the *bdnf* gene may be involved in mediating this persistent induction (Kumar et al., 2005).

Though fewer studies have been conducted to examine BDNF mRNA and protein levels after exposure to opiates, it appears that BDNF levels are regulated by opiates in certain reward-related brain regions. Acute morphine administration increases BDNF mRNA levels in the NAc, medial PFC (mPFC), VTA, and orbitofrontal cortex. In the VTA, chronic morphine, given via subcutaneous (s.c.) implants, is reported to be ineffective in altering BDNF mRNA expression (Numan et al., 1998). This, however, is in contrast to changes in BDNF protein observed after chronic morphine treatment. Using escalating doses of intraperitoneal (i.p.) morphine, it has been shown that the number of BDNF immunoreactive cells in the VTA is decreased (Chu et al., 2007), suggesting downregulation of BDNF function. Although no reports have examined BDNF expression in the hippocampus or caudate-putamen (CPu) after administration of stimulants or opiates, such studies are warranted since robust morphological changes have been observed in pyramidal neurons of the hippocampal CA3 region and medium spiny neurons (MSNs) of the CPu under these conditions ((Robinson and Kolb, 2004); see Table 1).

4. Drug-induced changes in BDNF signaling pathways in brain reward regions

Several proteins in neurotrophin signal cascades have been shown to be regulated within the mesolimbic dopamine system by opiates and stimulants; these include drug effects on IRS–PI3K–Akt, PLC γ , Ras–ERK, and NF κ B signaling (Figure 3). Stimulants dramatically increase ERK phosphorylation in numerous brain regions, including the NAc, VTA, and PFC, following acute or chronic drug administration (Jenab et al., 2005; Shi and McGinty, 2006, 2007; Sun et al., 2007; Valjent et al., 2004; Valjent et al., 2005). These findings are consistent with stimulant-induced increases in neuronal branching and spine number, given Ras–ERK's established role in neurite outgrowth. The effects of opiates on ERK signaling are less clear. Recently, it has been reported that ERK phosphorylation is decreased in the NAc (Muller and Unterwald, 2004), PFC (Ferrer-Alcon et al., 2004), and VTA (unpublished observations) after chronic morphine, an effect that is consistent with decreased neurite branching seen in these regions in morphine-dependent animals. However, earlier work from our group and others reported increased ERK activity, including increased ERK phosphorylation and catalytic activity, in the VTA after chronic morphine (Berhow et al., 1996b; Liu et al., 2007; Ortiz et al., 1995). Further studies are needed to determine the explanation for these discrepant findings. Moreover, it is important to use multiple approaches to measure protein activity so that biochemical events can be correlated with morphological and behavioral endpoints. For example, inhibition of ERK in VTA dopamine neurons does not affect cell size (Russo et al., 2007), such that future studies are required to address the functional relevance of drug-induced changes in ERK activity in this and other brain areas as they relate to addictive phenotypes.

Several recent reports have shown that IRS–PI3K–Akt signaling is influenced by drugs of abuse (Brami-Cherrier et al., 2002; McGinty et al., 2008; Muller and Unterwald, 2004; Russo et al., 2007; Shi and McGinty, 2007; Wei et al., 2007; Williams et al., 2007). Chronic opiate administration decreases Akt phosphorylation in both the NAc and VTA (Muller and Unterwald, 2004; Russo et al., 2007). These biochemical alterations correspond to decreased neuronal branching and dendritic spine density or, in the case of VTA dopamine neurons, decreased cell body size (Diana et al., 2006; Robinson et al., 2002; Robinson and Kolb, 1999b; Russo et al., 2007; Spiga et al., 2005; Spiga et al., 2003).

The effects of stimulants on IRS–PI3K–Akt signaling in these regions are less clear. For example, chronic cocaine increases PI3K activity in the NAc shell and decreases its activity

in the NAc core (Zhang et al., 2006). These data are in line with a previous report showing that chronic cocaine selectively increased BDNF mRNA levels in the NAc shell and decreased TrkB receptor mRNA in the NAc core (Filip et al., 2006). Thus, shell and core differences in PI3K activity could be explained by differential upstream regulation of BDNF and TrkB by cocaine. Interestingly, when a more general dissection of striatum is used (including NAc and CPu), it has been shown that amphetamine decreases Akt activity in synaptosome preparations (Wei et al., 2007; Williams et al., 2007), and we have observed similar effects of chronic cocaine in the NAc without distinguishing between core and shell (Pulipparacharuvil et al., 2008). Additionally, these studies are complicated by the time-course used to study Akt signaling changes, as recent work by McGinty and colleagues suggests that chronic amphetamine causes a transient and nuclear-specific change in Akt phosphorylation in striatum (McGinty et al., 2008). At early timepoints after amphetamine administration there is a nucleus-specific increase in Akt phosphorylation, however, after two hours Akt phosphorylation is decreased, suggesting a compensatory mechanism to turn off this activity. Understanding the dynamic relationship between stimulants and Akt signaling will be important to determine whether this signaling pathway is driving stimulant-induced structural plasticity in the NAc, as is the case for opiates in the VTA (see Section 6).

Alterations in the PLC γ and NF κ B signaling pathways in drug abuse have not been as well studied as ERK and Akt; however, recent work shows that both pathways are regulated by drugs of abuse. Chronic administration of morphine increases total levels of PLC γ protein as well as levels of its activated tyrosine-phosphorylated form (Wolf et al., 2007; Wolf et al., 1999). Moreover, viral-mediated PLC γ overexpression in the VTA was found to increase ERK activity in this brain region (Wolf et al., 2007), thereby mimicking a similar increase in ERK activity seen after chronic morphine in earlier studies (Berhow et al., 1996b). PLC γ overexpression in the VTA also regulates opiate reward and related emotional behaviors, with distinct effects seen in rostral vs. caudal VTA (Bolanos et al., 2003). Likewise, Graham and colleagues (Graham et al., 2007) observed increased phosphorylation of PLC γ in the NAc following acute, chronic yoked, and chronic self-administered cocaine, an effect that was dependent on BDNF.

