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RESEARCH****Research Report****Brain-derived neurotrophic factor and cocaine addiction***Jacqueline F. McGinty*, Timothy W. Whitfield Jr., William J. Berglund**Department of Neurosciences, Medical University of South Carolina, 173 Ashley Ave MSC 510, Charleston, SC 29425, USA*

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ABSTRACT

The effects of brain-derived neurotrophic factor (BDNF) on cocaine-seeking are brain region-specific. Infusion of BDNF into subcortical structures, like the nucleus accumbens and ventral tegmental area, enhances cocaine-induced behavioral sensitization and cocaine-seeking. Conversely, repeated administration of BDNF antiserum into the nucleus accumbens during chronic cocaine self-administration attenuates cocaine-induced reinstatement. In contrast, BDNF infusion into the dorsomedial prefrontal cortex immediately following a final session of cocaine self-administration attenuates relapse to cocaine-seeking after abstinence, as well as cue- and cocaine prime-induced reinstatement of cocaine-seeking following extinction. BDNF-induced alterations in the ERK–MAP kinase cascade and in prefronto-accumbens glutamatergic transmission are implicated in BDNF's ability to alter cocaine-seeking. Within 22 hours after infusion into the prefrontal cortex, BDNF increases BDNF protein in prefrontal cortical targets, including nucleus accumbens, and restores cocaine-mediated decreases in phospho-ERK expression in the nucleus accumbens. Furthermore, 3 weeks after BDNF infusion in animals with a cocaine self-administration history, suppressed basal levels of glutamate are normalized and a cocaine prime-induced increase in extracellular glutamate levels in the nucleus accumbens is prevented. Thus, BDNF may have local effects at the site of infusion and distal effects in target areas that are critical to mediating or preventing cocaine-induced dysfunctional neuroadaptations.

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1. BDNF is a neurotrophic peptide that mediates synaptic plasticity including drug-induced neuroadaptations

BDNF, a member of the neurotrophin polypeptide family that includes nerve growth factor, neurotrophin-3, and neurotrophin 4/5, is the most widely and abundantly expressed neurotrophin in the nervous system (Thoenen, 1995). Like other neuropeptides, BDNF is synthesized as a pro-peptide (32 kDa) that is proteolytically processed into a smaller

(13 kDa), mature form. This cleavage is thought by some to occur after secretion by the extracellularly located tissue plasminogen-activated plasmin (Lu, 2003); however, others have reported that CNS neurons store and secrete mature, not pro-BDNF, in response to excitatory input (Matsumoto et al., 2008). The significance is that pro-BDNF binds and activates p75 pro-apoptotic receptors, whereas mature BDNF binds and activates pro-survival tropomyosin receptor kinase B (TrkB) receptors (Bibel and Barde, 2000). The latter results in receptor dimerization, tyrosine phosphorylation that provides docking

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Abbreviations: BDNF, brain-derived neurotrophic factor; CREB, cAMP response element binding protein; DA, dopamine; ERK, extracellular-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated extracellular-regulated protein kinase; GDNF, glial cell line-derived neurotrophic factor; NAC, nucleus accumbens; PFC, prefrontal cortex; PLC γ , phospholipase C gamma; PI3K, phosphoinositide 3-kinase; Trk, tropomyosin receptor kinase; VTA, ventral tegmental area

BDNF may also regulate drug-induced long-term neuroadaptations that encompass alterations in molecular components at the synapse, changes in gene expression, and modifications of behavioral output. Integration of dopaminergic and glutamatergic input to medium spiny neurons in the striatum, including the nucleus accumbens (NAc), and pyramidal cells in the prefrontal cortex (PFC) generates molecular changes in dendritic spines that activate a variety of intracellular cascades important for associative learning (Kennedy, 2000; Hyman, 2005; Girault et al., 2007). Repeated alterations in calcium influx, phosphorylation–dephosphorylation events, and the activation of immediate early genes and transcription factors culminate in changes in neuronal structure and synaptic composition that ultimately modify behavior. Repeated exposure to psychostimulants increases dendritic branching and dysmorphic dendritic spines in the NAc and PFC in rats during extended abstinence (Robinson et al., 2001); however, the molecular cascades underlying these responses have not been elaborated. Thus, a significant question is how the cellular substrates of plasticity undergo dynamic alterations in drug addiction and how BDNF modifies these changes.

