Role of Dopamine and Glutamate Receptors in Intracellular Mechanisms for Cocaine Action

By

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Abstract

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Accumulating evidence suggests that cocaine exerts its behavioral and biochemical effects through dopamine and glutamate transmission. However, the underlying mechanisms are not well understood. The purpose of this proposal is to assess the role of dopamine D1 and NMDA receptors and related signal transduction pathways in response to acute cocaine. We hypothesize that cocaine-induced intracellular cascades are mediated by dopamine D1 and NMDA receptors. In addition, the receptor physical interaction between them is altered by cocaine. To study the intracellular mechanisms induced by cocaine, we measured extracellular signal-regulated kinase (ERK) and dopamine- and cAMP-regulated phosphoprotein (DARPP-32) pathways. Overall, a single cocaine administration increased ERK-mediated signaling proteins, phosphoryation of cAMP response element-binding protein kinase regulator, pp90 ribosomal S6 kinase, and c-Fos protein levels in the caudate-putamen (CPu). Acute cocaine administration also induced phosphorylation of the striatal-enriched protein tyrosine phosphatase and decreased the phosphorylation of DARPP-32 protein at the Thr-75 site.

To study the role of dopamine D1 and NMDA receptors on cocaine-induced early gene (IEG), c-Fos, FosB and p-MKP-1 protein levels were measured. Acute cocaine administration time dependently increased IEG protein expression and phosphorylation in

the CPu and nucleus accumbens (NAc). In the CPu, the cocaine-induced c-Fos and FosB proteins expression is totally abolished by pre-administration of dopamine D1 receptor antagonist, SCH23390. In the NAc, SCH23390 also inhibited cocaine-induced c-Fos protein expression. The pre-treatment of NMDA receptor antagonist, MK-801, partially reduced cocaine-activated c-Fos protein expression in the CPu. Furthermore, the escalation of p-MKP-1 after acute cocaine administration is dependent on both the activation of dopamine D1 and NMDA receptors in both brain regions examined.

At the receptor level, we found that acute cocaine reduced NMDA-NR1 and dopamine D1 receptor protein-protein interactions in the CPu. Interestingly, the administration of NMDA and MK-801 also decreased the physical interaction between these receptors. These results indicated that pharmacological receptors manipulation resulted in the dissociation of receptors interactions.

Taken together, we suggest that acute cocaine may regulate the intracellular transduction and underlying IEG through dopamine D1 and NMDA receptors as well as through their physical interactions.

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I dedicate this dissertation to Luyi Zhou. As my wife and colleague, your support, love and the existence of my life made it possible for me to complete personal and professional goals. Thank you with all my heart.

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Chapter 1: Introduction

I. Background:

A. Neurocircuitry in addiction

Figure 1 summarizes the interconnected circuit that is thought to be important for the development and manifestation of addictive behavior. The neurobiological mechanisms mediating both acute and chronic cocaine-induced behavior changes are associated with the dopamine pathway originating in the midbrain ventral tegmental area (VTA) and substantia nigra area, projecting to the ventral striatum/nucleus accumbens (NAc), dorsal striatum/caudate-putamen (CPu), prefrontal cortex (PFc) including the anterior cingulated/prelimbic cortex and the ventral orbital cortex, and amygdala (Berke and Hyman, 2000; Goldstein and Volkow, 2002; Koob, 1992; Neisewander et al., 2000; Nestler and Aghajanian, 1997; Self and Nestler, 1995; White and Kalivas, 1998). Behavioral studies have demonstrated that the NAc and CPu are important for the psychostimulant-induced locomotor and stereotypic behaviors, respectively (Delf et al., 1990; Kelly et al., 1975; Kelly and Iverson, 1976). Lesions of the mesolimbic dopamine pathway also can abolish cocaine self-administration (Roberts et al., 1977, 1980). In addition to the dopamine pathway, the cell body region in the VTA and terminal region of the NAc contain glutamatergic inputs from PFc, amygdala and hippocampus (Christe et al., 1987; Gorelova and Yang, 1997; Grovenewegen et al., 1987; Kelley et al., 1982). The PFc and the basolateral amygdala also form reciprocal glutamatergic connections (Kalivas, 2004). At the behavioral level, previous studies demonstrated that ibotenic acid lesions of PFc disrupted the development and expression

behavioral sensitization in response to chronic psychostimulants exposure (Li et al., 1999; Li and Wolf, 1997; Pierce et al., 1998). In addition, the CPu also receives glutamatergic innervations from the cortex and thalamus (Gerfen and Wilson, 1996). Furthermore, the γ-amino butyric acid (GABA)ergic and peptidergic neurons in the NAc project to the ventral pallidum and VTA as the primary output of this topographically organized circuitry (Groenewegen et al., 1996).

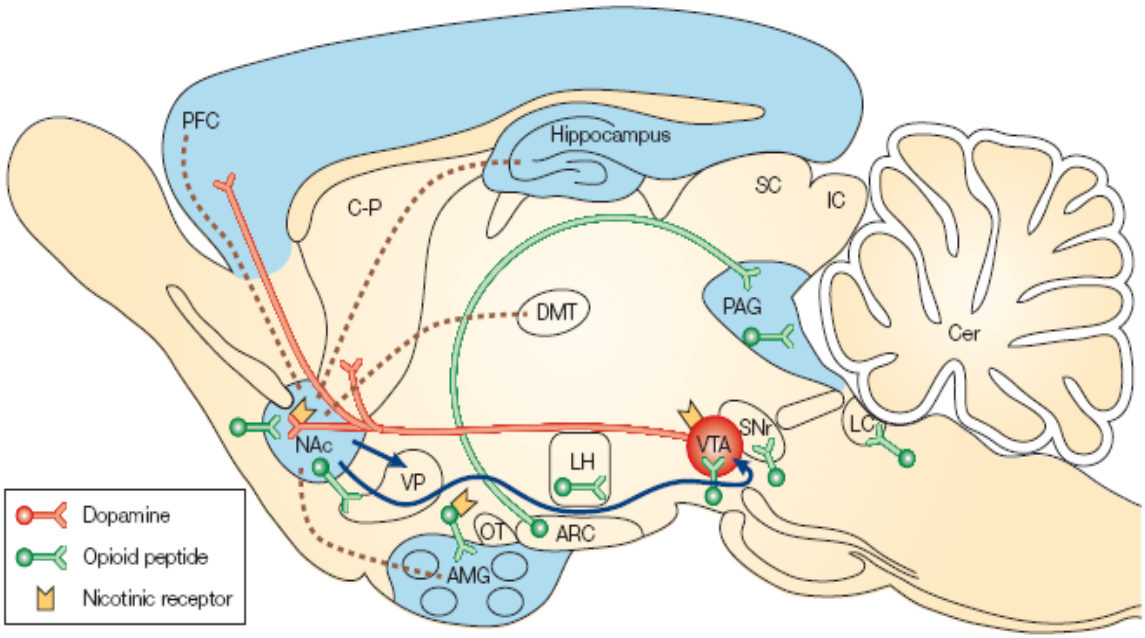


Figure 1. Illustration of circuitry involved in the development and expression of psychostimulant-induced addiction. C-P, caudate-putamen; NAc, nucleus accumbens; PFc, prefrontal cortex; VP, ventral palladium; VTA, ventral tegmental area (Adopted from Nestler, 2001).

B. Cocaine effects on dopaminergic and glutamatergic pathways

Cocaine acts as a monoamine uptake inhibitor, causing a buildup of the monoamine levels in the synaptic and extra-synaptic compartments (Church et al., 1987; Di Chiara and Imperato, 1988). For example, *in vitro* studies have shown that there is an enhanced dopamine release from accumbal and striatal tissue slices and synaptosomes from rats sensitized to psychostimulants (e.g. cocaine) (Castaneda et al., 1988; Kalivas and Duffy, 1988; Kolta et al., 1985; Robinson et al., 1982; Yamada et al., 1988). *In vivo* microdialysis studies demonstrated that acute cocaine administration leads to an immediate and dose-dependent increase in extracellular dopamine levels in various areas associated with the mesolimbic system (Carboni et al., 1989; Church et al., 1987; Kuczenski et al., 1991; Maisonneuve et al., 1995; Maisonneuve and Glick, 1992; Maisonneuve and Kreek, 1994). Studies using freely moving rats further demonstrated that, although with different profile of dopamine release, acute and chronic cocaine treatments can elevate the extracellular dopamine concentration in both CPu and VTA (Parsons and Justice, 1993; Reith et al., 1997).

Cocaine also can exert its influence on the glutamatergic system in the mesolimbic system. In the VTA, substantia nigra and/or NAc, acute cocaine injection induces transient extracellular glutamate concentration increases in a dose-dependent manner but with decreased glutamate level in the CPu (Kalivas and Duffy, 1995; Smith et al., 1995; Zhang et al., 2001). In a chronic cocaine treatment paradigm, sensitized rats show higher extracellular glutamate levels in VTA, NAc and CPu after subsequent challenge compared to relative control animals (Kalivas and Duffy, 1998; Pierce et al., 1996; Reid and Berger, 1996; Zhang et al., 2001).

Taken together, these findings indicated that cocaine increases the extracellular dopamine concentration in various areas of mesolimbic system via its direct interaction with dopamine receptors. In addition, cocaine may modulate the extracellular glutamate level through its influence on the dopamine system.

C. Dopamine D1 receptor and its interaction to cocaine

Dopamine D1 receptor:

Dopamine receptors can be divided into two classes, the D1-like (D1 and D5) and the D2-like (D2, D3 and D4) receptors based on their responses to distinct sets of ligands and their linkage to different intracellular transduction cascades (Civelli et al., 1993; Gingrich and Caron, 1993). Analysis of the primary structure of dopamine receptors reveals that they are members of the seven-transmenbrane G protein-coupled receptors (Missale et al., 1998). Among them, the D1 receptor mRNA is found throughout the forebrain, highly concentrated in the NAc, CPu, olfactory tubercle and amygdala, and with moderate levels in the hippocampus, septum, thalamus hypothalamus and cerebellum (Meador-Woodruff, 1994). However, no mRNA level has been detected in certain regions where the dopamine D1 receptor protein is highly expressed such as entopeducular and substantia nigra, suggesting that the dopamine D1 receptor in these areas is mainly present in projections (Dawson et al., 1988; Dearry et al., 1990; Fremeau et al., 1991; Weiner et al., 1991).

The effect of dopamine D1 receptor on cocaine-induced behavior:

Accumulating evidence indicates that dopamine D1 receptor is necessary for cocaine-mediated behavioral activation. For example, selective dopamine D1

receptor antagonists reverse cocaine-induced stereotypic behavior when administrated directly into the CPu (Arnt, 1985). Either systemic or intra-accumbens dopamine D1 receptor antagonists injection attenuates acute cocaine-induced locomotor and stereotypic behavior in rats (Becker at al., 1998; Kaddis et al., 1993; Neisewander et al., 1995; Ushijima et al., 1995) and in mice (Cabib et al., 1991). In addition, short-term intra-accumbens dopamine D1 receptor agonist infusion can induce enhanced locomotor response to subsequent cocaine challenge (cross-sensitization) that can be blocked by systemic dopamine D1 receptor antagonist injection (De Vries et al., 1998). Similarly, dopamine D1 receptor antagonists also prevent the expression of repeated cocaine-induced behavioral sensitization (McCreary and Marsden, 1993; Tella, 1994) and the acquisition of behavioral sensitization to cocaine during the early withdrawal (White et al., 1998). Previous studies using dopamine D1 receptor mutant mice also suggested that the dopamine D1 receptor is important for the behavioral activation induced by both acute and chronic cocaine treatments (Xu et al., 1994, 2000). Moreover, pretreatment of dopamine D1 receptor antagonist abolished acute and chronic cocaine-induced the elevation of glutamate concentration in VTA related to the behavioral activation in response to cocaine (Kalivas and Duffy, 1995, 1998).

Dopamine D1 receptors are also involved in the rewarding effect associated with cocaine. For instance, dopamine D1 receptor antagonists increase cocaine self-administration (Hubner and Moreton 1991; Hurd et al., 1997; Koob at al., 1987; McGregor and Roberts, 1993) and attenuate cocaine priming-induced reinstatement of drug-seeking behavior followed by cocaine self-administration in rats

(Alleweireldt et al., 2006; Anderson et al., 2003). In addition, dopamine D1 receptor antagonists have been reported to block the acquisition of the cocaine conditioned place preference (Baker et al., 1998; Cervo et al., 1995; Nazarian et al., 2004; Pruitt et al., 1995).

Cocaine effects on dopamine D1 receptors:

Several studies have indicated that cocaine may modulate the activity and expression of dopamine receptors in various brain areas related to cocaine addiction. In general, acute binge cocaine treatment does not induce any alternation on dopamine D1 binding site (Tsukada et al., 1996). However, acute cocaine administration may enhance dopamine D1 receptor mRNA expression in the striatum (Svensson and Hurd, 1998).

Previous studies have shown that the earliest physiological changes associated with cocaine-induced sensitization occur within in VTA including somatodendritic dopamine autoreceptor subsensitivity and increase dopamine neuron activity but both of which are relatively transient (Ackerman and White, 1990; Henry et al., 1989). Repeated cocaine injection increases the postsynaptic dopamine D1 receptor sensitivity in NAc with persistently behavioral sensitization (Henry and White, 1991, 1995). In addition, chronic dopamine D1 and D2 receptors agonists co-administration induces cross-sensitization to cocaine, dopamine D1 receptor supersensitivity in NAc, and decrease autoreceptor activity in VTA mimicking the cocaine-induced behavioral sensitization (Henry et al., 1998). Previous studies of alternation of dopamine binding site after repeated cocaine exposure are equivocal. Overall, repeated cocaine induces no change or delayed decrease of dopamine D1

receptor binding sites in PFc, NAc, and CPu as well as limbic regions in rats (Kleven et al., 1990; Kunko et al., 1998; Laurier et al., 1994; Peris et al, 1990; Tsukada et al., 1996). However, the down-regulation of dopamine D1 receptor bind site observed in CPu and PFc after withdrawal accompanies the increase of D1 mRNA expression, indicating a *de novo* protein synthesis after proteolytic degeneration of receptors in response to prolong elevation of extracellular dopamine concentration (Kleven et al., 1990; Schmidt-Mutter et al., 1999). In contrast, previous studies demonstrated that the dopamine D1 binding sites were increased in the CPu and mesolimbic regions followed by either chronic binge or twice daily cocaine injections (Alburges et al., 1993; Unterwald et al., 1994).

In summary, high dose acute cocaine administration may induce D1 dopamine mRNA increase in the CPu without any alternation in dopamine D1 binding site. The discrepancies of chronic cocaine exposure in D1 dopamine binding site are due to the different doses used in each injection, length of cocaine administration or duration of withdrawal in different experiment paradigms.

D. NMDA receptor and its interaction with cocaine

NMDA receptor:

The *N*-methyl-D-aspartate (NMDA) receptor, one of glutamatergic receptors, contains glycine and glutamate binding sites and is permeable to potassium and sodium as well as calcium (Ascher and Nowak, 1987; Hollmann and Heinemann, 1994; MacDermott et al., 1986). NMDA receptor is a heteromeric ligand-gated ion channel composed of multiple subunits including NR1, NR2 and NR3 to form a functional channel. The diversity of NR1 subunit is created by alternative splicing in

three terminal cassettes (C1, C2 and N) results in eight functional spice variants (NR1-4 a/b) (Hollmann et al., 1993; McBain and Mayer, 1994). The NR1 splice variants contain C1 terminal domain with two phosphorylation residues, Ser 896 and Ser 897, which can be phosphorylated by protein kinase C (PKC) and protein kinase A (PKA), respectively (Tingly et al., 1997). The C2 terminal domain has been observed mainly in intracellular pool in cell and related to long-term potentiation-induced insertion of NMDA receptors into the cell membrane (Chazot and Stephenson, 1997; Grosshans et al., 2002). Splice variants contain N-terminal cassette are equally distributed between the cell surface and the cytoplasm when transfected into fibroblasts (Okabe et al., 1999).

The effect of NMDA receptor on cocaine-induced behaviors:

Accumulating studies have suggested that the NMDA receptor can modulate the cocaine-induced behavioral response. For example, the non-competitive or competitive NMDA receptor antagonists can dose-dependently prevent acute cocaine or dopamine D1 receptor agonist-induced locomotor response in mice (Uzbay et al., 2000) and in rats (Kreipke and Walker, 2004; Pulvirenti et al., 1991). Intra-accumbens administration of (+)-HA966, a partial agonist of the glycine site of NMDA receptor, also attenuates acute cocaine-mediated locomotor augment (Khan and Shoaib, 1996). Thus, both the glutamate and the glycine binding sites of the NMDA receptor are important for acute cocaine-induced behavioral changes.

In the chronic treatment paradigm, the NMDA antagonists also prevent the development of repeated cocaine-induced locomotor and stereotypic behavior sensitization (De Montis et al., 1992; Haracz et al., 1995; Itzhak and Stein, 1992, Li

et al., 1999; Wolf and Jeziorski, 1993) or reverse tolerance (Karler et al., 1989) and tolerance (De Montis et al., 1992) as well as cocaine-induced conditioned place preference and postsynaptic dopamine receptors supersensitivity (Kim et al., 1996). Moreover, a single NMDA injection can induce drug-seeking behavior in cue-induced cocaine relapse model (Lu et al., 2005b).

Recently, evidence indicates that specific subunit of NMDA receptor is important for the cocaine-induced behavioral response. For instance, studies have demonstrated that mice lacking NMDA-NR1 subunit show attenuated behavioral response to acute psychostimulant treatments, blunted conditioned place preference and behavioral sensitization induced by repeated cocaine administration (Heuser and Palmiter, 2005; Miyamoto et al., 2004).

Cocaine effects on NMDA receptors:

Similar to dopamine D1 receptors, although acute cocaine administration dose not change NMDA protein levels (Fitzgerald et al., 1996), it decreases the NMDA-NR1 mRNA levels in NAc and CPu (Ghasmazadeh et al., 1999). After chronic cocaine administration, the NMDA-NR1 protein level is transiently increased in VTA and non-NMDA receptor (GluR1 subunit of AMPA receptor) protein levels are persistently increased in NAc which are correlated the development of cocaine-induced behavioral sensitization (Churchill et al., 1999; Fitzgerald et al., 1996). However, in prolonged cocaine self-administration paradigm, endurable NMDA-NR1 protein level elevations have been observed in NAc, VTA, and central nucleus of amygdala even after long-term abstain (Lu et al., 2003, 2005a). This persistent alternation in NMDA-NR1 subunit is followed by the initial increase and

subsequent decrease of NR1 mRNA levels in the mesolimbic system due to the neuroadaptation in response to fluctuations of glutamate extracellular concentration across the period of cocaine withdrawal (Ambrosio and Crespo, 2000; Crespo et al., 2002; Turchan et al., 2003). Furthermore, in the PFc, repeated cocaine treatment decreases the phosphorylation of NMDA-NR1 subunit through the reduction of the PKC activity (Loftis and Janowsky 2002; Steketee et al., 1998).

II. Intracellular mechanisms underlying cocaine addiction

A. cAMP/PKA pathway

By binding to the dopamine transporter, cocaine elevates the extracelluler dopamine concentration and activates the dopamine D1 receptor, subsequently. Adenylyl cyclase is a membrane bound protein which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Through the positively coupling to G-proteins, Gs and Golf, the activation of dopamine D1 receptor stimulates the adenylyl cyclase activity in neostriatum (Herve et al., 1993; Sibley et al., 1993; Stoof and Kebabian, 1981; Zhuang et al., 2000, Figure 2) and robustly accumulates the cAMP in various cell lines (Monsma et al., 1990; Zhou et al., 1990). Enhancement of cAMP activates PKA activity which may phosphorylate several substrates and receptors.

Studies have demonstrated that repeated cocaine administration leads to increasing activities of adenylyl cyclase and PKA (Hope et al., 2005; Terwilliger et al., 1991), as well as increasing dopamine D1 receptor-mediated adenylyl cyclase activation (Unterwald et al., 1996). Behavioral studies also demonstrated a positive

association between cAMP/PKA signaling changes and behavior effects after cocaine administration. Specifically, administration of PKA activator and inhibitor enhanced and dampened acute cocaine-mediated locomotor behavior, respectively, as well as modulated the cocaine-induced conditioned place preference (Cervo et al., 1997; Miserendino and Nestler, 1995). PKA and adenylyl cyclase activators also potentiate behavioral sensitization after chronic cocaine treatment (Miserendino and Nestler, 1995; Schroeder et al., 2004). Moreover, in rats, intra-accumbens PKA activator injection increases cocaine self-administration. In contrast, intra-accumbens PKA inhibitor infusion decreases cocaine self-administration (Self et al., 1998).

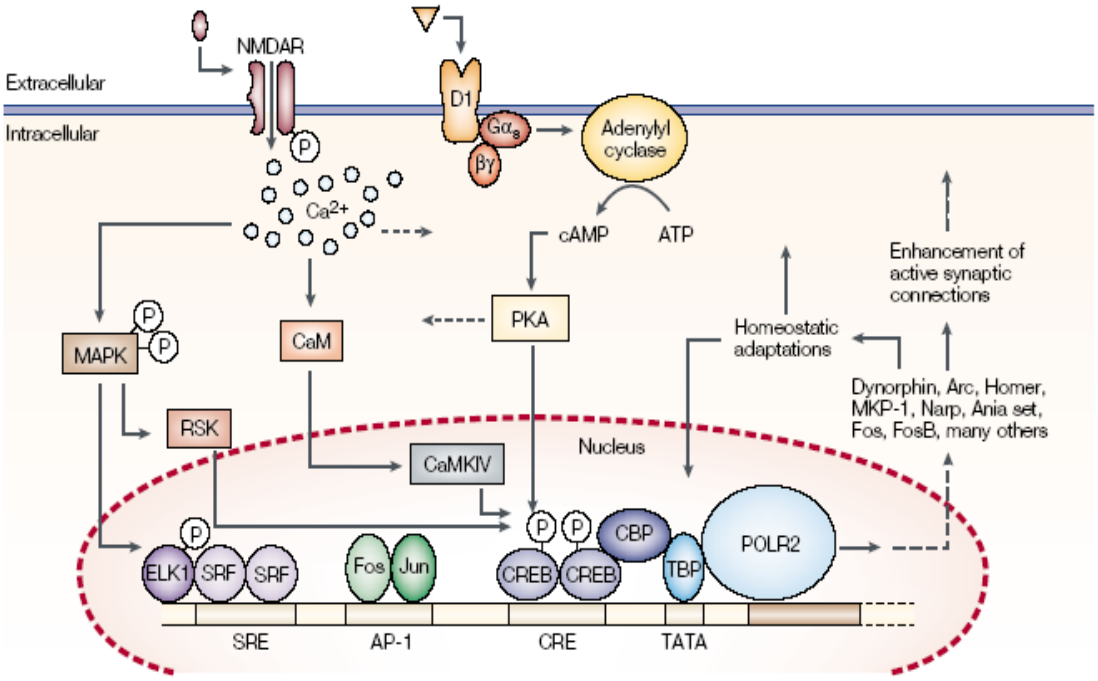


Figure 2. Intracellular signaling mechanisms mediated by dopamine D1 and NMDA receptors. Activation of both receptors stimulates multiple kinases leading to gene transcription and neuroadptations (Adopted from Hyman and Malenka 2001).

B. MEK/ERK/Elk-1 signaling

Initially, the intracellular extracelluler signal-regulated kinases (ERK1/2 or p44/p42 MAPKs) cascade, one of isoforms of mitogen-activated protein kinases (MAPK), has been characterized to respond to extracellular stimuli and regulates the cell proliferation and differentiation (Seger and Krebs, 1995). For example, once activating by growth factors, the receptor tyrosine kinases recruit Ras family G-proteins and lead to sequential activation Raf (MAPK kinase kinase), MEK (MAPK kinase), and ERKs. After activation, ERK proteins can translocate to the nucleus

(Chen et al., 1992), where they phosphorylate the ternary complex factor Elk-1 (Gille et al., 1992, 1995a) and thereby control gene transcription. Increasing evidence shows a glutamate linkage to ERKs phosphorylation in neurons both *in vivo* and *in vitro* (Figure 3). For instance, through the elevation of intracellular calcium (Ca^{2+})/calmodulin (CaM)/CaM kinases (CaMKs), the activation of the NMDA receptor can increase the phosphorylation of MEK/ERK/Elk-1 in hippicampal slice and neuronal culture (Kurino et al., 1995; Roberson et al., 1999), cortical cultured neurons (Xia et al., 1996), and striatal cultured neurons (Mao et al., 2004; Perkinton et al., 2002; Vincent et al., 1998). Inhibition of ERKs activation attenuates glutamate-mediated Elk-1 phosphorylation in striatal slice (Vanhoutte et al., 1999), striatum (Choe and McGinty 2001; Sgambato et al., 1998a, 1998b) and in hippocampus (Davis et al., 2000). Alternatively, in PC12 cell, Ca^{2+} may increase the intracellular cAMP through Ca^{2+}/CaM-sensitive adenylyl cyclase leading to the activation of PKA. Increase of cAMP and PKA phosphorylates the MEK via the activation Rap1/Raf (Grewal et al., 2000; Vossler et al., 1997). Consistent with these studies, pharmacological activation of dopamine D1 receptor or the adenylyl cyclase markedly stimulates ERKs activity in various neuronal cells (Choe and McGinty, 2000; Gerfen et al., 2002; Impey et al., 1998; Roberson et al., 1999; Zanassi et al., 2001).

Recently, studies have shown that the MEK/ERK/Elk-1 signaling is involved in psychostimulants addiction. In mice, acute cocaine treatment induces rapid and transient increase of the ERK protein phosphorylation (p-ERKs) in CPu, NAc, FCx and amygdala which can be abolished by the pretreatment of NMDA receptor

antagonist, dopamine D1 receptor antagonist or MEK inhibitor (Radwanska et al., 2005; Valjent et al., 2000, 2004). In addition, pharmacological inhibition of NMDA receptor also attenuated acute cocaine-induced p-ERK in rats CPu (Jenab et al., 2005). Repeated cocaine injections followed by subsequent challenge increases the phosphrylation of ERKs protein or ERKs catalytic activity in the CPu, NAc and VTA (Berhow et al., 1996; Valjent et al., 2000). Behaviorally, the cocaine-induced hyper-locomotor activity, the development of sensitization and conditioned place preference as well as underlying Elk-1 phosphorylation are attenuated by the treatment of MEK inhibitor (Mattson et al., 2005; Miller et al., 2005; Pierce et al., 1999; Valjent et al., 2000, 2005). Recent study on ERK1 mutant mice indicated that, due to the hyper-phosphorylation of ERK2 protein, mutated mice show stronger psychostimulant- (e.g. cocaine) induced conditioned place preference and behavioral sensitization than those of the wild-type controls (Ferguson et al., 2006; Mazzucchelli et al., 2002). Moreover, in the CPu, mice lacking of dopamine D1 receptor show attenuated ERKs phosphorylation in response to acute cocaine treatment suggesting the important role of dopamine D1 receptor-mediated MEK/ERK/Elk-1 signaling in cocaine addiction (Zhang et al., 2004).

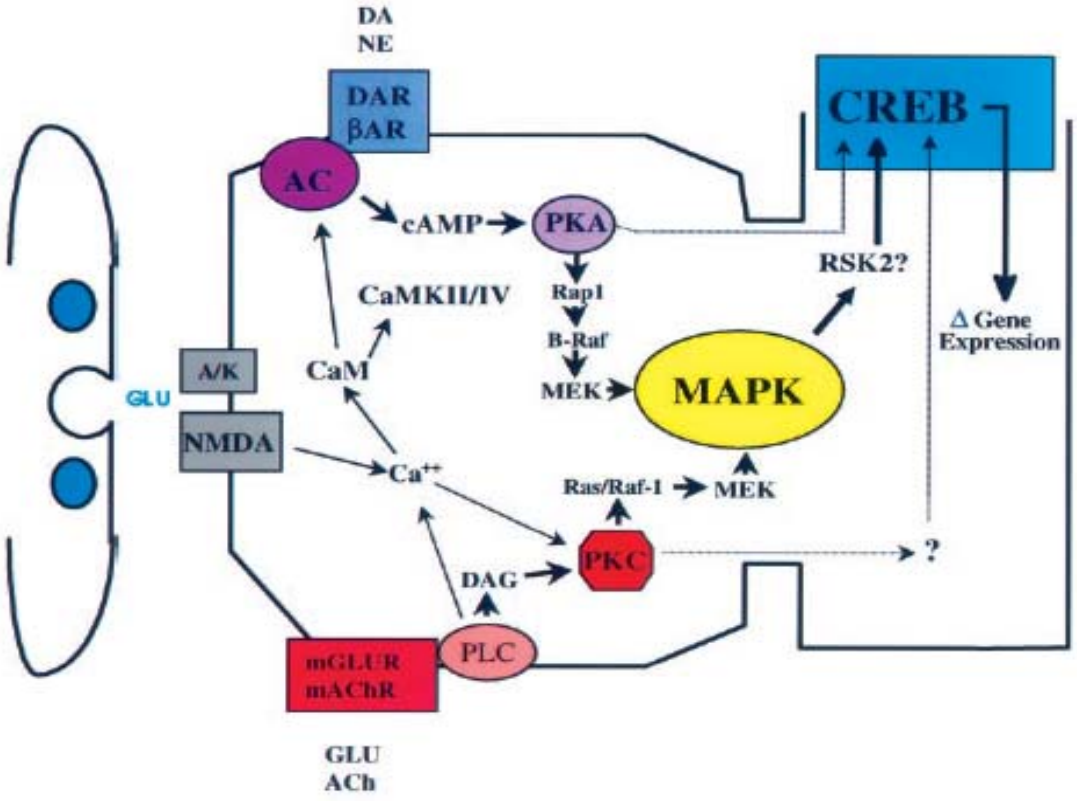


Figure 3. MEK/ERK/Elk intracellular signal transduction pathways. Stimulation of dopamine D1 receptor and NMDA receptor activates MAPK signaling and influences the cell gene transcription (Adopted from Roberson et al., 1999).

C. Interaction between dopamine D1 receptor- and NMDA receptor-signaling cascades

As noted in earlier sections, both dopamine D1 receptor and NMDA receptor mediate psychostimulant-induced intracellular signaling alternation. The MEK/ERK/Elk-1 cascade acts as an integrator to both dopamine and glutamate transmission. In addition, the activation of D1/cAMP/PKA pathway modulates NMDA receptor-mediated signals transduction. In this section, we review several

candidates may be responsible for the intracellular cascades interaction between dopamine D1 and NMDA receptors.

DARPP-32:

Dopamine- and cAMP-regulated phosphoprotein of Mr 32 kDa (DARPP-32) is a cytosolic protein that is enriched in medium spiny neurons in the CPu and NAc (Greengard, 2001; Greengard et al, 1999). Once DARPP-32 is phosphorylated at Thr-34 site (p-Thr 34 DARPP-32) by means of PKA, it becomes an inhibitor of protein phosphatase-1 (PP-1) and thereby potentiates dopaminergic transmission (Hemmings et al, 1984; Nishi et al, 1997, 2000; Svenningsson et al, 2004). On the other hand, cyclin-dependent kinase 5 phosphorylates DARPP-32 at Thr-75 site (p-Thr-75 DARPP-32) both *in vitro* and *in vivo*. P-Thr 75 DARPP-32 inhibits PKA in vitro by a competitive mechanism (Bibb et al., 1999). It is well established that the activation of dopamine D1 receptor leading to the accumulation of PKA activates p-Thr-34 and inhibits p-Thr-75 DARPP-32 via PKA-activated protein phosphatase 2A (PP-2A), respectively (Nishi et al., 2000). Dephosphorylation of p-Thr-75 DARPP32 leads to a disinhibitory effect on PKA and further amplifies the D1/cAMP/PKA/p-Thr-34 DARPP-32/PP-1 signaling. In contrast, when phosphatase groups are removed from p-Thr-34 DARPP-32 by protein phosphatase-2B (PP-2B), the DARPP-32 mediated-inhibitory effect on PP-1 is blocked and dampens the D1/cAMP/PKA pathway, subsequently (Gupta and Tsai, 2001; King et al., 1984). Stimulation of NMDA receptor has been reported to dephosphorylate the p-Thr-34 and p-Thr-75 DARPP-32 through Ca^{2+}-activated PP-2A and PP-2B, respectively

(Figure 4, Halpain et al., 1990; Nishi et al., 2000, 2002). Thus, DARPP-32 serves as an integrator for dopamine- and glutamate-mediated intracellular signaling.