An earlier study from our group showed that the NF κ B subunits p105, p65, and I κ B are increased in the NAc in response to chronic cocaine administration (Ang et al., 2001). This is consistent with findings from Cadet and colleagues (Asanuma and Cadet, 1998), who demonstrated that methamphetamine induces NF κ B binding activity in striatal regions. Given that some of the drug-regulated NF κ B proteins activate NF κ B signaling, whereas others inhibit it, it was unclear from these original studies whether the observed protein changes reflect an overall increase or decrease in NF κ B signaling. We have more recently resolved this question by showing that chronic cocaine administration upregulates NF κ B transcriptional activity in the NAc, based on findings in NF κ B-LacZ transgenic reporter mice (Russo, Soc. Neurosci. Abstr. 611.5, 2007). More recent evidence has directly implicated the induction of NF κ B signaling in the NAc in the structural and behavioral effects of cocaine (see Section 6). These early findings are intriguing and warrant further exploration including an examination of the effect of opiates on NF κ B signaling in brain reward regions.

5. Drug-induced structural plasticity in brain reward regions

The brain's reward circuitry has evolved to direct one's resources to obtain natural reward, but this system can be corrupted or hijacked by drugs of abuse. Within this circuit, structural plasticity is generally characterized by altered dendrite branching or arborization and by changes in the density or morphometry of dendritic spines. Although the direct behavioral relevance of experience-dependent morphological changes is still under investigation, it is believed that synaptic function is determined not only by the number, but also the size and

shape of each individual spine head. As spines form, they send out thin immature structures which take on either stubby, multisynaptic, filopodial, or branched shapes (for review see (Bourne and Harris, 2007; Tada and Sheng, 2006). In the adult brain, under basal conditions, it is estimated that at least 10% of spines have these immature shapes suggesting that plasticity is a continuous process throughout life (Fiala et al., 2002; Harris, 1999; Harris et al., 1992; Peters and Kaiserman-Abramof, 1970). These structures are transient and can form within hours of stimulation and persist as long as a few days in vivo (Holtmaat et al., 2005; Majewska et al., 2006; Zuo et al., 2005).

It is believed that stabilization of a transient, immature spine into a more permanent, functional spine occurs through an activity-dependent mechanism (for review see (Tada and Sheng, 2006). Stimulation protocols which induce long-term depression (LTD) are associated with shrinkage or retraction of spines on hippocampal and cortical pyramidal neurons (Nagerl et al., 2004; Okamoto et al., 2004; Zhou et al., 2004), whereas induction of long-term potentiation (LTP) is associated with the formation of new spines and enlargement of existing spines (Matsuzaki et al., 2004; Nagerl et al., 2004; Okamoto et al., 2004). At a molecular level, it is believed that LTP and LTD initiate changes in signaling pathways, and in the synthesis and localization of proteins, which eventually alter the polymerization of actin to affect spine maturation and stability and ultimately to produce a functional spine (LTP) or retraction of an existing spine (LTD) (for review see (Bourne and Harris, 2007; Tada and Sheng, 2006). Upon stabilization, spines become mushroom shaped, have larger postsynaptic densities (Harris et al., 1992), and have been shown to persist for months (Holtmaat et al., 2005; Zuo et al., 2005). These changes reflect a highly stable cellular event that may be a plausible explanation for at least some of the long-term behavioral changes associated with drug addiction.

Most classes of addictive substances, when administered chronically, alter structural plasticity throughout the brain's reward circuitry. Most of these studies are correlative and associate structural changes in specific brain regions with a behavioral phenotype indicative of addiction. Over the past decade, Robinson and colleagues have led the way in understanding how drugs of abuse regulate structural plasticity (for review see (Robinson and Kolb, 2004). Since these original observations, other drug abuse researchers have added to this growing literature to uncover drug class-specific effects on neuronal morphology. As depicted in Table 1 and Figure 3, opiates and stimulants differentially affect structural plasticity. Opiates have been shown to decrease the number and complexity of dendritic spines on NAc MSNs and mPFC and hippocampus pyramidal neurons, and to decrease the overall soma size of VTA dopaminergic neurons, with no effect seen on non-dopaminergic neurons in this brain region (Nestler, 1992; Robinson and Kolb, 2004; Russo et al., 2007; Sklair-Tavron et al., 1996). To date, there is a single exception to these findings, where it was reported that morphine increases spine number on orbitofrontal cortical neurons (Robinson et al., 2002). In contrast to opiates, stimulants such as amphetamine and cocaine have been shown to consistently increase dendritic spines and complexity in NAc MSNs, VTA dopaminergic neurons, and PFC pyramidal neurons, with no reports of decreased structural plasticity (Lee et al., 2006; Norrholm et al., 2003; Robinson et al., 2001; Robinson and Kolb, 1997, 1999a; Sarti et al., 2007).

Although the molecular mechanisms downstream of neurotrophic factor signaling which underlie these changes are poorly understood, many of these structural changes are accompanied by alterations in levels or activity of proteins well known to regulate the neuronal cytoskeleton. These include, but are not limited to, drug-induced changes in microtubule associated protein 2 (MAP2), neurofilament proteins, activity-regulated cytoskeletal-associated protein (Arc), LIM-kinase (LIMK), myocyte enhancer factor 2 (MEF2), cyclin-dependent kinase s5 (Cdk5), postsynaptic density 95 (PSD95), and cofilin, as well as changes in actin cycling, in the NAc or other brain reward regions (Beitner-Johnson et al., 1992; Bibb et al., 2001; Chase et al., 2007; Marie-Claire et al., 2004; Pulipparacharuvil et al., 2008; Toda

et al., 2006; Yao et al., 2004; Ziolkowska et al., 2005). Since many of the biochemical changes induced by stimulants and morphine are similar, it will be important to identify distinct opiate- and stimulant-regulated gene targets related to dendritic function, as they may provide insight into the generally opposite effects of opiate and stimulants on neurotrophic factor-dependent structural plasticity.