Key brain areas that are altered by drugs of abuse, particularly psychostimulants, are implicated in the reinstatement of drug-seeking. These structures include the PFC and its downstream target, the NAc. Both of these structures receive dopamine (DA) innervation from the ventral tegmental area (VTA) via the mesocorticolimbic DA ("reward") pathway. Many cocaine-induced neuroadaptations that are thought to drive reinstatement of cocaine-seeking are manifested by alterations in the plasticity of the PFC–NAc pathway. BDNF is

Under several conditions, endogenous *bdnf* mRNA and protein are differentially regulated in mesolimbic and cortical neurons in response either to acute administration of addictive drugs or during extended periods of abstinence from prolonged drug administration. For instance, acute administration of cocaine, amphetamine, or alcohol transiently induces *bdnf* mRNA expression in the medial PFC, cingulate cortex, and/or striatum 45 minutes to 4 hours after drug administration (McGough et al., 2004; Kerns et al., 2005; Le Foll et al., 2005; Fumagalli et al., 2007, 2009; Saylor and McGinty, 2008). This acute induction of *bdnf* mRNA may represent the initial stages of cortical plasticity that lay the foundation for subsequent psychostimulant-induced alterations in intracellular signaling and altered neurotransmission. However, repeated, random stress prevents this cocaine-induced increase (Fumagalli et al., 2009). After repeated administration of cocaine, amphetamine, or alcohol, the expressions of *bdnf* mRNA and BDNF protein are also transiently increased in forebrain structures (Meredith et al., 2002; McGough et al., 2004; Fumagalli et al., 2007). In contrast however, 22 hours, but not 21 days, after the end of cocaine self-administration, *bdnf* mRNA levels in the dorsomedial PFC

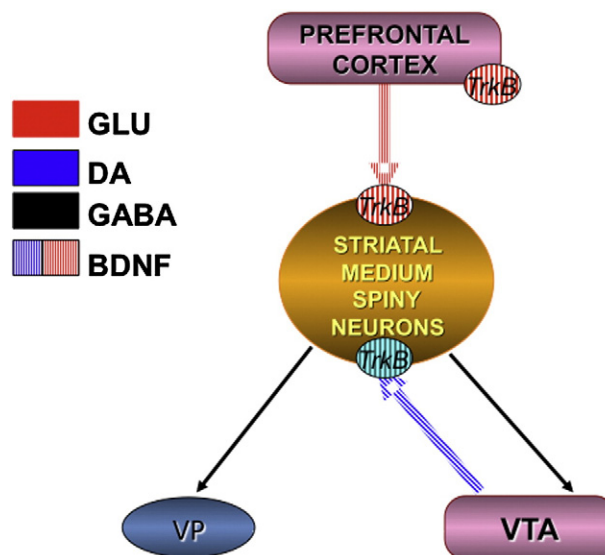


Fig. 1 – BDNF expression within the mesocorticolimbic system. BDNF is expressed in glutamatergic pyramidal neurons arising from the PFC as well as dopaminergic neurons arising from the VTA, both of which synapse on GABAergic medium spiny neurons within the NAC. BDNF itself is transported anterogradely to the striatum where it binds to TrkB receptors on medium spiny neurons. Labeling studies have demonstrated that a majority of BDNF expression within the NAC arises from the PFC, not the VTA. Glu = glutamate, DA = dopamine, GABA = gamma-aminobutyric acid.

are decreased (Figs. 2A and B). In addition, a persistent BDNF protein response develops in mesolimbic and cortical structures and lasts for extended durations during abstinence from cocaine. Following self-administration of cocaine, BDNF protein is induced in mesolimbic structures including the VTA, NAc, and the amygdala at extended time points of abstinence (Grimm et al., 2003). In addition, BDNF protein is induced in the dorsomedial PFC 21 days after cocaine self-administration whether or not a cocaine priming injection was administered 30 minutes prior to sacrifice (Fig. 2C). Given that BDNF is transported in the cortical glutamatergic projections from the PFC to the NAc (Altar et al., 1997) and that this pathway regulates relapse to drug-seeking (McFarland et al., 2003; Fuchs et al., 2004), variable expression of BDNF in this network of reciprocally interconnected structures suggests that BDNF may constitute a critical component of cocaine-induced plasticity in mesolimbic and cortical neurons.

3. BDNF modifies drug-induced behaviors

Ventral midbrain or NAc BDNF infusions enhance motor activity, cocaine reinstatement after extended abstinence, as well as sensitization to cocaine and cocaine-conditioned cues (Altar et al., 1992; Guillin et al., 2001; Martin-Iversen et al., 1994;

Martin-Iversen and Altar, 1996; Horger et al., 1999; Lu et al., 2004; Bahi et al., 2008). These reports suggest that elevated BDNF is associated with enhancement of dopamine neurotransmission, motor activation, and goal-directed behavior. Consistent with this idea, microinfusion of antisera against BDNF or TrkB receptors into NAc attenuated acute METH-induced increases in dopamine extracellular levels and psychostimulant-induced motor activity (Narita et al., 2003). Further, alcohol-induced increases in *bdnf* mRNA expression negatively regulate alcohol-associated behaviors and alcohol consumption (Janak et al., 2006). Consistent with these data, *bdnf* antisense oligonucleotide infusions into the central or medial, but not the basolateral, amygdala, increased alcohol intake, which was prevented by co-infusion of BDNF (Pandey et al., 2006). Conversely, *bdnf* heterozygous mice display increased conditioned-place preference and increased locomotor sensitization to alcohol and show a prolonged elevation in alcohol consumption following a period of abstinence (McGough et al., 2004). Similarly, acute amphetamine stimulates more locomotor behavior that lasts longer in *bdnf*^{+/−} mice than in WT mice (Dluzen et al., 2001; Saylor and McGinty, 2008). Furthermore, *bdnf*^{+/−} mice show similar depletions in tyrosine hydroxylase immunoreactivity and DA tissue concentrations in response to a neurotoxic regimen of METH (Boyer et al., in press; Dluzen et al., 2001). However, in one

