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PROGRESS IN MOLECULAR BIOLOGY AND TRANSLATIONAL SCIENCE

The Molecular Basis of Drug Addiction

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Edited by

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PREFACE

Drug addiction is the most complex and costly neuropsychiatric disorder affecting millions of people in the world. Recent surveys indicate that approximately 250 million people are illegal drug users which represent ~4% of the global population. Acute and chronic exposure to drugs of abuse produces numerous neurobiological effects, but the cellular and molecular processes involved are only partially understood. Neuroscientists around the world are searching for clues that underlie the molecular basis of drug addiction. While current scientific breakthroughs have increased the understanding on molecular determinants of drug addiction, limitations exist on effective treatment strategies for many forms of drug addiction. Thus, there is a need to translate the current knowledge regarding molecular mechanisms of drug addiction derived from neurobiological research into the discovery of new therapeutics.

This volume, *The Molecular Basis of Drug Addiction*, consists of eight chapters written by eminent experts in the field. The volume covers important aspects of neuroscience research on drug addiction associated with the neurotransmitter receptors, signaling molecules, and relevant mechanisms implicated in drug addiction. The chapters in this volume describe some of the latest concepts in emerging and innovative research, discuss new breakthrough findings, define innovative strategies, and target multiple signaling pathways and genes. The primary molecular targets discussed in this volume include extracellular signal-regulated kinase, glutamate-associated genes or proteins, S-glutathionylated proteins, cannabinoid receptor mediated signaling pathways, adenylyl cyclase/cyclic adenosine 3,5-monophosphate protein kinase A, neuronal nicotinic receptors, and nociceptin receptors involved in many forms of drug addiction. The first chapter presents and discusses the role of the extracellular signal-regulated kinase and its related intracellular signaling pathways in drug-induced neuroadaptive changes that are associated with drug-mediated psychomotor activity, rewarding properties, and relapse of drug-seeking behaviors (Zhu *et al.*). The second chapter reviews the role of glutamate neurotransmitter receptor system in mediating the development of alcohol dependence. The chapter discusses the expression levels of glutamate-associated genes and/or proteins, including metabotropic and ionotropic receptor subunits and glutamate transporters

in a genetic animal model of alcoholism and highlights the changes in glutamate receptors, transporters, enzymes, and scaffolding proteins involving alcohol dependence (Bell *et al.*). The third chapter presents and highlights the evidence for S-glutathionylation as a redox-sensing mechanism and how this may be involved in the response to drug-induced oxidative stress. The function of S-glutathionylated proteins involved in neurotransmission, dendritic spine structure, and drug-induced behavioral outputs are reviewed with specific reference to alcohol, cocaine, and heroin (*Uys and Reissner*). The fourth chapter provides a comprehensive account of the state of knowledge regarding mechanisms of *Cannabis* signaling in the brain and the modulation of key brain neurotransmitter systems involved in addiction and psychiatric disorders (Ronan *et al.*). The fifth chapter reviews the existing literature on the roles of nociception receptors and associated mechanisms in the rewarding and addictive actions of cocaine (Lutfy and Zaveri). The sixth chapter presents recent insights on the rewarding effects of alcohol as they pertain to different brain nicotinic receptor subtypes and associated signaling pathways that contribute to the molecular mechanisms of alcoholism and/or comorbid brain disorders (Rahman *et al.*). The seventh chapter focuses on and reviews the adenylyl cyclase and cyclic adenosine 3,5-monophosphate/protein kinase A system as a central player in mediating the acute and chronic effects of opioids in opiate abusers (Chan and Lutfy). The eighth chapter concentrates on *Caenorhabditis elegans*, a nonvertebrate model, to study the molecular and genetic mechanisms of drug addiction and to identify potential targets for medication development (Engleman *et al.*).

Together, this body of work not only provides a deeper understanding of our current knowledge on specific neurotransmitter systems, functional proteins, signaling molecules, genes, and additional targets for drug addiction, but also indicates complex interactions between drugs of abuse, endogenous neuromodulators, signaling molecules, and the mechanisms underlying the structural and functional plasticity in the brain. I hope that the molecular basis of drug addiction research summarized in this volume will generate new ideas on diverse targets and stimulate translational research for further mechanistic understanding and insight into effective strategies for novel therapeutics in the management of drug addiction.