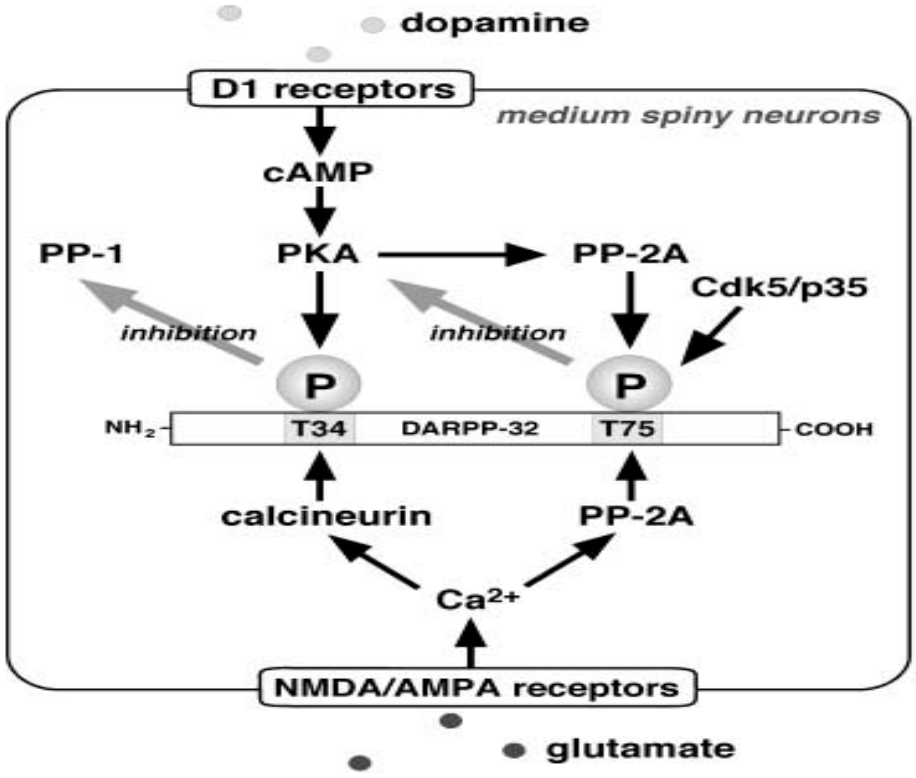


Figure 4. Scheme illustrating of the dopamine and glutamate signaling pathways in phosphorylation and dephosphorylation of DARPP-32 at Thr-34 and Thr-75 sites (Adopted from Nishi et al., 2002).

The NMDA-NR1 subunit C1 terminal contains the PKA phosphorylation site (Tingley et al., 1997). In striatal neuron, pharmacological stimulation of dopamine D1 receptor, through activation of PKA, phosphorylates NMDA-NR1 subunit (Dudman et al., 2003; Snyder et al., 1998) and enhances NMDA receptor-mediated current, subsequently (Cepeda et al., 1998; Flores-Hernandez et al., 2002; Westphal et al., 1999). P-Thr-34 DARPP-32 can be recruited to stabilize PKA-induced

NMDA receptor phosphorylation by inhibiting PP-1 cascade which may dephosphorylates the NMDA receptor and reduce NMDA receptor-mediated current (Blank et al., 1997; Flores-Hernandez et al., 2002; Greengard et al., 1999; Westphal et al., 1999). However, the increase of intracellular Ca^{2+} level by PKA-potentiated NMDA receptor current, in turn, activates PP-2B and dephosphrylated the p-Thr-34 DARPP-32 as a negative feedback loop (Greengard et al., 1999; Nishi et al., 2000).

Cocaine induced alternations of DARPP-32 phosphorylation have been studied *in vivo*. In the CPu, NAc and FCx, acute cocaine administration transiently increases p-Thr-34 and decrease p-Thr-75DARPP-32 with correspondent elevation of extracellular dopamine levels (Nishi et al, 2000; Rauggi et al., 2005). However, in cocaine-sensitized rats, a long-lasting and opposite DARPP-32 phosphrylation pattern (increase in p-Thr75 DARPP-32 and decrease in p-Thr 34 DARPP-32) has been reported (Scheggi et al., 2004a, 2004b). Recently, studies have been shown that mice with a targeted disruption of DARPP-32 gene (Fienberg et al, 1998) or with the site of Thr-34 DARPP-32 mutation (Valjent 2005; Zachariou et al, 2006) have attenuated locomotor activity and rewarding effect in response to cocaine treatment, and blunted ERK/Elk-1 protein phosphorylation after acute cocaine treatment. However, after chronic cocaine treatment paradigm, both Thr-34 and Thr-75 DARPP-32 mutant mice show augmented and reduced behavioral sensitization, respectively, indicating distinct role of p-Thr-34 and p-Thr-75 DARPP-32 in regulating short- and long-term behavioral actions of cocaine (Zachariou et al., 2006).

STEP:

Striatal-enriched protein tyrosine phosphatase (STEP) is preferentially expressed in the basal ganglia and related structures (Boulanger et al., 1995; Lombroso et al., 1993). Alternative splicing produces various STEP family members, and both cytosolic (STEP46) and membrane-associated (STEP61) variants exist (Bult et al., 1996). Through the protein-protein interaction via kinase-interacting motif (KIM), STEP and its non-neuronal homologs has been implicated in the dephosphorylation of ERKs protein and prevented its nuclear translocation (Nika et al., 2004; Zuniga et al., 1999). Both *in vitro* and *in vivo*, stimulation of D1/cAMP/PKA pathway has been reported to phosphorylate KIM and abolish STEP capability to dephosphorylate ERKs (Paul et al., 2000, Nika et al., 2004). However, the activation of PP-1 or PP-2B through NMDA receptor-induced Ca^{2+} influx dephosphorylates STEP and attenuates NMDA receptor-mediated ERKs protein phospohrylation (Paul et al., 2003; Nika et al., 2004).

Recently, in mice, amphetamine induces significant increase of STEP and ERKs phosphorylation in the striatum. In contrast, in DARPP-32 knockout mice, the phosphorylation of STEP and ERKs is attenuated after acute amphetamine treatment (Valjent et al., 2005). Taken together, dopamine D1 receptor-activated cAMP/PKA signaling influences the MEK/ERKs activations through STEP and its upstream DARPP-32/PP-1. Thus, in striatal neurons, STEP may serve as a coincidence detector to converge dopamine and glutamate signaling cascades induced by psychostimulants.

CREB:

Phosphorylation of Ser133 of the cAMP-regulated element-binding protein (CREB) increases a large number of genes that alter neuronal function and regulate synaptic plasticity (Frank and Greenberg, 1994; Mayr and Montiminy, 2001). Several kinase pathways can phosphorylate CREB, including PKA (Montminy and Bilezikjian 1987) and neuronal activity-induced CaMKs (Dash et al., 1991; Sheng et al., 1991; Tokumitsu et al., 2004). In addition, ERKs has been reported to phosphorylate CREB via pp90 ribosomal S6 kinase (p-RSKs) family (Frodin and Gammeltoft, 1999; Xing et al., 1998) and mitogen- and stress-activated protein kinase-1 (p-MSK-1, Authur and Cohen, 2000). In the primary striatal neuronal culture, dopamine application induces the phosphorylation of CREB with similar cAMP activity profile. Either dopamine D1 receptor antagonist or NMDA receptor antagonists can block the dopamine-induced CREB phosphorylation (Cole et al., 1995; Eaton et al., 2004; Konradi et al., 1996). In addition, in rats, pharmacological activation PKA induces CREB and Elk protein phosphorylation that can be blocked by NMDA receptor antagonist (Choe and McGinty, 2000). Similarly, in the striatal brain slice, the application of glutamate also induces the CREB and Elk-1 phosphorylation that can be attenuated by MEK and CaMKs inhibitor indicating the role of MEK/ERK/Elk-1 signaling cascade in the CREB activation (Vanhoutte et al., 1999).

Accumulating evidence has reported that cocaine activates CREB in various areas associated to drug addiction. For instance, in mice, acute cocaine treatment induces CREB phosphorylation in CPu (Karasinska et al., 2005). Cocaine-induced

conditioned place preference is associated with the increase of CREB phosphorylation in the NAc, and both behavioral and CREB alternations can by blocked by MEK inhibitor (Miller and Marshall, 2005). After chronic cocaine administration, the phosphorylation of CREB is increased in the NAc and CPu (Kano et al., 1995; Mattson et al., 2005; Terwilliger et al., 1991) and can be blocked by MEK inhibitor (Mattson et al., 2005). In addition, dopamine D1 receptor mutant mice exhibit attenuated CREB phosphorylation in CPu after acute and chronic cocaine administration (Karansinska et al., 2005). Viral-mediated overexpression of CREB in the NAc decreases the reinforcing properties of cocaine, whereas, overexpression a mutant form of CREB increases the reinforcing properties of cocaine (Carlezon et al., 1998). CREB knockout mice exhibit stronger cocaine-induced behavioral sensitization and conditioned place preference when compared to wild-type controls (Walters and Blendy, 2001). Using inducible knockout technology, mice with mutant CREB show blunted locomotor sensitization induced by repeated cocaine treatment (Sakai et al., 2002). Overall, in cAMP/PKA and MEK/ERK/Elk-1 pathways dependent manner, both acute and chronic cocaine administration increase the CREB phosphorylation in various brain areas in the mesolimbic system. The suppression and overexpression of CREB increase and decrease the rewarding effect of cocaine, respectively.

c-fos:

Neurotransmission in the brain activates the transcription of immediate-early genes, including *c-fos.* The *c-fos* promoter contains cAMP response elements (CRE) and serum response elements (SRE). Through the formation of homo- or

heterodimers with other CREB/ATF proteins at the leucine zipper domains, phosphorylated CREB binds to CRE of target gene regulates gene expression and leads to activation of transcription complexes and increasing gene expression (Hai and Curran, 1991; Montminy et al., 1990). On the other hand, the SRE is the second major cis-element responsible for the activation of *c-fos* and is constitutively occupied by a serum response factor (SRF) dimer. SRF dimer recruits the ternary complex factor that may include Elk-1 protein and promote *c-fos* transcription (Adams and Sweatt, 2002; Davis et al., 2000; Hill et al., 1993). In the strtatal neuronal culture, dopamine or dopamine D1 receptor agonists induce *c-fos* mRNA increase and can be diminished by the NMDA receptor antagonists (Konradi et al., 1996). In vivo, acute dopamine D1 receptor agonist treatment induces robust *c-fos* expression in rat CPu (Capper-Loup at al., 2002; Svenningsson et al., 2000). In addition, glutamate application may induce *c-fos* expression in striatal brain slice through activation of Elk-1 and CREB (Vanhoutte et al., 1999). Acute glutamate receptors agonist treatment also induces rapid Fos-like protein expression in rat CPu in dopamine D1 and NMDA receptors-dependent manners (Berretta et al., 1992).

Dopamine and glutamate transmissions have been implicated for cocaine-induced *c-fos* transcription. Acute cocaine induces *c-fos* mRNA expression and Fos-like protein immunoreactivity in rat CPu and NAc that can be blocked by the pretreatment of dopamine D1 receptor antagonists, NMDA receptor antagonists or MEK inhibitors. (Berretta et al., 1992; Grabus et al., 2004; Graybial et al., 1990; Johansson et al., 1994; Radwanska et al., 2005; Steiner and Gerfen, 1993; Torres and Rivier, 1992; Young et al., 1991). In addition, acute cocaine injection fails to

induce *c-fos* expression in dopamine D1 receptor deficient mice (Drago et al., 1996). Similarly, chronic cocaine treatment increases *c-fos* expression and Fos protein immunoreactivity in PFc, CPu, NAc, amygdala and the subthalamic nucleus correlated to behavioral sensitization (Brown et al., 1992; Crombag et al., 2002; Samaha et al., 2004; Steiner and Gerfen, 1993; Todtenkopf at al., 2002; Uslaner et al., 2003). Furthermore, recent study demonstrated that *c-fos* mutant mice show attenuated behavioral sensitization after chronic cocaine and more persistent memory of cocaine-induced conditioned place preference, suggesting that c-Fos is critical to long-term behavioral plasticity and rewarding effects of chronic cocaine (Zhang et al., 2006).

III. Directly physical interaction between dopamine D1 and NMDA receptors

In contrast with classical role of G-protein coupled receptors family functioning as monomers, accumulating evidence indicated that these receptors may form as oligodimers with other members to increase the signaling, trafficking and pharmacological complexities of interacting receptors (Anger et al., 2002; Devi, 2000). For example, the dopamine D2 and D3 receptors can form a heterodimeric complex with particular pharmacological and signaling properties (Scarselli et al., 2000) and dopamine D1 receptors bind to adenosine A1 receptors forming heterodimeric complexes with decreasing dopamine D1 receptor agonist-induced accumulation of cAMP (Gines et al., 2000). In addition, dopamine receptors also interact with ligand-gated ion receptors such as direct protein-protein coupling

between the dopamine D5 receptor and γ-aminobutyric acid A ($GABA_A$) receptor enables mutually inhibitory functional interactions (Liu et al., 2000).

Recently, studies have shown that dopamine D1 receptors bind to specific subunits of NMDA receptors in various cell lines (Lee et al, 2002; Fiorentini et al., 2003; Fiorentini and Missale, 2004; Lee and Liu, 2004; Pei et al., 2004). Dopamine D1 receptor C-terminal (CT) region regions, D1-t2 (Leu^{387}-Leu^{416}) and D1-t3 (Ser^{417}-Thr^{466}), mediate direct protein interactions with the C1 terminal of NR1-1a and NR2A subunits of NMDA receptor, respectively, in hippocampal neurons and COS-7 cells (Lee et al., 2002; Pei et al., 2004). Furthermore, *in vitro* studies also indicated that both NR1-1a/b and NR1-3a/3b isoforms may interact with dopamine D1 receptor in striatal neurons (Fiorentini et al., 2003).

The functional significance of dopamine D1 and NMDA receptors physical interactions:

In primary hippocampal neuron culture and human embryonic kidney 293 (HEK-293) cells co-expressing dopamine D1 receptors and NR1-1a/NR2A subunits, dopamine D1 receptor agonist application significantly reduced the physical association between dopamine D1 receptors and NR1 subunit of NMDA receptors but not NR2A subunits (Lee et al., 2002). In addition, the dopamine D1 agonist-induced receptors disassociation is related to the reduction of inward current and apoptosis mediated by NMDA receptors through the activation the PI-3 kinase (phosphoinositide 3-kinase) pathway (Figure 5), and is independent of traditional PKA or PKC intracellular signaling (Fischer et al., 1998; Hisatsune et al., 1997; Joyal et al., 1997; Lee and Liu, 2004; Lee et al., 2002; Wyszynski et al., 1997).

However, in the striatal post-synaptic density, dopamine D1 receptors form direct protein- protein interactions with the CT of NMDA receptors NR1 subunits but not with either NR2A or NR2B subunits (Fiorentini et al., 2003). Bioluminescence resonance energy transfer experiment indicated that D1/NR1 interaction is not influenced by administration of dopamine or glutamate in COS-7 co-expressing dopamine D1 receptor and NR1/NR2B subunits of NMDA receptors. Furthermore, the expression of NR2B subunits promotes the translocation of the NR1/D1 complexes from cytoplasmic compartments to plasma membrane and prevents SKF-81297 induced dopamine D1 receptors internalization in HEK-293 cells (Fiorentini et al., 2003; Fiorentini and Missale, 2004). This different D1/NMDA receptors interaction patterns in hippocampal and striatal neurons as well as the insensitivity of agonists-induced D1/NR1 disassociation and receptors translocation in the cells transfecting with NR2B subunits may reflect that differential functional NMDA receptor properties is dependent on NR2 subunit subtype composition and the NR2B is the prevalent subunits in striatal neurons (Brimecombe et al., 1997; Krupp at al., 1996; Sprengel et al., 1998; Standaert et al., 1994).

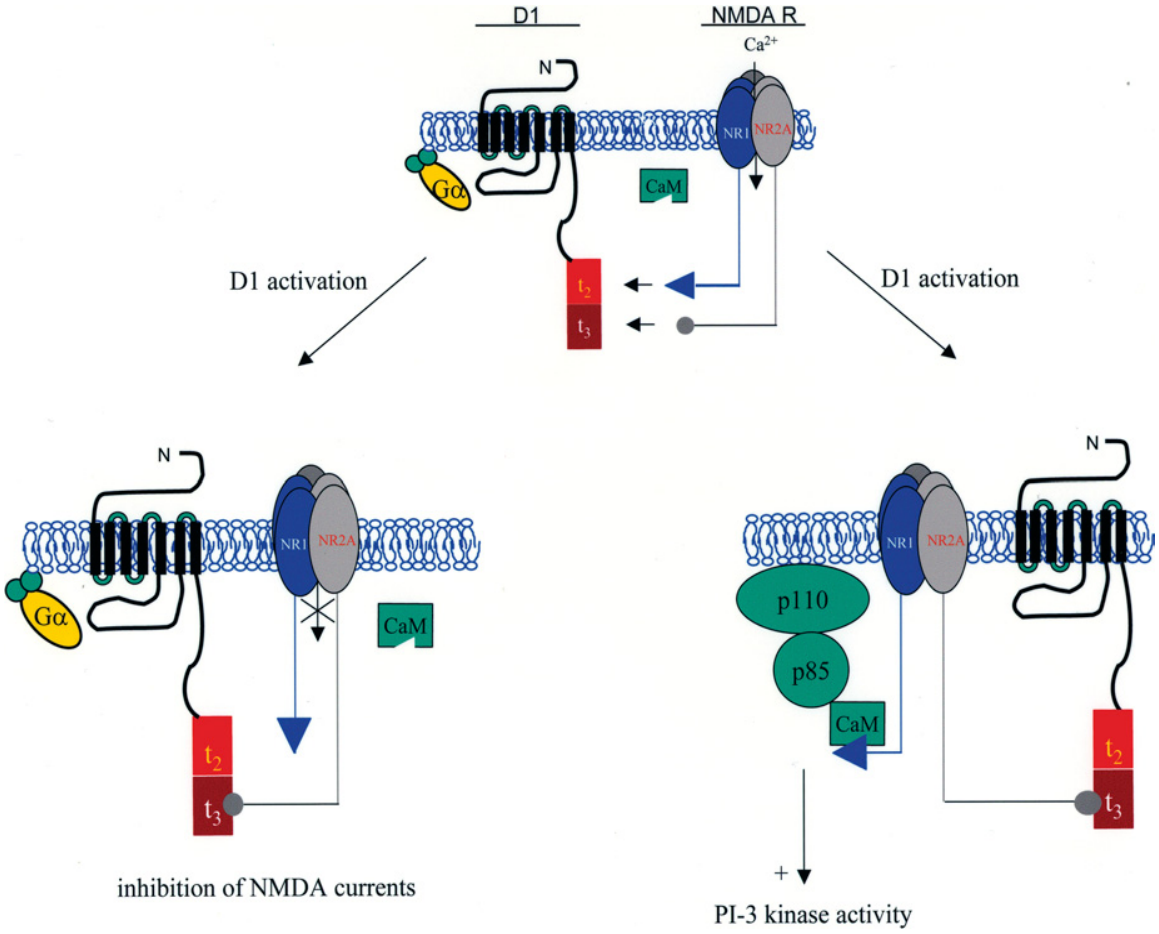


Figure 5. A model of the direct interactions between dopamine D1 and NMDA receptors. Through intracellular C-terminals, dopamine D1 receptors interact with NR1 and NR2A subunits of NMDA receptors. (Adopted from Lee and Liu, 2004)

On the other hand, in rat hippocampal tissue and cells co-transfecting dopamine D1 and NMDA receptors, pretreatment with NMDA significantly increased the dopamine D1 receptor-mediated cAMP accumulation by recruiting more dopamine D1 receptors to the plasma membrane via exocytotic process. This effect can be abolished through the application of dopamine D1 or NMDA receptors antagonists

(Pei et al., 2004). Furthermore, the potentiating effect of NMDA receptor on dopamine D1 receptor-activated cAMP accumulation and dopamine D1 receptor membrane insertion are mediated by D1-t2/NR1-1a interaction because of the inhibitory effect of the overexpression D1-t2 or NR1-C1 (Asp^{864}-Thr^{900}) of the NR1-1a CT mini-gene (Lee and Liu, 2004; Pei et al., 2004).

IV. Significance of work

Cocaine exposure leads to transient and long-term molecular molecular and neurochemical alternations that underlie addiction and associative behaviors. In the striatum including the CPu and NAc, effects of cocaine are mediated by dopamine and glutamate transmissions through cAMP/PKA and MEK/ERK/Elk-1 signaling cascades. Furthermore, activations of DARPP-32, CREB, STEP and *c-fos* serve as intermediate steps to converge cocaine-activated intracellular mechanisms as well as to modulate addictive behaviors. The objectives of present study can be divided into three parts. First, we will evaluate the intracellular mechanisms mediated by dopamine D1 and NMDA receptors after acute cocaine administration in the CPu, a region associated with cocaine-induced behavioral changes. We hypothesize that dopamine D1 and NMDA receptors-mediated intracellular signaling will be activated after acute cocaine administration. Second, through pharmacological manipulation of NMDA and dopamine D1 receptors, we will evaluate their effects on immediate early gene expression after acute cocaine. We postulate that both dopamine D1 and NMDA receptors are necessary for the induction and phosporylation of immediate early gene. Lastly, we will determine whether acute

cocaine administration or NMDA receptors activation/inhibition alters the physical interaction between dopamine D1 and NMDA receptors. We speculate that the physical receptor interaction will be decreased after manipulations.

Accomplishment of this study will provide novel information about the physical interaction between dopamine D1 receptors and NR1 subunit of NMDA receptors as well as the key molecules that are shared between these two pathways and regulate cocaine-induced gene expression. Thus, this approach will advance general knowledge regarding the development of novel pharmacotherapies for treatment of cocaine addiction.

Aims:

Specific Aim I: To determine the effect of acute cocaine administration on dopamine D1 and NMDA receptors-mediated intracellular mechanisms in rats CPu.

1A: We hypothesize that acute cocaine administration will induce the activation of MEK/ERK/ Elk-1 pathway signaling in rats.

1B: We hypothesize that acute cocaine administration will result in the activation of relevant regulators (e.g., DARPP-32 pathway and STEP) for the interaction between NMDA receptors- and dopamine D1 receptors-mediated intracellular signaling.

Specific Aim II: To determine the effect of pharmacological blockade of NMDA or dopamine D1 receptors on acute cocaine-induced immediate early genes in the CPu and NAc, brain regions are associated with cocaine-induced behavioral changes and rewarding effects.

2A: We postulate that acute cocaine administration will induce ERK and immediate early genes expression/phosphorylation in a time-dependent manner.

2B: We postulate that pharmacological inhibition of NMDA or dopamine D1 receptors will reduce acute cocaine-induced immediate early genes expression and phosphorylation.

Specific Aim Ⅲ: To determine the effects of dopamine and glutamate transmission on NMDA and dopamine D1 receptors physical interactions in rats CPu.

3A: We speculate that acute cocaine administration will lead to changes on NMDA and dopamine D1 receptors physical interactions in time course experimental design.

3B: We speculate that pharmacological activation and inhibition NMDA receptors will alter NMDA and dopamine D1 receptors physical protein-protein interaction.

Chapter 2: Effects of acute cocaine on ERK and DARPP-32 phosphorylation pathways in the dorsal striatum/caudate-putamen (CPu) of Fischer rats.

I. Introduction

Cocaine is primarily a drug of abuse in Western countries. The dopaminergic inputs from the ventral tegmental area to the nucleus accumbens and the nigrostriatal projections to the caudate-putamen (CPu) have been attributed to behavioral and biochemical effects of cocaine (reviewed in Hyman and Malenka 2001; Koob and Nestler 1997; Spanagel and Weiss 1999). For example, by binding to the dopamine transporter, cocaine leads to a buildup of extracellular dopamine levels in the mesocorticolimbic system (Maisonneuve and Kreek 1994; Reith et al., 1997). In addition, acute cocaine administration has been shown to alter synaptic glutamate concentration in the CPu (Kalivas and Duffy 1995; Smith et al., 1995; Zhang et al., 2001).

Accumulating studies have been demonstrated that extracellular signal-regulated kinase (ERK), a member of mitogen-activated protein kinase family, is critical in mediating cocaine-induced intracellular mechanisms. For instance, in rodents, both repeated and acute cocaine administration increase ERK phosphorylation (p-ERK) in the mesocorticolimbic system and CPu (Berhow and Nestler 1996; Jenab et al., 2005; Valjent et al., 2000, 2004, 2005, Zhang et al., 2004). ERK1 knockout mice show enhanced behavioral and reward responses to morphine and cocaine (Ferguson et al., 2006; Mazzucchelli et al., 2002). Pharmacological inhibition of mitogen-activated protein kinase/ERK kinase (MEK), the upstream activator of ERK, blunts

cocaine-induced p-ERK, ternary complex factor Elk-1 phosphorylation (p-Elk-1), and immediate early gene expression (such as c-Fos), suggesting that MEK activation is necessary for ERK activation and underlying transcriptional mechanisms (reviewed in Lu et al., 2006; Mattson et al., 2005; Miller and Marshall 2005; Valjent et al., 2000; Zhang et al., 2004). In addition, through pp90 ribosomal S6 kinase (p-RSK) family, p-ERK has been reported to result in cAMP response element-binding protein phosphorylation (p-CREB), a transcriptional factor that has been shown to regulate gene expression (Frodin and Gammeltoft 1999; Xing et al., 1998). Recently, studies have shown that prenatal cocaine treatment caused augmented p-RSKs and p-CREB protein levels in the heart tissue of neonatal rats (Sun and Quamina 2004).

Alternatively, dopamine- and cAMP-regulated phosphoprotein of Mr 32 kDa (DARPP-32), a cytosolic protein enriched in medium spiny neurons of CPu, is another important regulator in mediating cocaine-induced intracellular signaling (Greengard et al., 1999; Svenningsson et al., 2005). Once DARPP-32 is phosphorylated at Thr-34 site (p-Thr 34 DARPP-32) by means of protein kinase A (PKA), it becomes an inhibitor of protein phosphatase-1 (PP-1) thereby modulating several substrates (Hemmings et al., 1984; Nishi et al., 1997, 2000; Svenningsson et al., 2004). On the other hand, phosphoylation of DARPP-32 at the Thr75 site (p-Thr75 DARPP-32) inhibits PKA and attenuates its capability to increase p-Thr34 DARPP-32 (Bibb et al., 1999; Nairn et al., 2004). *In vitro*, dopamine D1 receptor activation has been shown to activate p-Thr34 and inhibit p-Thr75 DARPP-32 via PKA-activated protein phosphatase 2A (PP-2A; Nishi et al., 2000). In the

mesocorticolimbic system and CPu, acute cocaine administration transiently increases p-Thr34 and decreases p-Thr75 DARPP-32 with correspondent elevation of extracellular dopamine levels (Nishi et al., 2000; Rauggi et al., 2005). Furthermore, studies have shown that DARPP-32 mutant mice display attenuated behavioral effects and blunted p-ERK in response to cocaine treatment, suggesting that DARPP-32 is an upstream regulator for ERK-mediated signaling (Fienberg et al., 1998; Valjent et al., 2005; Zachariou et al., 2006).

One possible candidate to link the DARPP-32- and ERK-mediated cascades is striatal-enriched protein tyrosine phosphatase (STEP), preferentially expressed in mesocorticolimbic system and CPu (Boulanger et al., 1995; Lombroso et al., 1993). Through direct interaction of a kinase-interacting motif, STEP and its non-neuronal homologues have been implicated in the dephosphorylation of p-ERK and prevention of its nuclear translocation (Nika et al., 2004; Zuniga et al., 1999). Stimulation of PKA has been reported to phosphorylate STEP (p-STEP) and decrease its capability to dephosphorylate p-ERK (Nika et al., 2004; Paul et al., 2000). Recently, Valjent et al. (2005) demonstrated that, in the CPu, acute amphetamine administration increases p-STEP and p-ERK in mice, whereas, similar activation is abolished in DARPP-32 mutant mice. Thus, psychostimulant-activated DARPP-32 signaling may control p-ERK signaling through activation of STEP.

On this basis, the aim of our study was to systematically investigate ERK- and DARPP-32-mediated signaling in the CPu, an area known to regulate cocaine treatment response, after acute cocaine administration.

II. Methods

Animals:

60-day-old male Fischer rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chambers (20 × 20 × 41 cm). Rats were maintained on a 12-hour light/dark cycle (lights on at 9:00 a.m.) with free access to food and water. Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

Drug and antibodies:

Cocaine hydrochloride was purchased from Sigma chemical Co. (St. Louis, MO). Primary antibodies of p-Thr34 and p-Thr75 DARPP-32 were purchased from Phosphosolutions (Aurora, CO). Antibodies for p-MEK, MEK, p-ERK, ERK, DARPP-32, PP-2A B (PP-2A regulatory B subunit), PP-2A C (PP-2A catalytic subunit), p-RSK, RSK, p-Elk-1, Elk-1 and c-Fos were bought from Cell Signaling Technologies (Beverly, MA). Antibodies against STEP and PP-2A B' (PP-2A regulatory B' subunit) were from Upstate Group Inc. (Waltham, MA). α-tubulin antibody was purchased from Santa Cruz Technologies (Santa Cruz, CA). Both horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG were purchased from Amersham Pharmacia (Piscataway, NJ).

Drug administration:

Cocaine solutions were prepared by dissolution in physiological saline (0.9%) and injected intra-peritoneally (i.p.). Rats received an injection of saline (1 ml/kg) or cocaine (30 mg/kg) and sacrificed 5, 10, 15, 30, 45, or 60 min later. P-Thr34 DARPP-32 protein levels were evaluated by the time course design. c-Fos protein expression was determined 45 min after cocaine administration. The 10 min samples were used for detecting all the other proteins.

Protein preparation and measurement:

After decapitation (following a brief 20 s exposure to CO_2), rat brains were removed, flash frozen in 2-methylbutane (-40° C), and stored at -80° C until used. The coronal slices (1mm thick) were cut out in a matrix (ASI instruments, Warren, MI) and CPu was dissected out on a cold glass plate. CPu was homogenized by using a Polytron handheld homogenizer (Kinematica, Luzern, Switzerland) in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 10% Glycerol, 1% Triton X-100, 1% Igepal CA-630, 1% sodium dexycholic acid) containing phosphatase inhibitors mixture. After 30 min incubation, homogenates were centrifuged at 13,000 rpm for 15 min at 4° C. Supernatants were then collected and stored at -80° C until used. Total protein content was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

Western blot analysis:

Protein samples were analyzed by using Western blot as previously described (Jenab et al., 2005). Briefly, 40 μg of protein extracts were boiled in Lammeli buffer

containing 1% β-mercaptoethanol for 5 min and ran on SDS-PAGE gels, transferred to PVDF membranes. Membranes were then blocked with 5% nonfat dry milk for 1hr at room temperature and incubated with antibodies of MEK (1:1000), p-MEK (1:500), ERK (1:1000), p-ERK (1:1000), RSK (1:1000), p-RSK (1:1000), Elk-1 (1:1000), p-Elk-1 (1:500), c-Fos (1:1000), DARPP-32 (1:1000), p-Thr34 DARPP-32 (1:1000), p-Thr75 DARPP-32 (1:1000), PP-2A B (1:2000), PP-2A B' (1:1000), PP-2A C (1:5000), or STEP (1:2000) overnight at 4° C. After three washes with Tris-Tween-20 Buffer (TBST; pH = 7.4), membranes were incubated with their appropriate secondary antibodies (1:1000) for 1hr at room temperature followed by three more washes with TBST. Antibody binding was detected by using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia, Piscataway, NJ). Intensity of protein bands was quantified with a computer densitometer and Image Quant Program (Molecular Dynamics). For normalization of protein levels, all membranes were re-probed with α-tubulin antibody (1:1000).