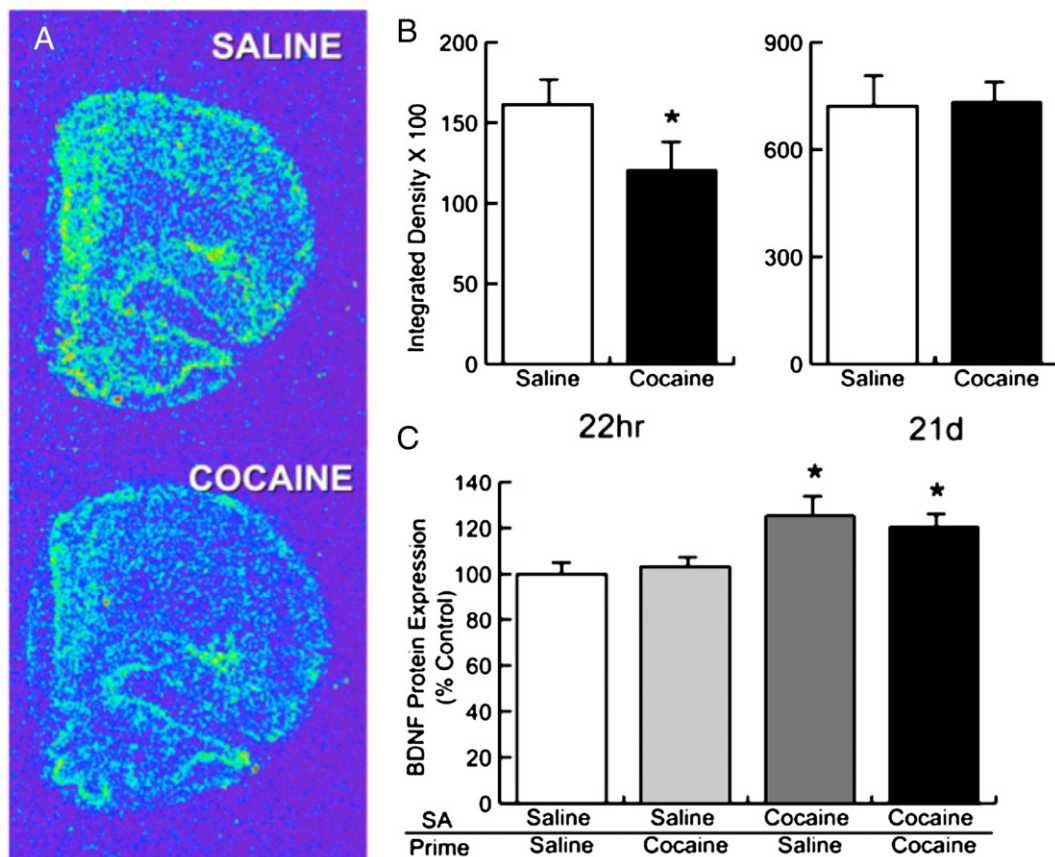


Fig. 2 – In situ hybridization histochemistry performed on frontal sections after the end of cocaine self-administration demonstrates that *Bdnf* mRNA in the dorsomedial PFC was downregulated after 22 hours (A, B – left), but not after 21 days (B – right), of cocaine abstinence. (C) BDNF protein expression was increased in the PFC 21 days after the end of cocaine self-administration in abstinent rats. SA=self-administration condition in which rats received cocaine in response to active lever presses or yoked-saline. Prime=i.p. injection of saline or cocaine given 30 minutes before euthanasia. *P<0.05.

report (Joyce et al., 2004), DAT binding and TH-ir were less affected in *bdnf*^{−/−} mice than in WT mice after a high-dose METH binge (mortality, thermal response, age, and sex of the mice were not reported).