I would like to thank all the authors for their outstanding contributions to this volume. I am very thankful to Dr. P. Michael Conn, the Editor-in-Chief of the Book Series, for his guidance. Finally, I also thank Ms. Mary Ann

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Shafiqur Rahman
Editor

CHAPTER ONE

Molecular Mechanism: ERK Signaling, Drug Addiction, and Behavioral Effects

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Abstract

Addiction to psychostimulants has been considered as a chronic psychiatric disorder characterized by craving and compulsive drug seeking and use. Over the past two decades, accumulating evidence has demonstrated that repeated drug exposure causes long-lasting neurochemical and cellular changes that result in enduring neuroadaptation in brain circuitry and underlie compulsive drug consumption and relapse. Through intercellular signaling cascades, drugs of abuse induce remodeling in the rewarding circuitry that contributes to the neuroplasticity of learning and memory associated with addiction. Here, we review the role of the extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinase, and its related intracellular signaling pathways in drug-induced neuroadaptive changes that are associated with drug-mediated psychomotor activity, rewarding properties and relapse of drug seeking behaviors. We also discuss the neurobiological

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and behavioral effects of pharmacological and genetic interferences with ERK-associated molecular cascades in response to abused substances. Understanding the dynamic modulation of ERK signaling in response to drugs may provide novel molecular targets for therapeutic strategies to drug addiction.

ABBREVIATIONS

AC	Adenylyl cyclase
AMPH	Amphetamine
Amy	Amygdala
BDNF	Brain-derived neurotrophic factor
BNST	Bed nucleus of the striatal terminals
Ca^{2+}	Calcium
CaM	Calcium/calmodulin
CaMK	CaM kinase
CB1-R	Cannabinoid receptor 1
CB2-R	Cannabinoid receptor 2
CPP	Conditioned place preference
CPu	Caudate putamen
CREB	cAMP response element-binding protein
DA	Dopamine-regulated phosphoprotein-32
D1-R	Dopamine D1 receptor
D2-R	Dopamine D2-Receptor
ERK	Extracellular signal-regulated kinase
Glu	Glutamate
HIPP	Hippocampus
IEG	Immediate early gene
MAPK	Mitogen-activated protein kinase
MEK	MAPK kinase
METH	Methamphetamine
mGluR1/5	Metabotropic glutamate receptor-1/5
MKP-1/3	MAPK phosphatases 1 and 3
MSK	Mitogen- and stress-activated protein kinase
NAc	Nucleus accumbens
nAChRs	Nicotinic acetylcholine receptors
pCREB	Phosphorylated CREB
pERK	Phosphorylated ERK
PFC	Prefrontal cortex
pGluN2B	Phosphorylation of glutamate receptor, ionotropic, *N*-methyl D-aspartate 2B
PKA	Protein Kinase A
PKC	Protein Kinase C
pMEK	Phosphorylation of MEK
PP2A	Protein phosphatase 2A

PP2B	Protein phosphatase 2B
pSTEP	Phosphorylation of STEP
pThr75 DARPP-32	Phosphorylation of DARPP-32 at threonine 75
Ras-GRF-1	Ras-guanine nucleotide-releasing factors 1
RSK	Ribosomal S6 kinase
SA	Self-administration
STEP	Striatal-enriched protein tyrosine phosphatase
THC	Δ9-Tetrahydrocannabinol
VTA	Ventral tegmental area

1. INTRODUCTION

Drug addiction is a chronic brain disease characterized by high relapse rates and compulsive drug use despite negative consequences. To date, there is no effective treatment for drug addiction. Understanding the neurobiologic aspects underlying substance abuse provide a basis for developing potential therapeutic strategies targeting to drug addiction. Accumulating evidence demonstrates that drugs of abuse alter dopamine (DA) and glutamate (Glu) neurotransmission in the mesocorticolimbic system to exert their molecular and behavioral effects.[1–3] DA neurons in the ventral tegmental area (VTA) and their descending projections to the nucleus accumbens, prefrontal cortex (PFC) and other limbic regions, including the hippocampus (HIPP) and amygdala (Amy), comprise the mesocorticolimbic system,[4] which is crucial for reward and reinforcement processing, motivation, and goal-directed behavior.[5,6] The NAc and VTA also receive Glu output from the PFC. In addition, a reciprocal Glu connection is found between the PFC and Amy. The nigrostriatal pathway containing the DA projection from the substantia nigra to the caudate putamen (CPu/dorsal striatum) has also been implicated in molecular events, rewarding effects, and habitual behavior of drug addiction.[7,8]

The extracellular signal-regulated kinases (ERK1/2 or p44/p42 MAPK) cascade, one of the isoforms of mitogen-activated protein kinases (MAPK), is associated with the pathology of diseases due to its role in cell proliferation, differentiation, survival, and death.[9,10] Since the identification the activation of ERK by chronic morphine and cocaine administration in the VTA in 1996,[11] several lines of studies have focused ERK-mediated molecular signaling in response to various drugs of abuse during the last two decades.