The opposite morphological changes induced in brain reward regions by opiates and stimulants are paradoxical since the two drugs cause very similar behavioral phenotypes. For example, specific treatment regimens of opiates and stimulants, both of which result in locomotor sensitization and similar patterns of escalation of drug self-administration, cause opposite changes in dendritic spine density in the NAc (Robinson and Kolb, 2004). Thus, if these morphological changes are important mediators of addiction, either they must have bidirectional properties, whereby a change from baseline in both directions produces the same behavioral phenotype, or they mediate distinct behavioral or other phenotypes that are not captured with the experimental tools used. Additionally, these findings must be considered in the context of the drug administration paradigm in question. In our studies, for instance, animals receive high doses of subcutaneous morphine, continuously released from pellet implants, a paradigm more consistent with opiate tolerance and dependence. In contrast, most stimulant paradigms utilize once to several times daily injections of the drug, allowing blood levels to peak and return to baseline before the next administration, paradigms more consistent with drug sensitization. Patterns of opiate and stimulant use by humans can vary widely from person to person. Therefore, future studies will need to address the behavioral relevance of drug-induced morphological changes in brain reward regions in the context of dose and drug administration paradigms that mirror exposures seen in humans.

6. Role of BDNF and its signaling cascades in drug-induced structural and behavioral plasticity

Changes in growth factor signaling are hypothesized to be a major factor influencing the structural and behavioral plasticity associated with drug addiction. Human studies are limited. Drug-induced changes in serum BDNF have been observed in humans addicted to cocaine, amphetamine, alcohol, or opiates (Angelucci et al., 2007; Janak et al., 2006; Kim et al., 2005), yet the source of this BDNF, and the relevance of these changes to the onset and maintenance of addiction have remained unclear. It would be interesting in future studies to examine BDNF and its signaling pathways in human postmortem brain tissue.

Over the past decade, work in rodents has established the influence of BDNF on various phases of the addiction process. Early studies showed that local infusion of BDNF into the VTA or NAc augments locomotor and rewarding responses to cocaine, while global loss of BDNF exerts the opposite effects (Hall et al., 2003; Horger et al., 1999; Lu et al., 2004). More recent work has shown that cocaine self-administration increases BDNF signaling in the NAc (Graham et al., 2007). In addition, an intra-NAc infusion of BDNF potentiates cocaine self-administration and cocaine-seeking and relapse, while infusion of antibodies against BDNF, or local knockout of the *bdnf* gene in the NAc (achieved via viral expression of Cre recombinase in floxed BDNF mice), blocks these behaviors. Based on these studies, Graham and colleagues (2007) concluded that BDNF release in the NAc during the initiation of cocaine self-administration is a necessary component of the addiction process.

These data support the view that BDNF is a candidate molecule to mediate the structural changes in NAc neurons produced by chronic exposure to cocaine or other stimulants. According to this hypothesis, stimulant-induced increases in BDNF signaling in the NAc would induce an increase in dendritic arborization of NAc neurons, which would underlie sensitized behavioral responses to the stimulants as well as strong drug-related memories crucial for

relapse and addiction. Consistent with this hypothesis are findings from cultured hippocampal neurons, where it has been shown that BDNF secretion induces protein synthesis-dependent enlargement of individual dendritic spines (Tanaka et al., 2008b). The weakness of this hypothesis is that there has been no direct experimental evidence that enhancement of dendritic spines of NAc neurons per se is necessary or sufficient for sensitized drug responses. In fact, there are data that suggest a more complex relationship between the two phenomena: inhibition of Cdk5 in the NAc blocks the ability of cocaine to increase dendritic spines on NAc neurons, despite the fact that such inhibition potentiates locomotor and rewarding responses to cocaine (Norrholm et al., 2003; Taylor et al., 2007). Clearly, further work is needed to study the relationship between this structural and behavioral plasticity.

Another important caveat to this hypothesis is that changes in BDNF signaling may produce profoundly different effects on neuronal morphology and behavior depending on the brain region examined. Recent reports have drawn clear distinctions between BDNF function in the hippocampus versus VTA (Berton et al., 2006; Eisch et al., 2003; Krishnan et al., 2007; Shirayama et al., 2002): BDNF infusions in the hippocampus are antidepressant-like, whereas infusions of BDNF in the VTA or NAc produce prodepressant-like effects. Similar patterns are emerging in the addiction field. Notably, increased BDNF in the NAc enhances cocaine-induced behaviors (Graham et al., 2007; Horger et al., 1999), whereas in the PFC BDNF suppresses these same behaviors (Berglind et al., 2007). Not surprisingly, the induction of BDNF by cocaine is also differentially regulated in these two brain regions, a pattern which further substantiates the behavioral differences (Fumagalli et al., 2007).

Preliminary evidence has implicated NF κ B signaling in the regulation of cocaine-induced structural and behavioral plasticity. Although the direct mechanism by which these changes occur is unknown, previous work has shown that the p75NTR, which is upstream of NF κ B, is localized at the synapse and that p75NTR activation by BDNF is necessary for LTD. Although BDNF-TrkB interactions have been extensively studied in drug abuse, these data suggest an alternative pathway through NF κ B that warrants further investigation. In line with this hypothesis, we have recently observed that viral-mediated overexpression of a dominant negative antagonist of the NF κ B pathway in the NAc prevents the ability of chronic cocaine to increase the density of dendritic spines on NAc MSNs. Such inhibition of NF κ B signaling also blunts sensitization to the rewarding effects of cocaine (Russo, Soc. Neurosci. Abstr. 611.5, 2007). These data, unlike the situation for Cdk5 cited above, support a link between increased dendritic arborization and behavioral sensitization to cocaine, further emphasizing the complexity of these phenomena and the need for further study.

Although limited work has addressed the relevance of neurotrophic factor signaling in opiate-induced behaviors, work from our laboratory has uncovered a role for BDNF and the downstream IRS2-PI3K-Akt pathway in the regulation of VTA dopaminergic cell size and subsequent reward tolerance (Russo et al., 2007; Sklair-Tavron et al., 1996). Specifically, chronic opiate administration in rodents produces a state of reward tolerance and physical dependence during relatively early periods of withdrawal that is thought to contribute to an escalation of drug-taking behavior. Early experiments found that intra-VTA infusion of BDNF prevents the morphine-induced decrease in VTA neuron size (Sklair-Tavron et al., 1996). More recently, we have shown that the timeline of reward tolerance, as measured by conditioned place preference, parallels the timeline of reduced dopaminergic cell size and that these phenomena are mediated via BDNF signaling cascades (Russo et al., 2007). As mentioned earlier, the biochemical signaling pathways in the VTA that are downstream of BDNF and the TrkB receptor are differentially regulated by chronic morphine: morphine activates PLC γ (Wolf et al., 2007; Wolf et al., 1999), decreases activity of the IRS-PI3K-Akt pathway (Russo et al., 2007; Wolf et al., 1999), and produces variable effects on ERK (see above). In light of recent evidence that Akt regulates the size of many cell types in the central nervous system