Statistical analysis:

Protein levels were expressed as a ratio to α-tubulin levels. For p-STEP, the protein level was normalized by its non-phophospholated isoforms. Data was expressed as mean±SEM relative to saline controls, which were arbitrarily set at 100%. Student's t-tests were used to determine differences between cocaine- and saline-treated animals. For time course experiments, p-Thr 34 DARPP-32 and DARPP-32 protein levels were analyzed by two-way ANOVA; treatment (saline or

cocaine) × time (5, 15, 30 or 60 min) followed by with LSD post hoc analysis when appropriate. Differences were considered statistically significant at 0.05 level.

III. Results

Effects of acute cocaine on ERK pathway:

Comparison of control animals treated with saline to animals treated with acute cocaine demonstrated that the administration of 30 mg/kg cocaine significantly increased p-MEK and p-ERK protein levels [$t(7) = 3.50$, $p< 0.01$; $t(7) = 7.57$, $p<0.0001$; Figure 6A and B, respectively]. In addition, cocaine also increased p-RSK, p-Elk-1, and c-Fos protein expression in the CPu [$t(7) = 3.60$, $p< 0.01$; $t(7) = 2.99$, $p< 0.01$; $t(6) = 2.66$, $p< 0.05$; Figure 6C-E, respectively]. As shown in Table 1, the acute cocaine administration did not alter total protein levels for all phospho-proteins that we were measured.

Effects of acute cocaine on DARPP-32 pathway:

For the DARPP-32-mediated cascades, animals with cocaine administration had lower p-Thr75 DARPP-32 protein level compared to the saline control [$t(7) = -2.37$, $p< 0.05$; Figure 7A]. However, the p-Thr34 DARPP-32 protein level was not significantly changed after cocaine (Figure 7B). No significant changes were observed for PP-2A protein levels including its regulatory subunits, B and B' subunits, and catalytic subunit, C-subunit (Figure 7C-E, respectively). Furthermore, time course experiments demonstrated that acute cocaine injection did not alter p-

Thr34 DARPP-32 or total DARPP-32 protein expression in the CPu compared to respective saline controls (Figure 8A and B, respectively).

Effect of acute cocaine on p-STEP:

Figure 9 demonstrates that the phosphorylation of STEP as shown by the decrease of mobility of the 46-kDa STEP, the cytosolic isoform of STEP presented in the CPu (Lombroso et al., 1993; Valjent et al., 2005), was significantly increased after acute cocaine administration [$t(6) = 3.27$, $p < 0.05$].

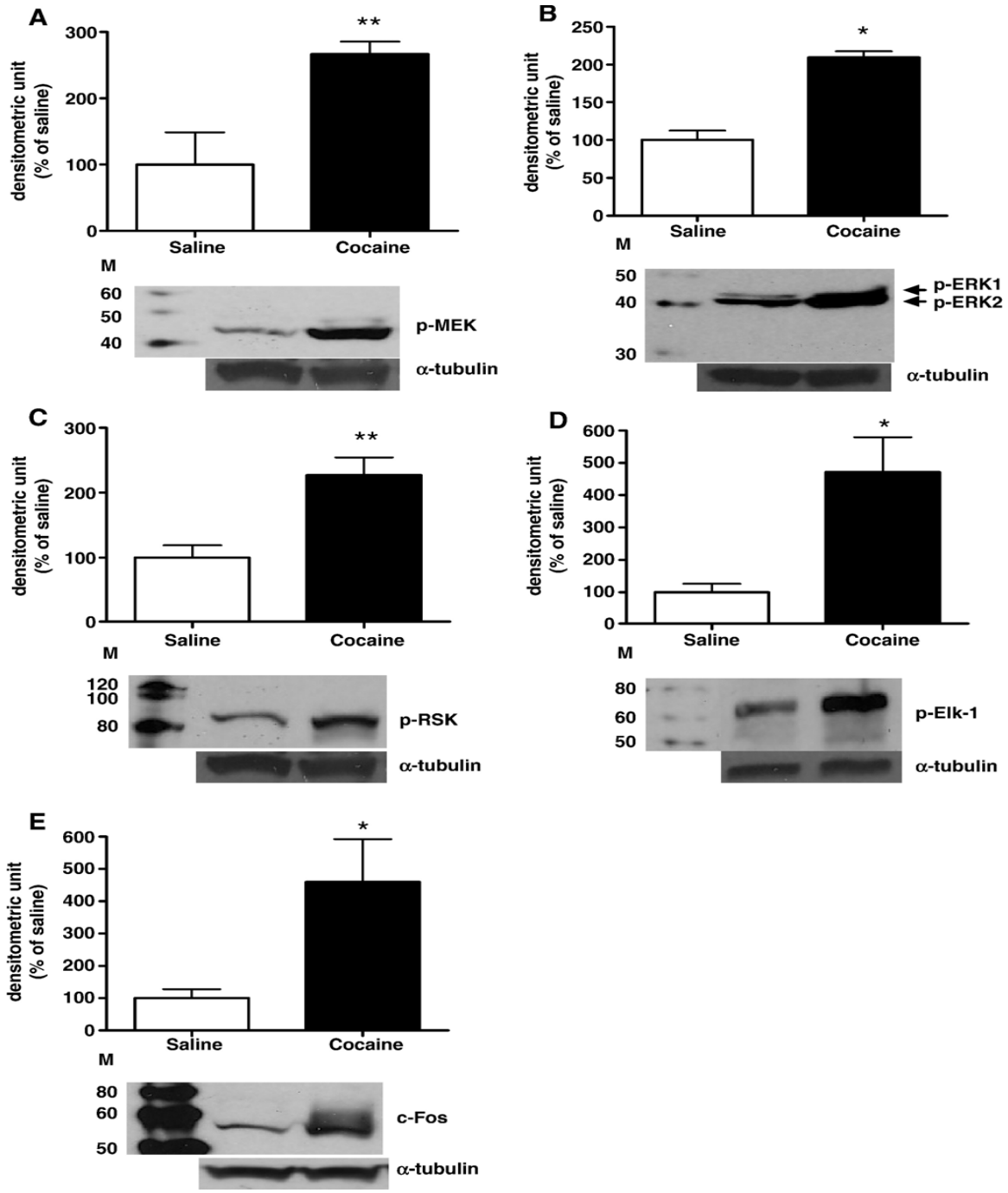


Figure 6. Effects of cocaine on ERK pathway. Results represent as protein levels over α-tubulin in the CPu expressed as percentage of saline control (4-5 animals per group) after rats were given injections: (A) p-MEK; (B) p-ERK (p-ERK1/2); (C) p-RSK; (D) p-Elk-1. 45 min after rats received injections: (E) c-Fos. M is the molecular marker in kDa. *p <0.05, and **p <0.01 as compared with saline group.

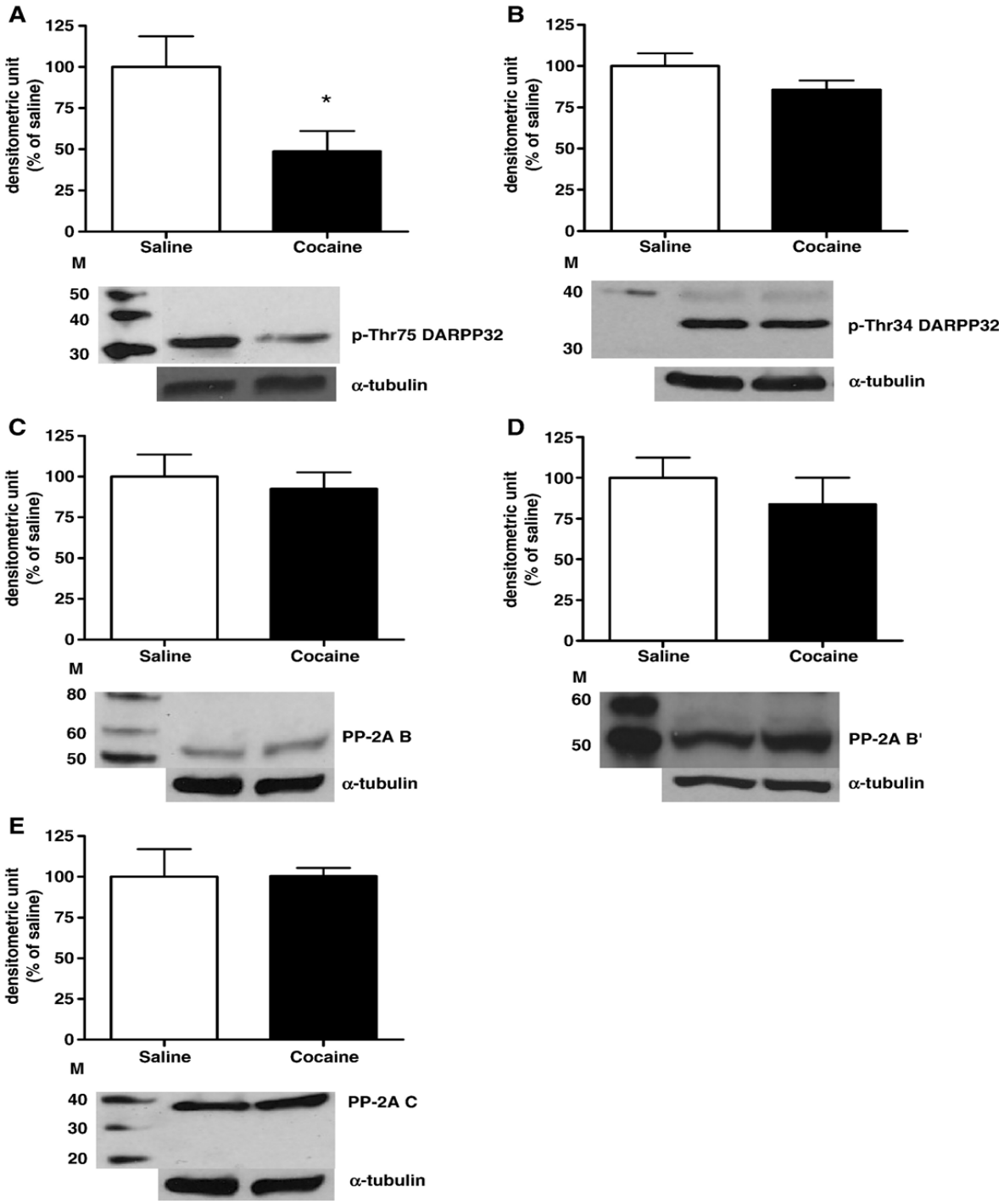


Figure 7. Effects of cocaine on DARPP-32 pathway. Results represent as protein levels over α-tubulin in the CPu expressed as percentage of saline control (4-5 animals per group) after rats were given injections: (A) p-Thr75 DARPP-32; (B) p-Thr34 DARPP-32; (C) PP-2A B; (D) PP-2A B'; (E) PP-2A C. M is the molecular marker in kDa. *p <0.05 as compared with saline group.

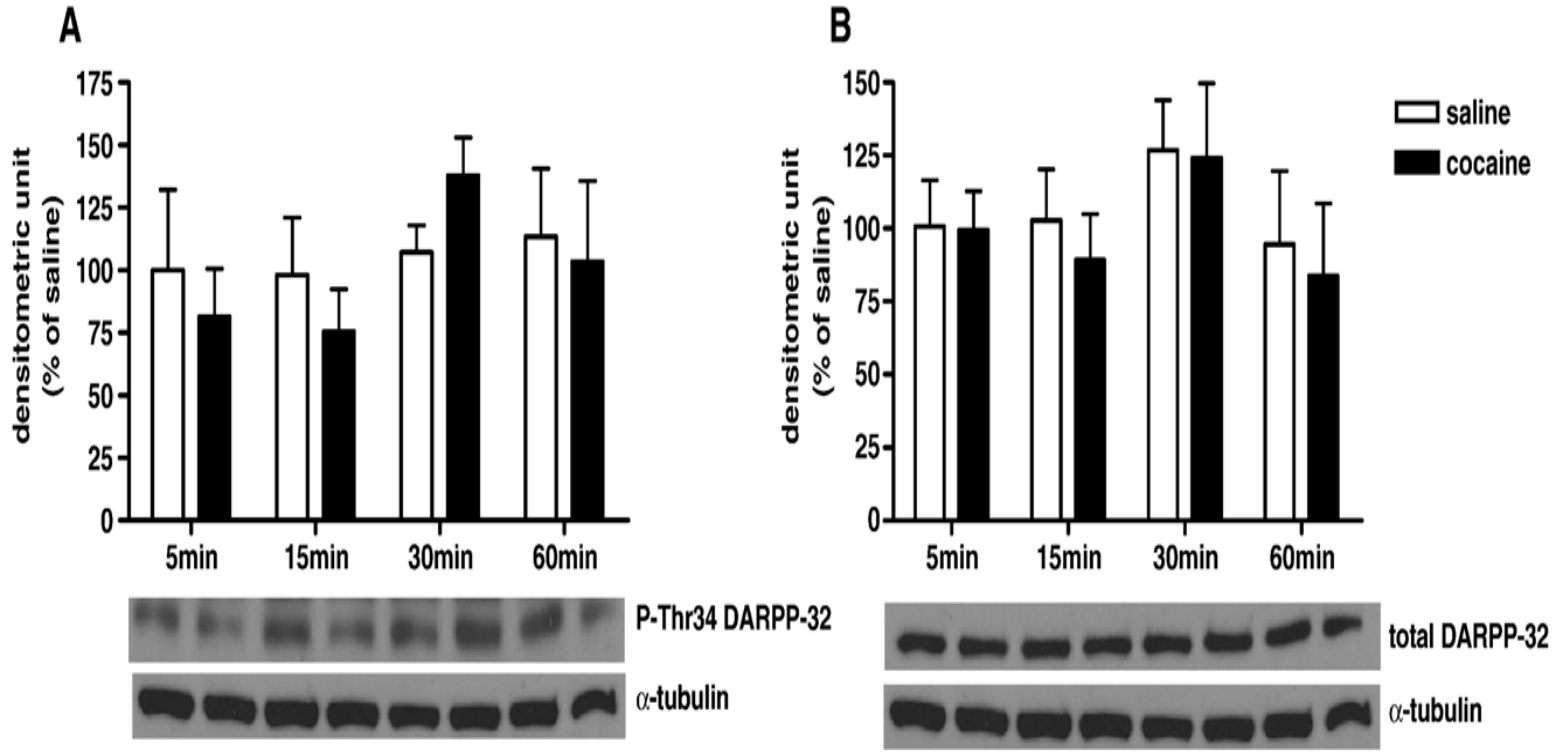


Figure 8. The time course of cocaine effects on p-Thr34 DARPP-32. Results represent as protein levels over α-tubulin in the CPu expressed as percentage of saline control (4-6 animals per group). 5, 15, 30, or 60 min after rats were given injections: (A) p-Thr34

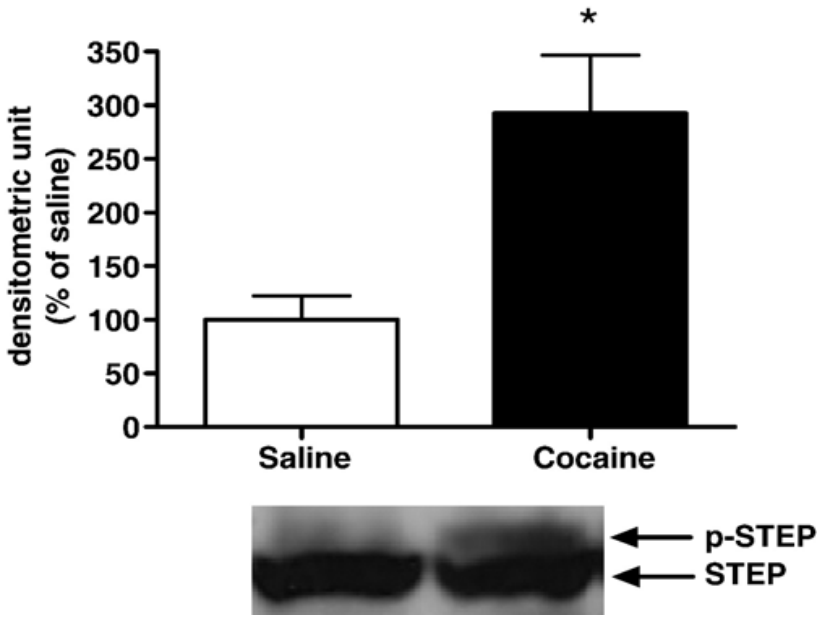
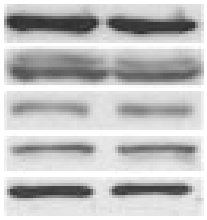


Figure 9. Effects of cocaine on STEP. Result represents as p-STEP protein levels over STEP in the CPu expressed as percentage of saline control (4 animals per group) 10 min after injections. *p <0.05 as compared with saline group.

Table I. Effects of acute cocaine on total proteins in the rat CPu

Proteins/ Treatment	Saline	Cocaine	Saline Cocaine
MEK	100.00±6.10	95.18±7.40	
ERK	100.00±7.91	91.47±6.14	
RSK	100.00±12.39	102.69±8.26	
Elk-1	100.00±6.74	124.38±16.48	
DARPP-32	100.00±10.45	107.66±6.49	

Left: The ratio of total proteins over α-tubulin is expressed relative to saline controls which are set at 100%. Data represent mean ± SEM. **Right**: Representative Western blots for respective proteins.

IV. Discussion

Consistent with previous studies demonstrating that acute cocaine induced rapid and transient ERK activation in mice (Valjent et al., 2000, 2004), we further showed that p-MEK, p-ERK and p-Elk-1 protein levels were increased in the CPu of rats. After phosphorylation by p-MEK, p-ERK is able to translocate to the nuclear compartment, where it phosphorylates the ternary complex factor Elk-1 (Gille et al., 1992, 1995b). Elk-1 and other ternary complex factors may associate with serum response factor (SRF), dimmerize with *c-fos* serum response element, and promote its transcription (Davis et al., 2000; Hill et al., 1993; Treisman, 1996). Recently, we also demonstrated that the cocaine-induced ERK-mediated signaling is dependent on both dopamine D1 and glutamate NMDA receptor activation (Jenab et al., 2005). Thus, in the CPu, both dopamine and glutamate transmission may converge on the elevation of MEK/ERK/Elk-1 activation, resulting in c-Fos expression after acute cocaine administration.

Consistent with previous studies showing that prenatal cocaine exposure resulted in elevated p-RSK in neonatal heart tissue (Sun and Quamina, 2004), p-RSK protein levels were also increased in the CPu after acute cocaine administration in the current study. Both *in vitro* and *in vivo* evidence have indicated that ERK activation is required for the phosphorylation of RSK (Alessi et al., 1995; Lazar et al., 1995; Sturgill et al., 1988). RSK has been shown to phosphorylate CREB (Pende et al., 1997; Xing et al., 1996) and up-regulate *c-fos* expression in an Elk-independent manner (Chen et al., 1993, 1996; De Cesare et al., 1998). Moreover, RSK and the CREB binding protein (CBP) have physical interactions in quiescent cells. After ERK activation, the RSK-CBP complex is

dissociated allowing p-RSK to phosphorylate CREB, recruit CBP to p-CREB, and subsequently modulate underlying transcriptional mechanisms (Merienne et al., 2001). Together, these results indicate that, instead of the ERK/Elk-1 signaling, ERK/RSK/CREB pathway may represent a distinct and/or redundant cascade to induce the c-Fos expression after acute cocaine administration.

Studies in PC12 and hippocampal neuronal cells have demonstrated that PKA-mediated signaling regulates ERK pathway activation (Impey et al., 1998; Roberson et al., 1999; Vossler et al., 1997). Recently, our laboratory and others have demonstrated that cocaine-induced p-ERK is dependent on dopamine D1 receptor stimulation, which accumulates PKA through the activation of adenylyl cyclase (Jenab et al., 2005; Valjent et al., 2000; Zhang et al., 2004; Zhang and Xu, 2006). To evaluate the influence of D1/PKA on ERK signaling, we systemically analyzed the DARPP-32 pathway in response to acute cocaine injections. Previous studies have shown that acute cocaine administration increases p-Thr34 DARPP-32 in the mice neostriatum or in the rat prefrontal cortex and nucleus accumbens (Nishi et al., 2000; Rauggi et al., 2005). However, we did not detect any changes in the CPu of Fischer rats. A recent study by D'Addario et al. (2007) demonstrated that acute cocaine (10 mg/kg) induced p-Thr34 DARPP-32 in Sprague-Dawley caudate extracts. However, in their study, rats received 5 days of vehicle injections before cocaine administration. In addition, they also used a different strain of rats, the Sprague-Dawley, which have been shown to differ in their response to cocaine self-administration than our Fischer rats (Kosten et al., 2007). Strains and/or cocaine injection schedule differences may contribute to the differential p-Thr34 DARPP-32 phosphorylation in the dorsal striatum of rats. On the other hand,

the p-Thr75 DARPP-32 was decreased in response to acute cocaine administration. The PKA-activated PP-2A is the major protein phosphatase to downregulate p-Thr75 DARPP-32 in the striatum (Ahn et al., 2007; Nishi et al., 2000). Interestingly, in the current study, the PP-2A protein levels were not changed in the CPu, suggesting that during the early stage after acute cocaine (e.g., 10 min) the reduction of p-Thr75 DARPP-32 is not mediated by PKA activation. However, an inhibitory effect of PP-2A on p-Thr75 DARPP-32 at later time point after acute cocaine cannot be excluded. In addition, other protein phosphatases including PP-1 and PP-2C have been implicated in the dephosphorylation of p-Thr75 DARPP-32 *in virto* and neostriatal slice (Nishi et al., 2000). The activation of NMDA receptors is essential for acute cocaine-mediated ERK phopsphorylation; however, such receptors activation may dephosphorylate p-Thr75 DARPP-32 in Ca^{2+}-dependent mechanisms (Jenab et al., 2005; Nishi et al., 2002). Thus, the inhibitory effects of NMDA-mediated Ca^{2+} influx and other protein phosphatases on p-Thr75 DARPP-32 should be further elucidated in the CPu after acute cocaine administration. Taken together, since p-Thr75 DARPP-32 is constitutively activated and has been shown to be a potential inhibitor of PKA, it is reasonable to postulate that disinhibition on PKA activity by attenuated p-Thr75 DARPP-32 may contribute to the ERK-dependent transcription after acute cocaine administration.

By direct protein-protein interaction, STEP has been shown to dephosphorylate p-ERK providing a time-limited activation of ERK (Paul et al., 2000, 2003; Pulido et al., 1998). Phosphorylation of STEP reduces its affinity to p-ERK and causes dissociation between them via activation of D1 receptor and PKA (Blanco-Aparicio et al., 1999; Paul et al., 2000). Veljent et al. (2005) demonstrated that acute amphetamine injections

increased p-STEP protein level, the decreased mobility of the 46-kDa isoform of STEP, with the activation of ERK in the CPu of mice. In the present study, we also showed that acute cocaine induced elevated p-STEP protein level, indicating that release of the inhibitory effect of STEP may promote the ERK phosphorylation stimulated by psychostimulants.

Finally, previous studies indicated that the induction of p-STEP is presumably dependent on the activation of p-Thr34 DARPP-32/PP-1 pathway (Girault et al., 2006; Valjent et al., 2005). However, in our analysis of CPu extracts, we did not detect any changes in p-Thr34 DARPP-32, suggesting that other molecular signaling cascades may contribute to STEP phosphorylation. In addition, application of protein phosphatase including PP-2A has been shown to dephosphorylate hematopic protein tyrosine phosphatase, a non-neuronal homolog of STEP, in T-cells (Nika et al., 2004). Thus, the unaltered PP-2A protein levels after acute cocaine administration in CPu could be partially explained by the net balance between the PKA-driven PP-2A elevation (an inhibitory effect on p-Thr75 DARPP-32) and reduction of PP-2A inhibitory effects on p-STEP.

In summary, at least two intracellular signaling pathways, the PKA/DARPP-32 and ERK pathways, have been shown to participate in the regulation of locomotor behavior by dopamine transmission (Greengard et al., 1999; Valjent et al., 2005). Here, we demonstrated that both pathways were modulated by acute cocaine administration, and immediate early gene, c-Fos, expression was also enhanced in the CPu which may contribute to the initiation of cocaine induced behavioral effects.

Chapter 3: Effects of dopamine and NMDA receptors on cocaine-induced Fos expression in the dorsal and ventral striatum of Fischer rats

I. Introduction

Cocaine is a major drug of abuse in Western countries and induces its psychomotor effects by blocking monoamine transporters. Among three monoaminergic systems, the dopaminergic inputs from the ventral tegmental area (VTA) to the ventral striatum/nucleus accumbens (NAc) and the nigrostriatal projections to the dorsal striatum/caudate-putamen (CPu) have been postulated to be the main regulator of cocaine's behavioral and biochemical effects (reviewed in Hyman and Malenka 2001; Koob and Nestler 1997; Spanagel and Weiss 1999). For example, *in vitro* and *in vivo*, cocaine administration causes a buildup of synaptic dopamine (DA) levels and increases DA neuronal activity in the CPu and NAc (Carboni et al., 1989; Kalivas and Duffy 1988; Maisonneuve and Kreek 1994; Reith et al., 1997). Cocaine also exerts its influence on the glutamatergic system. For instance, in the CPu and NAc, studies have shown that single or repeated cocaine injection modulates extracellular glutamate concentration (Pierce et al., 1996; Reid and Berger, 1996; Smith et al., 1995; Zhang et al., 2001).

Extracellular signal-regulated kinases (ERK), one of isoforms of mitogen-activated protein kinases, has been characterized to respond to extracellular stimuli and regulates cell proliferation and differentiation (Seger and Krebs, 1995). In both CPu and NAc, acute cocaine administration induces ERK phosphorylation (p-ERK; Corbille et al., 2007; Jenab et al., 2005; Sun et al., 2007; Valjent, et al., 2000; 2005; Zhang et al., 2004;

review in Zhai et al., 2008). p-ERK, in turn, translocates to the nucleus and controls gene expression through regulating cAMP response element binding protein (CREB) and ternary complex factor Elk-1 (Adams and Sweatt, 2002; Davis et al., 2000; Hill et al., 1993). Pharmacological inhibition of mitogen-activated protein kinase/ERK kinase (MEK), an upstream activator of ERK, attenuates cocaine-induced immediate early gene (IEG) expression in mesocorticolimbic brain regions (Ferguson et al., 2006; Valjent et al., 2000); suggesting that ERK-mediated cascades are important for cocaine-regulated transcriptional mechanisms.

Mitogen-activated protein kinase phosphatase-1 (MKP-1) belongs to the family of dual specificity phosphatase that is highly regulated by ERK activation. For example, p-ERK directly induces MKP-1 expression *in vivo* and *in vitro* (Brondello et al., 1997; Sgambato et al., 1998). After induction, MKP-1 dephosphorylates p-ERK, inactivating it, as a negative feedback mechanism (Duff et al., 1995; Sun et al., 1993). Corticostriatal stimulation coincidently increases *MKP-1* and c-*fos* mRNA in an ERK-dependent manner (Sgambato et al., 1998). In the striatum, *MKP-1* mRNA and protein levels were increased after either acute or chronic methamphetamine administration (Takaki et al., 2001; Ujike et al., 2002). In addition, chronic amphetamine injection also induces *MKP-1* mRNA in the ventral VTA (Rajadhyaksha et al., 2004). Furthermore, there is evidence that MKP-1 is phosphorylated (p-MKP-1) by p-ERK at two extreme C-terminal Ser residues *in vitro* (Brondello et al., 1999). The phosphorylation stabilizes MKP-1 protein but dose not influence its ability to dephosphorylate p-ERK.

Recently, our group and others have shown that cocaine induction of p-ERK may be mediated via DA-D1 and NMDA receptors activation (Jenab et al., 2005; Zhang et al.,

2004). Although *MPK-1* and c-*fos* mRNA induction in response to ERK activation has been documented, the parallel p-MKP-1 and Fos-related protein expression after acute cocaine administration has not been elucidated. To this end, we examined the activation profile of p-ERK, p-MKP-1 and Fos-related proteins expression after a single cocaine injection. In a second study, we determined the effects of DA-D1 and NMDA receptor blockade on cocaine-increased p-MKP-1 and Fos protein expression in the CPu and NAc.

II. Methods

Animals:

60-day-old male Fischer rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chambers (20 × 20 × 41 cm). Rats were maintained on a 12-hour light/dark cycle (lights on at 9:00 a.m.) with free access to food and water. Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

Drug and antibodies:

Cocaine hydrochloride, MK-801, and SCH23390 hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Primary antibodies for ERK (#9102), p-ERK (#9101), p-MKP-1 (#2857), c-Fos (#4384) and FosB (#2251) were purchased from Cell Signaling Technologies (Beverly, MA). Antibodies against MKP-1 (sc-370) and α-tubulin (sc-8035) were purchased from Santa Cruz Technologies (Santa Cruz,

CA). Both horseradish peroxidase-conjugated anti-rabbit IgG (NA-934) and anti-mouse IgG (NA-931) were from Amersham Pharmacia (Piscataway, NJ).

Drug administration:

Cocaine solutions were prepared by dissolution in physiological saline (0.9%) and injected intra-peritoneally (i.p.). One week after arrival, rats received an injection of saline (1 ml/kg) or cocaine (30 mg/kg). For evaluating p-ERK protein levels, rats were sacrificed 5, 15, 30, or 60 min after drug treatment. For the measurement of p-MKP-1, c-Fos and FosB protein expression, rats received same doses of saline or cocaine administration and sacrificed 45, 90, 180 or 360 min later. For experimental paradigm involving antagonists, rats were administered MK801 (0.25 mg/kg in 0.9% saline, i.p.) or SCH23390 (0.25 mg/kg in 0.9% saline, i.p.) 30 min before single cocaine (30 mg/kg) or saline injections and sacrificed 60 min after the last injection. The doses of MK801 and SCH23390 were previously reported to inhibit the acute cocaine-induced p-ERK in the striatum (Jenab et al., 2005).

Protein preparation and measurement:

After decapitation (following a brief 20 s exposure to CO_2), rat brains were removed, flash frozen in 2-methylbutane (-40° C), and stored at -80° C until used. The coronal slices (1mm thick) were cut out in a matrix (ASI instruments, Warren, MI) and areas of CPu and NAc were simultaneously dissected out on a cold glass plate. Tissue samples were homogenized by using a Polytron handheld homogenizer (Kinematica, Luzern, Switzerland) in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 10%

Glycerol, 1% Triton X-100, 1% Igepal CA-630, 1% sodium dexycholic acid) containing a mixture of phosphatase inhibitors. After 30 min incubation, homogenates were centrifuged at 13,000 rpm for 15 min at 4° C. Supernatants were then collected and stored at -80° C until used. Total protein content was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

Western blot analysis:

Protein samples were analyzed by using Western blot analysis as previously described (Jenab et al., 2005). Briefly, equal amount of protein extracts were boiled in Lammeli buffer containing 1% β-mercaptoethanol for 5 min and ran on SDS-PAGE gels, then transferred to PVDF membranes. Membranes were blocked with 5% nonfat dry milk for 1hr at room temperature and then incubated with primary antibodies of ERK (1:1000), p-ERK (1:1000), MKP-1 (1:500), p-MKP-1 (1:1000), c-Fos (1:1000), and FosB (1:1000), individually, overnight at 4° C. After three washes with Tris-Tween-20 Buffer (TBST; pH = 7.4), membranes were incubated with their appropriate secondary antibodies (1:1000) for 1hr at room temperature followed by three more washes with TBST. Antibody binding was detected by using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia, Piscataway, NJ). Intensity of protein bands was quantified with a computer densitometer and Image Quant Program (Molecular Dynamics). For normalization of protein levels, all membranes were re-probed with α-tubulin antibody (1:1000).

Statistical analysis:

Protein levels were expressed as a ratio to α-tubulin levels. Data was expressed as mean %±SEM relative to respective saline controls, which were arbitrarily set at 100%. For time course experiments, protein levels were analyzed by two-way ANOVA; treatment (saline or cocaine) × time (5, 15, 30 or 60 min/45, 90, 180 or 360 min). To determine the effect of antagonist treatment on cocaine-induced activation, a one-way ANOVA was used. For Post hoc analysis, LSD tests were conducted when appropriate. Differences were considered significant at 0.05 level [$P<0.05$].