Herein, we review the alterations of ERK signaling induced by abused substances including cocaine, amphetamine (AMPH), methamphetamine, marijuana, nicotine, and alcohol. In addition, most of these drugs have been shown to induce psychomotor changes, the ERK-associated molecular changes underlying drug-induced behaviors are also discussed. Further, due to the critical role of ERK in the neuroplasticity of learning and memory associated with addiction,[12] its influence on the reinforcing, rewarding, and relapse/reinstatement of drug addiction is also described.

2. ERK SIGNALING PATHWAY

Initially, intracellular ERK signaling has been characterized to respond to extracellular stimuli and regulate cell proliferation and differentiation.[13] For example, once ERK is activated by growth factors or neurotrophins, the tyrosine kinase receptors recruit Ras family G-proteins and lead to sequential activation of Raf (MAPK kinase kinase), MEK (MAPK kinase), and ERK. Once ERK is activated, the phosphorylated ERK (pERK) protein can translocate to the nucleus,[14] where they phosphorylate the ternary complex factor Elk-1.[15,16] The activated Elk-1 and other ternary complex factors associate with serum response factor, bind to the serum response element site, and promote immediate early gene (IEG) transcription related to neuroadaptation.[17–19] In addition to Elk-1, through phosphorylating ribosomal S6 kinases and mitogen- and stress-activated protein kinases (pRSKs and pMSKs, respectively), ERK has been shown to indirectly result in cAMP response element-binding protein phosphorylation (pCREB), a transcription factor that has been shown to regulate gene expression.[20–24] Increasing evidence shows a Glu linkage to ERK signaling in neurons both *in vivo* and *in vitro.* For instance, through the elevation of intracellular calcium (Ca^{2+})/calmodulin (CaM)/CaM kinases (CaMK), the activation of the Glu NMDA receptor can increase the phosphorylation of MEK (pMEK)/ERK/Elk-1 in hippocampal slices, neuronal culture,[25–27] cortical cultured neurons,[28] and striatal cultured neurons.[29–31] Inhibition of ERK activation attenuates Glu-mediated pElk-1 in the striatal slice,[32] striatum,[33–35] and in the HIPP.[17] Alternatively, in PC12 cells, Ca^{2+} may increase the intracellular cAMP through Ca^{2+}/CaM-sensitive adenylyl cyclase (AC) leading to the activation of PKA. Increase of cAMP and PKA induces pMEK via the activation of Rap1/Raf.[36,37] Consistent with these studies, pharmacologic activation of D1-R or the AC markedly stimulates ERK activity and its phosphorylation

in various neuronal cells.[33,38–41] In addition, activation of group 1 metabotropic Glu receptors (mGluR1/5) has been shown to increase the intracellular Ca^{2+} and activate ERK signaling.[42–45] Although the activation of DA D2 receptor (D2-R) inhibits PKA activity, D2-R stimulation also increases ERK signaling through PKC activation.[46]

There are several families of ERK-related phosphatases. Among these, protein phosphatase 2A (PP2A) and striatal-enriched protein tyrosine phosphatase (STEP) are the best characterized. PP2A is a major serine/threonine phosphatase containing two regulatory subunits and one catalytic subunit. PP2A mediates a rapid inactivation of pERK *in vitro*. STEP is another phosphatase that regulates ERK activation. Although it is enriched in the striatum, STEP is expressed abundantly in the mesocorticolimbic system.[47,48] Through direct interaction of a kinase-interacting motif, STEP and its nonneuronal homologues have been shown to dephosphorylate pERK and prevent its nuclear translocation.[49,50] Phosphorylation of STEP (pSTEP) reduces its activity and its capacity to inhibit pERK.[49] STEP is regulated through D1-R/PKA/DARPP-32 signaling.[51] *In vitro*, D1-R activation has been shown to activate pThr34 and inhibit pThr75 DARPP-32 via PKA-activated PP2A,[52] which inhibits protein phosphatase 1 and thereby increasing pSTEP.[53] In addition, stimulation of NMDA-R has been reported to induce Ca^{2+}-activated PP2A and protein phosphatase 2B (PP2B), which inhibit DARPP-32 signaling[52,54,55] and indirectly modulate ERK activity. Therefore, the protein phosphatases of pERK are regulated by DA- and Glu-mediated transmission. Further, dual specificity MAPK phophatases 1 and 3 (MKP-1/3) are also implicated in pERK deactivation. Both *in vitro* and *in vivo* studies indicated that MKP-1/3 expression and activation is dependent on ERK signaling. Once induced and activated, MKP-1/3 reduces the ERK activation as an inhibitory feedback loop.[34,56–61] Furthermore, there is evidence demonstrating that MKP-1 is phosphorylated (pMKP-1) by pERK leading to MKP-1 protein stabilization without altering its ability to dephosphorylate pERK.[62]