(Backman et al., 2001; Kwon et al., 2006; Kwon et al., 2001; Scheidenhelm et al., 2005), we utilized viral gene transfer techniques to directly show that morphine produces reward tolerance through inhibition of the IRS2–PI3K–Akt pathway and reduced size of VTA dopamine neurons. These effects were not observed by altering ERK or PLC γ signaling, again pointing to the importance of IRS–PI3K–Akt signaling for this phenomenon. Future studies will address the relevance of BDNF and IRS–PI3K–Akt pathways in the escalation of opiate self-administration, a more clinically relevant paradigm to measure addiction. A greater understanding of the upstream changes in neurotrophic factors or their receptors and downstream targets of Akt will address the specific mechanisms of opiate reward tolerance in addiction models. Moreover, it will be important to understand the role of BDNF signaling in the regulation of VTA function within a neural circuit context. In this regard, it is interesting to note that Pu et al. (2006) showed that following withdrawal from repeated cocaine exposure, excitatory synapses onto dopamine neurons in the VTA are more responsive to potentiation by weak presynaptic stimuli, an effect requiring endogenous BDNF–TrkB signaling.

7. Role of other neurotrophic factors in drug-induced structural and behavioral plasticity

While the above discussion focuses on BDNF and its signaling cascades, there is evidence that several other neurotrophic factors and their downstream signaling pathways also influence behavioral or biochemical responses to drugs of abuse. NT3, like BDNF, has been shown to promote sensitized responses to cocaine at the level of the VTA (Pierce and Bari, 2001; Pierce et al., 1999). Chronic administration of morphine or cocaine up-regulates glial cell line-derived neurotrophic factor (GDNF) signaling in the VTA–NAc circuit, which in turn feeds back and suppresses the behavioral effects of these drugs of abuse (Messer et al., 2000). Amphetamine induces basic fibroblast growth factor (bFGF) in the VTA–NAc circuit and bFGF knockout mice have a blunted response to locomotor sensitization induced by repeated amphetamine injections (Flores et al., 2000; Flores and Stewart, 2000). The cytokine, ciliary neurotrophic factor (CNTF), administered directly into the VTA, enhances the ability of cocaine to induce biochemical adaptations in this brain region; cocaine increases intracellular signaling cascades through Janus kinase (JAK) and signal transducers and activators of transcription (STATs), an effect that was potentiated by an acute infusion of CNTF (Berhow et al., 1996a). There is also evidence that chronic morphine alters levels of insulin-like growth factor 1 (IGF1) in the VTA and other brain regions (Beitner-Johnson et al., 1992). These isolated findings indicate that a diverse array of neurotrophic mechanisms control VTA–NAc function to regulate plasticity to drugs of abuse in complex ways and highlight the need for much future research in this area.

8. Conclusions

Over the past decade, we have expanded our understanding of how drugs of abuse regulate neurotrophic signaling pathways and the morphology of diverse neuronal populations throughout the brain's reward circuitry. Recent advances in viral gene transfer allow for manipulations of specific downstream neurotrophic signaling proteins within a given brain region of interest of fully developed adult animals to study the relationships among drug abuse, neuronal morphology, and behavioral plasticity. With novel bicistronic viral vectors, it is possible to express a protein that manipulates neurotrophic signaling pathways as well as a fluorescent protein to visualize neuronal morphology (Clark et al., 2002). Thus, with improved immunohistochemical techniques to label specific neuronal populations, it is possible to assess drug-induced morphological changes and associated biochemical adaptations in neurotrophic signaling in a cell type-specific manner, and therefore provides crucial information for drug-induced regulation of heterogeneous brain reward regions. Using multidisciplinary approaches with behavioral, physiological, biochemical, and morphological endpoints, it will be increasingly possible to define the mechanisms of addiction with far greater precision,

including the precise role of neurotrophic factor signaling in experience-dependent plasticity and the addiction process. This knowledge may lead to the development of novel medical interventions to normalize the maladaptive plasticity induced by drugs of abuse in brain reward regions and to thereby reverse the addiction process in humans.

References

- Ang E, Chen J, Zagouras P, Magna H, Holland J, Schaeffer E, Nestler EJ. Induction of nuclear factor-kappaB in nucleus accumbens by chronic cocaine administration. *J Neurochem* 2001;79:221–224. [PubMed: 11595774]
- Angelucci F, Ricci V, Pomponi M, Conte G, Mathe AA, Attilio Tonali P, Bria P. Chronic heroin and cocaine abuse is associated with decreased serum concentrations of the nerve growth factor and brain-derived neurotrophic factor. *J Psychopharmacol* 2007;21:820–825. [PubMed: 17715210]
- Asanuma M, Cadet JL. Methamphetamine-induced increase in striatal NF-kappaB DNA-binding activity is attenuated in superoxide dismutase transgenic mice. *Brain Res Mol Brain Res* 1998;60:305–309. [PubMed: 9757070]
- Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO, Mak TW. Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. *Nat Genet* 2001;29:396–403. [PubMed: 11726926]
- Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *Embo J* 1982;1:549–553. [PubMed: 7188352]
- Beitner-Johnson D, Guitart X, Nestler EJ. Neurofilament proteins and the mesolimbic dopamine system: common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. *J Neurosci* 1992;12:2165–2176. [PubMed: 1376774]
- Berglind WJ, See RE, Fuchs RA, Ghee SM, Whitfield TW Jr, Miller SW, McGinty JF. A BDNF infusion into the medial prefrontal cortex suppresses cocaine seeking in rats. *Eur J Neurosci* 2007;26:757–766. [PubMed: 17651427]
- Berhow MT, Hiroi N, Kobierski LA, Hyman SE, Nestler EJ. Influence of cocaine on the JAK-STAT pathway in the mesolimbic dopamine system. *J Neurosci* 1996a;16:8019–8026. [PubMed: 8987828]
- Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J Neurosci* 1996b;16:4707–4715. [PubMed: 8764658]
- Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel DV, Rosenthal A. Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. *Neuron* 1991;7:857–866. [PubMed: 1742028]
- Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* 2006;311:864–868. [PubMed: 16469931]
- Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 2001;410:376–380. [PubMed: 11268215]
- Bolanos CA, Nestler EJ. Neurotrophic mechanisms in drug addiction. *Neuromolecular Med* 2004;5:69–83. [PubMed: 15001814]
- Bolanos CA, Perrotti LI, Edwards S, Eisch AJ, Barrot M, Olson VG, Russell DS, Neve RL, Nestler EJ. Phospholipase Cgamma in distinct regions of the ventral tegmental area differentially modulates mood-related behaviors. *J Neurosci* 2003;23:7569–7576. [PubMed: 12930795]
- Bourne J, Harris KM. Do thin spines learn to be mushroom spines that remember? *Curr Opin Neurobiol* 2007;17:381–386. [PubMed: 17498943]
- Brami-Cherrier K, Valjent E, Garcia M, Pages C, Hipkind RA, Caboche J. Dopamine induces a PI3-kinase-independent activation of Akt in striatal neurons: a new route to cAMP response element-binding protein phosphorylation. *J Neurosci* 2002;22:8911–8921. [PubMed: 12388598]
- Chakravarthy S, Saiepour MH, Bence M, Perry S, Hartman R, Couey JJ, Mansvelder HD, Levelt CN. Postsynaptic TrkB signaling has distinct roles in spine maintenance in adult visual cortex and hippocampus. *Proc Natl Acad Sci U S A* 2006;103:1071–1076. [PubMed: 16418274]

- Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci* 2003;4:299–309. [PubMed: 12671646]
- Chase T, Carrey N, Soo E, Wilkinson M. Methylphenidate regulates activity regulated cytoskeletal associated but not brain-derived neurotrophic factor gene expression in the developing rat striatum. *Neuroscience* 2007;144:969–984. [PubMed: 17156936]
- Chu NN, Zuo YF, Meng L, Lee DY, Han JS, Cui CL. Peripheral electrical stimulation reversed the cell size reduction and increased BDNF level in the ventral tegmental area in chronic morphine-treated rats. *Brain Res* 2007;1182:90–98. [PubMed: 17945205]
- Clark MS, Sexton TJ, McClain M, Root D, Kohen R, Neumaier JF. Overexpression of 5-HT1B receptor in dorsal raphe nucleus using Herpes Simplex Virus gene transfer increases anxiety behavior after inescapable stress. *J Neurosci* 2002;22:4550–4562. [PubMed: 12040062]
- Cohen S, Levi-Montalcini R, Hamburger V. A Nerve Growth-Stimulating Factor Isolated from Sarcom as 37 and 180. *Proc Natl Acad Sci U S A* 1954;40:1014–1018. [PubMed: 16589582]
- Danzer SC, Kotloski RJ, Walter C, Hughes M, McNamara JO. Altered morphology of hippocampal dentate granule cell presynaptic and postsynaptic terminals following conditional deletion of TrkB. *Hippocampus*. 2008
- Diana M, Spiga S, Acquas E. Persistent and reversible morphine withdrawal-induced morphological changes in the nucleus accumbens. *Ann N Y Acad Sci* 2006;1074:446–457. [PubMed: 17105943]
- Eisch AJ, Bolanos CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. *Biol Psychiatry* 2003;54:994–1005. [PubMed: 14625141]
- Everitt BJ, Dickinson A, Robbins TW. The neuropsychological basis of addictive behaviour. *Brain Res Brain Res Rev* 2001;36:129–138. [PubMed: 11690609]
- Fahnestock M, Michalski B, Xu B, Coughlin MD. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Mol Cell Neurosci* 2001;18:210–220. [PubMed: 11520181]
- Ferrer-Alcon M, Garcia-Fuster MJ, La Harpe R, Garcia-Sevilla JA. Long-term regulation of signalling components of adenylyl cyclase and mitogen-activated protein kinase in the pre-frontal cortex of human opiate addicts. *J Neurochem* 2004;90:220–230. [PubMed: 15198681]
- Fiala JC, Allwardt B, Harris KM. Dendritic spines do not split during hippocampal LTP or maturation. *Nat Neurosci* 2002;5:297–298. [PubMed: 11896399]
- Filip M, Faron-Gorecka A, Kusmider M, Golda A, Frankowska M, Dziedzicka-Wasylewska M. Alterations in BDNF and trkB mRNAs following acute or sensitizing cocaine treatments and withdrawal. *Brain Res* 2006;1071:218–225. [PubMed: 16423334]
- Flores C, Samaha AN, Stewart J. Requirement of endogenous basic fibroblast growth factor for sensitization to amphetamine. *J Neurosci* 2000;20:RC55. [PubMed: 10632621]
- Flores C, Stewart J. Basic fibroblast growth factor as a mediator of the effects of glutamate in the development of long-lasting sensitization to stimulant drugs: studies in the rat. *Psychopharmacology (Berl)* 2000;151:152–165. [PubMed: 10972461]
- Fumagalli F, Di Pasquale L, Caffino L, Racagni G, Riva MA. Repeated exposure to cocaine differently modulates BDNF mRNA and protein levels in rat striatum and prefrontal cortex. *Eur J Neurosci* 2007;26:2756–2763. [PubMed: 18001273]
- Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci* 2007;10:1029–1037. [PubMed: 17618281]
- Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci* 2003;23:742–747. [PubMed: 12574402]
- Hall FS, Drgonova J, Goeb M, Uhl GR. Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. *Neuropsychopharmacology* 2003;28:1485–1490. [PubMed: 12784114]
- Hallbook F, Wilson K, Thorndyke M, Olinski RP. Formation and evolution of the chordate neurotrophin and Trk receptor genes. *Brain Behav Evol* 2006;68:133–144. [PubMed: 16912467]