4. The effects of exogenous BDNF on drug-seeking are brain region-specific

Infusion of BDNF into subcortical structures, like the NAc and VTA, enhances cocaine- and opioid-seeking (Lu et al., 2004; Graham et al., 2007; Vargas-Perez et al., 2009). Conversely, repeated administration of BDNF antiserum into the NAc during chronic cocaine self-administration attenuates cocaine-induced reinstatement (Graham et al., 2007). Collectively, these studies implicate BDNF activity in the VTA and NAc in long-term modulation of cocaine-induced behavior, possibly by interacting with enhanced dopamine neurotransmission or VTA GABAergic signaling (Vargas-Perez et al., 2009). These findings and the drug-induced alterations in BDNF expression observed during drug abstinence (described above) have led some investigators to speculate that BDNF activity promotes vulnerability to drug addiction and that therapeutic approaches that inhibit BDNF signaling may decrease individuals' motivation to seek cocaine (Graham et al., 2007; Tsai, 2007). However, this conclusion is based only on altered BDNF expression and effects in subcortical brain regions and does not take into consideration that the effects of BDNF on drug-seeking are site-specific. For example, in models of depression, BDNF has an anti-depressant profile when infused into the hippocampus and a pro-depressant profile when infused into the VTA (Shirayama et al., 2002; Eisch et al., 2003). Similarly, in contrast to the pro-drug-seeking effects of subcortical BDNF infusion described above, our data indicate that BDNF infusion into the dorsomedial PFC suppresses cocaine-seeking. Specifically, intra-PFC infusion immediately

following a final session of cocaine self-administration attenuates relapse to cocaine-seeking after abstinence, as well as cue- and cocaine prime-induced reinstatement of cocaine-seeking following extinction (Fig. 3 – Berglind et al., 2007). In this paradigm, rats self-administered 0.6 mg/kg, i.v. cocaine 2 hours/day for 10 days. Immediately after the final cocaine self-administration session, rats received a single, bilateral infusion of BDNF or vehicle within the prelimbic cortex (0.75 µg/side). The rats that received an intra-PFC BDNF infusion demonstrated significant attenuation in extinction responding, cue- and cocaine-induced reinstatement of drug seeking. Furthermore, intra-PFC BDNF infusions decreased extinction responding and prevented a cocaine-induced decrease in phospho-ERK activity in the NAc, but not in the caudate-putamen (CPu), detected after 1 day of abstinence, revealing a trans-synaptic regulation of NAc neurons by BDNF activity in the PFC (Fig. 4). Taken in isolation, this evidence suggests that BDNF, rather than a BDNF antagonist, would have possible therapeutic value in cocaine addiction. However, considering our findings in combination with Graham et al. (2007) and Lu et al. (2004) powerfully argues for a diverse and complex role of BDNF in relapse to cocaine-seeking. Our findings should not be viewed as contradictory to those of Graham or Lu et al.; rather they illustrate the site-specific effects of exogenous BDNF. Furthermore, they underscore the complexity of endogenous BDNF activity and serve to caution the promotion of theories about BDNF's possible therapeutic value in the treatment of cocaine addiction.

5. BDNF regulates glutamatergic neurotransmission in the prefronto-accumbens pathway

The susceptibility to drug relapse and other addictive behaviors is thought to depend on long-term neuroadaptations in

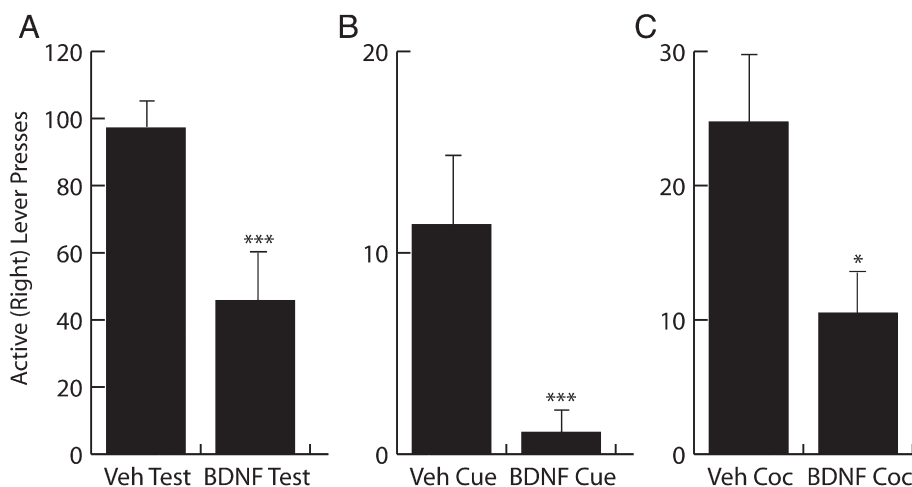


Fig. 3 – An intra-PFC infusion of BDNF attenuated extinction responding, cue- and cocaine-induced reinstatement of cocaine-seeking. (A) After 6 days of abstinence, the BDNF-treated rats exhibited significantly fewer active lever presses during the post-abstinence test than the vehicle-treated rats (*** $P=0.001$). **(B)** The BDNF-treated rats exhibited significantly fewer active (*** $P<0.001$) lever presses during the cue-induced reinstatement test than vehicle-treated rats. **(C)** The BDNF-treated rats exhibited significantly fewer active lever presses during the cocaine-induced reinstatement test than the vehicle-treated rats (* $P<0.05$). Modified from Berglind et al. (2007).