3. ERK SIGNALING AND DRUG ADDICTION

ERK signaling is responsive to various abused drugs in the mesocorticolimbic system. Both acute and chronic exposure to drugs results in alteration of ERK-mediated signaling in specific brain regions underlying neuronal plasticity and drug-induced behavioral changes. Therefore, we

focus on the effects of the most prevalent abused substances on ERK signaling and its relationship of drug-mediated behavioral changes across different paradigms including locomotor activity/sensitization, conditioned place preference (CPP), and self-administration (SA), if applicable. Since pharmacologic and genetic approaches have been used to interfere with the ERK signaling cascade, their effects on abused drug-mediated behaviors were summarized in Table 1 and Table 2, respectively.

3.1 Cocaine

Numerous studies have demonstrated that acute cocaine administration increases pERK in the CPu, NAc, PFC, central and basolateral Amy (CeA and BLA, respectively), HIPP, and bed nucleus of the striatal terminals (BNST).[98–112] The increased pERK and its downstream targets including pMSK-1, pElk-1, pCREB, phosphorylation of GluN2B (pGluN2B) and IEGs by acute cocaine, are dependent on the activation of MEK, D1-R/DARPP-32, and NMDA-R.[51,69,71,97–99,102,103,106,107,111] In addition to pMSK-1 induction, the pRSKs in the striatum are also increased by acute cocaine leading to the indirect activation of CREB by pERK.[97,112] In terms of protein phosphatases of pERK, acute cocaine has been shown to result in an increase of MKP-1 mRNA in the striatum and cortex.[113] In addition, depending on D1- and NMDA-Rs, the phosphorylation of MKP-1 was also enhanced in the CPu and NAc 45–60 min after acute cocaine, contributing to the transient pERK induction.[111] Further, the pSTEP was also downregulated after acute cocaine in the CPu with corresponding pERK induction.[112] Together, in a time-dependent manner, the activation and inactivation of protein phosphatases are critical for controlling the acute cocaine–augmented pERK. Behaviorally, the acute cocaine–induced locomotor activity was not affected by MEK inhibitor, SL327 (30 or 40 mg/kg), but partially inhibited or not altered with a higher dose injection (50 mg/kg), which has nonspecific sedative effect on basal locomotion.[51,69,71,75,114] Similar to acute cocaine, MEK/ERK activation is necessary for the chronic cocaine-induced IEG expression in the CPu, NAc, and Amy in a time-dependent manner.[102,103] In cocaine-sensitized animals, 7–21 days but not 1 day withdrawal resulted in increased AMPA-R subunit surface insertion and NDMA-R subunit expression with paralleled pERK induction in the NAc.[115–119] AMPA-R expression in the NAc after prolonged withdrawal from repeated cocaine injection is dependent on the activation of both GluN2B and pERK, which contributes to the development of behavioral sensitization.[117] This conclusion is further supported by a study that D1-R/Src