III. Results

Effects of acute cocaine administration on p-ERK:

In the CPu, 5 and 15 min after acute cocaine administration, p-ERK protein levels were increased by approximately 65% and 40%, respectively [Figure 10A]. However, two-way ANOVA only revealed marginal treatment × time interaction [$F(3, 24) = 3.98$, $P = 0.57$]. No treatment and time main effects were observed. In the NAc, acute cocaine time-dependently increased p-ERK protein levels [treatment main effect: $F(1, 24) = 9.68$, $P<0.05$; time main effect: $F(3, 24) = 5.93$, $P<0.05$; treatment × time interaction: $F(3, 24) = 5.93$, $P<0.05$; Figure 10B]. Post hoc test demonstrated that, compared to saline controls, acute cocaine increased p-ERK protein levels at 5 and 15 min [both $P<0.05$]. In cocaine-treated animals, p-ERK protein levels were higher at 5 min when compared to other time points [all $P<0.05$]. Acute cocaine also induced higher p-ERK protein levels 15 min than those at 60 min [$P<0.05$]. In addition, acute cocaine administration did not alter total protein levels of ERK in both brain regions [Table IIA].

Effects of acute cocaine on c-Fos and FosB:

Overall, in the CPu, cocaine increased c-Fos protein levels expression in a time dependent manner [treatment main effect: $F(1, 24) = 24.19$, $P<0.05$; time main effect: $F(3, 24) = 3.79$, $P<0.05$; treatment × time interaction: $F(3, 24) = 3.79$, $P<0.05$; Figure 11A]. Cocaine induced higher c-Fos protein expression from 45 to 180 min when compared to saline controls [all $P<0.05$]. Furthermore, in cocaine-treated animals, c-Fos protein levels were higher at 45 and 90 min than those at 360 min [both $P<0.05$]. In the NAc, a significant treatment main effect was found [$F(1, 24) = 32.92$, $P<0.05$; Figure 11B]. In comparison with saline-treated animals, acute cocaine induced higher c-Fos protein levels from 45 to 180 min [all $P<0.05$].

In the CPu, acute cocaine increased FosB protein expression [treat main effect: $F(1, 24) = 32.67$, $P<0.05$; Figure 11C]. Further analysis indicated that, 90 to 360 min after cocaine administration; FosB protein levels in cocaine-treated groups were higher than those in saline controls [all $P<0.05$]. On the other hand, in the NAc, acute cocaine time dependently increased FosB protein expression [treatment main effect: $F(1, 24) = 28.02$, $P<0.05$; time main effect: $F(3, 24) = 4.80$, $P<0.05$; treatment × time interaction: $F(3, 24) = 5.16$, $P<0.05$; Figure 11D]. In comparison with saline-treated animals, acute cocaine induced higher FosB protein levels from 180 to 360 min [all $P<0.05$]. In cocaine-treated animals, FosB protein levels from 180 to 360 min were significant higher than those at 45 min [all $P<0.05$].

Effects of acute cocaine on p-MKP-1:

In the CPu, 45 and 90 min after acute cocaine administration, p-MKP-1 protein levels were increased by approximately 210% and 35%, respectively [Figure 12A]. Two-way ANOVA showed that p-MKP-1 protein levels were time-dependently increased by acute cocaine administration [treatment main effect: $F(1, 24) = 13.01$, $P<0.05$; time main effect: $F(3, 24) = 6.76$, $P<0.05$; treatment × time interaction: $F(3, 24) = 6.76$, $P<0.05$]. p-MKP-1 protein levels were higher 45 and 90 min after acute cocaine when compared to respective saline controls [both $P<0.05$]. In addition, in cocaine-treated animals, p-MKP-1 protein levels at 45 min were significantly higher than those at other time points [all $P<0.05$]. In the NAc, acute cocaine administration also increased p-MKP-1 in a time dependent manner [treatment main effect: $F(1, 24) = 7.74$, $P<0.05$; time main effect: $F(3, 24) = 4.03$, $P<0.05$; treatment × time interaction: $F(3, 24) = 4.03$, $P<0.05$; Figure 12B]. In comparison with saline-treated animals, acute cocaine induced higher p-MKP-1 protein levels at 45 and 90 min [both $P<0.05$]. In cocaine-treated animals, p-MKP-1 protein levels at 45 min were higher than those at 360min [$P<0.05$]; protein levels of p-MKP-1 at 90 min were also higher than those at 180 and 360 min [both $P<0.05$]. In addition, acute cocaine administration did not alter total protein levels of MKP-1 in both brain regions [Table IIB].

Effects of MK801 and SCH23390 pre-treatments on cocaine-induced Fos protein expression and p-MKP-1:

In the CPu, a significant antagonist pre-treatment effect was found on c-Fos induced by cocaine [$F(5, 29) = 3.06$, $P<0.05$; Figure 13A]. Both MK801 and SCH23390 blocked acute cocaine-induced c-Fos protein induction [$P<0.05$]. However, in the NAc,

although an effect of antagonist pre-treatment was observed in c-Fos protein expression [$F(5, 29) = 2.92$, $P<0.05$; Figure13B], only SCH23390 attenuated cocaine-induced c-Fos expression when compared with saline/cocaine-treated rats [$P<0.05$].

In the CPu, a significant effect of antagonist pre-treatment on cocaine-induced FosB protein expression was found [$F(5, 29) = 3.77$, $P<0.05$; Figure 13C]. SCH23390 blocked cocaine induction of FosB protein levels [$P<0.05$]. However, neither MK801 nor SCH23390 altered cocaine effects on FosB in the NAc [Figure 13D].

Similar to Figure12, acute cocaine administration significantly increased p-MKP-1 protein levels in the CPu. A significant effect of antagonist pre-treatment on cocaine-induced p-MKP-1 protein levels was obtained [$F(5, 29) = 3.06$, $P<0.05$; Figure 13E]. SCH23390 blocked the increase of p-MKP-1 after cocaine injection [$P<0.05$]. Although MK801 pre-treatment decreased cocaine-induced p-MKP-1 protein levels by approximately 40%, it failed to reach the significant level [$P>0.05$]. In the NAc, a significant effect of antagonist pre-treatment on cocaine-induced p-MKP-1 was also observed [$F(5, 29) = 3.54$, $P<0.05$; Figure13F]; where both MK801 and SCH23390 pre-treatments reduced cocaine-induced p-MKP-1 activation [$P<0.05$]. As shown in Table II C, antagonists pre-treatment and/or acute cocaine injections had no effect on non-phosphorylated MKP-1 protein levels in both CPu and NAc.

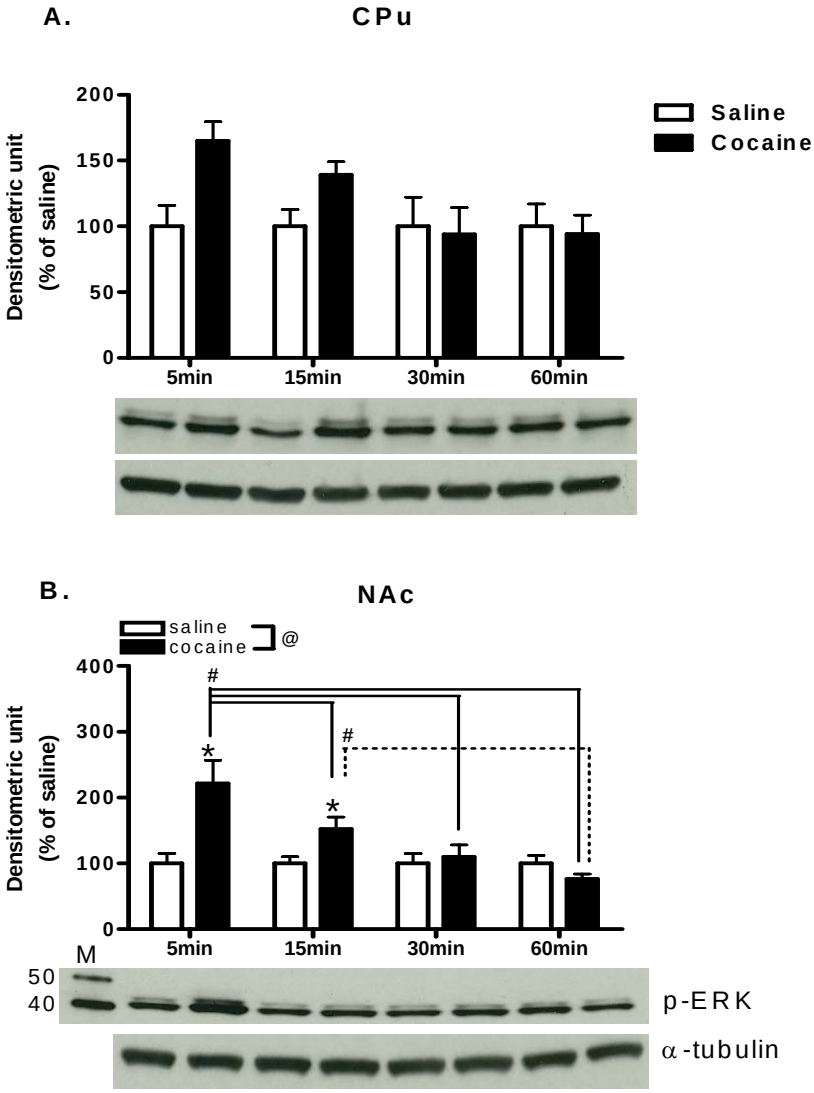


Figure 10. The time course of cocaine effects on p-ERK in (A) CPu and (B) NAc. Results represent as protein levels over α-tubulin expressed as percentage of saline control (4 animals per group). 5, 15, 30, or 60 min after rats were given injections. M is the molecular marker in kDa. $^{@}p$ <0.05 as a significant treatment main effect; *p <0.05 as compared with respective saline group; $^{\#}$ p <0.05 as compared with cocaine groups at different time points.

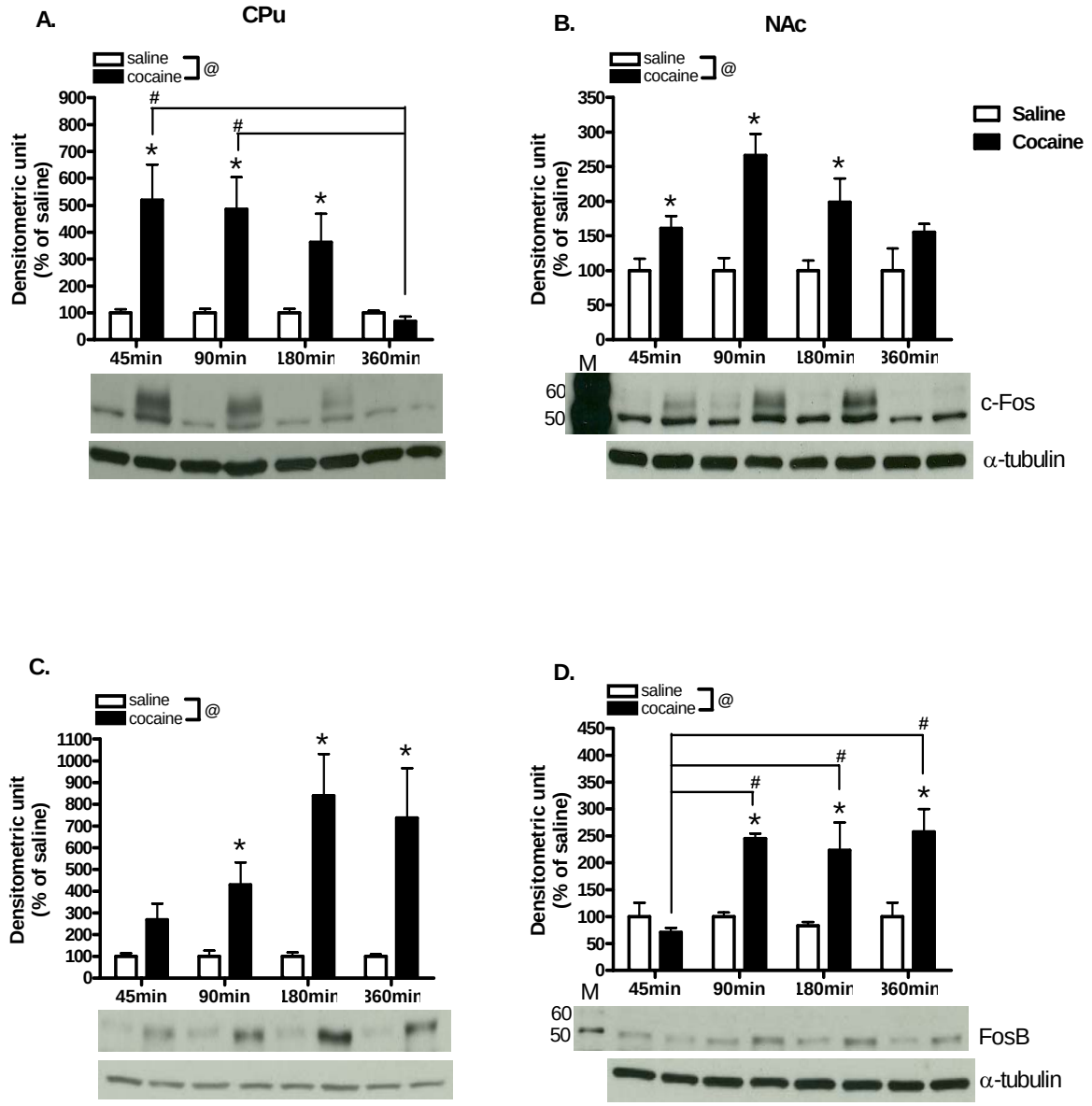


Figure 11. The time course of cocaine effects on Fos-like protein expression. c-Fos in (A) CPu and (B) NAc; FosB in (C) CPu and (D) NAc. Results represent as protein levels over α-tubulin expressed as percentage of saline control (4 animals per group). 45, 90, 180, or 360 min after rats were given injections. M is the molecular marker in kDa. $^{@}p$ <0.05 as a significant treatment main effect; *p <0.05 as compared with respective saline group; $^{\#}$ p <0.05 as compared with cocaine groups at different time points.

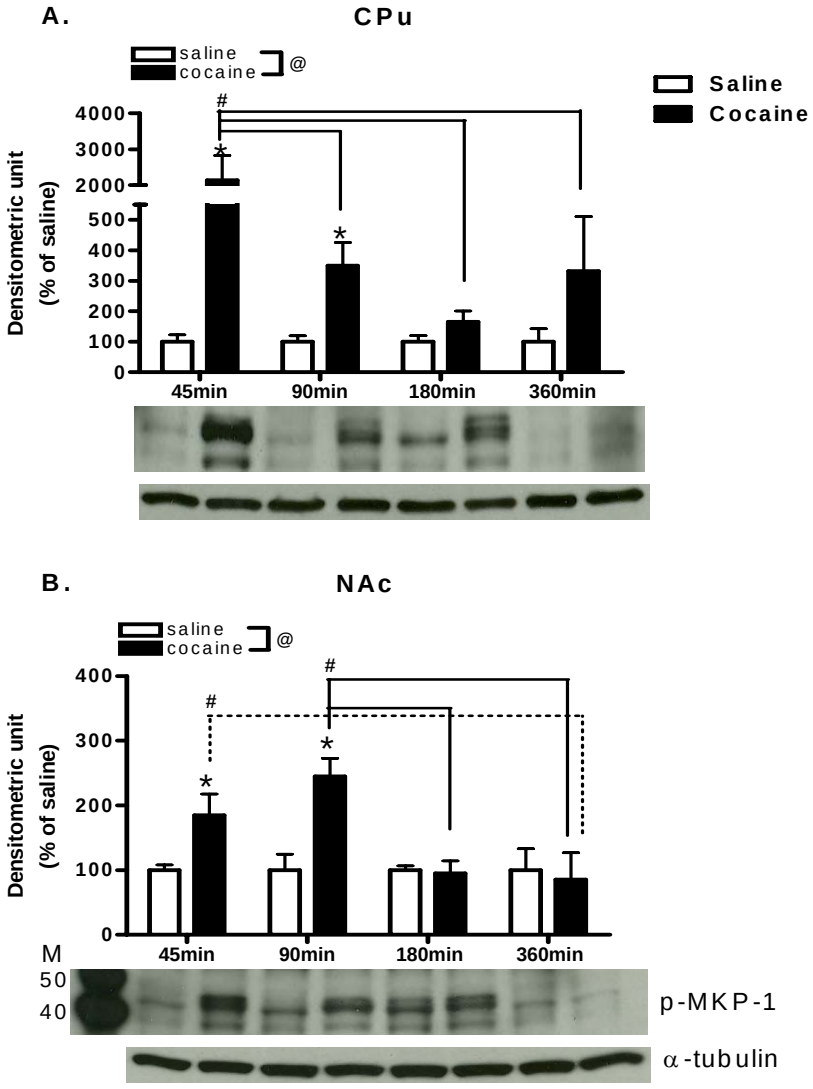


Figure 12. The time course of cocaine effects on p-MKP-1 in (A) CPu and (B) NAc. Results represent as protein levels over α-tubulin in the CPu expressed as percentage of saline control (4 animals per group). 45, 90, 180, or 360 min after rats were given injections. M is the molecular marker in kDa. $^{@}p$ <0.05 as a significant treatment main effect; *p <0.05 as compared with respective saline group; $^{\#}$ p <0.05 as compared with cocaine groups at different time points.

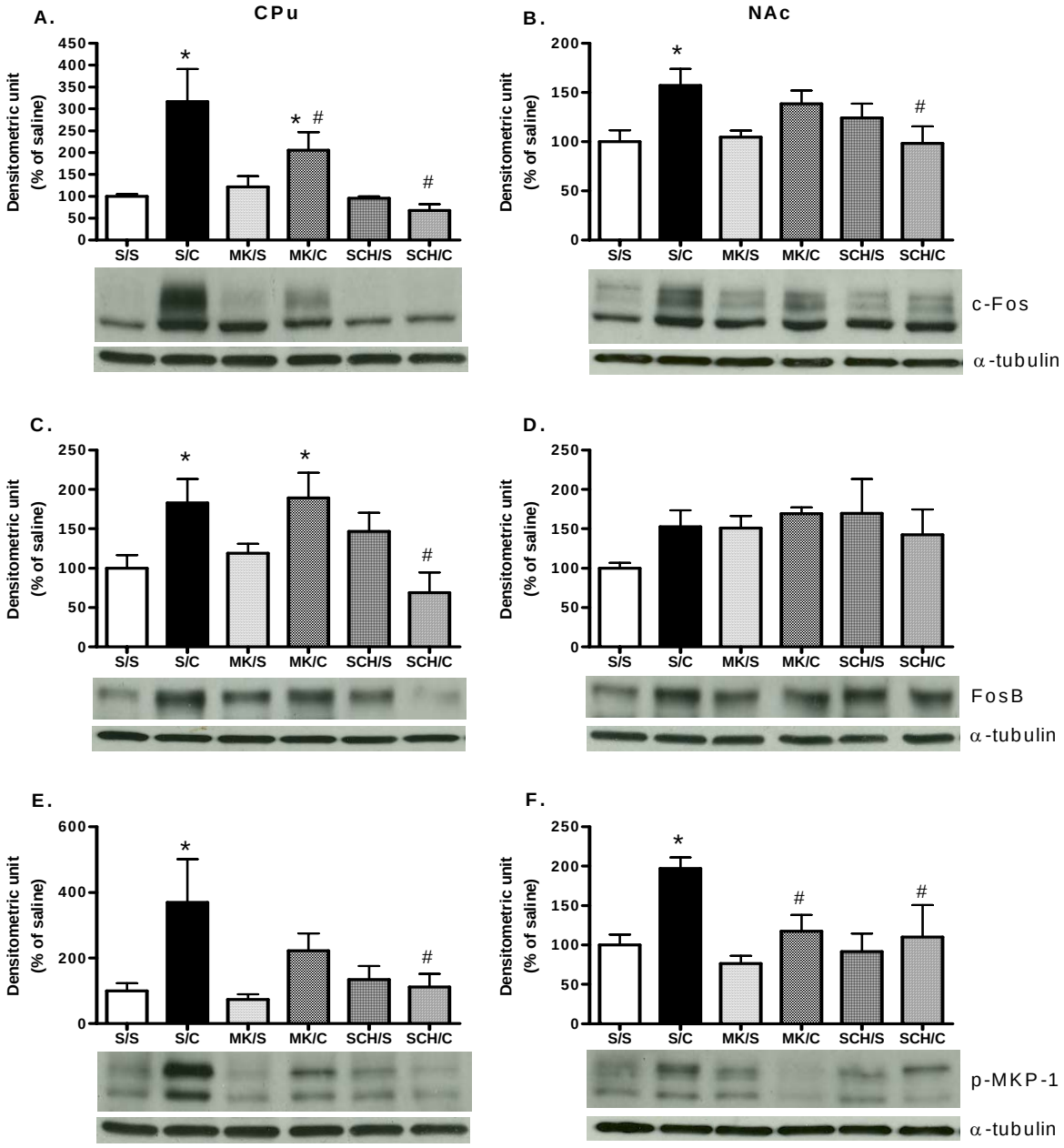


Figure 13. Effects of MK801 and SCH23390 on cocaine-induced Fos-like protein expression and p-MKP-1. c-Fos in (A) CPu and (B) NAc; FosB in (C) CPu and (D) NAc; p-MKP-1 in (E) CPu and (F) NAc. Rats were pre-administrated saline, MK801, or SCH23390 30 min before a single saline or cocaine injection. Results represent as protein levels over α-tubulin expressed as percentage of saline/saline control (5 animals per group). **p* <0.05 as compared with saline/saline group and #*p*<0.05 as compared with saline/cocaine group. [S/S= saline/saline; S/C=saline/cocaine;MK/S=MK801/saline; MK/C=MK801/cocaine; SCH/S=SCH23390/ saline; SCH/C=SCH23390/cocaine].

Table Ⅱ. Total protein levels after cocaine/antagonist treatment in the rat CPu and NAc.

Time	5 min		15 min		30 min		60 min	
Treatment	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
A. Cocaine effect on ERK								
CPu	100.00±4.62	94.99±10.24	100.00±11.50	82.11±8.73	100.00±20.41	92.48±26.17	100.00±31.50	101.86±24.81
NAc	100.00±8.18	88.16±5.85	100.00±1.78	94.94±6.10	100.00±1.94	97.65±10.45	100.00±9.72	111.89±2.73
Time	45 min		90 min		180 min		360 min	
Treatment	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
B. Cocaine effect on MKP-1								
CPu	100.00±5.35	133.10±15.12	100.00±9.06	100.22±11.90	100.00±14.46	120.56±19.35	100.00±26.65	85.47±9.59
NAc	100.00±6.50	103.72±17.51	100.00±5.57	99.94±13.64	100.00±11.25	75.04±9.15	100.00±7.97	92.41±7.54
Treatment	S/S	S/C	MK/S	MK/C	SCH/S	SCH/C		
C. Cocaine/antagonist effects on MKP-1								
CPu	100.00±11.44	110.00±13.29	114.37±23.36	100.36±9.04	124.23±13.75	98.21±15.74		
NAc	100.00±12.21	99.53±5.53	99.47±9.30	109.39±9.06	107.22±10.92	110.45±14.90		

The ratio of total proteins over α-tubulin is expressed relative to saline controls which are set at 100%. Data are represented as mean±SEM. (A) Time course of ERK protein levels after acute cocaine in CPu and NAc. (B) Time course of MKP-1 protein levels after acute cocaine in CPu and NAc. (C) MKP-1 levels after antagonist (MK801 or SCH23390) pre-treatment followed by cocaine or saline injections. [S/S = saline/saline; S/C = saline/cocaine; MK/S = MK801/saline; MK/C = MK801/cocaine; SCH/S = SCH23390/ saline; SCH/C = SCH23390/cocaine].

IV. Discussion

In the present study, after a single cocaine injection, a rapid and transient increase of p-ERK protein levels was observed in the CPu and NAc. This result is consistent with studies demonstrating that acute cocaine induced ERK activation in drug reward associated areas (Corbille et al., 2007; Jenab et al., 2005; Sun et al., 2007; Valjent, et al., 2000; 2005; Zhang et al., 2004). In addition, we further showed the delayed elevation of IEG protein expression and phosphorylation in response to acute cocaine administration. Two transcription factors, Elk-1 and CREB, have been characterized as nuclear targets of ERK activation (Adams and Sweatt, 2002; Davis et al., 2000; Hill et al., 1993). Previous studies have reported that acute cocaine administration increases Elk-1 phosphorylation in the striatum (Jenab et al., 2005; Sun et al., 2007; Valjent et al., 2000). Pharmacological inhibition of ERK pathway signaling also attenuates acute cocaine-induced c-Fos and FosB protein rexprssion in the striatum (Radwanska et al., 2006; Guan et al., 2008). In mice CPu and NAc, acute cocaine injection augments CREB phosphorylation in an ERK-dependent manner (Brami-Cherrier et al., 2005; Kano et al., 1995; Karasinska et al., 2005; Walters et al., 2003). To the best of our knowledge there is no evidence showing that acute cocaine increases CREB phosphorylation in the CPu of rats. However, in rats, we have observed an elevation of CREB phosphorylation in the NAc after acute cocaine injection (Nazarian et al., unpublished observation). Furthermore, ERK-regulated phosphorylation of pp90 ribosomal S6 kinase, a kinase mediating CREB phosphorylation, was increased in response to acute cocaine administration in the CPu of Fischer rats (Sun et al., 2007). Thus, based on the current

results and those of Nazarian, it can be hypothesized that ERK-activated Elk-1 and/or CREB may underlie cocaine-induced gene expression in both CPu and NAc.

Numerous studies have demonstrated that acute cocaine induces *c-fos, FosB* mRNA and Fos-like protein expression (Brown et al., 1992; Chen et al., 1995; Graybiel et al., 1990; Hope et al., 1992; Jenab et al., 2003; Steiner and Gerfen, 1993; Sun et al., 2007; Young et al., 1991). Herein, similar to previous studies suggesting distinct cocaine effects on Fos-like protein induction (Nestler et al., 2001; Ranwanska et al., 2006; Young et al., 1991), we also found that acute cocaine administration induces differential activation profiles in immediate early gene protein expression in both areas examined: a rapid and transient c-Fos protein expression (45-180 min) and delayed activation of FosB (90-360 min). A recent study has indicated that acute cocaine induced robust and moderate histone H4 acetylation in *c-fos* and *FosB* promoters, respectively (Kumar et al., 2005). In addition, after chronic cocaine injection, the histone H3 acetylation is evident in *FosB* but not in *c-fos*. Therefore, distinct protein expression and histone modification may further promote different transcriptional regulation after cocaine exposure.

Accumulating evidence has established that the interaction between DA-D1 and NMDA receptors in the postsynaptic region is necessary for Fos-like protein induction in the striatum (Berretta et al., 1992; Das et al., 1997). Previous studies have shown that, in the CPu, the acute cocaine-induced *c-fos* mRNA and protein expression is dependent on both DA-D1 and NMDA receptor activation (Jenab et al., 2003; Torres and Rivier, 1993; Young et al., 1991). We also demonstrated that DA D1 antagonist, SCH23390, abolished cocaine-mediated c-Fos protein expression in the CPu and NAc. However,

only in the CPu, the NMDA receptor antagonist, MK801, partially inhibited c-Fos expression. Three plausible explanations may underlie the regional discrepancy in response to NMDA receptor antagonism. First, microdialysis studies in freely moving rats have indicated that systemic MK801 administration (0.2-0.5 mg/kg) significantly increases extracellular DA levels in the prefrontal cortex and the NAc but not in the CPu (Mathe et al., 1996; Wedzony et al., 1993; Wolf et al., 1993). In addition, previous studies indicated that moderate to high doses of MK801 (0.5-8.0 mg/kg) may induce Fos-like protein expression in various brain regions including the striatum (Draunow and Faull, 1990; Liu et al., 1994; Storvik et al., 2006). However, in both CPu and NAc, low doses of MK801 (0.1-0.3 mg/kg) itself had no effect on *c-fos* mRNA induction (De Leonibus et al., 2002; Dalia and Wallace, 1995). Similarly, although MK801 (0.25 mg/kg) itself did not change basal c-Fos protein expression in present study, it is possible that both MK801 and cocaine administration may elevate dopamine outflow in the NAc by a synergistic action and result in inefficiency of MK801 blockade of c-Fos protein expression. Nevertheless, the relationship between MK801-induced extracellular DA level and postsynaptic IEG protein expression should be further elucidated. Secondly, as postulated by Zhai et al (2008), glutamate signaling may play a subsidiary role to DA's dominant signaling role. In our CPu extract, NMDA antagonism partially blocked cocaine-induced c-Fos protein expression similar to a previous study using higher dose of MK801 or CPP (Torres and River, 1993). On the other hand, administration of SCH23390 abolished cocaine-induced c-Fos protein expression in both brain regions. In the CPu, DA-D1 but not NMDA receptor blockade also prevents the early development of FosB expression after acute cocaine injection. Since a high

dose of cocaine was used in present study, it is tempting to suggest that dopaminergic signaling cascades are the major component mediating IEG expression. Finally, the CPu and NAc mediate distinct cocaine-induced behavioral responses (Hyman et al., 2006). With a detailed examination, we have demonstrated that acute cocaine induced high and moderate magnitude IEG proteins expression in the CPu and NAc, respectively (Figure 11). A different time course for activation was also observed in both brain regions. One hour after cocaine administration, fully developed c-Fos protein expression may occur in the CPu depending on both DA and glutamate signaling. However, DA-D1 receptors activation is necessary for premature/initial c-Fos development in the NAc. Thus, the different activation profile and sensitivity in response to receptor antagonism may reflect a shift in neuronal activity between both regions underlying cocaine's psychomotor responses.

MKP-1 protein, an IEG product, is the phosphatase involved in the inactivation of ERK in several transfected cells (Alessi et al., 1993; Sun et al., 1993). P-ERK has been reported to induce and stabilize p-MKP-1 without altering its intrinsic capability to downregulate ERK activation (Brondello et al., 1999). Previous studies have shown that acute methamphetamine administration induces *MKP-1* mRNA in the striatum (Ujike et al., 2002; Takaki et al., 2001). In the CPu and NAc, we further demonstrate an elevation of p-MKP-1 protein levels in DA-D1 and NMDA receptor-dependent manner. After acute cocaine, increasing p-MKP-1 protein levels are followed by the downregulation of p-ERK suggesting that p-MKP-1 may regulate the time-limited activation of ERK. Interestingly, acute cocaine administration coincidentally induces c-Fos protein expression and p-MKP-1. Although a different experimental paradigm, Sgambato et al

(1998) showed a spatially coincident distribution of *c-fos* and *MKP-1* mRNAs in the striatum via electrical stimulation of the glutamatergic corticostriatal pathway. In addition, blocking Elk-1 and CREB activation using intrastriatal infusion of the ERK inhibitor, PD 98059, completely abolished *c-fos* and *MKP-1* induction. Since the dose of antagonists used in present study has been shown to attenuate acute cocaine-induced p-ERK in the CPu (Jenab et al., 2005), it is reasonable to suggest that the decrease in cocaine-activated p-MKP-1 after DA D1 and NMDA receptor antagonism is due to the inactivation of ERK signaling. Taken together, in both CPu and NAc, we demonstrated that p-MKP-1 protein levels were significantly increased after single cocaine injection. Perhaps, via the ERK-mediated intracellular cascade, DA-D1 and NMDA receptor antagonist pretreatment abolished cocaine-induced p-MKP-1. In addition, administration of abused drug and/or striatal stimulation may regulate *MKP-1* gene induction and its protein phosphorylation underlying the synaptic plasticity in the striatum. However, the contribution of total MKP-1 and p-MKP-1 protein to p-ERK inactivation after cocaine administration should be further examined.

In summary, several drugs, including cocaine, stimulate the expression of IEG in specific regions resulting in the modulation of downstream genes expression. Our data demonstrate that acute cocaine administration induces IEG protein expression (c-Fos and FosB) and phosphorylation (p-MKP-1). However, such protein activation profiles vary in the CPu and NAc, indicating that cocaine exerts differential influence on the two brain regions. In addition, the activation of DA-D1 and/or NMDA receptors seems to converge on ERK-mediated signaling and to control IEG protein expression underlying the cocaine-induced neuronal plasticity and psychomotor effects.

Chapter 4: The role of NMDA and dopamine D1 receptors in NMDA-NR1/D1 physical interactions in the dorsal striatum/caudate-putamen of Fischer rats.