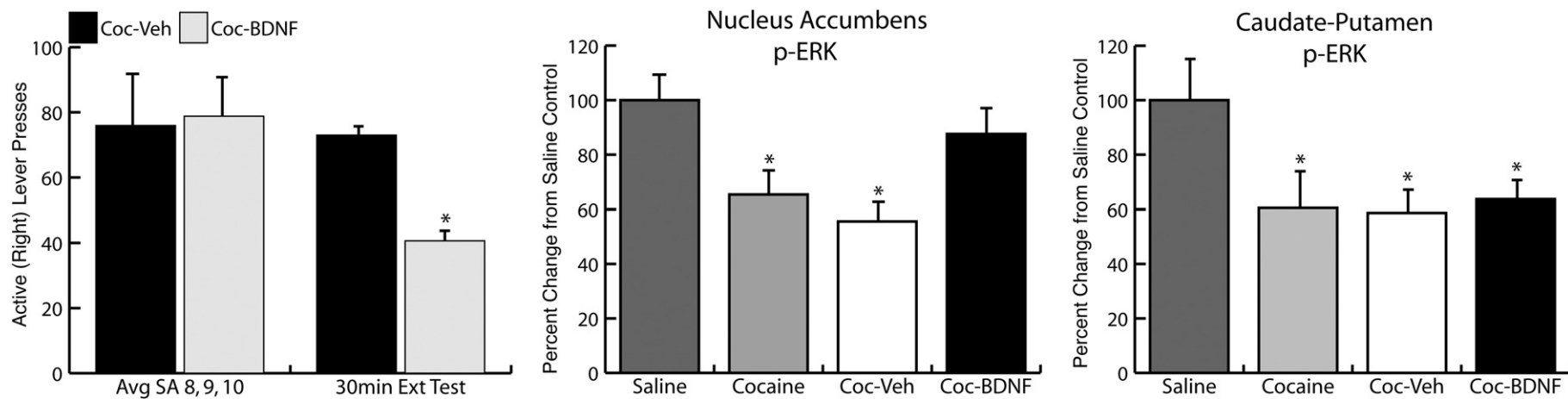


Fig. 4 – Intra-PFC BDNF infusion suppressed cocaine-seeking behavior (A) and normalized phospho-(p)ERK expression levels in the NAc (B), but not the caudate-putamen (C) of rats after a 30-minute extinction test 22 hours after the final cocaine SA session. *P<0.05. Avg SA 8, 9, 10=average number of active lever presses on the last 3 days of cocaine self-administration; 30 min Ext Test=30 minutes of extinction test 22 hours after the 10th self-administration session; Coc-Veh=cocaine self-administration and intra-PFC vehicle infusion; Coc-BDNF=cocaine self-administration and intra-PFC BDNF infusion. Modified from [Berglind et al., 2007](#).

mRNA, proteins, and phospho-proteins in mesocortico-basal ganglia circuitry (Nestler, 2005; Kalivas and Volkow, 2005). These persistent neuroadaptive changes encompass alterations in molecular components at the synapse, changes in gene expression, and altered behavioral output. The PFC, a critical region for goal-directed behaviors and impulse control, is one of the brain regions in which these changes take place. Imaging studies of addicts have revealed decreased basal activity during drug withdrawal (Goldstein and Volkow, 2002) and large increases in metabolic activity in the PFC following exposure to drug cues (Grant et al., 1996; Maas et al., 1998; Childress et al., 1999).

Reinstatement of drug-seeking after extinction in rodent studies appears to be driven primarily by disturbances in glutamatergic transmission in the prefronto-accumbens pathway, specifically a suppression of basal levels that is associated with an excessive cocaine prime-induced overshoot of glutamate release in the NAc (McFarland et al., 2003; Kalivas, 2004). BDNF augments glutamatergic transmission in the cerebral cortex and hippocampus where its expression is closely linked to activity-dependent synaptic plasticity (Lessmann, 1998; Lu, 2003; Matsumoto et al., 2001). Although dopaminergic afferents to the striatum, including NAc, also contain BDNF, the primary source of BDNF protein levels in the striatum is the cortico-accumbens pathway. BDNF potentiates excitatory neurotransmission (Lu and Chow, 1999) by increasing synaptic vesicle docking at excitatory synapses (Tyler and Pozzo-Miller, 2001), enhancing glutamate release (Lessmann, 1998), and postsynaptic NMDA receptor activity (Levine et al., 1995, 1998). BDNF regulation of glutamatergic signaling contributes to the neuronal plasticity underlying learning and memory (Tyler et al., 2002) by inducing early- and late-phase LTP (Kang and Schuman, 1996; Massaoudi et al., 1998) and dendritic spine growth (Horch et al., 1999).