Table 1 Effects of MEK Inhibitors on Drug-Induced Behaviors

Drugs	MEK Inhibitors (Dose, Area)	Behavioral Effects	References
None	SL327 (50 mg/kg, i.p.)	↑ Basal locomotor activity	[63]
	SL327 (50–100 mg/kg, i.p.)	↓ Basal locomotor activity	[64–67]
	PD98059 (50 μM, continuous infusion into the PFC)	↑ Basal locomotor activity↓	[68]
Cocaine	SL327 (50 mg/kg, i.p.)	↓ Acute cocaine–induced locomotion	[69]
	SL327 (30 mg/kg, i.p.); PD98059 (10 μM, VTA)	↓ Development of locomotor sensitization (inhibitors were injected/infused before each cocaine injection)	[64,70]
	SL327 (40 mg/kg, i.p.); PD98059 (2 μg) or U0126 (1 μg, NAc)	↓ Expression of locomotor sensitization (inhibitors were injected/infused before cocaine challenge)	[71,72]
	SL327 (30 mg/kg, i.p.)	↓ Conditioned locomotor response (inhibitor was injected before each cocaine injection during conditioning)	[64]
	SL327 (50 mg/kg, i.p.); U0126 (0.1 μg, VTA)	↓ Development of CPP (inhibitors injected/infused before each cocaine injection during conditioning)	[69,73]
	U0126 (1 μg, NAc core)	↓ Expression of CPP (inhibitor was infused before CPP test)	[74]
	SL327 (30 mg/kg, i.p.); PD98059 (2 μg) or U0126 (1 μg, NAc core); U0126 (1 μg, BLA)	↓ Context- and cocaine priming-induced expression of CPP and ↓ context-induced reinstatement after SA by impairing memory reconsolidation (inhibitors were injected/infused either before or after reconsolidation phase)	[74–77]
	U0126 (1 μg, CeA)	↓ Context + cues-induced relapse after abstinence from SA (inhibitor was infused before relapse testing)	[78]
	U0126 (1 μg, VTA)	↓ BDNF/GDNF-enhanced relapse by context + cues after abstinence from SA (infusions were conducted immediately after the end of the last SA session)	[79,80]
	U0126 (0.5 μg, dmPFC)	↓ BDNF's inhibitory effect on context-, cues-, and cocaine priming-induced drug seeking after abstinence/extinction	[81]

(*Continued*)

Table 1 Effects of MEK Inhibitors on Drug-Induced Behaviors—cont'd.

Drugs	MEK Inhibitors (Dose, Area)	Behavioral Effects	References
		of SA (infusions were conducted immediately after the end of the last SA session)	
Amphetamine	SL327 (50–100 mg/kg, i.p.)	↓ Acute AMPH–induced locomotion	[69,82,83]
	PD98059 (50 μM, continuous infusion into the PFC)	↑ Acute AMPH–induced locomotor activity	[68]
	SL327 (40 mg/kg, i.p.)	↓ Expression of locomotor sensitization (inhibitors were injected/infused before AMPH challenge)	[71]
	SL327 (30 mg/kg, i.p.)	↓ Conditioned locomotor response (inhibitor was injected before each AMPH injection during conditioning)	[64]
	PD98059 (2.5 μg, NAc)	↓ Development of intra-NAc AMPH-induced CPP (inhibitor was infused before or after each intra-NAc AMPH infusion during conditioning)	[84]
	PD98059 (2 μg, NAc)	↓ Expression of AMPH-CPP (inhibitor was infused before CPP testing)	[85]
Marijuana (THC)	SL327 (50 mg/kg, i.p.)	↓ Development of THC-induced locomotion tolerance (inhibitor was injected before each THC administration)	[86]
	SL327 (50 mg/kg, i.p.)	↓ Development of THC-CPP (inhibitor was injected before each conditioning session)	[87]
Alcohol	PD98059 (30 or 90 μg, i.c.v.)	↓ Development of ACD-CPP (inhibitor was infused before each conditioning session)	[88]
	SL327 (30 mg/kg, i.p.)	↑ Ethanol SA (inhibitor was injected before SA session)	[67]
	U0216 (0.5 μg, VTA)	↓ GDNF's inhibitory effect on ethanol SA (infusions were conducted before SA session)	[89]

i.p., intraperitoneal injection; i.c.v., intracerebroventricular infusion; ↑, enhancing effect; ↓, inhibiting effect.