- Harris KM. Structure, development, and plasticity of dendritic spines. *Curr Opin Neurobiol* 1999;9:343–348. [PubMed: 10395574]
- Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *J Neurosci* 1992;12:2685–2705. [PubMed: 1613552]
- Hohn A, Leibrock J, Bailey K, Barde YA. Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature* 1990;344:339–341. [PubMed: 2314473]
- Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, Svoboda K. Transient and persistent dendritic spines in the neocortex in vivo. *Neuron* 2005;45:279–291. [PubMed: 15664179]
- Horch HW, Kruttgen A, Portbury SD, Katz LC. Destabilization of cortical dendrites and spines by BDNF. *Neuron* 1999;23:353–364. [PubMed: 10399940]
- Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci* 1999;19:4110–4122. [PubMed: 10234039]
- Janak PH, Wolf FW, Heberlein U, Pandey SC, Logrip ML, Ron D. BIG news in alcohol addiction: new findings on growth factor pathways BDNF, insulin, and GDNF. *Alcohol Clin Exp Res* 2006;30:214–221. [PubMed: 16441270]
- Jenab S, Festa ED, Nazarian A, Wu HB, Sun WL, Hazim R, Russo SJ, Quinones-Jenab V. Cocaine induction of ERK proteins in dorsal striatum of Fischer rats. *Brain Res Mol Brain Res* 2005;142:134–138. [PubMed: 16271798]
- Johnson D, Lanahan A, Buck CR, Sehgal A, Morgan C, Mercer E, Bothwell M, Chao M. Expression and structure of the human NGF receptor. *Cell* 1986;47:545–554. [PubMed: 3022937]
- Kalivas PW, O'Brien C. Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology* 2008;33:166–180. [PubMed: 17805308]
- Kaplan DR, Hempstead BL, Martin-Zanca D, Chao MV, Parada LF. The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science* 1991;252:554–558. [PubMed: 1850549]
- Kim DJ, Roh S, Kim Y, Yoon SJ, Lee HK, Han CS, Kim YK. High concentrations of plasma brain-derived neurotrophic factor in methamphetamine users. *Neurosci Lett* 2005;388:112–115. [PubMed: 16039058]
- Klein R, Jing SQ, Nanduri V, O'Rourke E, Barbacid M. The trk proto-oncogene encodes a receptor for nerve growth factor. *Cell* 1991;65:189–197. [PubMed: 1849459]
- Koob GF, Le Moal M. Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. *Nat Neurosci* 2005;8:1442–1444. [PubMed: 16251985]
- Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 2007;131:391–404. [PubMed: 17956738]
- Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 2005;48:303–314. [PubMed: 16242410]
- Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF. Pten regulates neuronal arborization and social interaction in mice. *Neuron* 2006;50:377–388. [PubMed: 16675393]
- Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, Eberhart CG, Burger PC, Baker SJ. Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. *Nat Genet* 2001;29:404–411. [PubMed: 11726927]
- Lamballe F, Klein R, Barbacid M. trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell* 1991;66:967–979. [PubMed: 1653651]
- Le Foll B, Diaz J, Sokoloff P. A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning. *Neuroreport* 2005;16:175–178. [PubMed: 15671872]

- Lee KW, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P. Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc Natl Acad Sci U S A* 2006;103:3399–3404. [PubMed: 16492766]
- Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. *Science* 2001;294:1945–1948. [PubMed: 11729324]
- Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengeler B, Masiakowski P, Thoenen H, Barde YA. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 1989;341:149–152. [PubMed: 2779653]
- Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR. Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res* 2006;1067:1–12. [PubMed: 16376315]
- Liu Y, Wang Y, Jiang Z, Wan C, Zhou W, Wang Z. The extracellular signal-regulated kinase signaling pathway is involved in the modulation of morphine-induced reward by mPer1. *Neuroscience* 2007;146:265–271. [PubMed: 17320300]
- Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. *J Neurosci* 2004;24:1604–1611. [PubMed: 14973246]
- Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD. Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science* 1990;247:1446–1451. [PubMed: 2321006]
- Majewska AK, Newton JR, Sur M. Remodeling of synaptic structure in sensory cortical areas in vivo. *J Neurosci* 2006;26:3021–3029. [PubMed: 16540580]
- Marie-Claire C, Courtin C, Roques BP, Noble F. Cytoskeletal genes regulation by chronic morphine treatment in rat striatum. *Neuropsychopharmacology* 2004;29:2208–2215. [PubMed: 15199374]
- Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. *Nature* 2004;429:761–766. [PubMed: 15190253]
- McGinty JF, Shi XD, Schwendt M, Saylor A, Toda S. Regulation of psychostimulant-induced signaling and gene expression in the striatum. *J Neurochem* 2008;104:1440–1449. [PubMed: 18221378]
- Messer CJ, Eisch AJ, Carlezon WA Jr, Whisler K, Shen L, Wolf DH, Westphal H, Collins F, Russell DS, Nestler EJ. Role for GDNF in biochemical and behavioral adaptations to drugs of abuse. *Neuron* 2000;26:247–257. [PubMed: 10798408]
- Muller DL, Unterwald EM. In vivo regulation of extracellular signal-regulated protein kinase (ERK) and protein kinase B (Akt) phosphorylation by acute and chronic morphine. *J Pharmacol Exp Ther* 2004;310:774–782. [PubMed: 15056728]
- Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* 2004;44:759–767. [PubMed: 15572108]
- Nestler EJ. Molecular mechanisms of drug addiction. *J Neurosci* 1992;12:2439–2450. [PubMed: 1319476]
- Nestler EJ. Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* 2001;2:119–128. [PubMed: 11252991]
- Norrholm SD, Bibb JA, Nestler EJ, Ouimet CC, Taylor JR, Greengard P. Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. *Neuroscience* 2003;116:19–22. [PubMed: 12535933]
- Numan S, Lane-Ladd SB, Zhang L, Lundgren KH, Russell DS, Seroogy KB, Nestler EJ. Differential regulation of neurotrophin and trk receptor mRNAs in catecholaminergic nuclei during chronic opiate treatment and withdrawal. *J Neurosci* 1998;18:10700–10708. [PubMed: 9852605]
- Okamoto K, Nagai T, Miyawaki A, Hayashi Y. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nat Neurosci* 2004;7:1104–1112. [PubMed: 15361876]
- Ortiz J, Harris HW, Guitart X, Terwilliger RZ, Haycock JW, Nestler EJ. Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. *J Neurosci* 1995;15:1285–1297. [PubMed: 7532701]
- Peters A, Kaiserman-Abramof IR. The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *Am J Anat* 1970;127:321–355. [PubMed: 4985058]