The glutamatergic pathway arising in the dorsomedial PFC and projecting to the NAc core is a critical component of the reward circuitry that underlies reinstatement to cocaine-

seeking. In addition to attenuating reinstatement to cocaine-seeking, BDNF infusion into the dorsomedial PFC elevates BDNF and phospho-ERK expression in the NAc (Berglind et al., 2007). Moreover, intra-PFC BDNF prevents the cocaine self-administration-induced reduction in basal extracellular glutamate, as well as the cocaine prime-induced increase in extracellular glutamate levels in the NAc up to 3 weeks after BDNF infusion (Fig. 5 – Berglind et al., 2009). These data suggest that intra-PFC BDNF attenuates reinstatement to cocaine-seeking by normalizing cocaine-induced neuroadaptations that alter glutamate neurotransmission within the NAc.

6. Prefrontal cortical Trk receptor binding is necessary for the expression of BDNF's suppressive effect on cocaine-seeking

Despite the finding that intra-PFC BDNF infusion suppresses cocaine relapse, the specific site(s) of action and molecular-cellular substrates of BDNF's behavioral effects are not fully understood. It is possible that BDNF has local effects at the site of infusion (PFC) as well as distal effects in PFC target areas like the NAc. Infusion of exogenous BDNF into the PFC can have implications for local postsynaptic neuroplasticity in PFC neurons. Interactions between extracellular BDNF and TrkB can promote postsynaptic survival and growth signaling (Goggi et al., 2003), prolonged neuroplasticity in the form of LTP (Bramham and Messaoudi, 2005), and synaptic scaling (Rutherford et al., 1998; Leslie et al., 2001). However, there is evidence that BDNF may also have more widespread effects via its neurotransmitter-like activities (Blum and Konnerth, 2005). It has been demonstrated that exogenous BDNF can be internalized and become available for activity-dependent secretion (Santi et al., 2006; Dean et al., 2009) and that intracellular BDNF can undergo axonal transport (Altar and DiStefano, 1998) and calcium-dependent release (Sadakata et al., 2004) from presynaptic terminals. Taken together, this

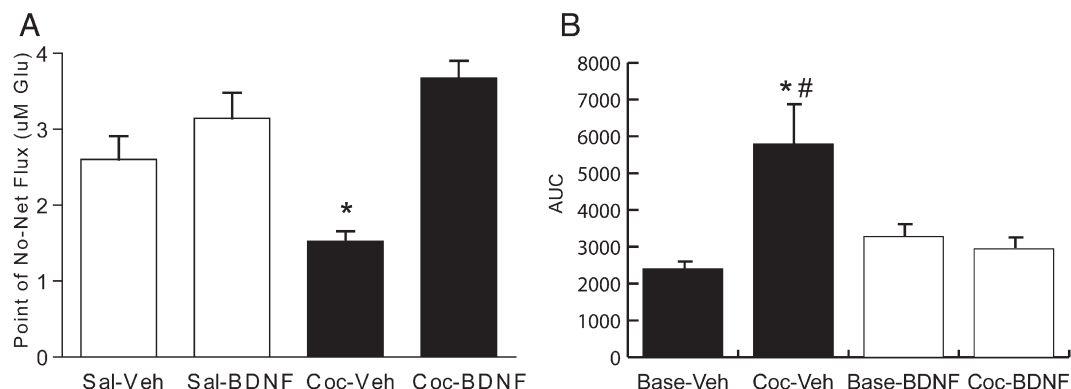


Fig. 5 – (A) BDNF-normalized basal extracellular glutamate levels in the NAc of rats with a cocaine self-administration history. Intra-PFC BDNF treatment significantly increased the point of no net flux, indicating an increase in the basal extracellular glutamate concentrations in the rats compared with those that received intra-PFC vehicle infusions, * $P < 0.05$. (B) Average area under the curve (AUC) values before and after a cocaine challenge in rats with a cocaine history. Base-Veh: average baseline AUC value for rats that received an intra-PFC vehicle infusion; Coc-Veh: average AUC value after the cocaine challenge for rats that received a vehicle infusion; Base-BDNF: average baseline AUC value for rats that received an intra-PFC BDNF infusion; Coc-BDNF: average AUC value after the cocaine challenge for rats that received an intra-PFC BDNF infusion. * $P < 0.05$ vs. Coc-BDNF; # $P < 0.05$ vs. Base-Veh. Modified from Berglind et al. (2009).

evidence suggests that BDNF has the capability to regulate neuroplasticity both locally in the PFC as well as distally in target regions of PFC projections.

Information obtained to date indicates that exogenous BDNF infused into the PFC 1) increases BDNF protein expression in the NAc and other PFC targets such as the amygdala (Fig. 6), (2) restores cocaine-mediated decreases in p-ERK expression in the NAc, and (3) prevents cocaine-mediated aberrations in extracellular glutamate levels in the NAc (Berglind et al., 2007, 2009). These data support the notion that intra-PFC BDNF's suppressive effect on cocaine-seeking depends on normalization of cocaine-mediated plasticity in the PFC–NAc pathway. However, this does not rule out the possibility that local BDNF–TrkB interactions in the PFC precede subsequent distal effects in target structures such as the NAc. Therefore, we investigated the site and mechanism of action of intra-PFC BDNF's suppressive effects on cocaine-seeking behavior.