Table 2 Effects of Interfering ERK Signaling-Related Genes/Proteins on Drug-Induced Behaviors

Target Genes/Proteins	Behavioral Effects	References
Ca^{2+}-stimulated AC1/AC8 (KO)	↑ Acute cocaine–induced locomotion ↓ Development of cocaine locomotor sensitization	[90]
Ras-GRF-1 (KO)	↓ Development and expression of cocaine locomotor sensitization ↓ Cocaine-CPP ↓ Repeated THC-induced behavioral tolerance	[86,91,92]
Ras-GRF-1 (OE)	↑ Development and expression of cocaine locomotor sensitization ↑ cocaine-CPP	[91]
Ras-GRF-2 (KO)	↓ Ethanol intake and preference (two bottle-free choice task)	[93]
ERK1 (KO)	↑ Basal locomotor activity ↑ AMPH-induced locomotion ↑ Development of cocaine locomotor sensitization ↑ cocaine-CPP	[34,63,94,95]
ERK1 (KD in the PFC)	↑ Basal locomotor activity ↑ AMPH-induced locomotion	[68]
ERK2 (OE in the VTA)	↑ development and expression of cocaine locomotor sensitization ↑ Cocaine-CPP	[96]
ERK2 (KD in the VTA)	↓ Development and expression of cocaine locomotor sensitization ↓ Cocaine-CPP	[96]
MSK-1 (KO)	↓ Development and expression of cocaine locomotor sensitization ↑ Cocaine-CPP	[97]
Inhibition of pElk-1	↓ Development and expression of cocaine locomotor sensitization ↓ The establishment of cocaine-CPP	[98]

KO, knockout; KD, knockdown; OE, overexpression; ↑, enhancing effect; ↓, inhibiting effect.

kinase-mediated pGluN2B is necessary for the pERK induction in response to repeated cocaine administration.[106] In addition, cocaine challenge after withdrawal from repeated cocaine administration also resulted in sensitized pERK in the NAc and CPu compared to the acute cocaine effect.[108,120,121] The cocaine behavioral sensitization-induced pERK and pCREB in the NAc is dependent on ERK activation.[122] Further, the induction and expression of cocaine behavioral sensitization can be inhibited by systemic SL327 injection or intra-NAc MEK inhibitor infusion.[64,71,72] Similarly through MEK activation, the pERK induction in the VTA is required for the development of behavioral sensitization to cocaine.[11,70] Lastly, studies have indicated that, in response to D1- and NMDA-R activation, pERK induced by cocaine is responsible for the chronic cocaine-enhanced dendritic spine density and dendritic length in the CPu and NAc[123,124] providing the morphologic evidence mediated by ERK signaling after repeated cocaine administration.

Repeated pairing of a specific environment with drug administration leads to a memory association between contextual cues and the drug rewarding effect. Subsequently, the context itself directly motivates drug-seeking behavior as a measurement of the reinforcing effect of the drug,[125,126] which is associated with ERK signaling. For example, the acquisition of cocaine-CPP is accompanied by pERK induction in the NAc and PFC in a D1-R-dependent manner.[127] Systemic preadministration of SL327 (50 mg/kg) and a GluN2B antagonist inhibited the development of cocaine-CPP,[69,106] indicating the requirement of NMDA-R-mediated ERK activation in the formation of context–drug association memory. ERK activation in the VTA is necessary for the development of cocaine-CPP.[73] Cocaine challenge in the drug-paired environment resulted in pERK and pCREB induction in the subset of neurons of the NAc.[128] In animals with repeated cocaine administration, the saline challenge enhanced pERK induction in the D1-positive neurons in NAc and CPu, indicating context conditioning-induced ERK activity.[108] Similarly, after the establishment of CPP, CPP testing or re-exposure to the cocaine-associated context induced pERK, pCREB, and/or ΔFosB in the CPu, HIPP, VTA, and NAc as well as in D1-R-containing neurons of the NAc.[73,129–133] The CPP test-induced pERK expression in the VTA is dependent on mGluR1 activation and protein synthesis.[133] Further, Miller and Marshall demonstrated that CPP test-elevated pERK and drug-seeking behavior were blocked by intra-NAc core infusion of U0126 (2 μg/side).[74] In the cocaine SA paradigm, context-induced relapse is also associated with enhanced pERK in the NAc core

and CPu.[134] Altogether, these results imply that, through ERK signaling, the NAc core and VTA are important for the memory formation of context–drug association. pERK in the NAc core and CPu also involve the retrieval of CPP memory and a general motor activation driven by drug-associated context, respectively.