I. Introduction

Cocaine, a psychostimulant, is one of the most widely abused drugs in Western countries. By acting as an indirect dopamine agonist, cocaine regulates dopamine and glutamate release in the striatum (Carboni et al., 1989; Kalivas and Duffy, 1988; Lee et al., 2008; Maisonneuve and Kreek, 1994; Reith et al., 1997; Shin et al., 2007; Williams and Steketee, 2004). At the receptor level, it has been shown that either pharmacological or genetic manipulation of dopamine D1 and NMDA receptor attenuates acute cocaine-induced locomotor activity (Jenab et al., 2005; Karasinska et al., 2005; Ramsey et al., 2008; Uzbay et al., 2000; Xu et al., 1994), reinforcing and rewarding effects of cocaine (Caine et al., 2007; Heusner and Palmiter, 2005; Nazarian et al., 2004; Ramsey et al., 2008) and cocaine-induced intracellular signaling alternations (e.g., extracellular signal-regulated kinase (ERK) and cyclic AMP response element-binding protein phosphorylation) in response to acute cocaine in the dorsal striatum/caudate-putamen (CPu; Jenab et al., 2005; Karasinska et al., 2005; Valjent et al., 2000; Zhang et al., 2004). These results suggested that both dopamine D1 and NMDA receptors are involved in cocaine-induced behavioral and biochemical changes.

Accumulating evidence demonstrated that chronic but not acute cocaine alters dopamine D1 receptor binding density in the striatum (Kleven et al., 1990; Laurier at al., 1994; Tsukada et al., 1996; Unterwald et al., 1994). Similarly, protein levels

of the NR1 subunit of NMDA receptor (NMDA-NR1) are not changed after acute cocaine administration (Fitzgerald et al., 1996). On the other hand, repeated cocaine exposure decreases NMDA-NR1 mRNA levels and increases its protein levels in the CPu and the ventral tegmental area (VTA), respectively (Churchill et al., 1999; Fitzgerald et al., 1996; Ghasmazadeh et al., 1999). Thus, it shows that acute cocaine may play a limited role in dopamine D1 and NMDA-NR1 protein levels. However, recent *in vitro* and *in vivo* studies demonstrated that dopamine D1 and NMDA-NR1 physically interact with each other via protein-protein interactions at their carboxyl terminal (CT) regions (Fiorentini et al., 2003; Lee et al., 2000; Lee and Liu 2004; Pei et al., 2004). In hippocampal neurons and dopamine D1 and NMDA-NR1 co-transfected cell lines, the stimulation of dopamine D1 receptor results in a disassociation between dopamine D1/NMDA-NR1 CT regions (Lee et al., 2000; Figure 18). In addition, the NMDA receptor-mediated dopamine D1 receptor membrane surface insertion is also dependent on dopamine D1/NMDA-NR1 physical interactions (Pei et al., 2004). Taken together, it suggests that dopamine and glutamate transmission may subtly modulate physical interactions between dopamine D1 and NMDA-NR1.

Previously, we have demonstrated that acute cocaine-induced p-ERK and immediate early gene are dependent on the activation of dopamine D1 and NMDA receptor (Jenab et al., 2005; Sun et al., 2008). However, these receptors interactions in response to acute cocaine are not well understood. Thus, the present study was designed to evaluate the effect of acute cocaine in dopamine D1/NMDA-NR1 protein-protein interactions in the CPu. Based on Lee et al (2000) showed that

dopamine reduces receptors interactions *in vitro*, we hypothesize that these receptors interactions may also dissociate after acute cocaine through the elevation of extracellular dopamine levels. In a second study, we further determined the effect of NMDA receptor activation and blockade on dopamine D1/NMDA-NR1 physical interactions.

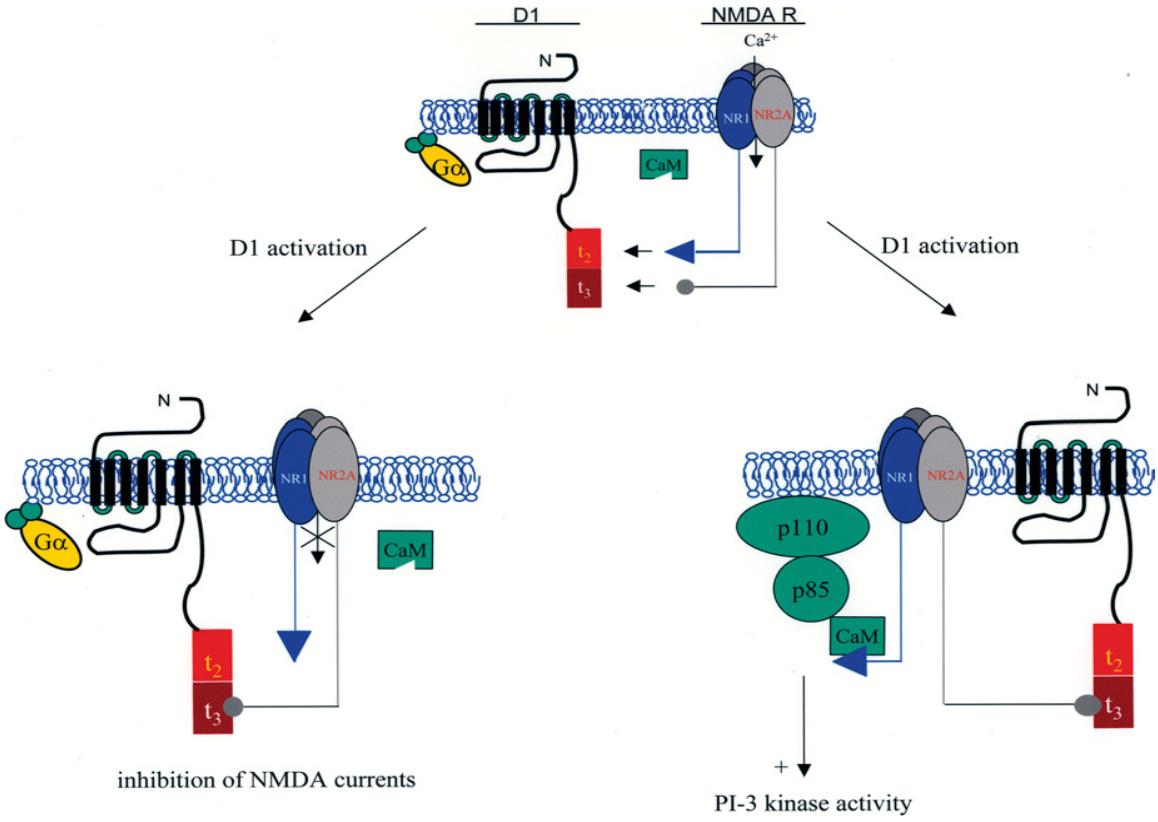


Figure 14. Physical interactions between dopamine D1 and NMDA receptors. Through t_2 and t_3 regions at the intracellular C-terminal, dopamine D1 receptor forms physical interactions with C-terminals of NMDA-NR1 and NMDA-NR2A subunits, respectively. Activation of dopamine D1 receptors reduces physical interactions with NMDA-NR1 and NMDA receptor-mediated Ca^{2+} influx subsequently. (Adopted from Lee and Liu, 2004)

II. Methods

***Animals**:*

60-day-old male Fischer rats (Charles River, Raleigh, NC) were individually housed in Plexiglas chambers (20 × 20 × 41 cm). Rats were maintained on a 12-hour light/dark cycle (lights on at 9:00 a.m.) with free access to food and water. Animal care and use was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

Drugs and antibodies:

Cocaine hydrochloride, NMDA and MK-801 were purchased from Sigma chemical Co. (St. Louis, MO). Antibody for dopamine D1a receptor was bought from Chemicon International (Temecula, CA). Mouse monoclonal antibody for NMDA-NR1 subunit was purchased form BD Pharmingen (San Diego, CA). α-tubulin antibody and protein A/G agarose were from Santa Cruz Technologies (Santa Cruz, CA). Both horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG were purchased from Amersham Pharmacia (Piscataway, NJ).

Drugs administration:

For cocaine time course study, cocaine solutions were prepared by dissolution in physiological saline (0.9%) and injected intra-peritoneally (i.p.). Rats were received an injection of saline (1 ml/kg) or cocaine (30 mg/kg) and sacrificed 5, 15, 30 or 60

min later. A separate cohort of rats was received saline, NMDA (25 mg/kg, i.p.) or MK-801 (0.25 mg/kg, i.p) administration and sacrificed 30 min later.

Protein preparation and measurement:

After decapitation (following a brief 20 s exposure to CO_2), rat brains were removed, flash frozen in 2-methylbutane (-40° C), and stored at -80° C until used. The coronal slices (1mm thick) were cut out in a matrix (ASI instruments, Warren, MI) and CPu was dissected out on a cold glass plate. CPu was homogenized by using a Polytron handheld homogenizer (Kinematica, Luzern, Switzerland) in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 10% Glycerol, 1% Triton X-100, 1% Igepal CA-630, 1% sodium dexycholic acid) containing phosphatase inhibitors mixture. After 30 min incubation, homogenates were centrifuged at 13,000 rpm for 15 min at 4° C. Supernatants were then collected and stored at -80° C until used. Total protein content was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

Western blot and immunoprecipitation analyses:

Western blot: Protein samples were analyzed by using Western blot as previously described (Jenab et al., 2005). Briefly, 40 μg of protein extracts were boiled in Lammeli buffer containing 1% β-mercaptoethanol for 5 min and ran on SDS-PAGE gels, transferred to PVDF membranes. Membranes were then blocked with 5% nonfat dry milk for 1hr at room temperature and incubated with antibodies of D1a (1:1000) or NMDA-NR1 (1:1000) overnight at 4° C. After three washes with Tris-

Tween-20 Buffer (TBST; pH = 7.4), membranes were incubated with their appropriate secondary antibodies (1:1000) for 1hr at room temperature followed by three more washes with TBST. Antibody binding was detected by using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia, Piscataway, NJ). Intensity of protein bands was quantified with a computer densitometer and Image Quant Program (Molecular Dynamics). For normalization of protein levels, all membranes were re-probed with α-tubulin antibody (1:1000).

Immunoprecipitation: For immunoprecipition experiments, equal amount of protein (400-500μg) extracts were incubated in lysis buffer containing protease inhibitors mixture with anti-NMDA-NR1 or anti-dopamine D1a primary antibody (1:50-100) for overnight at 4°C, followed by the addition of protein A/G agarose (20 μl) for 4hrs. For control samples, protein extracts were incubated in absence of primary antibody. Beads were washed in lysis buffer, and centrifuged (2,500 rpm, 5 min) for 4 times. After final centrifugation, same amount of Lammeli buffer containing 1% β-mecraptoethanol (50-70 μl) was added and boiled for 5 min. Samples were re-centrifuged at 13,000 rpm for 5 min. Equal amount of supernatants (10-20μl) were run on SDS-PAGE gels for Western blot analysis.

Statistical analysis:

Protein levels were expressed as a ratio to α-tubulin levels in western blot analysis. For immunoprecipitation analysis, NMDA-NR1 protein levels were expressed as a ratio over optic density of NMDA-NR1 levels obtained from western blot experiments. Data was expressed as mean % ± SEM relative to respective

saline controls, which were arbitrarily set at 100%. For time course experiments, protein levels were analyzed by two-way ANOVA; treatment (saline or cocaine) × time (5, 15, 30 or 60 min). Additional planned multiple comparisons were used to determine the difference between saline- and cocaine-treated groups at each time point. To determine effects of NMDA and MK-801 administration on receptors interactions, a one-way ANOVA was used. For Post hoc analysis, LSD tests were conducted when appropriate. Differences were considered significant at 0.05 level [$P<0.05$].

III. Results

Effects of acute cocaine on dopamine D1 and NMDA-NR1 receptors interactions:

Because a large amount of protein is necessary for immunoprecipitation assay, only the dorsal striatum/CPu but not the ventral striatum/nucleus accumbens was used in the present study. First, through western blot experiments, we evaluated whether total protein levels of dopamine D1 and NMDA-NR1 receptors were changed by a single cocaine administration. As shown in Figure 15, acute cocaine did not alter either dopamine D1 receptor or NMDA-NR1 protein levels in the CPu. In the immunoprecipitation experiment for receptors interactions, two-way ANOVA did not reveal any significant main effects or interaction [all $P>0.05$]. However, planned multiple comparisons indicated that 30 min after cocaine administration, the NMDA-NR1 and dopamine D1 receptors interactions was decreased when compared to the saline control [$P< 0.01$; Fig. 16A]. No significant changes were found at other time points. In order to validate the disassociation of receptors

interactions induced by acute cocaine, an opposite immunoprecipitation experimental procedure was used: immunoprecipitated by NMDA-NR1 antibody followed by probing with dopamine D1a antibody. As shown in Figure 16B, a decreasing of receptors interactions was also observed from 15 to 30 min after acute cocaine administration.

Effects of NMDA and MK-801 administration on NMDA-NR1 and D1 receptors interactions:

Since acute cocaine modulated the NMDA-NR1 and dopamine D1 receptor dimerization, we conducted a separate experiment to determine whether pharmacological activation or inhibition of NMDA receptor may alter the physical interactions between NMDA-NR1 and dopamine D1 receptor in the CPu. Similar to the effect of acute cocaine, 30 min after NMDA or MK-801 injection did not change the total protein levels of NMDA-NR1 subunit and dopamine D1 receptor [Fig. 17A and B, respectively]. However, immunoprecipitation experiment showed that there was a significant treatment effect on receptors interactions [$F(2, 6) = 22.23$, $P<0.05$; Fig. 18A]. Further post hoc test revealed that both NMDA and MK-801 treatments significantly reduced NMDA-NR1 and dopamine D1 receptors interactions compared to saline-treated animals [$P<0.01$ and $P<0.01$, respectively]. No difference was found between NMDA- and MK-801-treated groups. On the other hand, by using NMDA-NR1 antibody to pull down the dimerization complex, a similar reduction in receptors interactions was also observed in our CPu extract [Fig. 18B].

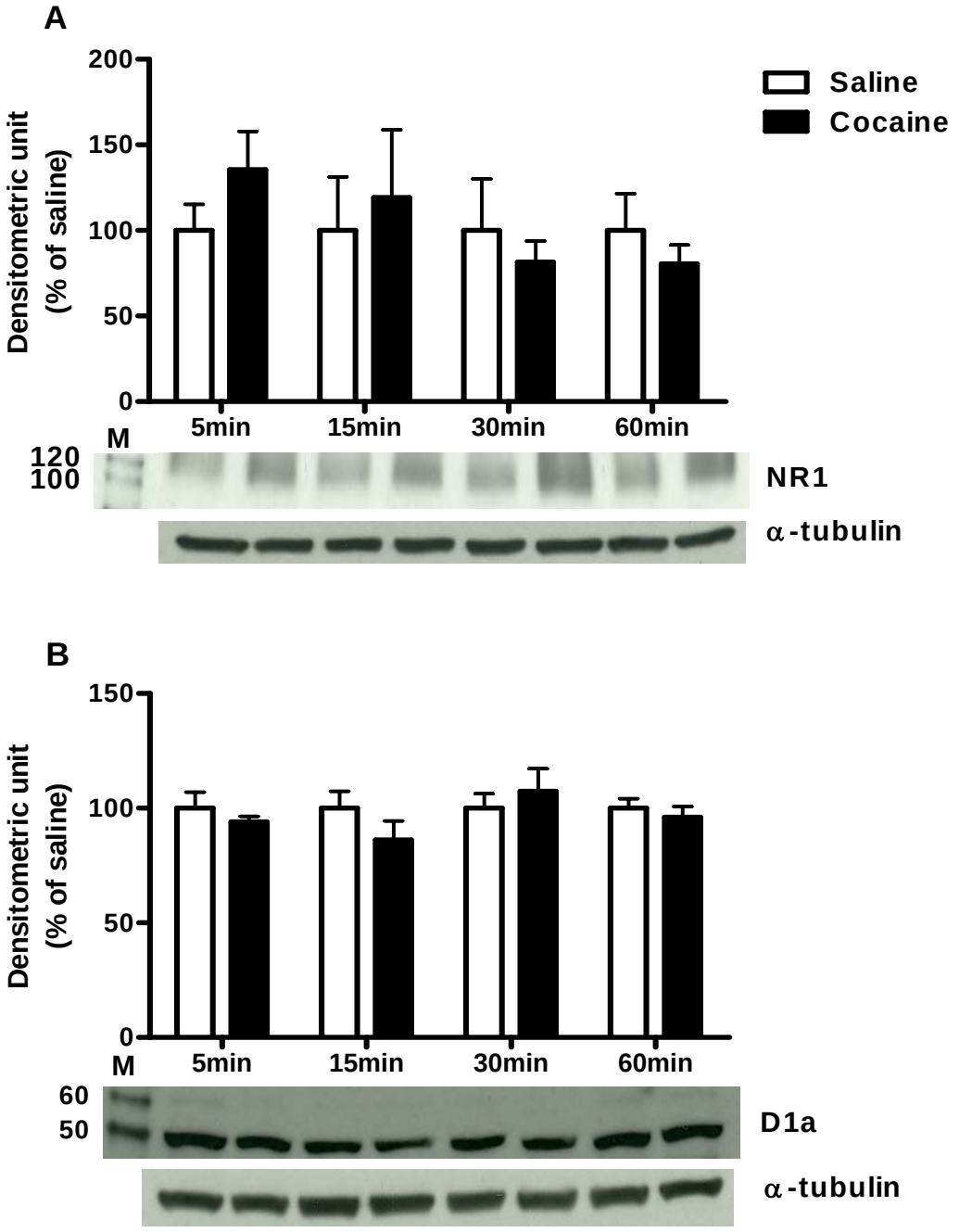


Figure 15. The time course of cocaine effects on total NMDA and D1 receptor in the CPu. (A) NMDA-NR1; (B) D1a. Results represent as protein levels over α-tubulin expressed as percentage of saline control (4 animals per group). 5, 15, 30, or 60 min after rats were given injections. M is the molecular marker in kDa.

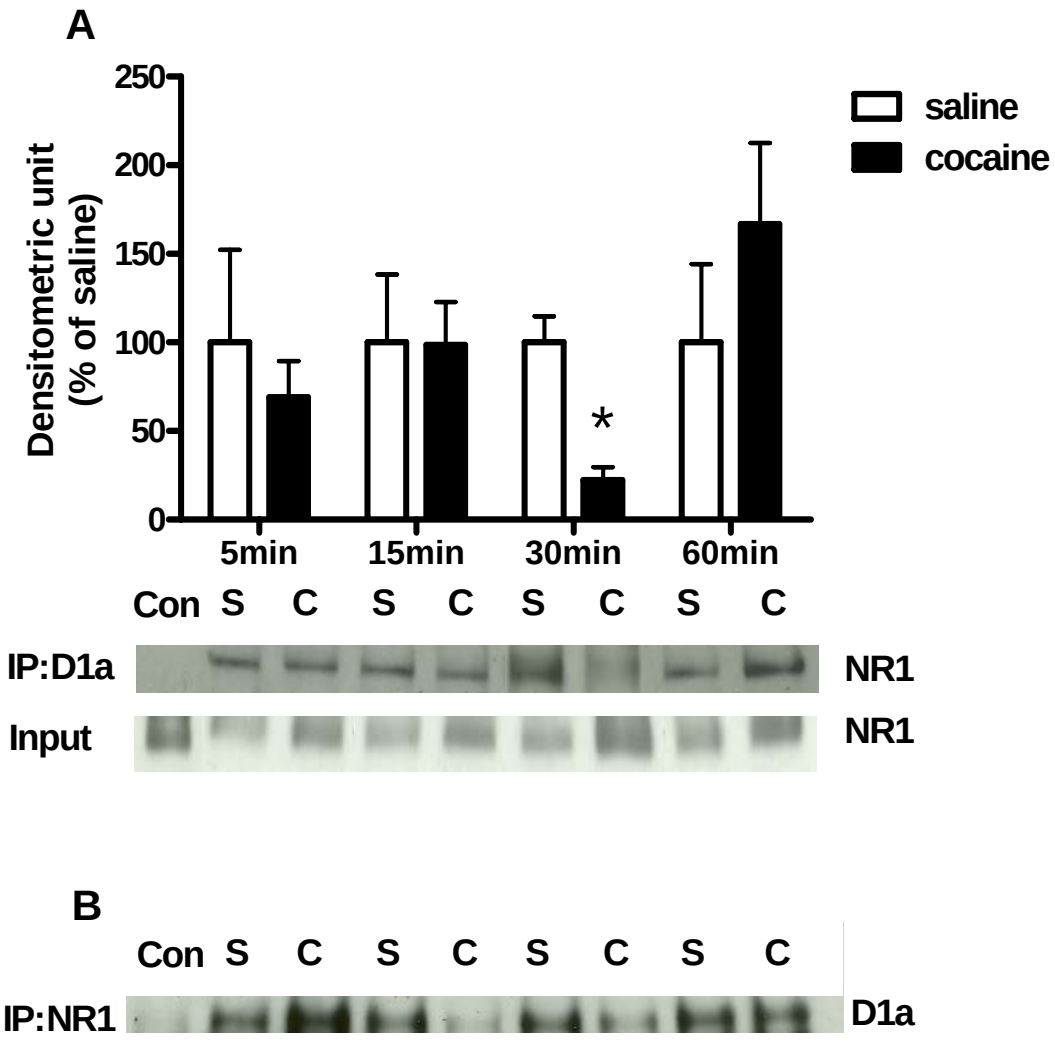


Figure 16. The time course of cocaine effects on NMDA and D1 receptor physical interactions in the CPu. (A) NR1 protein levels by using D1a antibody to pull down. Results represent as protein levels over total NR1 levels expressed as percentage of saline control (3 animals per group). 5, 15, 30, or 60 min after rats were given injections. **p* <0.05 as compared with respective saline group. (B) Representative D1a protein levels by using NR1 antibody to pull down. [Con = control; S = Saline; C = Cocaine]

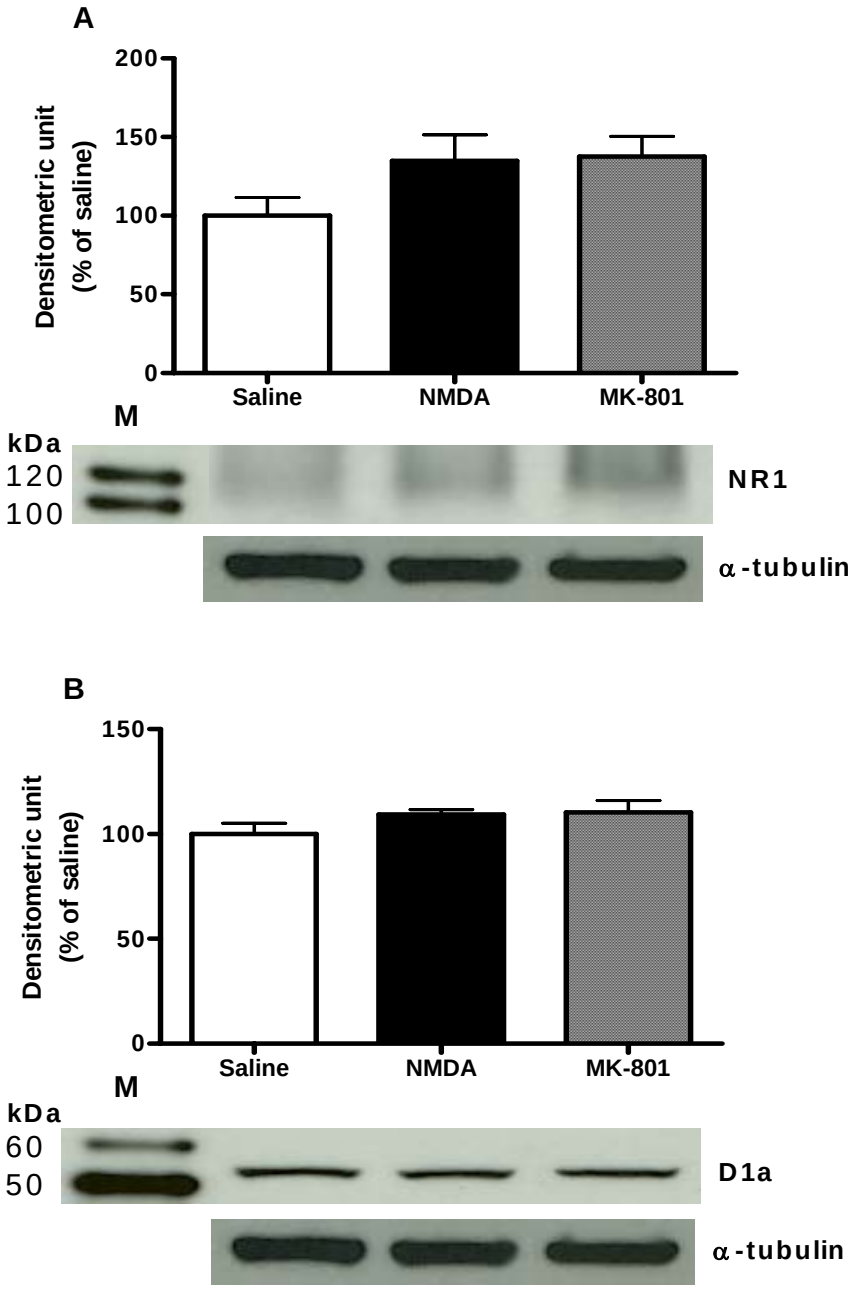


Figure 17. Effects of NMDA and MK-801 administration on total NMDA and D1 receptor in the CPu. (A) NMDA-NR1; (B) D1a. Results represent as protein levels over α-tubulin expressed as percentage of saline control (3 animals per group). 30 min after rats were given saline, NMDA or MK-801 injection. M is the molecular marker in kDa.

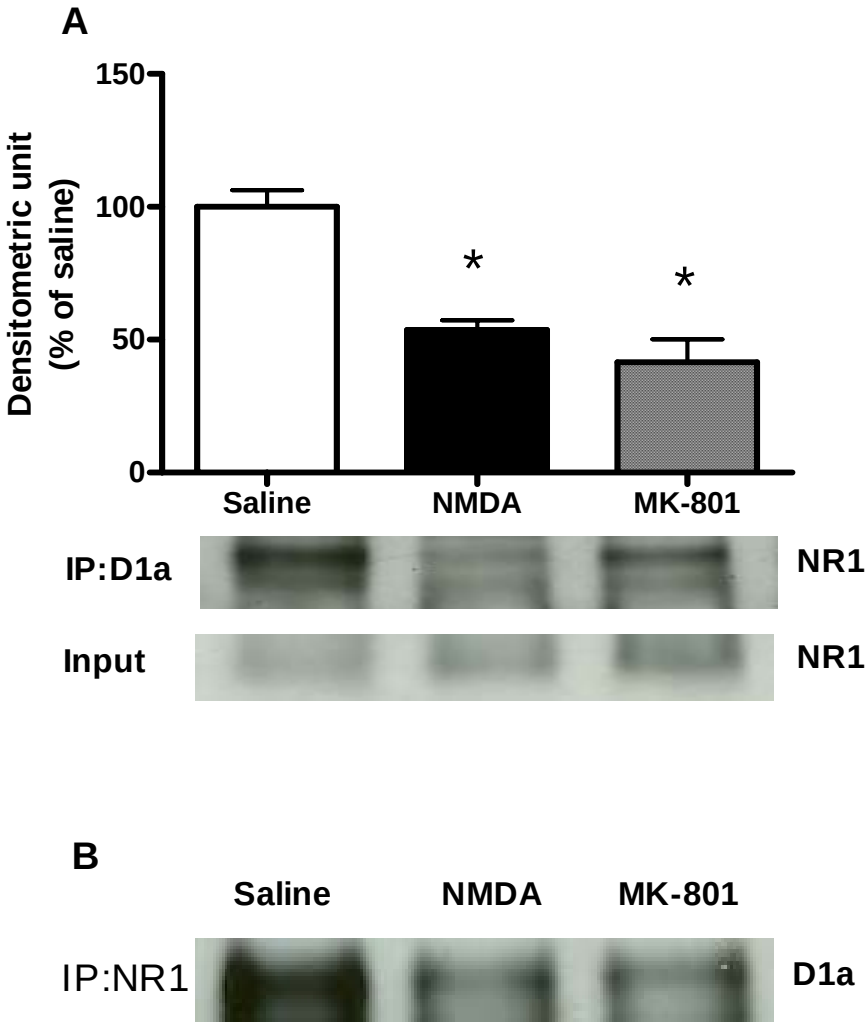


Figure 18. Effects of NMDA and MK-801 administration on NMDA and D1 receptor physical interactions in the CPu. (A) NR1 protein levels by using D1a antibody to pull down. Results represent as protein levels over total NR1 levels expressed as percentage of saline control (3 animals per group). 30 min after rats were given saline, NMDA or MK-801 injection. **p* <0.05 as compared with saline control. (B) Representative D1a protein levels by using NR1 antibody to pull down.

IV. Discussion

Results presented here demonstrated that acute cocaine injection time-dependently reduced NMDA-NR1 and dopamine D1 receptor protein-protein interactions in the CPu. In line with previous studies, we also showed that acute cocaine did not change protein levels of NMDA-NR1 and dopamine D1 receptor, suggesting the reduction of physical interactions between theses receptors is not due to total proteins alternation. In addition, after NMDA and MK-801 treatment the association between NMDA-NR1 and dopamine D1 receptor was also decreased. Thus, either activation or inhibition of NMDA receptor disrupts these receptors physical interactions.

Numerous studies indicated a complex interaction between NMDA and dopamine D1 receptors. For example, in hippocampal and striatal cultures, the stimulation of dopamine D1 receptor and PKA results in the phosphorylation of NMDA-NR1 subunits and increasing of cytosolic calcium which may lead to the phosphorylation of ERK proteins (Choe et al., 2001; Dudman et al., 2003; Leveque et al., 2000; Sweatt et al., 2001; Vanhoutte et al., 1999; Xia et al., 1996). In the striatal culture and VTA, a rapid NMDA-NR1 subunits postsynaptic membrane insertion has been also reported after dopamine D1 receptor agonists or a single cocaine administration (Dunah and Standaert, 2001; Schilstrom et al., 2006). The membrane insertion of NMDA-NR1 subunits may contribute to the potentiation of NMDA receptor-mediated current by dopamine D1 receptor. However, Lee et al (2002) showed that the dopamine D1 receptor activation by dopamine application disrupted the NMDA-NR1/D1 physical interactions and reduced the NMDA

receptor-mediated calcium influx via NMDA-NR2A/D1 CT interactions. Previously, we have demonstrated that single cocaine injection (30 mg/kg) transiently induced ERK phosphorylation, which gradually returned to basal levels 30 min after injections (Sun et al., 2008; see Figure. 10). In present study, although the functional significance of cocaine-induced disassociation on NMDA-NR1/D1 interactions is still unknown, it is tempting to postulate that the disassociation of receptors interactions may act as a time-limited gating mechanism on ERK activation is response to acute cocaine: (1) a rapid ERK phosphorylation due to potentiated NMDA-mediated calcium influx and/or NMDA-NR1 redistribution through cocaine-elevated extracellular dopamine levels and dopamine D1 receptor activation followed by (2) a deactivation of ERK protein because of the decreasing in intracellular calcium via the disruption between NMDA-NR1 and dopamine D1 receptors interactions.