Given that TrkB receptor binding and activation have been shown to be necessary for the expression of BDNF's behavioral effects *in vivo* (Ou and Gean, 2006), we determined the extent to which the attenuation of cocaine-seeking following intra-PFC BDNF depends on local TrkB signaling. Infusion of the extracellular BDNF scavenger TrkB-Fc 20 minutes prior to BDNF in the dorsomedial PFC completely reversed BDNF's suppressive effect on cocaine-seeking 22 hours post-infusion (Fig. 7). However, lever pressing of rats that received an intra-PFC infusion of TrkB-Fc followed by vehicle was not significantly different from vehicle–vehicle-treated rats, indicating that scavenging endogenous BDNF in the PFC did not affect cocaine-seeking. This lack of effect of TrkB-Fc alone is consistent with the finding that *bdnf* mRNA is decreased in the PFC 22 hours after the end of cocaine self-administration (see Fig. 2).

In further experiments, we assessed whether BDNF's suppressive effect on cocaine relapse is mediated by Trk receptor

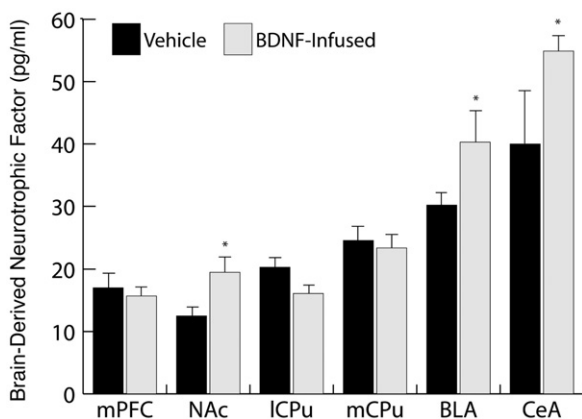


Fig. 6 – BDNF protein expression levels, detected by ELISA, are significantly elevated in the NAc, basolateral amygdala (BLA), and central nucleus of the amygdala (CeA) of naive rats 22 hours after infusion of BDNF in to the dorsomedial PFC. N=6 per group. mPFC=medial prefrontal cortex; NAc=nucleus accumbens; ICPu=lateral caudate–putamen; mCPu=medial caudate–putamen. *P<0.05 vs. Vehicle-infused.

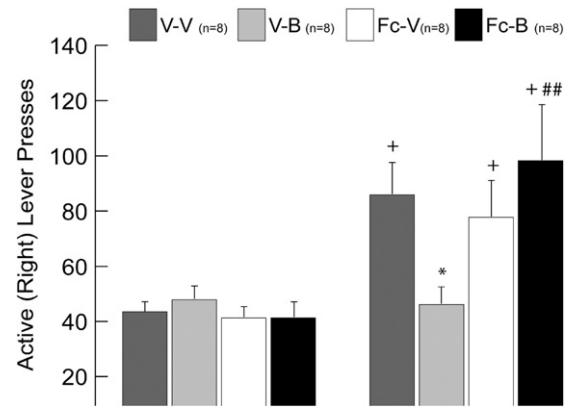


Fig. 7 – Pre-infusion with a BDNF scavenger, TrkB/Fc, prevented the suppressive effect of intra-PFC BDNF on cocaine-seeking during day 1 of extinction responding. V–V=vehicle–vehicle; V–B=vehicle–BDNF; Fc–V=TrkB/Fc–vehicle; Fc–B=TrkB/Fc–BDNF. Avg SA 8, 9, 10=average number of active lever presses on the last 3 days of cocaine self-administration; 2 hr Ext Test=2 hours extinction test 22 hours after the 10th self-administration session. *P<0.05 vs. V–V; *P<0.005 vs. V–B; *P<0.05 vs. Avg SA 8, 9, 10 within each group.**

binding by infusing the Trk inhibitor, K252a, into the dorsomedial PFC 20 minutes before BDNF. K252a blocked BDNF's suppressive effect on cocaine-seeking following 6 days of abstinence from cocaine self-administration and in tests of cue- and cocaine prime-induced reinstatement (Whitfield et al., 2008). These data suggest that exogenous BDNF infused into the PFC binds surface-expressed Trk receptors and that this BDNF–Trk interaction is necessary for BDNF's suppressive effect on cocaine-seeking. Collectively, the reversal of the suppressive behavioral effects of intra-PFC BDNF with both the TrkB-Fc and K252a compounds demonstrates that BDNF's effects are, in part, local to the PFC and depend on TrkB receptor activation.

7. BDNF suppresses cocaine-seeking by altering TrkB-mediated intracellular signaling in the prefrontal cortex

A critical point at which BDNF likely intersects with the effects of psychostimulants is at the level of intracellular signaling. Alterations in TrkB-mediated activation of several distinct intracellular signaling cascades, including the mitogen-activated protein kinase (MAPK), the phospholipase C- γ (PLC- γ), and phosphoinositide 3-kinase (PI3K) cascades, provide a means by which BDNF mediates persistent neuroplasticity (Patapoutian and Reichardt, 2001). These cascades converge on nuclear transcription factors such as CREB and ELK to regulate gene expression and induce neuroadaptations. Fig. 8 illustrates the downstream signaling cascades of TrkB activation in neurons.