Memory reconsolidation occurs when well-established drug-associated memories are recalled by re-exposure to drug-associated context, cues, or the drug itself during which memories can be destabilized by adding new information or subjected to manipulation.[135–137] The ability to disrupt drug-related memories provides an opportunity to promote treatment outcome and prevent relapse. The general procedure to test the memory reconsolidation on drug-seeking behavior contains two phases: re-exposing animals to drug-associated context (phase 1) followed by testing drug-seeking behavior after withdrawal (phase 2). A previous study demonstrated that, before or immediately after phase 1, intra-NAc core MEK inhibition through U0126 (1 μg/side) or PD98059 (2 μg/side) reduced cocaine-CPP during the phase 2. The protein expression of pERK, pCREB, pElk-1, and c-Fos induced by phase 2 is also attenuated with inhibiting ERK during phase 1.[74] Systemic SL327 injection after phase 1 also decreased subsequent context-induced CPP in animals conditioned by escalating doses of cocaine.[76] Similar to reactivation of CPP memory by context, the memory reconsolidation in response to cocaine is also accompanied by ERK activation in the PFC, NAc, and CPu. With or without cocaine priming, the systemic SL327 (20 mg/kg) pretreatment before phase 1 inhibits the subsequent drug-seeking behavior.[75] However, the effect of ERK on cocaine-induced memory reconsolidation is still dependent on the presence of context. Thus, the contribution of cocaine itself on memory reconsolidation is still ambiguous. After the establishment of cocaine SA, U0126 (1 μg/side) infusion into the BLA immediately after phase 1 inhibited context-induced reinstatement and the pERK induction after phase 2.[77] Taken together, these studies indicate that ERK signaling activated during memory reconsolidation is necessary for cocaine-seeking behavior. However, a critical time window, 6 h after the reactivation of memories, has been documented during which the memory is susceptible to alteration in the fear-conditioning paradigm.[138] The pretreatment before phase 1 may influence the memory retrieval instead of reconsolidation. If the ERK signaling actually involves drug-related memory reconsolidation, the difference should be found when treatment is conducted within and beyond the critical time window in terms of both behavioral and molecular aspects.

Unlike pERK sensitization in cocaine-induced behavioral sensitization, immediately after the cessation of cocaine SA, there is a dissociation between pERK induction and cocaine intake indicating the failure of developing pERK sensitization or tolerance, although with enhanced pERK expression in several brain regions.[139] However, ERK activation has been implicated in relapse after withdrawal. For example, the extinction test (conditioned cues + context) significantly increased pERK in the CeA and cocaine-seeking behavior after 30 days of withdrawal. Both enhanced pERK and relapse are dependent on MEK and NMDA-R activation.[78] Similarly, the pERK induction in the ventromedial PFC has been shown to mediate extinction-test-induced cocaine-seeking behavior after 1- or 30-day withdrawal from cocaine SA.[140] Through ERK activation, direct intra-VTA glial cell line-derived neurotrophic factor (GNDF) or brain-derived neurotrophic factor (BDNF) infusion immediately after the last session of cocaine SA induced robust drug-seeking behavior after 3 or 10 days withdrawal.[79,80] These results demonstrated that the potentiated ERK signaling underlies relapse behavior after cocaine SA. In contrast to augmented pERK induction in the PFC after 1-day abstinence of cocaine SA,[140] 2 h after the last cocaine SA session, we have demonstrated a transient reduction of pERK in the PFC.[81,141,142] The reduction of pERK is associated with an increase of STEP but not PP2A activity accompanied by decreased total GluN2B protein expression and phosphorylation, suggesting the inhibitory effect of STEP on pERK and NMDA-R.[143] Through MEK activation and normalization of pERK in the PFC, direct BDNF infusion into the dorsomedial PFC immediately after the end of the last cocaine SA session resulted in a long-term inhibition on context-, cue-, or cocaine-induced relapse.[81] Thus, it indicated that rescuing the ERK signaling or hypofunction in the PFC during early withdrawal might provide a potential therapeutic strategy for preventing cocaine relapse.

Several animal models have been used to dissect the ERK signaling cascade in cocaine-induced behavioral changes. For example, double knock-out (KO) type 1 and type 8Ca^{2+}-stimulated AC resulted in a reduction of basal pERK in medium spiny neurons in the striatum with blunted acute cocaine–induced pERK, pMSK-1, and pCREB. Behaviorally, these double KO AC mice are supersensitive to low-dose acute cocaine–induced locomotion and fail to develop behavioral sensitization in response to repeated cocaine administration.[90] Ras-guanine nucleotide-releasing factors 1 (Ras-GRF1), the upstream activator of Ras, can increase ERK signaling. In the striatum, the protein expression of Ras-GRF-1 is increased by acute psychostimulants including cocaine.[144,145] D1-R agonist and Glu-induced