Alternatively, the NMDA-NR1/D1 physical associations may regulate the dopamine D1 receptor surface expression. Recently, NMDA-NR1/D1 oligomerization was also found in cells co-transfected with dopamine D1 and NMDA receptors including NR1/NR2B subunits as well as in the striatal post-synaptic density (PSD; Fiorentini et al., 2003; Fiorentini and Missale, 2004). In the presence of the NMDA-NR2B subunit, the NMDA-NR1/D1 complex was delivered to membrane surface and then prevents the dopamine D1 agonist-induced D1 receptor internalization, suggesting these receptors interactions may represent as a mechanism to recruit dopamine D1 receptor to the sriatal PSD (Fiorentini et al., 2003, Missale et al., 2006). However, in the present study, we demonstrated a

decreasing of NMDA-NR1 and dopamine D1 receptor physical interactions after acute cocaine which is opposite to the finding from Missale's group. In part, two plausible explanations may resolve this discrepancy. First, evidence has shown that dopamine D1 receptor stimulation results in NMDA receptor membrane insertion in the striatum (Dunah and Standaert, 2001; Dunah et al., 2004). It is plausible that through dopamine D1 activation, acute cocaine recruits NMDA receptor to the membrane region. The newly inserted NMDA receptor may not physically associate with dopamine D1 receptor immediately but form receptors interactions at later time point. This temporal change in receptors interactions is consistent with present results in that transient reduction of NMDA-NR1/D1 interactions was observed only 30 min after acute cocaine administration. Secondly, previous *in vivo* study has shown that a single intrastriatal dopamine D1 receptor agonist infusion or amphetamine injection (i.p.) rapidly induced dopamine D1 receptor internalization in the striatum (Dumartin et al., 1998). The modification of dopamine D1 receptor distribution was found in cell bodies and dendrites but not in dendritic spines at the periphery of synaptic clefts, where PSD is located (Dumartin et al., 1998). Thus, after cocaine, the disassociation on NMDA-NR1/D1 physical interactions may indicate dopamine D1 receptor segregation and the reduction of receptor availability for extracellular ligands in a region-specific manner. However, further biochemical fractionation procedure and immunohistochemical labeling at the ultrastructural level should be conducted to examine cocaine-induced dopamine D1 receptor internalization and decreasing of receptors interactions in different neuronal compartments.

In the CPu, we demonstrated that the NMDA-NR1/D1 receptors physical interactions were decreased 30 min after MK-801 administration. Similarly, *in vitro* study showed that NMDA receptor antagonist, AP-5, pre-application results in the disassociation between NMDA-NR1 and dopamine D1 receptors in cells, suggesting that the NMDA receptor activation is necessary to maintain the protein-protein interaction between theses receptors (Pei et al., 2004). In the striatum, a recent study showed that MK-801 (0.1 mg/kg) did not modify NMDA-NR1 and dopamine D1 receptors interactions (Fiorentini et al., 2004). However, a higher dose of MK-801 (0.25 mg/kg) was used in the present study. In addition, previous studies indicated that MK-801 (0.2-0.5 mg/kg) injection induced the elevation of extracellular dopamine levels in the striatum (Mathe et al., 1996; Wedzony et al., 1993; Wolf et al., 1993). Thus, through the augment of synaptic dopamine levels, MK-801 itself may result in the reduction of NMDA-NR1 and dopamine D1 receptors interactions which is similar to the effect obtained from acute cocaine administration. Surprisingly, NMDA treatment also reduced NMDA-NR1/D1 physical interactions, suggesting that the activation of NMDA receptor also disrupts the receptors interaction. In cortical neurons, study has demonstrated that chronic neuronal activity promotes the degradation of NMDA-NR1 through ubiquitin-proteasome system (Ehlers, 2003). However, in the present study, NMDA-NR1 protein levels were not altered after NMDA administration, indicating that the reduction in NMDA-NR1/D1 physical interactions is not due to decreasing of NMDA-NR1 abundance. In hippocampal neurons, PKC-induced rapid NMDA receptors dispersal and lateral mobility between the synaptic and extrasynaptic domains have been

documented (Fong et al., 2002; Groc et al., 2004). NR2B-containing NMDA receptors exhibit faster mobility than those contain NR2A subunit (Groc et al., 2006). In addition, NR2B-containing receptors are prominently expressed in the striatum (Standaert et al., 1994). Thus, through NMDA receptors, the NMDA treatment may induce PKC activation and lateral mobility of NMDA receptors and then contribute to the decrease of NMDA-NR1/D1 physical interactions.

In summary, the present study demonstrated that acute cocaine reduced the NMDA-NR1 and dopamine D1 receptor physical protein-protein interactions in a time-dependent manner. We postulate that the disassociation may regulate cocaine- and/or dopamine-mediated intracellular signaling cascades and dopamine D1 receptor distribution underlying the cocaine-induced behavioral changes. In addition, we also showed that the homeostatic state of NMDA receptor is necessary for the NMDA-NR1/D1 physical interactions, since both NMDA receptor activation and inhibition reduced receptors associations. However, the function significance of their receptors interactions on psychostimulant addiction as well as NMDA receptor- and dopamine D1 receptor-mediated intracellular signaling transduction should be further elucidated in the future.

Chapter 5: Conclusion

The focus of this dissertation was to investigate the dopamine D1 and NMDA receptor-mediated intracellular mechanisms as well as the physical receptors interactions between dopamine D1 and NMDA receptors in response to acute cocaine. Overall, the findings of the present experiments have extended more information and understanding about the cocaine action. In summary, the first study used to address *Aim I* suggested that a single cocaine (30 mg/kg) administration increased ERK-mediated signaling proteins, pp90 ribosomal S6 kinase (RSK) which is the regulator for the phosphoryation of cAMP response element-binding protein (p-CREB) kinase, and c-Fos protein levels in the dorsal striatum/CPu of Fischer rats. Acute cocaine administration also induced phosphorylation of the striatal-enriched protein tyrosine phosphatase (p-STEP) and decreased the phosphorylation of DARPP-32 protein at the Thr-75 site. The phosphorylation states of these inhibitors of ERK and DARPP-32 proteins thus may contribute to the effects of cocaine on ERK- and DARPP-32-mediated cascades, on gene expression and on behaviors.

Previous studies demonstrated that DARPP-32 knockout mice showed attenuated locomotor response after acute cocaine but have higher locomotor sensitization in response to chronic cocaine (Fienberg et al., 1998; Hiroi et al., 1999). In addition, mice with alanine mutations introduced into DARPP-32 at the Thr-34 site have lower acute cocaine-induced locomotor response, cocaine-induced conditioned place preference, higher behavioral sensitization, and less sensitivity in response to cocaine self-administration (Zachariou et al., 2006; Zhang et al., 2006). On the other hand, Thr-75 site mutated mice have lower behavioral sensitization

after chronic cocaine (Zachariou et al., 2006). Taken together, these results suggest that the activation on of Thr-34 and Thr-75 DARPP-32 may mediate different behavioral and rewarding effect of cocaine. Interestingly, previous studies showed that the p-Thr-34 DARPP-32 was increased after acute cocaine administration (D'Addario et al., 2007; Nishi et al., 2000; Rauggi et al., 2005). However, from the cocaine time course experiment, we did not find any change of p-Thr-34 DARPP-32 in the CPu (Fugure 8A). The discrepancy could be due to species/strains differences and experiment manipulations (e.g. vehicle injection before cocaine administration). Alternatively, the acute cocaine-induced p-Thr-34 DARPP-32 may be restricted a specific neuronal population. For example, in the dorsal striatum, two neuronal populations, striatonigral and striatopallidal neurons, mainly contain dopamine D1 or D2 receptors as direct and indirect pathways, respectively (Gerfen et al., 1990; LeMoine and Bloch, 1995). Behaviorally, activation of the direct pathway is related to the facilitation of locomotor behavior. In contrast, the stimulation of the indirect pathway may result in the inhibition of locomotion (Albin et al., 1989). At the molecular level, activation of dopamine D1 receptor couples to Gs proteins lead to the accumulation of cAMP, whereas dopamine D2 receptor activation inhibits cAMP by coupling to Gi proteins suggesting that the two populations have opposing functional properties in cAMP/PKA pathway as well as p-Thr-34 DARPP-32 (Alexander et al., 1986; Fitzgerald and Nestler, 1995; Nishi et al., 1997; Svenningsson et al., 2000a; Lindskog et al., 1999). A recent study further demonstrated that acute cocaine induced p-Thr-34 DARPP-32 in the dopamine D1-containing neurons but has opposite effects on dopamine D2-containing neurons

(Bateup et al., 2008). Thus, in our western blot analysis, it is possible that the lack of change on p-Thr-34 DARPP-32 after acute cocaine is due to the total protein homogenate from both neuronal populations nullifying the dopamine D1 receptor-mediated signaling. On the other hand, after chronic cocaine the increasing sensitivity on dopamine D1 receptor and decreasing sensitivity on D2 receptor may reveal the augmentation of p-Thr-34 DARPP-32. However, further double labeling immunochemistry analysis is needed to test the hypothesis. In addition, via PP-2B activation, evidence demonstrated that glutamate receptor-mediated signaling inhibits the p-Thr-34 DARPP-32 in both dopamine D1 and D2 containing neurons population (Narian et al., 2004). Thus, the glutamate transmission may negatively regulate the p-Thr-34 DARPP-32 in response to acute cocaine.

In addition to the DARPP-32 mediated signaling, the ERK-mediated cascade is also involved in cocaine action. Within this pathway, we first showed that p-RSK protein levels were increased after cocaine, suggesting that ERK protein activation may indirectly regulate the transcription regulator p-CREB. This viewpoint was also supported by recent findings that after acute cocaine, the phosphorylated mitogen- and stress-activated protein kinase, the ERK direct nuclei target, and p-CREB were also increased in an ERK-dependent manner in mice CPu (Brami-Cherrier et al., 2005; Bertran-Gonzalez et al., 2008). However, to the best of our knowledge there is no evidence showing that acute cocaine increases p-CREB in the CPu of rats. An elevation of p-CREB protein levels was only observed in the NAc after acute cocaine injection (Nazarian et al., unpublished observation). Thus, the ERK-modulatory effect on p-CREB should be further elucidated in the CPu.

Since previous literatures demonstrated that both dopamine D1 and NMDA receptors are necessary for cocaine-activated p-ERK (Jenab et al., 2005; Zhang et al., 2004), we further explore their role on immediate early gene (IEG) expression and phosphorylation after acute cocaine administration. In summary, the second study used to address *Aim II* suggested that acute cocaine administration time-dependently increases ERK phosphorylation, c-Fos and FosB protein expression, and MKP-1 phosphorylation (p-MKP-1), in the dorsal stiratum/CPu and ventral striatum/NAc of Fischer rats. In the CPu, one hour after cocaine injection, the increase in c-Fos and FosB proteins expression is totally abolished by pre-administration of dopamine D1 receptor antagonist, SCH23390. In the NAc, SCH23390 also inhibits cocaine-induced c-Fos protein expression. The pre-treatment of NMDA receptor antagonist, MK801, partially reduces cocaine-activated c-Fos protein expression in the CPu. Furthermore, the escalation of p-MKP-1 after acute cocaine administration is dependent on both dopamine D1 and NMDA receptors activation in both brain regions examined. Our data suggest that cocaine may modulate ERK pathway signaling through the activation of dopamine D1 and NMDA receptors, subsequently influencing the IEG protein expression.

The induction of IEG has been implicated in psychostimulant-induced neuronal plasticity and long-term behavioral change. For instance, *c-fos* mutant mice show attenuated behavioral sensitization, dendritic branching, and dendritic spine density in the medium spiny neurons after chronic cocaine administration (Zhang et al., 2006). *FosB* mutant mice demonstrate higher locomotor behavior in response to both acute and chronic cocaine, and cocaine-induced place conditioned place

preference (Hiroi et al., 1997). In the present dissertation, we further demonstrated that both dopamine D1 and NMDA receptors are important for the IEG protein expression in acute cocaine paradigm. Previously, we have showed that acute cocaine-induced p-ERK is dependent on dopamine D1 and NMDA receptors activation (Jenab et al., 2005). Pharmacological inhibition of MEK also attenuates acute cocaine-induced c-Fos and FosB protein expression in the striatum (Radwanska et al., 2006; Guan et al., 2008). Thus, it is possible that both dopamine D1 and NMDA receptors-mediated signaling cascades converge on ERK pathway to elevate the Fos-like protein expression after cocaine. Alternatively, protein kinase B (PKB/Akt) may be a critical mediator responsible for drug-induced neuronal plasticity. Akt is the member of serine/threonine kinase family and a major target of phosphoinosotode 3-kinase (PI3-kinase, Alessi et al., 1997; Burgering and Coffer 1995; Franke et al., 1995). In the striatal neuronal culture, NMDA receptor-induced p-ERK and phosphorylation of Akt (p-Akt) are dependent on PI3-kinase activation (Perkinton et al., 2002). In contrast, in a PI3-kinase insensitive manner, application of MEK inhibitor, U0126, also blocked dopamine D1 receptor agonist-induced p-ERK, p-Akt and p-CREB in striatal cultures (Bram-Cherrier et al., 2002). Furthermore, overexpression of a dominant-negative form of Akt attenuated p-CREB by dopamine D1 agonists. Taken together, these results indicate a complicated crosstalk between Akt and ERK signaling in response to dopamine D1 and NMDA receptor activation. Recent *in vivo* study showed that acute cocaine activated p-Akt in the mice CPu (Bram-Cherrier et al., 2002). In addition, acute amphetamine induced a bi-directional change on Akt across time: a rapid dopamine

D1 receptor-elevated nuclear Akt activity (>30 min) and a late deactivation/decrease of its activity by dopamine D2 receptor at later time points (30-120 min, Beaulieu et al., 2004, 2005; McGinty et al., 2008; Shi and McGinty 2007). Thus, through the change of p-Akt and its potential substrates (e.g., p-CREB), psychostimulants may differently modulate the transcriptional mechanisms. However, whether the same effect could be obtained in cocaine paradigm and its role on cocaine-induced sensitization should be further examined.

In addition to the Fos-like protein, we further investigated the effect of acute cocaine on p-MKP-1, a transcriptional substrate and inhibitor of p-ERK. Forty-five minutes after acute cocaine, p-MKP-1 protein levels were increased by approximately 210% and 85% in the CPu and NAc, respectively (Figure 12). An elevation of p-ERK was also observed during early time points after cocaine (5-15 min, Figure 10). Thus, acute cocaine-induced p-ERK may accumulate p-MKP-1 protein levels and then subsequently inhibit ERK activation as a positive feedback mechanism. However, in contrast to previous study indicating that the MKP-1 mRNA and protein levels were increased after acute methampheatmine administration in dopamine D1 and NMDA receptors dependent manners (Takaki et al., 2001), we did not observe any MKP-1 protein levels change after cocaine. The different pharmacological effects between these drugs (mathamphetamine and cocaine) can in part explain the discrepancy. On the other hand, since MKP-1 is constitutively expressed, it may suggests that the activational effect on MKP-1 is more important than the induction of this IEG in inhibiting ERK activation. Additionally, one hour after acute methamphetamine, an increase in MKP-3 mRNA

expression was detected in the striatum (Takaki et al., 2001). Previous studies have showed that MKP-3 has more selective effects on the inactivation of p-ERK (Muda et al., 1996a, 1996b; Hafen 1998). Thus, whether there is an augmentation of protein expression and phosphorylation of MKP-3 in response to acute cocaine remain to be determined.

The third study used to address *Aim III* revealed that acute cocaine time-dependently reduced the NMDA-NR1 and dopamine D1 receptors physical protein-protein interactions. Since total protein levels of both receptors were not altered, it is unlikely that the reduction is mediated cocaine-induced receptors protein syntheses. Activation of dopamine D1 receptor can potentiate NMDA function and therefore regulate subsequent intracellular signaling (e.g., ERK-mediated signaling). Evidence has shown that tyrosine phosphorylation of NMDA receptor promotes its membrane insertion, which may underlie the dopamine D1 receptor enhancing effect on NMDA receptor-mediated current (Dunah and Standaert 2001). Recently, in the CPu, we also demonstrated that p-STEP protein levels, a potential protein phosphatase on p-ERK, were increased after acute cocaine administration (Sun et al., 2007). Because phosphorylation of STEP results in a decrease of its tyrosine dephosphorylated capability, it is plausible that the elevation of p-STEP can simultaneously enhance the NMDA receptor trafficking/insertion and p-ERK activation during the early stage of acute cocaine (e.g. 10 min after acute cocaine). However, the newly inserted NMDA receptors may not physically interact with dopamine D1 receptors immediately and then result in the decrease of receptors interaction at later time point (e.g. 30 min after acute cocaine).

On the other hand, the reduction of NMDA-NR1 and dopamine D1 receptor physical interactions decreases the NMDA receptor-mediated calcium influx (Lee et al., 2002). Thus, the lack of NMDA-NR1 and dopamine D1 receptor protein-protein physical interactions may contribute to the rapid deactivation of p-ERK after cocaine. However, further studies are needed to investigate the functional significance of NMDA-NR1 and dopamine D1 receptor physical interactions in response to cocaine. Specifically, a functional NMDA receptor requires NR1/NR2 assemblies. Different NR2 subunits confer diverse biophysical and pharmacological properties of NMDA receptors (Cull-Candy and Leszkiewicz 2004). Thus, it is worthy to evaluate the contribution of NR2 subunits on NMDA-NR1/D1 protein-protein interaction and their specific influences on cocaine-mediated signaling.

Based on our finding, we propose that acute cocaine administration alters receptors physical protein-protein interactions and intracellular mechanisms (Figure 19). At the receptor levels, acute cocaine resulted in the reduction of the NMDA-NR1 and dopamine D1 receptors protein-protein interactions, which may have influence on the underlying intracellular signaling cascades mediated by dopamine D1 and NMDA receptors including ERK-phosphorylation pathways. Behaviorally, studies have demonstrated that ERK activation is important for behavioral sensitization and rewarding effects after chronic cocaine (Ferguson et al., 2006; Miller and Marshall, 2005). Furthermore, a prolonged ERK activation was also observed after chronic psychostimulants administration (Berhow and Nestler, 1996; Shi et al., 2007). If NMDA-NR1 and dopamine D1 receptors interactions act as the mechanism in gating ERK activation, intra-striatal infusion of mini-gene interfering

to the receptors interactions C-terminal regions may be a potential therapeutic to abrogate cocaine-induced behavioral change and underlying intracellular signaling cascades. In addition to cocaine, we also demonstrated that pharmacological manipulations of NMDA receptor resulted in the decrease of receptors interactions. Thus, NMDA-NR1 and dopamine D1 receptors interactions are dependent on both receptors activational statuses. It could provide new strategies for the development of bi-functional compounds targeting to both dopamine D1 and NMDA receptors without fully affecting the individual of each receptors, includes different combinations of antagonistic and agonistic properties.

At the intracellular level, we further demonstrated ERK-phosphorylation pathways as well as underlying IEG protein expression/phosphorylation were induced by cocaine in NMDA and dopamine D1 receptors-dependent manners. For DARPP-32 pathway, although we did not find significant changes on p-Thr-34 DARPP-32, the p-Thr-75 DARPP-32 was increased after acute cocaine. This augmentation of p-Thr-75 DARPP-32 may potentiate dopamine D1/PKA signaling and further increase ERK-mediated signaling. Previous studies have indicated that the IEG transcription including c-fos and fosB is necessary for chronic cocaine-induced behavioral and morphological changes (Hiroi et al., 1997; Zhang et al., 2007). Thus, direct manipulation of ERK-mediated signaling transduction could provide another therapeutic opportunity. In particular, the administration of MEK inhibitors or ERK short inhibitory RNA (siRNA) could serve as an approach to dampen cocaine induced transcriptional and behavioral alternations.

In conclusion, the purpose of the research conducted here was to better understand the acute cocaine action from the receptor level to transcriptional mechanisms. Understanding the complicated interactions between the NMDA and dopamine D1 receptors as well as the underlying intracellular mechanisms provides new information about the cocaine-induced behavioral change and neuronal plasticity. Ultimately, the findings of these studies are critical for the scientific community to develop improved approaches in treatments for cocaine abuse.

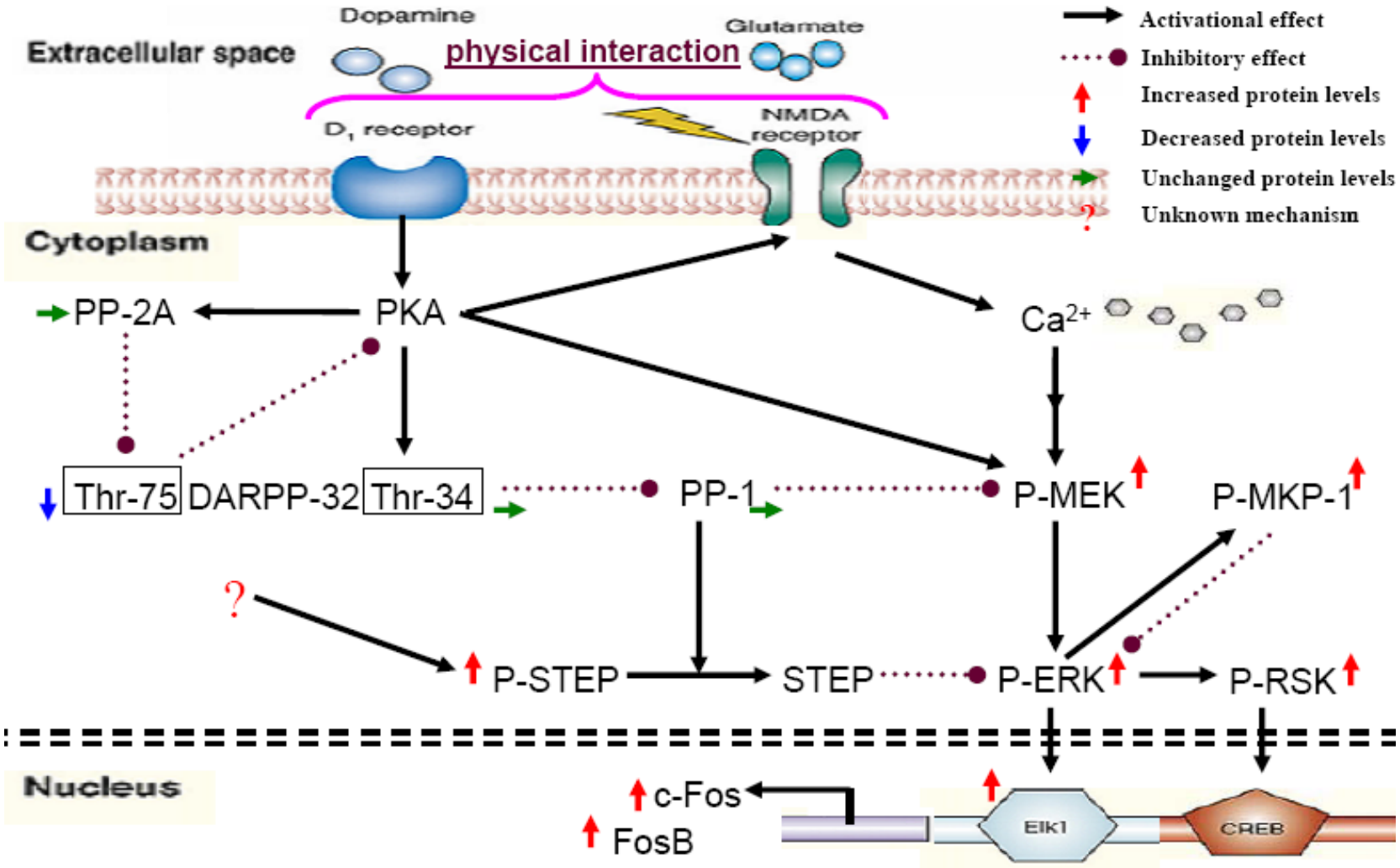


Figure 19. Proposed model of intracellular mechanisms and physical receptors interactions in response to acute cocaine.

References

Ackerman, J.M., White, F.J., 1990. A10 somatodendritic dopamine autoreceptor sensitivity following withdraw from repeated cocaine treatment. Neurosci. Lett. 117, 181-187.

Adams, J.P., Sweatt, J.D., 2002. Molecular psychology: Roles for the ERK MAP kinase cascade in memory. Annu. Rev. Pharmacol. Toxicol. 42, 135-63.

Ahn, J.H., McAvoy, T., Rakhilin, S.V., Nishi, A., Greengard, P., Nairn AC, 2007. Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56{delta} subunit. Proc. Acad. Sci. USA 104, 2979-2984.

Albin, R.L., Young, A.B., Penney, J.B., 1989. The functional anatomy of basal ganglia disorders. Trends. Neurosci. 12, 366-375.

Alburges, M.E., Narang, N., Wamsley, J.K., 1993. Alterations in the dopaminergic receptor system after chronic administration of cocaine. Synapse 14, 314-323.

Alessi, D.R., Cuenda, A., Cohen, P., Dudley, D.T., Saltiel, A.R., 1995. PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase in vitro and in vivo. J. Biol. Chem. 270, 27489-27494.

Alessi, D.R., James, S.R., Downes, C.P., Holmes, A.B., Gaffney, P.R., Reese, C.B., Cohen, P., 1997. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr. Biol. 7, 261-269.

Alessi, D.R., Smythe, C., Keyse, S.M., 1993. The human CL100 gene encodes a Tyr/Thr-protein phosphatase which potently and specifically inactivates MAP kinase and suppresses its activation by oncogenic ras in Xenopus oocyte extracts. Oncogene 8, 2015-2020.

Alexander, G.E., DeLong, M.R., Strick, P.L., 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu. Rev. Neurosci. 9, 357–381.

Alleweireldt, A.T., Hobbs, R.J., Taylor, A.R., Neisewander, J.L., 2006. Effects of SCH-23390 Infused into the Amygdala or Adjacent Cortex and Basal Ganglia on Cocaine Seeking and Self-Administration in Rats. Neuropsychopharmacology 31, 363-374.

Ambrosio, E., Crespo, J.A., 2000. Neuroadaptative changes in dopaminergic and glutamatergic systems during cocaine withdarw. In Recent Advances in Neurobiology of Drug Addiction. Proceedings of 6th Internet World Conference for Biomedical Sciences-Internet Association for Biomedical Sciences (INABIS)-2000. M., Garcia-Rojo, Ed. Presentation 25.

Anderson, S.M., Bari, A.A., Pierce, R.C., 2003. Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. Psychopharmacology 168, 132-138.

Angers, S., Salahpour, A., Bouvier, M., 2004. Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. Annu. Rev. Pharmacol. Toxicol. 42, 409-435.

Arnt, J., 1985. Antistereotypic effects of dopamine D-1 and D-2 antagonists after intrastriatal injection in rats. Pharmacological and regional specificity. Naunyn. Schmiedebergs. Arch. Pharmacol. 330, 97-104.

Ascher, P., Nowak, L., 1987. Electrophysiological studies of NMDA receptors. Trends. Neurosci. 16, 284-287.

Arthur, J.S., Cohen, P., 2000. MSK1 is required for CREB phosphorylation in response to mitogens in mouse embryonic stem cells. FEBS Lett. 482, 44-48.

Baker, D.A., Fuchs, R.A., Specio, S.E., Khroyan, T.V., Neisewander, J.L., 1998. Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. Synapse 30, 181-193.

Bateup, H.S., Svenningsson, P., Kuroiwa, M., Gong, S., Nishi, A., Heintz, N., Greengard, P., 2008. Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. Nat. Neurosci. 11, 932-939.

Beaulieu, J.M., Sotnikova, T.D., Marion, S., Lefkowitz, R.J., Gainetdinov, R.R., Caron, M.G., 2005. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 122, 261-273.

Beaulieu, J.M., Sotnikova, T.D., Yao, W.D., Kockeritz, L., Woodgett, J.R., Gainetdinov, R.R., Caron, M.G., 2004. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. Proc. Natl. Acad. USA 101, 5099-5104.

Berhow, M.T., Hiroi, N., Nestler, E. J., 1996. 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. 16, 4707-4715.

Berke, J.D., Hyman, S.E., 2000. Addition, dopamine and the molecular mechanisms of memory. Neuron 25, 515-532.

Berretta, S., Robertson, H.A., Graybiel, A.M., 1992. Dopamine and glutamate agnosts stimulate neuron-specific expression of Fos-like protein in the striatum. J. Neurophysiol. 68 767-777.

Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Hervé, D., Valjent, E., Girault, J.A., 2008. Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J. Neurosci. 28, 5671-5685.

Bibb, J.A., Snyder, G.L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A.A., Tsai, L.H., Kwon, Y.T., Girault, J.A., Czernik, A.J., Huganir, R.L., Hemmings, H.C. Jr., Nairn, A.C., Greengard, P., 1999. Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. Nature 402, 669-671.

Blank, T., Nijholt, I., Teichert, U., Kugler, H., Behrsing, H., Fienberg, A., Greengard, P., Spiess, J., 1997. The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. Proc. Natl. Acad. Sci. USA 94, 14859-14864.

Blanco-Aparicio, C., Torres, J., Pulido, R., 1999. A novel regulatory mechanism of MAP kinases activation and nuclear translocation mediated by PKA and the PTP-SL tyrosine phosphatase. J. Cell Biol. 147, 1129-1136.

Boulanger, L.M., Lombroso, P.J., Raghunathan, A., During, M.J., Wahle, P., Naegele, J.R., 1995. Cellular and molecular characterization of a brain-enriched protein tyrosine phosphatase. J. Neurosci. 15, 1532-1544.

Brami-Cherrier, K., Valjent, E., Garcia, M., Pagès, C., Hipskind, R.A., Caboche, J., 2005. Dopamine induces a PI3-kinase-independent activation of Akt in striatal neurons: a new route to cAMP response element-binding protein phosphorylation. J. Neurosci. 22, 8911-8921.

Brami-Cherrier, K., Valjent, E., Hervé, D., Darragh, J., Corvol, J.C., Pages, C., Simon, A.J., Girault, J.A., Caboche, J., 2005. Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J. Neurosci. 25, 11444-11454.

Brimecombe, J.C., Boeckman, F.A., Aizenman, E., 1997. Functional consequences of NR2 subunit composition in single recombinant N-methyl-D-aspartate receptors. Proc. Natl. Acad. Sci. USA 94, 11019-11024.

Brondello, J.M., Brunet, A., Pouysségur, J., McKenzie, F.R., 1997. The dual specificity mitogen-activated protein kinase phosphatase-1 and -2 are induced by the p42/p44MAPK cascade. J. Biol. Chem. 272, 1368-1376.

Brondello, J.M., Pouysségur, J., McKenzie, F.R., 1999. Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. Science 286, 2414-2517.

Brown, E.E., Robertson, G.S., Fibiger, H.C., 1992. Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. J. Neurosci. 12, 4112-4121.

Bult, A., Zhao, F., Dirkx, R. Jr., Sharma, E., Lukacsi, E., Solimena, M., Naegele, J.R., Lombroso, P.J., 1996. STEP61: A member of a family of brain-enriched PTPs is localized to the endoplasmic reticulum. J. Neurosci. 16, 7821-7831.

Burgering, B.M., Coffer, P.J., 1995. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature 37, 599-602.

Cabib, S., Castellano, C., Cestari, V., Filibeck, U., Puglisi-Allegra, S., 1991. D1 and D2 receptor antagonists differently affect cocaine-induced locomotor hyperactivity in the mouse. Psychopharmacology (Berl). 105, 335-339.

Caine, S.B., Thomsen, M., Gabriel, K.I., Berkowitz, J.S., Gold, L.H., Koob, G.F., Tonegawa, S., Zhang, J., Xu, M., 2007. Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. J. Neurosci. 27, 13140-13150.

Capper-Loup, C., Canales, J.J., Kadaba, N., Graybiel, A.M., 2002. Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. J. Neurosci. 22, 6128-6627.

Carboni, E., Imperato, A., Perezzani, L., Di Chiara, G., 1989. Amphetamine, cocaine phencyclidine and nomifensin increase extracellular dopamine concentration preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28, 653-661.

Carlezon, Jr. W.A., Thome, J., Olson, V.G., Lane-Ladd, S.B., Brodkin, E.S., Hiroi, N., Duman, R.S., Neve, R.L., Nestler, E.J., 1998. Regulation of cocaine reward by CREB. Science 282, 2272-2275.

Castaneda, E., Becker, J.B., Robinson, T.E., 1988. The long-term effects of repeated amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro. Life Sci. 42, 2447-2456.

Cepeda, C., Colwell, C.S., Itri, J.N., Chandler, S.H., Levine, M.S., 1998. Dopaminergic modulation of NMDA-induced whole cell current in neostriatal neurons in slice: Contribution of calcium conductances. J. Neurophysiol. 79, 82-94.

Cervo, L., Mukherjee, S., Bertaglia, A., Samanin, R., 1997. Protein kinase A and C are involved in the mechanisms underlying consolidation of cocaine place conditioning. Brain Res. 775, 30-36.

Cervo, L., Samanin, R., 1995. Effects of doperminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. Brain Res. 673, 242-250.

Chazot, P.L., Stephenson, F.A., 1997. Biochemical evidence for the existence of a pool of unassembled C2 exon-containing NR1 subunits of mammalian forebrain NMDA receptor. J. Neurochem. 68, 507-516.