- Pierce RC, Bari AA. The role of neurotrophic factors in psychostimulant-induced behavioral and neuronal plasticity. *Rev Neurosci* 2001;12:95–110. [PubMed: 11392459]
- Pierce RC, Pierce-Bancroft AF, Prasad BM. Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the Ras/Mitogen-activated protein kinase signal transduction cascade. *J Neurosci* 1999;19:8685–8695. [PubMed: 10493769]
- Pu L, Liu QS, Poo MM. BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. *Nat Neurosci* 2006;9:605–607. [PubMed: 16633344]
- Pulipparacharuvil S, Renthal W, Hale CF, Taniguchi M, Xiao G, Kumar A, Russo SJ, Sikder D, Dewey CM, Davis M, Greengard P, Nairn AC, Nestler EJ, Cowan CW. Cocaine Regulates MEF2 to Control Synaptic and Behavioral Plasticity. *Neuron*. 2008in press
- Robinson TE, Gorny G, Mitton E, Kolb B. Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse* 2001;39:257–266. [PubMed: 11169774]
- Robinson TE, Gorny G, Savage VR, Kolb B. Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. *Synapse* 2002;46:271–279. [PubMed: 12373743]
- Robinson TE, Kolb B. Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *J Neurosci* 1997;17:8491–8497. [PubMed: 9334421]
- Robinson TE, Kolb B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci* 1999a;11:1598–1604. [PubMed: 10215912]
- Robinson TE, Kolb B. Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. *Synapse* 1999b;33:160–162. [PubMed: 10400894]
- Robinson TE, Kolb B. Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* 47 Suppl 2004;1:33–46.
- Rodriguez-Tebar A, Dechant G, Barde YA. Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* 1990;4:487–492. [PubMed: 2157470]
- Rodriguez-Tebar A, Dechant G, Gotz R, Barde YA. Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. *Embo J* 1992;11:917–922. [PubMed: 1547788]
- Russo SJ, Bolanos CA, Theobald DE, DeCarolis NA, Renthal W, Kumar A, Winstanley CA, Renthal NE, Wiley MD, Self DW, Russell DS, Neve RL, Eisch AJ, Nestler EJ. IRS2-Akt pathway in midbrain dopamine neurons regulates behavioral and cellular responses to opiates. *Nat Neurosci* 2007;10:93–99. [PubMed: 17143271]
- Sarti F, Borgland SL, Kharazia VN, Bonci A. Acute cocaine exposure alters spine density and long-term potentiation in the ventral tegmental area. *Eur J Neurosci* 2007;26:749–756. [PubMed: 17686047]
- Scheidenhelm DK, Cresswell J, Haipek CA, Fleming TP, Mercer RW, Gutmann DH. Akt-dependent cell size regulation by the adhesion molecule on glia occurs independently of phosphatidylinositol 3-kinase and Rheb signaling. *Mol Cell Biol* 2005;25:3151–3162. [PubMed: 15798201]
- Scott J, Selby M, Urdea M, Quiroga M, Bell GI, Rutter WJ. Isolation and nucleotide sequence of a cDNA encoding the precursor of mouse nerve growth factor. *Nature* 1983;302:538–540. [PubMed: 6336309]
- Segal RA. Selectivity in neurotrophin signaling: theme and variations. *Annu Rev Neurosci* 2003;26:299–330. [PubMed: 12598680]
- Shi X, McGinty JF. Extracellular signal-regulated mitogen-activated protein kinase inhibitors decrease amphetamine-induced behavior and neuropeptide gene expression in the striatum. *Neuroscience* 2006;138:1289–1298. [PubMed: 16459022]
- Shi X, McGinty JF. Repeated amphetamine treatment increases phosphorylation of extracellular signal-regulated kinase, protein kinase B, and cyclase response element-binding protein in the rat striatum. *J Neurochem* 2007;103:706–713. [PubMed: 17635666]
- Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* 2002;22:3251–3261. [PubMed: 11943826]

- Sklair-Tavron L, Shi WX, Lane SB, Harris HW, Bunney BS, Nestler EJ. Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. *Proc Natl Acad Sci U S A* 1996;93:11202–11207. [PubMed: 8855333]
- Spiga S, Puddu MC, Pisano M, Diana M. Morphine withdrawal-induced morphological changes in the nucleus accumbens. *Eur J Neurosci* 2005;22:2332–2340. [PubMed: 16262671]
- Spiga S, Serra GP, Puddu MC, Foddai M, Diana M. Morphine withdrawal-induced abnormalities in the VTA: confocal laser scanning microscopy. *Eur J Neurosci* 2003;17:605–612. [PubMed: 12581178]
- Sun WL, Zhou L, Hazim R, Quinones-Jenab V, Jenab S. Effects of acute cocaine on ERK and DARPP-32 phosphorylation pathways in the caudate-putamen of Fischer rats. *Brain Res* 2007;1178:12–19. [PubMed: 17920048]
- Tada T, Sheng M. Molecular mechanisms of dendritic spine morphogenesis. *Curr Opin Neurobiol* 2006;16:95–101. [PubMed: 16361095]
- Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H. Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* 2008a;319:1683–1687. [PubMed: 18309046]
- Tanaka JI, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H. Protein Synthesis and Neurotrophin-Dependent Structural Plasticity of Single Dendritic Spines. *Science*. 2008b
- Taylor JR, Lynch WJ, Sanchez H, Olausson P, Nestler EJ, Bibb JA. Inhibition of Cdk5 in the nucleus accumbens enhances the locomotor-activating and incentive-motivational effects of cocaine. *Proc Natl Acad Sci U S A* 2007;104:4147–4152. [PubMed: 17360491]
- Thomas MJ, Kalivas PW, Shaham Y. Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *Br J Pharmacol*. 2008
- Toda S, Shen HW, Peters J, Cagle S, Kalivas PW. Cocaine increases actin cycling: effects in the reinstatement model of drug seeking. *J Neurosci* 2006;26:1579–1587. [PubMed: 16452681]
- Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *Eur J Neurosci* 2004;19:1826–1836. [PubMed: 15078556]
- Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc Natl Acad Sci U S A* 2005;102:491–496. [PubMed: 15608059]
- von Bohlen Und Halbach O, Minichiello L, Unsicker K. TrkB but not trkC receptors are necessary for postnatal maintenance of hippocampal spines. *Neurobiol Aging*. 2007
- Wei Y, Williams JM, Dipace C, Sung U, Javitch JA, Galli A, Saunders C. Dopamine transporter activity mediates amphetamine-induced inhibition of Akt through a Ca²⁺/calmodulin-dependent kinase II-dependent mechanism. *Mol Pharmacol* 2007;71:835–842. [PubMed: 17164407]
- Williams JM, Owens WA, Turner GH, Saunders C, Dipace C, Blakely RD, France CP, Gore JC, Daws LC, Avison MJ, Galli A. Hypoinsulinemia regulates amphetamine-induced reverse transport of dopamine. *PLoS Biol* 2007;5:2369–2378.
- Wolf DH, Nestler EJ, Russell DS. Regulation of neuronal PLCgamma by chronic morphine. *Brain Res* 2007;1156:9–20. [PubMed: 17524370]
- Wolf DH, Numan S, Nestler EJ, Russell DS. Regulation of phospholipase Cgamma in the mesolimbic dopamine system by chronic morphine administration. *J Neurochem* 1999;73:1520–1528. [PubMed: 10501197]
- Yao WD, Gainetdinov RR, Arbuckle MI, Sotnikova TD, Cyr M, Beaulieu JM, Torres GE, Grant SG, Caron MG. Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. *Neuron* 2004;41:625–638. [PubMed: 14980210]
- Zhang D, Zhang L, Lou DW, Nakabeppu Y, Zhang J, Xu M. The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression. *J Neurochem* 2002;82:1453–1464. [PubMed: 12354293]
- Zhang X, Mi J, Wetsel WC, Davidson C, Xiong X, Chen Q, Ellinwood EH, Lee TH. PI3 kinase is involved in cocaine behavioral sensitization and its reversal with brain area specificity. *Biochem Biophys Res Commun* 2006;340:1144–1150. [PubMed: 16414349]