Most notably, activation of each of these cascades has been independently implicated in the CNS response to acute and chronic administration of psychostimulants, and in many cases, governs the expression of psychostimulant-related

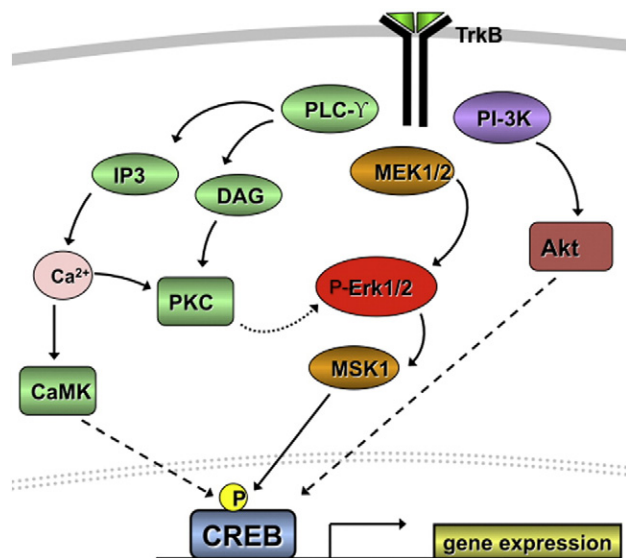


Fig. 8 – Schematic of BDNF/TrkB signal transduction. Activation of the TrkB receptor initiates downstream changes in CREB-dependent gene transcription via PI3K, ERK/MAPK, and PLC- γ signaling.

behaviors. For example, MAPK signaling in the ventral striatum is a necessary contributor to the expression of cocaine-mediated induction of immediate early genes, behavioral sensitization, and reward (Girault et al., 2007; Valjent et al. 2000, 2004, 2005), as well as to the consolidation and reactivation of cocaine conditioned-place preference (Miller and Marshall, 2005; Valjent et al., 2006). Moreover, VTA MAPK signaling is necessary for a BDNF-mediated inhibition of morphine or cocaine-induced tyrosine hydroxylase expression (Berhow et al., 1995, 1996) and enhancement of cocaine-seeking during abstinence (Lu et al., 2004). Additionally, it has been shown that acute administration of cocaine induces PLC- γ in the NAc and that anti-BDNF infusion blocks this effect (Graham et al., 2007). PFC and NAc PI3K signaling may also be involved, given that experimenter-administered cocaine-induced PI3K in the NAc (Zhang et al., 2006), and antagonism of PI3K with LY294002 intracerebroventricularly decreased cocaine-mediated behavioral sensitization (Izzo et al., 2002).

Although these and other studies have characterized the involvement of MAPK, PI3K, and PLC- γ signaling at the mesolimbic level, there is little information about the role of prefrontal cortical intracellular signaling in the expression of psychostimulant-mediated behaviors during self-administration and relapse. Therefore, we investigated the contribution of PFC MAPK and PI3K signaling to the expression of BDNF's suppressive effect on cocaine-seeking behavior. Infusion of the MAPK/ERK kinase (MEK1/2) inhibitor, U0126, 20 minutes prior to intra-PFC BDNF blocked BDNF's suppressive effect on cocaine-seeking following 6 days of abstinence and in tests of cue- and cocaine prime-induced reinstatement (Whitfield et al., 2009). In contrast, infusion of the PI3K inhibitor, wortmannin, had no effect on BDNF's suppression of cocaine-seeking during these tests. Interestingly, neither intra-PFC infusion of U0126 nor infusion of wortmannin followed by vehicle infusion uniquely altered cocaine-seeking behavior. These

data indicate that the intracellular mechanisms by which intra-PFC BDNF suppresses cocaine-seeking depend on MAPK, but not PI3K, signaling in PFC neurons. In summary, these studies elucidate a mechanism whereby intra-PFC BDNF binds and activates TrkB receptors, which, in turn, predominantly activates MAPK, not PI3K, signaling in PFC neurons in order to normalize cocaine-mediated plasticity and suppress addictive cocaine-seeking behavior.

8. Summary

In summary, BDNF expression is associated with neuronal activity and synaptic plasticity. BDNF enhances synaptic transmission and promotes LTP by increasing dendritic protein synthesis and dendritic spine formation. BDNF also regulates drug-induced long-term neuroadaptations that encompass alterations in molecular components at the synapse, changes in gene expression, and modifications of behavioral output. The effects of BDNF on cocaine-seeking are brain region-specific. BDNF may have local effects at the site of infusion and distal effects in target areas that are critical to mediating or preventing cocaine-induced dysfunctional neuroadaptations.

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