Chen, J., Nye, H.E., Kelz, M.B., Hiroi, N., Nakabeppu, Y., Hope, B.T., Nestler, E.J., 1995. Regulation of delta FosB and FosB-like proteins by electroconvulsive seizure and cocaine treatments. Mol. Pharmacol. 48, 880-889.

Chen, R.H., Abate, C., Blenis, J., 1993. Phosphorylation of the c-Fos transrepression domain by mitogen-activated protein kinase and 90-kDa ribosomal S6 kinase. Proc. Acad. Sci. USA 90, 10952-10956.

Chen, R.H., Juo, P.C., Curran, T., Blenis, J., 1996. Phosphorylation of c-Fos at the C-terminus enhances its transforming activity. Oncogene 12, 1493-1502.

Chen, R.H., Sarnecki, C., Blenis, J., 1992. Nuclear localization and regulation of erk- and rsk-encoded protein kinases. Mol. Cell Biol. 12, 915-927.

Choe, E.S., McGinty, J.F., 2000. N-methyl-D-aspartate receptors and p38-activated protein kinase are requires for cAMP-dependent cyclase response element binding and Elk-1 phophorylation in the striatum. Neuroscience 101, 607-617.

Choe, E.S., McGinty, J.F., 2001. Cyclic AMP and mitogen-activated protein kinases are required for glutamate-dependent cyclic AMP response element binding protein and Elk-1 phosphorylation in the dorsal striatum in vivo. J. Neurochem. 7, 401-412.

Christie, M.J., Summers, R.J., Stephenson, J.A., Cook, C.J., Beart, P.M., 1987. Excitatory amino acid projections to nucleus accumbens septi in the rat: a retrograde transport study utilizing D [3H]asparatate and [3H]GABA. Neuroscience. 22, 425-239.

Church, W.H., Justice, J.B. Jr., Byrd, L.D., 1987. Extracellular dopamine in rat striatum following uptake inhibition by cocaine, nomifensine and benztropine. Eur. J. Pharmacol. 139, 345-348.

Churchill, L., Swanson, C.J., Urbina, M., Kalivas, P.W., 1999. Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that development behavioral sensitization. J. Neurochem. 72, 2397-2403.

Civelli, O., Bunzow, J.R., Grandy, G.K., 1993. Molecular diversity of dopamine receptors. Annu. Rev. Pharmacol. Toxicol. 32, 281-307.

Cole, R.L., Konradi, C., Douglass, J., Hyman, S.E., 1995. Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. Neuron 14, 513-823.

Corbillé, A.G., Valjent, E., Marsicano, G., Ledent, C., Lutz, B., Hervé, D., Girault, J.A., 2007. Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. J. Neurosci. 27, 6937-6947.

Crespo, J.A., Oliva, J.M., Ghasemzadeh, M.B., Kalivas, P.W., Ambrosio, E., 2002. Neuroadaptative changes in NMDAR1 gene expression after extinction of cocaine self-administration. Ann. N.Y. Acad. Sci. 9656, 78-91.

Crombag, H.S., Jedynak, J.P., Redmond, K., Robinson, T.E., Hope, B.T., 2002. Locomotor sensitization to cocaine is associated with increased Fos expression in the accumbens, but not in the caudate. Behav. Brain Res. 136, 455-462.

Cull-Candy, S.G., Leszkiewicz, D.N., 2004. Role of distinct NMDA receptor subtypes at central synapse. Sci. STKE re 16.

D'Addario, C., Di Benedetto, M., Candeletti, S., Romualdi, P., 2007. The kappa-opioid receptor agonist U-69593 prevents cocaine-induced phosphorylation of DARPP-32 at Thr(34) in the rat brain. Brain Res. Bull. 73, 34-39.

Dalia, A., Wallace, L.J., 1995. Amphetamine induction of c-fos in the nucleus accumbens is not inhibited by glutamate antagonists. Brain Res. 694, 299-307.

Das, S., Grunert, M., Williams, L., Vincent, S.R., 1997. NMDA and D1 receptors regulate the phosphorylation of CREB and the induction of c-fos in striatal neurons in primary culture. Synapse 25, 227-233.

Dash, P.K., Karl, K.A., Colicos, M.A., Prywes, R., Kandel, E.R., 1991. cAMP response element-binding protein is activated by Ca2+/calmodulin-as well as cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA 88, 5061-5065.

Davis, S., Vanhoutte, P., Pages, C., Caboche, J., Laroche, S., 2000. The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. J. Neurosci. 20, 4563-4572.

Dawson, T.M., Barone, P., Sidhu, A., Wamsley, J.K., Chase, T.N., 1998. The D1 dopamine receptor in the rat brain: Quantitive autoradiographic localization using an iodinated ligand. Neuroscience 26, 83-100.

De Cesare, D., Jacquot, S., Hanauer, A., Sassone-Corsi, P., 1998. Rsk-2 activity is necessary for epidermal growth factor-induced phosphorylation of CREB protein and transcription of c-fos gene. Proc. Acad. Sci. USA 95, 12202-12207.

De Leonibus, E., Mele, A., Oliverio, A., Pert, A., 2002. Distinct pattern of c-fos mRNA expression after systemic and intra-accumbens amphetamine and MK-801. Neuroscience 115, 67-78.

De Montis, M.G., Devoto, P., Meloni, D., Gambarna, C., Giogi, G., Tanliamonte, A., 1992. NMDA receptor inhibition prevents tolerance to cocaine. Pharmacol. Biochem. Behav. 42, 179-182.

De Vries, T.J., Cools, A.R., Shippenberg, T.S., 1998. Infusion of a D-1 receptor agonist into the nucleus accumbens enhances cocaine-induced behavioural sensitization. Neuroreport 9, 1763-1768.

Dearry, A., Gigrich, J.A., Falardeau, P., Fremeau, R.T., Bates, M.D., Caron, M.G., 1990. Molecular cloning and expression of the gene of a human D1 dopamine receptor. Nature 347, 72-76.

Delf, J.M., Schreiber, L., Kelly, A.E., 1990. Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J. Neurosci. 10, 303-310.

Devi, L.A., 2000. G-protein-coupled receptor dimers in the lime light. Trends Pharmacol. Sci. 21, 324-326.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85, 5274-5278.

Drago, J., Gerfen, C.R., Westphal, H., Steiner, H., 1996. D1 dopamine receptor-deficient mouse: Cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. Neuroscience 74, 813-823.

Dragunow, M., Faull, R.L., 1990. MK801 induces c-fos protein in the thalamic and neocortical neurons of rat brain. Neurosci. Lett. 113, 144-150.

Dudman, J.T., Eaton, M.E., Rajahyaksha, A., Macias, W., Tacher, M., Barzak A., Kameyama, K., Huganir, R., Konradi, C., 2003. Dopamine D1 receptors mediate CREM phosphrylation via phorphorylation of the NMDA receptor at Ser897-NR1. J. Neurochem. 87, 922-934.

Duff, J.L., Monia, B.P., Berk, B.C., 1995. Mitogen-activated protein (MAP) kinase is regulated by the MAP kinase phosphatase (MKP-1) in vascular smooth muscle cells. Effect of actinomycin D and antisense oligonucleotides. J. Biol. Chem. 270, 7161-7166.

Dumartin, B., Caillé, I., Gonon, F., Bloch, B., 1998. Internalization of D1 dopamine receptor in striatal neurons in vivo as evidence of activation by dopamine agonists. J. Neurosci. 18, 1650-1661.

Dunah, A.W., Standaert, D.G., 2001. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. J. Neurosci. 21, 5546-5558.

Dunah, A.W., Sirianni, A.C., Fienberg, A.A., Bastia, E., Schwarzschild, M.A., Standaert, D.G., 2004. Dopamine D1-dependent trafficking of striatal N-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. Mol. Pharmacol. 65, 121-129.

Eaton, M.E., Macias, W., Youngs, R.M., Rajadhyaksha, A., Dudman, J.T., Konradi, C., 2004. L-type Ca2+ channel blockers promote Ca^{2+} accumulation when dopamine receptors are activated in striatal neurons. Mol. Brain Res. 131, 65-72.

Ehlers, M.D., 2003. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. Nat. Neurosci. 6, 231-242.

Ferguson, S.M., Fasano, S., Yang, P., Brambilla, R., Robinson, T.E., 2006. Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. Neuropsychopharmacology 31, 2660-2668.

Flores-Hernandez, J., Cepeda, C., Hernandez-Echeagaray, E., Calvert, C.R., Jokel, E.S., Fienberg, A.A., Greengard, P., Levine, M.S., 2002. Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: Role of D1 receptors and DARPP-32. J. Neurophysol. 88, 3010-3020.

Fienberg, A.A, Hiroi, N., Mermelstein, P.G., Song, W., Snyder, G.L., Nishi, A., Cheramy, A., O'Callaghan, J.P., Miller, D.B., Cole, D.G., Corbett, R., Haile, C.N., Cooper, D.C., Onn, S.P., Grace, A.A., Ouimet, C.C., White, F.J., Hyman, S.E., Surmeier, D.J., Girault, J., Nestler, E.J., Greengard, P., 1998. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science 281, 838-842.

Fiorentini, C., Gardoni, F., Spano, P., Di Luca, M., Missale, C., 2003. Regulation of dopamine D1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors. J. Biol. Chem. 278, 20196-20202.

Fiorentini, C., Missale, C., 2004. Oligomeric assembly of dopamine D1 and glutamate NMDA receptors: molecular mechanisms and functional implications. Biochem. Soc. Trans. 32, 1025-1028.

Fischer, R., Julsgart, J., Berchtold, M.W., 1998. High affinity calmodulin target sequence in the signalling molecule PI 3-kinase. FEBS Lett. 425, 175-177.

Fitzgerald, L.W., Nestler, E.J., Cocaine regulation of signal transduction pathways. In: Hammer R, ed. Neurobiology of cocaine: molecular and cellular aspects. Boca Raton, FL: CRC; 1995: 219-240

Fitzgerald, L.W., Ortiz, J., Hamedani, A.G., Nestler, E.J., 1996. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: Common adaptation among cross-sensitizing agents. J. Neurosci. 16, 274-282.

Fong, D.K., Rao, A., Crump, F.T., Craig, A.M., 2002. Rapid synaptic remodeling by protein kinase C: Reciprocal translocation of NMDA receptors and calcium/calmodulin dependent kinase II. J. Neurosci. 22, 2153-2164.

Frank, D.A., Greenberg, M.E., 1994. CREB: A mediator of long-term memory from mollusks to mammals. Cell 79, 5-8.

Franke, T.F., Yang, S.I., Chan, T.O., Datta, K., Kazlauskas, A., Morrison, D.K., Kaplan, D.R., Tsichlis, P.N., 1995. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. Cell 81, 727-736.

Fremeau, R.J., Duncan, G.E., Fornaretto, M.G., Dearry, A., Gigrich, J.A., Breese, G.R., Caron, M.G., 1991. Localization of D1 receptor mRNA in brain supports a role in cognitive, affective and neuroendorine aspects of dopaminergic neurotransmission. Proc. Natl. Acad. Sci. USA 91, 12564-12568.

Frodin, M., Gammeltoft, S., 1999. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol. Cell. Endocrinol. 151, 65-77.

Gerfen, C.R., Enber, T.M., Manhan, L.C., Susel, Z., Chase, T.N., Monsma, Jr. F.J., Sibley, D.R., 1990. D1 and D2 dopamine receptor-regulated gene expression of stiratonigral and striatopallidal neurons. Science 250, 1429-1432.

Gerfen, C.R., Miyachi, S., Paletzki, R., Brown, P., 2002. D1 dopamine receptor supersentivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J. Neurosci. 22, 5042-5054.

Gerfan, C.R., Wilson, C.J., 1996. The basal ganglia. In handbook of chemical neuroanatomy: integrated systems of the CNS (eds Swason, L.W., Bjorklund, A., and Hokfelt, T.), Vol. 12 Part Ⅲ. Elsevier, Amesterdam.

Ghasemzadeh, M.B., Nelson, L.C., Lu, X.Y., Kalivas, P.W., 1999. Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. J. Neurochem. 72, 157-165.

Gines, S., Hillion, J., Torvinen, M., Le Crom, S., Casado, V., Canela, E.I., Rondin, S., Lew, J.Y, Watson, S., Zoli, M., Agnati, L.F., Verniera, P., Lluis, C., Ferre, S., Fuxe, K.,

Franco, R., 2000. Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. Proc. Natl. Acad. Sci. USA 97, 8606-8611.

Gille, H., Sharrocks, A.D., Shaw, P.E., 1992. Phosphorylation of transcription factor p62TCF by MAP kinase stimulates ternary complex formation at c-fos promoter. Nature 358, 414-417.

Gille, H., Kortenjann, M., Thomae, O., Moomaw, C., Slaughter, C., Cobb, M.H., Shaw, P.E., 1995a. ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation. EMBO J. 14, 951-962.

Gille, H., Sharrocks, A.D., Shaw, P.E., 1992. Phosphorylation of transcription factor p62TCF by MAP kinase stimulates ternary complex formation at c-fos promoter. Nature 358, 414-417.

Gille, H., Strahl, T., Shaw, P.E., 1995b. Activation of ternary complex factor Elk-1 by stress-activated protein kinases. Curr Biol. 5, 1191-1200.

Gingrich, J.A., Caron, M.G., 1993. Recent advances in the molecular biology of dopamine receptors. Annu. Rev. Neurosci. 16, 299-231.

Girault, J.A., Valjent, E., Caboche, J., Herve, D., 2006. ERK2: A logical AND gate critical for drug-induced plasticity? Curr. Opin. Pharmacol. 7, 77-85.

Goldstein, R.A., Volkow, N.D., 2002. Drug addition and its underlying neurobiological basis: Neuroimaging evidence for the involvement of the frontal cortex. Am. J. Psychiatry 159, 1642-1652.

Gorelova, N., Yang, C.R., 1997. The course of neural projection from the prefrontal cortex to the nucleus accumbens in the rat. Neuroscience 76, 689-706.

Grabus, S.D., Glowa, J.R., Riley, A.L., 2004. Morphine- and cocaine-induced c-Fos levels in Lewis and Fisher rat strains. Brain Res. 998, 20-28.

Graybial, A.M., Moratalla, R., Robertson, H.A., 1990. Amphetamine and cocaine induced drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc. Natl. Acad. Sci. USA 87, 6912-6916.

Greengard, P., 2001. The neubiology of slow synaptic transmission. Science 294, 1024-1030.

Greengard, P., Allen, P.B., Nairn, A.C., 1999. Beyond the dopamine receptor: The DARPP-32/protein phosphatase-1 cascade. Neuron 23, 435-477.

Grewal, S.S., Horgan, A.M., York, R.D., Withers, G.S., Banker, G.A., Stork, P.J.S., 2000. Neuronal calcium activates a Rap1 and B-Raf signaling pathway via the cyclic adenosine monophosphate-dependent protein kinase. J. Biol. Chem. 275, 3722-3728.

Groc, L., Heine, M., Cognet, L., Brickley, K., Stephenson, F.A., Lounis, B., Choquet, D., 2004. Differential activity-dependent requlation of lateral mobilities of AMPA and NMDA receptors. Nat. Neurosci. 7, 695-696.

Groc, L., Heine, M., Cousins, S.L., Stephenson, F.A., Lounis, B., Cognet, L., Choquet, D., 2006. NMDA receptor surface mobility depends on NR2A-2B subunits. Proc. Natl. Acad. Sci. USA. 103, 18769-18774.

Groenewegen, H.J., Vermeulen-Van der Zee, A., te Kortschot, A., Witter, M.P., 1987. Organization of the projections from subiculum to ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris-leucoagglutinin. Neuroscience 23, 103-112.

Groenewegen, H.J., Wright, C.I., Beijer, V.J., 1996. The nucleus accumbens: Gateway for limbic structures to reach the motor system? Prog. Brain Res. 107, 485-551.

Grosshans, D.R., Clayton, D.A., Coultrap, S.J., Browing, M.D., 2002. LTP leads to rapid surface expression NMDA but no AMPA receptors in adult rat CA1. Nat. Neurosci. 5, 27-33.

Guan, X., Hu, J., Li, S., 2008. Involvement of extracellular signal-regulated protein kinase in acute cocaine-induced c-fos in nucleus accumbens. Neurosci. Lett. 438, 155-158.

Gupta, A., Tsai, L.H., 2001. A kinase to dampen the effects of cociane? Science 292, 236-237.

Hafen, E., 1998. Kinases and phosphatases - a marriage is consummated. Science 280, 1212-1213.

Hai, T., Curran, T., 1991. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. USA 88, 3720-3724.

Halpain, S., Girault, J.A., Greengard, P., 1990. Activation of NMDA receptors induces dephosphorylation of DARPP-32 in rat striatal slices. Nature 343, 369-372.

Haracz, J.L., Belanger, S.A., MacDonall, J.S., Sircar, R., 1995. Antagonists of N-Methyl-D-Asparate receptors partially prevent the development of cocaine sensitization. Life Sci. 57, 2347-2357.

Hemmings, H.C., Greengard, P., Tung, H.Y., Cohen, P., 1984. DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. Nature 310, 503-505.

Henry, D.J., Greene, M.A., White, F.J., 1989. Electrophysiological effects of cocaine in mesoaccumbens dopamine system: repeated administration. J. Pharmacol. Exp. Ther. 251, 833-839.

Henry, D.J., Hu, X.T., White, F.J., 1998. Adaptations in mesoaccumbens dopamine system resulting from repeated administration of dopamine D1 and D2 receptor-selective agonist: Relevance to cocaine sensitization. Psychopharmacology 140, 233-242.

Henry, D.J., White, F.J., 1991. Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor sensitivity within rat nucleus accumbens. J. Exp. Ther. 258, 882-890.

Henry, D.J., White, F.J., 1995. The persistence of behavioral sensitization to cocaine parallels enhanced inhibition of nucleus accumbens neurons. J. Neurosci. 15, 6287-6299.

Herve, D., Levi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J.P., Glowinski, J., Girault J.A., 1993. Golf and Gs in rat basal ganglia: possible involvement of Golf in the coupling of dopamine D1 receptor with adenylyl cyclase. J. Neurosci. 13, 2237-2248.

Heusner, C.L., Palmiter, R.D., 2005. Expression of mutant NMDA receptors in dopamine D1 receptor-containing cells prevents cocaine sensitization and decreases cocaine preference. J. Neurosci. 25, 6651-6657.

Hill, C.S., Marais, R., John, S., Wynne, J., Dalton, S., Treisman, R., 1993. Functional analysis of a growth factor-responsive transcription factor complex. Cell 73, 395-406.

Hiroi, N., Brown J.R., Haile, C.N., Ye, H., Greenberg, M.E., Nestler, E.J., 1997. FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. Proc. Acad. Sci. USA 94, 10397-10402.

Hiroi, N., Fienberg, A.A., Haile, C.N., Alburges, M., Hanson, G.R., Greengard, P., Nestler, E.J., 1999. Neuronal and behavioural abnormalities in striatal function in DARPP-32-mutant mice. Eur. J. Neurosci. 11, 1114-1118.

Hisatsune, C., Umemori, H., Inoue, T., Michikawa, T., Kohda, K., Mikoshiba, K., Yamamoto, T., 1997. Phosphorylation-dependent regulation of N-methyl-D-aspartate receptors by calmodulin. J. Biol. Chem. 272, 20805-20810.

Hollmann, M., Boulter, J., Maron, C., Beasley, L., Sullivan, J., Pecht, G., Heinemann, S., 1993. Zinc potentiates agonist-induced currents at certain splice variants of NMDA receptor. Neuron 10, 943-954.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31-108.

Hope, B., Kosofsky, B., Hyman, S.E., Nestler, E.J., 1992. Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc. Acad. Sci. USA 89, 5764-5768.

Hope, B.T., Crombag, H.S., Jedynak, J.P., Wise, R.A., 2005. Neuroadaptations of total levels of adenylate cyclase, protein kinase A, tyrosine hydroxylase, cdk5 and neurofilaments in the nucleus accumbens and ventral tegmental area do not correlate with expression of sensitized or tolerant locomotor responses to cocaine. J. Neurochem. 92, 536-545.

Hubner, C.B., Moreton, J.E., 1991. Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology 105, 151-156.

Hurd, Y.L., Mcgregor, A., Ponten, M., 1997. In vivo amygdala dopamine levels modulate cocaine self-administration behaviour in the rat: D1 dopamine receptor involvement. Eur. J. Neurosci. 9, 2541-2548.

Hyman, S.E., Malenka, R.C., 2001. Addiction and the brain: The neurobiology of compulsion and its persistence. Nat. Rev. Neurosci. 10, 695-703.

Hyman, S.E., Malenka, R.C., Nestler, E.J., 2006. Neural mechanisms of addiction: The role of reward-related learning and memory. Annu. Rev. Neurosci. 29, 565-598.

Impey, S., Obrietan, K., Wong, S.T., Poser, S., Yano, S., Wayman, G., Deloulme, J.C., Chan, G., Storm, D.R., 1998. Cross talk between ERK and PKA is required for $Ca2^{+}$ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21, 869-883.

Itzhak, Y., Stein, I., 1992. Sensitization to the toxic effect of cocaine in mice is associated with the regulation of N-methyl-D-aspartate receptors in the cortex. J Pharmacol. Exp. Ther. 262, 464-470.

Jenab, S., Festa, E.D., Nazarian, A., Wu, H.B., Sun, W.L., Hazim, R., Russo, S.J., Quinones-Jenab, V., 2005. Cocaine induction of ERK proteins in dorsal striatum of Fischer rats. Brain Res. Mol. Brain Res. 142, 134-138.

Jenab, S., Festa, E.D., Russo, S.J., Wu, H.B., Inturrisi, C.E., Quinones-Jenab, V., 2003. MK-801 attenuates cocaine induction of *c-fos* and preprodynorphin mRNA levels in Fischer rats. Brain Res. Mol. Brain Res. 117, 237-239.

Johansson, B., Lindstrom, K., Fredholm, B.B., 1994. Differences in the regional and cellular localization of c-fos messenger RNA induced by amphetamine, cocaine and caffeine in the rat. Neuroscience 59, 837-849.

Joyal, J.L., Burks, D.J., Pons, S., Matter, W.F., Vlahos, C.J., White, M.F., Sacks, D.B., 1997. Calmodulin activates phosphatidylinositol 3-kinase. J. Biol. Chem. 272, 28183-28186.

Kaddis, F.G., Wallace, L.J., Uretsky, N.J., 1993. AMPA/kainate antagonists in the nucleus accumbens inhibit locomotor stimulatory response to cocaine and dopamine agonists. Pharmacol. Biochem. Behav. 46, 703-708.

Kalivas, P.W., 2004. Glutamate systems in cocaine addiction. Curr. Opin. Pharmacol. 4, 23-29.

Kalivas, P.W., Duffy P., 1995. D1 receptors modulate glutamate transmission in the ventral tegmental area. J. Neurosci. 15, 5379-5388.

Kalivas, P.W., Duffy, P., 1998. Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. J. Neurochem. 70, 1497-1502.

Kano, T., Suzuki, Y., Shibuya, M., Kiuchi, K., Hagiwara, M., 1995. Cocaine-induced CREB phosphorylation and c-Fos expression are suppressed in Parkinsonism model mice. Neuroreport 6, 2197-2200.

Karasinska, J.M., George, S.R., Cheng, R., O'Dowd, B.F., 2005. Deletion of dopamine D1 and D3 receptors differentially affects spontaneous behaviour and cocaine-induced locomotor activity, reward and CREB phosphorylation. Eur. J. Neurosci. 22, 1741-50.

Karler, R., Calder, L.D., Chaudhry, I.A., Turkanis, S.A., 1989. Blockade of "reverse tolerance" to cocaine and amphetamine by MK801. Life Sci. 46, 599-606.

Kelley, A.E., Domesick, V.B., Nauta, W.J.H., 1982. The amygdalostriatal projection in the rat – an anatomical study by anterograde and retrograde tracing methods. Neuroscience 7, 615-630.

Kelly, P.H, Iversen, S.D., 1976. Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in the rat. Eur. J. Pharmacol. 40, 45-56.

Kelly, P.H., Seviour, P.W., Iversen, S.D., 1975. Amphetamine and apomorphine responses in rat following 6-OHDA lesions of nucleus accumbens septi and corpus striatum. Brain Res. 94, 507-523.

Khan, M.A., Shoaib, M., 1996. Neuroanatomical localization of the effects of (+)-HA966 on locomotor activity after cocaine injection to the nucleus accumbens of rats. Brain Res. 719, 198-202.

Kim, H.K., Park, W.K., Jang, C.G., Oh, S., 1996. Inhibition by MK-801 of cocaine-induced sensitization, conditioned place preference, and dopamine-receptor supersensitivity in mice. Brain Res. Bull. 40, 201-207.

King, M.M., Huang, C.Y., Chock, P.B., Nairn, A.C., Hemmings, Jr. H.C., Chan, K.F., Greengard, P., 1984. Mammalian brain phosphoproteins as substrates for calcineurin. J. Biol. Chem. 259, 8080-8083.

Kleven, M.S., Perry, B.D., Woolverton, W.L., Seiden, L.S., 1990. Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. Brain Res. 532, 265-270.

Kolta, M.G., Shreve, P., De Souza, V., Uretsky, N.J., 1985. Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. Neuropharmacology 24, 823-829.

Konradi, C., Leveque, J.C., Hyman, S.E., 1996. Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. J. Neurosci. 16, 4231-4239.

Koob, G.F., 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13, 177-184.

Koob, G.F., Le, H.T., and Creese, I., 1987. The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci. Lett. 79, 315-320.

Koob, G.F., Nestler, E. J., 1997. The neurobiology of drug addiction. J. Neuropsychiatry Clin. Neurosci. 9, 482-497.

Kosten, T.A., Zhang, X.Y., Haile, C.N., 2007. Strain Differences in maintenance of cocaine self-administration and their relationship to novelty activity responses. Behav. Neurosci. 121, 380-388.

Kreipke, C.W., Walker, P.D., 2004. NMDA receptors blockade attenuates locomotion elicited by intrastriatal dopamine D1-receptor stimulation. Synapse 53, 28-35.

Krupp, J.J., Vissel, B., Heinemann, S.F., Westbrook, G.L., 1996. Calcium-dependent inactivation of recombinant N-methyl-D-aspartate receptors is NR2 subunit specific. Mol. Pharmacol. 50, 1680-1688.

Kuczenski, D., Segel, D.S., Aizenstein, M.L., 1991. Amphetamine, cocaine and fencafamine: relationship between locomotor and stereotype response profiles and caudate and accumbens dopamine dynamics. J. Neurosci. 11, 2703-2712.

Kumar, A., Choi, K.H., Renthal, W., Tsankova, N.M., Theobald, D.E., Truong, H.T., Russo, S.J., Laplant, Q., Sasaki, T.S., Whistler, K.N., Neve, R.L., Self, D.W., Nestler, E.J., 2005. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48, 303-314.

Kunko, P.M., French, D., Izenwasser, S., 1998. Alternations in locomotor activity during chronic cocaine administration: Effects on dopamine receptors and interactions with opioids. J. Pharmacol. Exp. Ther. 285, 277-284.

Kurino, M., Fukunaga, K., Ushio, Y., Miyamoto, E., 1995. Activation of mitogen-activated protein kinase in cultured rat hippocampal neurons by stimulation of glutamate receptors. J. Neurochem. 65, 1282-1289.

Laurier, L.G., Corrigall, W.A., George, S.R., 1994. Dopamine receptor density, sensitivity, and mRNA levels are altered following self-administration of cocaine in the rat. Brain Res. 634, 31-40.

Lazar, D.F., Wiese, R.J., Brady, M.J., Mastick, C.C., Waters, S.B., Yamauchi, K., Pessin, J.E., Cuatrecasas, P., Saltiel, A.R., 1995. Mitogen-activated protein kinase kinase inhibition does not block the stimulation of glucose utilization by insulin. J. Biol. Chem. 270, 20801-20807.

Le Moine, C., Bloch, B., 1995. D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J. Comp. Neurol. 355, 418–426.

Lee, D.K., Bian, S., Rahman, M.A., Shim, Y.B., Shim, I., Choe, E.S., 2008. Repeated cocaine administration increases N-methyl-D-asparate NR-1 subunit, extracellular signal-regulated kinase and cyclic AMP response element-binding protein phosphorylation and glutamate release in the rat dorsal striatum. Eur. J. Pharmacol. 590, 157-162.

Lee, F. J., Liu, F., 2004. Direct interactions between NMDA and D1 receptors: A tale of tails. Biochem. Soc. Trans. 32, 1032-1036.

Lee, F. J., Xue, S., Pei, L., Vukusic, B., Chery, N., Wang, Y., Wang, Y.T., Niznik, H.B., Yu, X.M., Liu, F., 2002. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 111, 219-230.

Leveque, J.C., Macias, W., Rajahyaksha, A., Carlson, R.R., Barczak, A., Kang, I.S., Li, X.M., Coyle, J.T., Huganir, R., Heckers, S., Konradi, C., 2000. Intracellular modulation of NMDA receptor function by antipsychotic drugs. J. Neurosci. 20, 807-820.

Li, Y., Hu, X.T., Berney, T.G., Vartanian, A.J., Stien, C.D., Wolf, M.E., White, F.J., 1999. Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. Synapse 34, 169-180.

Li, Y., Wolf, M.E., 1997. Ibotenic acid lesions of prefrontal cortex do not prevent expression of behavioral sensitization to amphetamine. Behav. Brain Res. 84, 285-289.

Lindskog, M., Svenningsson, P., Fredholm, B.B., Greengard, P., Fisone, G., 1999. Activation ofdopamin e D2 receptors decreases DARPP-32 phosphorylation in striatonigral and striatopallidal projection neurons via different mechanisms. Neuroscience 88, 1005–1008.

Liu, F., Wan, Q., Pristupa, Z.B., Yu, X.M., Wang, Y.T., Niznik, H.B., 2000. Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 403, 274-280.

Liu, J., Nickolenko, J., Sharp, F.R., 1994. Morphine induces c-fos and junB in striatum and nucleus accumbens via D1 and N-methyl-D-aspartate receptors. . Proc. Acad. Sci. USA 91, 8537-8541.

Loftis, J.M., Janowsky, A., 2002. Cocaine treatment-and withdraw-induced alternations in the expression and serine phosporylation of the NR1 NMDA receptor subunit. Psychopharmacology 164, 349-359.

Lombroso, P.J., Naegele, J.R., Sharma, E., Lerner, M., 1993. A protein tyrosine phosphatase expressed within dopaminoceptive neurons of the basal ganglia and related structures. J. Neurosci.13, 3064-3074.

Lu, L., Dempsey, J., Shaham, Y., Hope, B.T., 2005a. Differential long-term neuroadaptations of glutamate receptors in basolateral and central amygdala after withdrawal for cocaine self-administration in rtas. J. Neurochem. 94, 161-168.

Lu, L., Grimm, J.W., Shaham, Y., Hope, T.B., 2003. Molecular neuroadaptations in the nucleus accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. J. Neurochem. 85, 1604-1613.

Lu, L., Hope, B.T., Dempsey, J., Liu, S.Y., Bosset, J.M., Shaham, Y., 2005b. Central amygdala ERK signaling is critical to incubation of cocaine craving. Nat. Neurosci. 8, 212-219.

Lu, L., Koya, E., Zhai, H., Hope, B.T., Shaham, Y., 2006. Role of ERK in cocaine addiction. Trends Neurosci. 29, 695-703.

MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J., Barker, J.L., 1986. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurons. Nature 321, 519-522.

Maisonneuve, I.M., Glick, S.D., 1992. Interactions between ibogaine and cocaine in rats: in vivo microdialysis and motor behavior. Eur. J. Pharmacol. 212, 263-266.

Maisonneuve, I.M., Ho, A., Kreek, M.J., 1995. Chronic administration of a cocaine "binge" alters basal extracellular levels in male rats: An in vivo microdialysys study. J. Pharmacol. Exp. Ther. 272, 652-657.

Maisonneuve, I.M., Kreek, M.J., 1994. Acute tolerance to the dopamine response induced by a "binge" pattern of cocaine administration in male rats: An in vivo microdialysis study. J. Pharmacol. Exp. Ther. 268, 916-921.

Mao, L., Tang, Q., Samdani, S., Liu, Z., Wang, J.Q., 2004. Regulation of MAPK/ERK phosphorylation via ionotropic glutamate receptors in cultured rat striatal neurons. Eur. J. Neurosci. 19, 1207-1216.