- Zhou Q, Homma KJ, Poo MM. Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* 2004;44:749–757. [PubMed: 15572107]
- Ziolkowska B, Urbanski MJ, Wawrzczak-Bargiela A, Bilecki W, Przewlocki R. Morphine activates Arc expression in the mouse striatum and in mouse neuroblastoma Neuro2A MOR1A cells expressing mu-opioid receptors. *J Neurosci Res* 2005;82:563–570. [PubMed: 16211563]
- Zuo Y, Lin A, Chang P, Gan WB. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron* 2005;46:181–189. [PubMed: 15848798]

Figure 1. Major cell types in the neural circuitry underlying addiction

Projections of VTA dopamine neurons (shown in solid red lines) impinge directly on NAc and mPFC neurons, as well as on amygdala and hippocampal neurons (the latter projections are not shown in the figure). The solid purple line represents GABAergic afferents (some direct, some indirect) from the NAc to the VTA, which provide feedback to VTA dopamine neurons. The dotted purple lines represent glutamatergic afferents to the NAc from mPFC, amygdala, and hippocampus. Each structure contains specialized neuronal cell types thought to play an integral role in the complex behavioral phenotypes associated with drug addiction. These cell types, color-coded in the key, include amygdala (green) and NAc (purple) spiny neurons, PFC (black) and hippocampal CA3 (blue) pyramidal neurons, and VTA dopamine neurons (red). Each of these cell types has been shown to be structurally altered by drugs of abuse (Table 1).

Figure 2. Intracellular signaling pathways downstream of neurotrophins

Neurotrophins (NT), which include BDNF, NGF, NT-3, and NT4/5, signal through Trk receptors (TrkB is the preferred receptor for BDNF) to activate 3 major pathways, IRS–PI3K–Akt, Ras–MEK–ERK, and PLC γ . The neurotrophins also signal through the p75 receptor (p75NTR) to activate several intracellular cascades, including TNF (tumor necrosis factor) receptor-associated factor 4/6 (TRAF4/6) or receptor interacting protein 2 (RIP2) and NF κ B. Activation of these pathways affects a wide range of cellular functions, including differentiation, survival, apoptosis, growth, and cytoskeleton reorganization. There is considerable crosstalk among these pathways, not shown in the figure. For example, upon activation of the Trk receptor by these neurotrophins, transcription factors downstream of Ras–MEK–ERK, PLC γ , and IRS–PI3K–Akt activity can bind DNA and regulate a number of gene targets involved in cytoskeleton reorganization.

Figure 3. Adaptations in BDNF signaling cascades associated with opiate and stimulant-induced structural plasticity in the VTA-NAc circuit

A VTA dopamine neuron is shown innervating a NAc medium spiny neuron. Opiates and cocaine produce generally opposite effects on the morphology of these two neuronal cell types. Chronic opiate administration decreases the surface area of VTA dopamine neurons, which is associated with decreased levels of neurofilament proteins and decreased axoplasmic transport to the NAc, however, their effects on dendritic spines and synaptic connectivity in the VTA are not known. The decrease in VTA neuron cell size is mediated by decreased activity of the IRS-PI3K-Akt pathway; chronic morphine also increases activity of the PLC γ pathway and causes variable effects on ERK in this brain region. In the NAc, opiates decrease neuronal

branching and synapse number, effects that correspond with decreases in IRS–PI3K–Akt and ERK activity, although the molecular basis of the structural changes remain unknown. In stark contrast to opiates, stimulants increase dendritic branching and spine number in VTA dopamine neurons and NAc medium spiny neurons. This structural plasticity is associated with activation of ERK in both regions and with activation of PLC γ and NK κ B in the NAc, with variable effects reported for IRS–PI3K–Akt. However, the molecular adaptations responsible for this dendritic growth in both regions are poorly understood.

Table 1
Drug -induced morphology changes

Decrease ←			Increase →		
Opiates	Neuron Function			Stimulants	
?	Amygdala spiny neuron		Emotional memory processing and encoding	?	
Dendrite complexity and spine density/morphometry	NAc medium spiny neuron		Integration of positive and negative stimuli	Dendrite complexity and spine density/morphometry	
Cell body size	VTA dopamine neuron		Arousal and parsing of relevant emotional stimuli	Spine density/morphometry	
Spine density	CA3 pyramidal neuron		Encoding contextual association memory	?	
Dendrite complexity and spine density/morphometry	PFC pyramidal neuron		Executive functioning	Dendrite complexity and spine density/morphometry	

The table shows that in several regions of the central nervous system opiates are observed to reduce neuronal size or extensions, while stimulants (e.g., cocaine and amphetamine) exert the opposite types of changes.