Mathe, J.M., Nomikos, G.G., Hildebrand, B.E., Hertel, P., Svensson, T.H., 1996. Prazosin inhibits MK-801-induced hyperlocomotion and dopamine release in the nucleus accumbens. Eur. J. Pharmacol. 309, 1-11.

Mattson, B.J., Bossert, J.M., Simmons, D.E., Nozaki, N., Nagarkar, D., Kreuter, J.D., Hope, B.T., 2005. Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. J. Neurochem. 95, 1481-94.

Mayr, B., Montminy, M., 2001. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat. Rev. Mol. Cell Biol. 2, 599-609.

Mazzucchelli, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W., Welzl, H., Wolfer, D.P., Pages, G., Valverde, O., Marowsky, A., Porrazzo, A., Orban, P. C., Maldonado, R., Ehrengruber, M.U., Cestari, V., Lipp, H.P., Chapman, P.F., Pouyssegur, J., Brambilla, R., 2002. Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. Neuron 34, 807-820.

McBain, C.J., Mayer, M.L., 1994. N-Methyl-D-aspartic acid receptor structure and function. Physio. Rev. 74, 723-760.

McCreary, A.C., Marsden, C.A., 1993. Cocaine-induced behaviour: dopamine D1 receptor antagonism by SCH 23390 prevents expression of conditioned sensitisation following repeated administration of cocaine. Neuropharmacology 32, 387-391.

McGinty, J.F., Shi, X.D., Schwendt, M., Saylor, A., Toda, S., 2008. Regulation of psychostimulant-induced signal and gene expression in the striatum. J. Neurochem. 104, 1440-1449.

McGregor, A., Roberts, D.C., 1993. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Res. 624, 245-252.

Meador-Woodruff, J.H., 1994. Update on dopamine receptors. Ann. Clin. Psychiatry 6, 79-90.

Merienne, K., Pannetier, S., Harel-Bellan, A., Sassone-Corsi, P., 2001. Mitogen-regulated RSK2-CBP interaction controls their kinase and acetylase activities. Mol. Cell. Biol. 21, 7089-7096.

Miller, C.A., Marshall, J.F., 2005. Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron 47, 873-884.

Miserendino, M.J.D., Nestler, E.J., 1995. Behavioral sensitization to cocaine: Modulation by the cyclic AMP system in the nucleus accumbens. Brain Res. 674, 299-306.

Missale, C., Fiorentini, C., Busi, C., Collo, G., Spano, P.F., 2006. The NMDA/D1 receptor complex as a new target in drug development. Curr. Top. Med. Chem. 6, 801-808.

Missale, C., Nash, S.R., Robison, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. Physiol. Rev. 78, 189-225.

Miyamoto, Y., Yamada, K., Nagai, T., Mori, H., Mishina, M., Furukawa, H., Noda, Y., Nabeshima, T., 2004. Behavioral adaptations to addictive drugs in mice lacking NMDA receptor ε 1 subunit. Eur. J. Neurosci. 19, 151-158.

Monsma, F.J. Jr., Manan, L.C., McVittie, L.D., Gerfen, C.R., Sibley, D.R., 1990. Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. Proc. Natl. Acad. Sci. USA 87, 6723-6727.

Montminy, M.R., Bilezikjian, L.M., 1987. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature 328, 175-178.

Montminy, M.R., Gonzalez, G.A., Yamamoto, K.K., 1990. Regulation of cAMP-inducible genes by CREB. Trends Neurosci. 13, 184-188.

Muda, M., Boschert, U., Dickinson, R., Martinou, J.C., Martinou, I., Camps, M., Schlegel, W., Arkinstall, S., 1996a. MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. J. Biol. Chem. 271, 4319-4326.

Muda, M., Theodosiou, A., Rodrigues, N., Boschert, U., Camps, M., Gillieron, C., Davies K., Ashworth, A., Arkinstall, S., 1996b. The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. J. Biol. Chem. 271, 27205-27208.

Nairn, A.C., Svenningsson, P., Nishi, A., Fisone, G., Girault, J.A., Greengard, P., 2004. The role of DARPP-32 in the actions of drugs of abuse. Neuropharmacology 47 Suppl. 1, 14-23.

Nazarian, A., Russo, S.J., Festa, E.D., Kraish, M., Quinones-Jenab, V., 2004. The role of D1 and D2 receptors in the cocaine conditioned place preference of male and female rats. Brain Res. Bull. 63, 295-299.

Neisewander, J.L., Baker, D.A., Fuchs, R.A., Tran-Nguyen, L.T.L., Palmer, A., Marshall, J.F., 2000. Fos protein expression and cocaine seeking behavior in rats after exposure to cocaine self-administration. J. Neurosci. 20, 798-805.

Neisewander, J.L., O'Dell, L.E., Redmond, J.C., 1995. Localization of dopamine receptor subtypes occupied by intra-accumbens antagonists that reverse cocaine-induced locomotion. Brain Res. 67, 201-212.

Nestler, E.J., 2001. Molecular basis of long-term plasticity underlying addiction, Nat. Rev. Neurosci 2,119-128.

Nestler, E.J., Aghajanian, G.K., 1997. Molecular and cellular basis of addition. Science 278, 58-63.

Nestler, E.J., Barrot, M., Self, D.W., 2001. DeltaFosB: a sustained molecular switch for addiction. Proc. Acad. Sci. USA 98, 11042-11046.

Nika, K., Hyunh, H., Williams, S., Paul, S., Bottini, N., Tasken, K., Lombroso, P.J., Mustelin, T., 2004. Haematopoietic protein tyrosine phosphatase (HePTP) phosphorylation by cAMP-dependent protein kinase in T-cells: Dynamics and subcellular location. J. Biochem. 378, 335-342.

Nishi, A., Bibb, J.A., Matsuyama, S., Hamada, M., Higashi, H., Nairn, A.C., Greengard, P., 2002. Regulation of DARPP-32 dephosphorylation at PKA- and Cdk5-sites by

NMDA and AMPA receptors: Distinct roles of calcineurin and protein -2A. J. Neurochem. 81, 382-841.

Nishi, A., Bibb, J.A., Snyder, G.L., Higashi, H., Narin, A.C., Greengard, P., 2000. Amplication of dopaminergic signaling by a positive feedback loop. Proc. Natl. Acad. Sci. USA 97, 12840-12845.

Nishi, A., Snyder, G.L., Greengard, P., 1997. Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J. Neurosci. 17, 8147-8155.

Okabe, S., Miwa, A., Okada, H., 1999. Alternative splicing of C-terminal domain regulates cell surface expression of the NMDA receptors. J. Neurosci. 20, 4452-4461.

Parsons, L.H., Justice, J.B. Jr., 1993. Sereotine and sopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. J. Neurochem. 61, 1611-1619.

Paul, S., Nairn, A.C., Wang, P., Lombroso, P.J., 2003. NMDA-mediated activation of the tyrosine phosphatase STEP regulates the duration of ERK signaling. Nat. Neurosci. 6, 34-42.

Paul, S., Snyder, G.L., Yokakura, H., Picciotto, M.R., Nairn, A.C., Lombroso, P.J., 2000. The dopamine/D1 receptor mediates the phosphorylation and inactivation of protein tyrosine phosphatase STEP via a PKA-dependent pathway. J. Neurosci. 20, 5630-5638.

Pei, L., Lee, F.J.S., Moszczynska, A., Vukusic, B., Liu, F., 2004. Regulation of dopamine D1 receptor function by physical interaction with the NMDA receptors. J. Nsurosci. 24, 1149-1158.

Pende, M., Fisher, T.L., Simpson, P.B., Russell, J.T., Blenis, J., Gallo, V., 1997. Neurotransmitter- and growth factor-induced cAMP response element binding protein phosphorylation in glial cell progenitors: role of calcium ions, protein kinase C, and mitogen-activated protein kinase/ribosomal S6 kinase pathway. J. Neurosci. 17, 1291-1301.

Peris, J., Boyson, S.J., Cass, W.A., Curella, P., Dwoskin, L.P., Larson, G., Lin, L.H., Yasuda, R.P., Zahniser N.R., 1990. Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmacol. Exp. Ther. 253, 38-44.

Perkinton, M.S., Ip, J.K., Wood, G.L., Crossthwaite, A.J., Williams, R.J., 2002. Phosphatidylinositol 3-kinase is a central mediator of NMDA receptor signalling to MAP kinase (Erk1/2), Akt/PKB and CREB in striatal neurones. J. Neurochem. 80, 239-254.

Pierce, R.C., Bell, K., Duffy, P., Kalivas, P.W., 1996. Repeated cocaine augments excitatory amino acid transmission in nucleus accumbens only in rats have developed behavioral sensitization. J. Neurosci. 16, 1550-1560.

Pierce, R.C., Pierce-Bancroft, A.F., Prasad, B.M., 1999. Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the Ras/Mitogen-activated protein kinase signal transduction cascade. J. Neurosci. 19, 8685-8695.

Pierce, R.C., Reeder, D.C., Hicks, J., Morgan, Z.R., Kalivas, P.W., 1998. Ibonetic acid lesions of the dorsal prefrontal cortex disrupt the expression of behavioral sensitization to cocaine. Neuroscience 82, 1103-1114.

Pruitt, D.L., Bolanos, C.A., McDougall, S.A., 1995. Effects of dopamine D1 and D2 receptor antagonists on cocaine-induced place preference conditioning in preweanling rats. Eur. J. Pharmacol. 283, 125-131.

Pulido, R., Zuniga, A., Ullrich, A., 1998. PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. EMBO J. 17, 7337-7350.

Pulvirenti, L., Swerdlow, N. R., Koob, G.F., 1991. Nucleus accumbens NMDA antagonist decreases locomotor activity produced by cocaine, heroin or accumbens dopamine, but not caffeine. Pharmacol. Biochem. Behav. 40, 841-845.

Ramsey, A.J., Laakso, A., Cyr, M., Sotnikova, T.D., Salahpour, A., Medvedev, I.O., Dykstra, L.A., Gainetdinov, R.R., Caron, M.G., 2008. Genetic NMDA receptor deficiency disrupts acute and chronic effects of cocaine but not amphetamine. Neuropsychopharmacology doi:10.1038/sj.npp.1301663.

Rauggi, R., Scheggi, S., Cassanelli, A., De Montis, M.G., Tagliamonte, A., Gambarana, C., 2005. The mesolinbic dopaminergic response to novel palatable food consumption increases dopamine-D1 receptor-mediated signaling with complex modifications of the DARPP-32 phosphorylation pattern. J. Neurochem. 92, 867-877.

Radwanska, K., Caboche, J., Kaczmarek, L., 2005. Extracellular signal-regulated kinases (ERKs) modulate cocaine-induced gene expression in the mouse amygdala. Eur. J. Neurosci. 22, 939-948.

Radwanska, K., Valjent, E., Trzaskos, J., Caboche, J., Kaczmarek, L., 2006. Regulation of cocaine–induced activator protein 1 transcription factors by the extracellular signal-regulated kinase pathway. Neuroscience 137, 253-264.

Rajadhyaksha, A., Husson, I., Satpute, S.S., Küppenbender, K.D., Ren, J.Q., Guerriero, R.M., Standaert, D.G., Kosofsky, B.E., 2004. L-type Ca2+ channels mediate adaptation of extracellular signal-regulated kinase 1/2 phosphorylation in the ventral tegmental area after chronic amphetamine treatment. J. Neurosci. 24, 7464-7476.

Reid, M.S., Berger, S.P., 1996. Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. Neuroreport 7, 1325–1329.

Reith, M.E., Li, M.Y., Yan, Q.S., 1997. Extracellular dopamine, norepinephrine and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. Psychopharmacology 134, 309-317.

Roberson, E.D., English, J.D., Adams, P., Selcher, J.C., Kondratick, C., Sweatt, J.D., 1999. The mitogen-activated protein cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J. Neurosci. 19, 4337-4348.

Roberts, D.C.S., Corcoran, M.E., Fibiger, H.C., 1977. On the role of ascending catecholamine systems in intravenous self-administration of cocaine. Pharmacol. Biochem. Behav. 6, 615-620.

Roberts, D.C.S., Koob, G.F., Klonoff, P., Fibiger, H.C., 1980. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 12, 781-787.

Robinson, T. E., Becker, J. B., 1982. Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur. J. Pharmacol. 85, 253-254.

Sakai, N., Thome, J., Newton, S.S., Chen, J., Kelz, M.B., Steffen, C., Nestler, E.J., Duman, R.S., 2002. Inducible and brain region-specific CREB transgenic mice. Mol. Pharmacol. 61, 1453-1464.

Samaha, A.N., Mallet, N., Ferguson, S.M., Gonon, F., Robinson, T.E., 2004. The rate of cocaine administration alters gene regulation and behavioral plasticity: Implications for addiction. J. Neurosci. 24, 6362-6370.

Scarselli, M., Novi, F., Schallmach, E., Lin, R., Baragli, A., Colzi, A., Griffon, N., Corsini, G.U., Sokoloff, P., Levenson, R., Vogel, Z., Maggio, R., 2001. D2/D3 dopamine receptor heterodimers exhibit unique functional properties. J. Biol. Chem. 276, 30308-30314.

Scheggi, S., Rauggi, R., Gambarana, C., Tagliamonte, A., De Montis, M.G., 2004a. Dopamine and cyclic AMP-regulated phosphoprotein-32 phosphorylation pattern in cocaine and morphine-sensitized rats. J. Neurochem. 90, 792-799.

Scheggi, S., Rauggi, R., Nanni, G., Tagliamonte, A., Gambarana, C., 2004b. Repeated acetyl-l-carnitine administration increases phospho-Thr34 DARPP-32 levels and antagonizes cocaine-induced increase in Cdk5 and phospho-Thr75 DARPP-32 levels in rat striatum. Eur. J. Neurosci. 19, 1609-1620.

Schilström, B., Yaka, R., Argilli, E., Suvarna, N., Schumann, J., Chen, B.T., Carman, M., Singh, V., Mailliard, W.S., Ron, D., Bonci, A., 2006. Cocaine enhances NMDA receptor-mediated currents in ventral tegmental area cells via dopamine D5 receptor-dependent redistribution of NMDA receptors. J. Neurosci. 26, 8549-8558.

Schmidt-Mutter, C., Muller, C., Zwiller, J., Gobaille, S., Maitre, M., 1999. Gamma-Hydroxyburate and cocaine administration increase mRNA expression of dopamine D1 and D2 receptors in rat brain. Neuropsychopharmacology 21, 662-669.

Schroeder, J.A., Hummel, M., Unterwald, E.M., 2004. Repeated intracerebroventricular forskolin administration enhances behavioral sensitization to cocaine. Behav. Brain Res. 153, 255-260.

Self, D.W., Genova, L.M., Hope, B.T., Barnhart, W.J., Spencer, J.J., Nestler, E.J., 1998. Involvement of cAMP-dependent protein kinase in the nuclus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J. Neurosci. 18, 1848-1859.

Self, D.W., Nestler, E.J., 1995. Molecular mechanisms of drug reinforcement and addition. Annu. Rev. Neurosci. 18, 463-495.

Seger, R., Krebs, E.G., 1995. The MAPK signaling cascade. FASEB J. 9, 726-735.

Sgambato, V., Pages, C., Rogard, M., Besson, M.J., Caboche, J., 1998a. Extracellular signal-regulated kinase (ERK) controls immediate early gene induction on corticostriatal stimulation. J. Neurosci. 18, 8814-8825.

Sgambato, V., Vanhoutte, P., Pages, C., Rogard, M., Hipskind, R., Besson, M.J., Caboche, J., 1998b. In vivo expression and regulation of Elk-1, a target of the extracellular-regulated kinase signaling pathway, in the adult rat brain. J. Neurosci. 18, 214-226.

Sheng, M., Thompson, M.A., Greenberg, M.E., 1991. CREB: A Ca^{2+}-regulated factor phosphorylated by calmodulin-dependent kinases. Science 252, 1427-1430.

Sibley, D.R., Monsma, F.J., Shen, Y., 1993. Molecular neubiology of dopaminergic receptors. Int. Rev. Neurobiol. 35, 391-415.

Shi, X., McGinty, J.F., 2007. 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. 103, 706-713.

Shin, E.H., Bain, S., Shim, Y.B., Rahman, M.A., Chung, K.T., Kim, J.Y., Wang, J.Q., Choe, E.S., 2007. Cocaine increases endoplasmic reticulum stress protein expression in striatal neurons. Neuroscience 145, 621-630.

Standaert, D.G., Testa, C.M., Young, A.B., Penney, J.B. Jr., 1994. Organization of N-methyl-D-aspartate glutamate receptor gene expression in the basal ganglia of the rat. J. Comp. Neurol. 343, 1-16.

Steketee, J.D., Rowe, L.A., Chandler, L.J., 1998. The effects of acute and repeated cocaine injections on protein kinase C activity and isoform levels in dopaminergic brain regions. Neuropharmacology 37, 339-347.

Smith, J.A., Mo, Q., Guo, H., Kunko, P.M., Robinson, S.E., 1995. Cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens. Brain Res. 683, 264–269.

Snyder, G.L., Fienberg, A.A., Huganir, R.L., Greengard, P., 1998. A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J. Neurosci. 18, 10297-10303.

Spanagel, R., Weiss, F., 1999. The dopamine hypothesis of reward: Past and current status, Trends Neurosci. 22, 521-527.

Sprengel, R., Suchanek, B., Amico, C., Brusa, R., Burnashev, N., Rozov, A., Hvalby, O., Jensen, V., Paulsen, O., Andersen, P., Kim, J.J., Thompson, R.F., Sun, W., Webster, L.C., Grant, S.G., Eilers, J., Konnerth, A., Li, J., McNamara, J.O., Seeburg, P.H., 1998. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell 92, 279-289.

Steiner, H., Gerfen, C.R., 1993. Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. J. Neurosci. 13, 5066-5081.

Stoof, J.C., Kebabian, J.W., 1981. Opposing-roles for D1 and D2 dopamine receptors in efflux of cyclic AMP from rat stritum. Nature 294, 366-368.

Storvik, M., Tiikkainen, P., van Iersel, M., Wong, G. 2006. Distinct gene expression profiles in adult rat brain after acute MK-801 and cocaine treatments. Eur. Neuropsychopharmacol. 16, 211-219.

Sturgill, T.W., Ray, L.B., Erikson, E., Maller, J.L., 1988. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. Nature 334, 715-718.

Sun, H., Charles, C.H., Lau, L.F., Tonks, N.K., 1993. MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. Cell 75, 487-493.

Sun, L.S., Quamina, A., 2004. Extracellular receptor kinase and cAMP response element binding protein activation in the neonatal rat heart after perinatal cocaine exposure. Pediatr. Res. 56, 947-952.

Sun, W.L., Zhou, L., Hazim, R., Quinones-Jenab, V., Jenab, S., 2007. Effects of acute cocaine on ERK and DARPP-32 phosphorylation pathways in the caudate-putamen of Fischer rats. Brain Res. 1178, 12-19.

Sun, W.L., Zhou, L., Hazim, R., Quinones-Jenab, V., Jenab, S., 2008. Effects of dopamine and NMDA receptors on cocaine-induced Fos expression in the striatum of Fischer rats. Brain Res. In press.

Svenningsson, P., Fredholm, B.B., Blochi, B., Le Moine, C., 2000a. Co-stimulation of D1/D5 and D2 dopamine receptors leads to and increase in c-fos messenger RNA in cholinergic interneurons and a redistribution of c-fos messenger RNA in striatal projection neurons. Neuroscience 98, 749-757.

Svenningsson, P., Lindskog, M., Ledent, C., Parmentier, M.,Greengard, P., Fredholm, B.B., Fisone, G., 2000b. Regulation of the phosphorylation ofthe dopamine- and cAMP-regulated phosphoprotein of 32 kDa in vivo by dopamine D1, dopamine D2, and adenosine A2A receptors. Proc. Natl. Acad. Sci. USA 97,1856–1860.

Svenningsson, P., Nishi, A., Fisone, G., Girault, J.A., Nairn, A.C., Greengard, P., 2004. DARPP-32: An integrator of neurotransmission. Annu. Rev. Pharmacol. Toxicol. 44, 269-296.

Svenningsson, P., Nairn, A.C., Greengard, P., 2005. DARPP-32 mediates the actions of multiple drugs of abuse. AAPS J. 7, E353-360.

Svensson, P., Hurd, Y.L., 1998. Specific reductions of striatal prodynorphin and D1 dopamine receptor messenger RNAs during cocaine abstinence. Mol. Brain Res. 56, 162-168.

Sweatt, J.D., 2001. The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. J. Neurochem. 76, 1-10.

Takaki, M., Ujike, H., Kodama, M., Takehisa, Y., Nakata, K., Kuroda, S., 2001. Two kinds of mitogen-activated protein kinase phosphatases, MKP-1 and MKP-3, are differently activated by acute and chronic methamphetamine treatment in the rat brain. J. Neurochem. 79, 679-688.

Tella, S.R., 1994. Differential blockade of chronic versus acute effects of intravenous cocaine by dopamine receptor antagonists. Pharmacol. Biochem. Behav. 48, 151-159.

Terwilliger, R.Z., Beitner-Johnson, D., Sevarino, K.A., Crain, S.M., Nestler, E.J., 1991. A general role for adaptations in G-proteins and cyclic AMP system in mediating the chronic actions of morphin and cocaine on neuronal function. Brain Res. 579, 181-186.

Tingley, W.G., Ehlers, M.D., Kameyama, K., Doherty, C., Ptak, J.B., Riley, C.T., Huganir, R.L., 1997. Characterization of protein kinase A and protein kinase C phosphorylation of the N-methyl-D-aspartate receptor NR1 subunit using phosphrylation site-specific antibodies. J. Biol. Chem. 272, 5157-5166.

Todtenkopf, M.S., Mihalakopoulos, A., Stellar, J.R., 2002. Withdrawal duration differentially affects c-fos expression in the medial prefrontal cortex and discrete subregions of the nucleus accumbens in cocaine-sensitized rats. Neuroscience 114, 1061-1069.

Torres, G., Rivier, C., 1992. Differential effects of intermittent or continuous exposure to cocaine on the hypothalamic-pituitary-adrenal axis and c-fos expression. Brain Res. 571, 204-11.

Treisman, R., 1996. Regulation of transcription by MAP kinase cascade. Curr. Opin. Cell Biol. 8, 205-215.

Tsukada, H., Kreuter, J., Maggos, C.E., Unterwald, E.M., Kakiuchi, T., Nishiyama, S., Futatsubashi, M., Kreek, M.J., 1996. Effect of binge pattern cocaine administration on dopamine D1 and D2 receptors in the rat brain: An in vivo study using positron emission tomography. J. Neurosci. 16, 7670-7677.

Tokumitsu, H., Hatano, N., Inuzuka, H., Yokokura, S., Nozaki, N., Kobayashi, R., 2004. Mechanism of the generation of autonomous activity of Ca2+/calmodulin-dependent protein kinase IV. J. Biol. Chem. 279, 40296-40302.

Turchan, J., Maj, M., Przewlocka, B., 2003. The effect of drug of abuse on NMDAR1 receptor expression in the rat limbic system. Drug Alcohol Depend. 72, 193-196.

Ujike, H., Takaki, M., Kodama, M., Kuroda, S., 2002. Gene expression related to synaptogenesis, neuritogenesis, and MAP kinase in behavioral sensitization to psychostimulants. Ann. N.Y. Acad. Sci. 965, 55-67.

Unterwald, E.M., Fillmore, J., Kreek, M.J., 1996. Chronic repeated cocaine administration increases dopamine D1 receptor-mediated signal transduction. Eur. J. Pharmacol. 318, 31-35.

Unterwald, E.M., Ho, A., Rubenfeld, J.M., Kreek, M.J., 1994. Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. J. Pharmacol. Exp. Ther. 270, 1387-1396.

Ushijima, I., Carino, M.A., Horita, A., 1995. Involvement of D1 and D2 dopamine systems in the behavioral effects of cocaine in rats. Pharmacol. Biochem. Behav. 52, 737-741.

Uslaner, J.M., Cromgab, H.S., Ferguson, S.M., Robinson, T.E., 2003. Cocaine-induced psychomotor activity is associated with its ability to induce c-fos mRNA expression in the subthalamic nucleus: effects of dose and repeated treatment. Eur. J. Neurosci. 17, 2180-2186.

Uzbay, I.T., Wallis, C.J., Lal, H., Forster, M.J., 2000. Effects of NMDA receptor blockers on cocaine-stimulated locomotor activity in mice. Behav. Brain Res. 108, 57-61.

Valjent, E., Corvol, J.C., Pages, C., Besson, M.J., Maldonado, R., Caboche, J., 2000. Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. J. Neurosci. 20, 8701-8709.

Valjent, E., Pages, C., Herve, D., Girault, J.A., Caboche, J., 2004. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. Eur. J. Neurosci. 19, 1826-1836.

Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Herve, E., Corvol, J.C., Stipanovich, A., Caboche, J., Lombroso, P.J., Nairn, A.C., Greengard, P., Herve, D., Girault, J.A., 2005. Regulation of a protein phophatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc. Natl. Acad. Sci. USA 102, 491-496.

Vanhoutte, P., Barnier, J.V., Guibert, B., Pages, C., Besson, M.J., Hipskind, R.A., Caboche, J., 1999. Glutamate induces phophorylation of ElK-1 and CREB, along with *c-fos* activation, via an extracelullar signal-regulated kinase-dependent pathway in brain slices. Mol. Cell. Biol. 19, 136-146.

Vincent, S.R., Sebben, M., Dumuis, A., Bockaert, J., 1998. Neurotransmitter regulation of MAP kinase signaling in striatal neurons in primary culture. Synapse 29, 29-36.

Vossler, M.R., Yao, H., York, R.D., Pan, M.G., Rim, C.S., Stork, P.J., 1997. cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. Cell 89, 73-82.

Walters, C.L., Blendy, J.A., 2001. Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. J. Neurosci. 21, 9438-9444.

Walters, C.L., Kuo, Y.C., Blendy, J.A., 2003. Differential distribution of CREB in the mesolimbic reward pathway. J. Neurochem. 87, 1237-1244.

Wedzony, K., Klimek, V., Gołembiowska, K., 1993. MK-801 elevates the extracellular concentration of dopamine in the rat prefrontal cortex and increases the density of striatal dopamine D1 receptors. Brain Res. 622, 325-329.

Weiner, D.M., Levey, A.I., Sunahara, R.K., Olsen, M.A., Brancheck, T.A., Hartig, P.R., 1991. Dopamine D1 and D2 receptor mRNA expression in rat brain. Proc. Natl. Acad. Sci. USA 88, 1859-1863.

Westphal, R.S., Tavalin, S.J., Lin, J.W., Alto, N.M., Fraser, I.D., Langeberg, L.K., Sheng, M., Scott, J.D., 1999. Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. Science 285, 93-96.

White, F.J., Joshi, A., Koeltzow, B.A., Hu, X.T., 1998. Dopamine receptor antagonists fail to prevent induction of cocaine sensitization. Neuropsychopharmacology 18, 26-40.

White, F.J., Kalivas, P.W., 1998. Neuroadaptions involved in amphetamine and cocaine addition. Drug Alcohol Depend. 51, 141-153.

Williams, J.M., Steketee, J.D., 2004. Cocaine increases medical prefrontal cortical glutamate overflow in cocaine-sensitized rats: A time course study. Eru. J. Neurosci. 20, 1639-1649.

Wolf, M.E., Jeziorski, M., 1993. Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. Brain Res. 613, 291-294.

Wolf, M.E., White, F.J., Hu, X.T., 1993. Behavioral sensitization to MK-801 (dizocilpine): neurochemical and electrophysiological correlates in the mesoaccumbens dopamine system. Behav. Pharmacol. 4, 429-442.

Wyszynski, M., Lin, J., Rao, A., Nigh, E., Beggs, A.H., Craig, A.M., Sheng, M., 1997. Competitive binding of alpha-actinin and calmodulin to the NMDA receptor. Nature 385, 439-442.

Xia, Z., Dudek, H., Miranti, C.K., Greenberg, M.E., 1996. Calcium influx via NMDA receptor induces immediate early gene transcription by a MAP linase/ERK-dependent mechanism. J. Neurosci. 16, 5425-5436.

Xing, J., Ginty, D.D., Greenberg, M.E., 1996. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 273, 959-963.

Xing, J., Kornhauser, J.M., Xia, Z., Thiele, E.A., Greenberg, M.E., 1998. Nerve growth factor activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways to stimulate CREB serine 133 phosphorylation. Mol. Cell. Biol. 18, 1946-1955.

Xu, M., Guo, Y., Vorhees, C.V., Zhang, J., 2000. Behavioral responses to cocaine and amphetamine administration in mice lacking the dopamine D1 receptor. Brain Res. 852, 198-207.

Xu, M., Hu, X.T., Cooper, D.C., Moratalla, R., Tonegawa, S., 1994. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. Cell 79, 945-955.

Yamada, S., Kojima, H., Yokoo, H., Tsutsumi, T., Takamuki, K., Anraku, S., Nishi, S., Inanaga, K., 1988. Enhancement of dopamine release from striatal slices of rats that were subchronically treated with methamphetamine. Biol. Psychiatry 24, 399-408.

Young, S.T., Porrino, L.J., Iadarola, M.J., 1991. Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. Proc. Natl. Acad. Sci. USA 88, 1291-1295.

Zachariou, V., Sgambato-Faure, V., Sasaki, T., Svenningsson, P., Berton, O., Fienberg, A.A., Nairn, A.C., Greengard, P., Nestler, E.J., 2006. Phosphorylation of DARPP-32 at threonine-34 is required for cocaine action. Neuropsychopharmacology 31, 555-562.

Zanassi, P., Paolillo, M., Feliciello, A., Avvedimento, E.V., Galloi, V., Schinelli, S., 2001. cAMP-dependent protein kinase induces cAMP-response element-binding protein phosphorylation via an intracellular calcium release/ERK-dependent pathway in striatal neurons J. Biol. Chem. 276, 11487-11495.

Zhai, H., Li, Y., Wang, X., Lu, L., 2008. Drug-induced alterations in the extracellular signal-regulated kinase (ERK) signalling pathway: Implications for reinforcement and reinstatement. Cell Mol. Neurobiol. 28, 157-172.

Zhang, J., Xu, M., 2006. Opposite regulation of cocaine-induced intracellular signaling and gene expression by dopamine D1 and D3 receptors. Ann. N.Y. Acad. Sci. 1074, 1-12.

Zhang, J., Zhang, L., Jiao, H., Zhang, Q., Lou, D., Katz, J.L., Xu, M., 2006. c-Fos facilitate the acquisition and extinction of cocaine-induced persistent changes. J. Neurosci. 26, 13287-13296.

Zhang, L., Lou, D., Jiao, H., Zhang, D., Wang, X., Xia, Y., Zhang, J., Xu, M., 2004. Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. J. Neurosci. 24, 3344-4454.

Zhang, Y., Loonam, T.M., Noailles, P.A., Angulo, J.A., 2001. Comparison of cocaine- and methamphetamine-evoked dopamine and glutamate overflow in somatodendritic and terminal field regions of the rat brain during acute, chronic, and early withdrawal conditions. Ann. N.Y. Acad. Sci. 937, 93-120.

Zhang, Y., Svenningsson, P., Picetti, R., Schlussman, S.D., Nairn, A.C., Ho, A., Greengard, P., Kreek, M.J., 2006. Cocaine self-administration in mice is inversely related to phosphorylation at Thr34 (protein kinase A site) and Ser130 (kinase CK1 site) of DARPP-32. J. Neurosci. 26, 2645-2651.

Zhou, Q.Y., Grandy, D.K., Thambi, L., Kushner, J.A., Van Tol, H.H.M., Cone, R., Pribnow, D., Salon, J., Bunzow, J.R., Civelli, O., 1990. Cloning and expression of human and rat D1 dopamine receptors. Nature 347, 76-80.

Zhuang, X., Belluscio, L., Hen ,R., 2000. Golfα mediates dopamine D1 receptor signaling. J. Neurosci. 20, 1-5.

Zuniga, A., Torres, J., Ubeda, J., Pulido, R., 1999. Interaction of mitogen-activated protein kinases with the kinase interaction motif of the tyrosine phosphatase PTP-SL provides substrate specificity and retains ERK2 in the cytoplasm. J. Biol. Chem. 274, 21900-21907.