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Glutamate Signaling to Ras-MAPK in Striatal Neurons

Mechanisms for Inducible Gene Expression and Plasticity

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Abstract

Extracellular signals can regulate mitogen-activated protein kinase (MAPK) cascades through a receptor-mediated mechanism in postmitotic neurons of adult mammalian brain. Both ionotropic and metabotropic glutamate receptors (mGluRs) are found to possess such an ability in striatal neurons. NMDA and AMPA receptor signals seem to share a largely common route to MAPK phosphorylation which involves first activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) via Ca^{2+} influx, followed by subsequent induction of phosphoinositide 3-kinase (PI3-kinase). Through its lipid and protein kinase activity, active PI3-kinase may transduce signals to Ras-MAPK cascades via at least two distinct pathways. A novel, Ca^{2+}-independent pathway is believed to mediate mGluR signals to Ras-MAPK activation. As an information superhighway between the surface membrane and the nucleus, Ras-MAPK cascades, through activating their specific nuclear transcription factor targets, are actively involved in the regulation of gene expression. Emerging evidence shows that MAPK-mediated genomic responses in striatal neurons to drug exposure contribute to the development of neuroplasticity related to addictive properties of drugs of abuse.

Index Entries: NMDA; mGluR; MAPK; ERK; PI3-kinase; JNK; p38; *fos;* nucleus accumbens.

Introduction

The excitatory amino acid L-glutamate (glutamate) is a major neurotransmitter in the developing and adult central nervous system (CNS) and participates in the regulation of a variety of synaptic and cellular activities related to signal transmission, survival, and neuroplasticity. The glutamate action is achieved by its interactions with two specific families of surface receptors: ionotropic and metabotropic receptors *(1,2).* The former is

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classified into *N*-methyl-D-aspartate (NMDA), (+/–)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate receptors *(2–4).* The latter includes eight subtypes of metabotropic glutamate receptors (mGluRs) that are further grouped into three functional subgroups according to their connections to intracellular signaling systems *(5).* The NMDA receptor allows endogenous glycine and glutamate to interact with *(6)* and is permeable to Ca^{2+} *(7)* as well as other ions (Na^+ and K^+) *(6).* The AMPA/kainate receptor is also an ion channel that promotes Na^+ influx and, to a lesser extent, Ca^{2+} influx *(8).* MGluRs are G protein-coupled receptors and, depending upon the subgroups, connect to different second messenger systems *(5).* For example, group I mGluRs (mGluR1 and 5 subtypes) are positively linked to phosphoinositide hydrolysis, resulting in an increase in Ca^{2+} signals and activation of protein kinase C (PKC). Group II (mGluR2 and 3) and III (mGluR4, 6, 7, and 8) receptors are negatively coupled to adenylate cyclase, leading to the inhibition of cAMP/protein kinase A (PKA).

Mitogen-activated protein kinases (MAPKs) refer to a large number of cytosolic and nuclear kinases that function in signal transduction cascades and mediate cellular growth, differentiation, and survival in mammalian proliferative cells *(9,10).* In postmitotic neuronal cells of adult mammalian brain, MAPKs are also highly expressed. In those mature neurons, MAPKs are sensitive to diverse extracellular signals, including those derived from synaptic stimulation of glutamate receptors. The Ca^{2+}-dependent signaling cascades are believed to be prime signaling pathways linking ionotropic receptors to MAPKs. However, a novel signaling pathway may be involved in processing mGluR signals to MAPK activation. The existence of multiple glutamate receptors and receptor subtype-specific signaling pathways to the MAPK regulation suggests distinct roles that MAPKs may play in regulating synaptic strength, gene expression, and many other cellular activities. Moreover, the existence of multiple pathways could allow either generation of a specific MAPK response or integration of complex MAPK responses to enhanced glutamatergic transmission.

The Ras-MAPK Pathway

The Ras-MAPK cascade involves a consecutive and sequential activation of four signaling proteins: Ras, Raf, MAPK kinase (MEK), and MAPK (*see* Fig. 1), and the last three are organized hierarchically into three-tiered protein kinase modules *(9,10).* The initial link Ras ($p21^{ras}$) is a small guanine nucleotide-binding protein that localizes to the inner face of the plasma membrane. Four genes encode the mammalian *ras* family: Ki-*ras,* H-*ras,* N-*ras,* and R-*ras* and they function as molecular switches that transmit extracellular or intracellular signals to downstream components of MAPK cascades. Prior to activation, Ras resides at the plasma membrane and is bound to GDP (GDP-Ras), a form of inactive Ras. In response to stimulation, such as growth factor-binding that induces growth factor receptors to dimerize and undergo tyrosine autophosphorylation (Tyr1068) within their cytoplasmic domains, Ras is activated after converting its GDP to GTP via a cascade involving a Grb2/Shc/Sos adapter protein complex. Once active, Ras transmits its signals to Raf-1 by phosphorylating it at Ser259 and/or Ser621 via an unknown mechanism. Phosphorylated Raf-1 serves as a serine kinase subsequently phosphorylating MEK at its Ser217/221 sites, which in turn activates MAPKs.

MAPKs are a widely conserved family of serine/threonine protein kinases, while they are also substrates for upstream protein kinases (MEKs). The first subfamily of MAPKs was identified by Ray and Sturgill in 1987 as insulin-induced protein kinases *(11).* In mammalian cells, these insulin-induced protein kinases were demonstrated to be p44 and p42 proteins which now are two well-characterized MAPKs: extracellular signal-regulated kinases 1 and 2 (ERK1/2), respectively, in addition to a number of less related and relatively uncharacterized

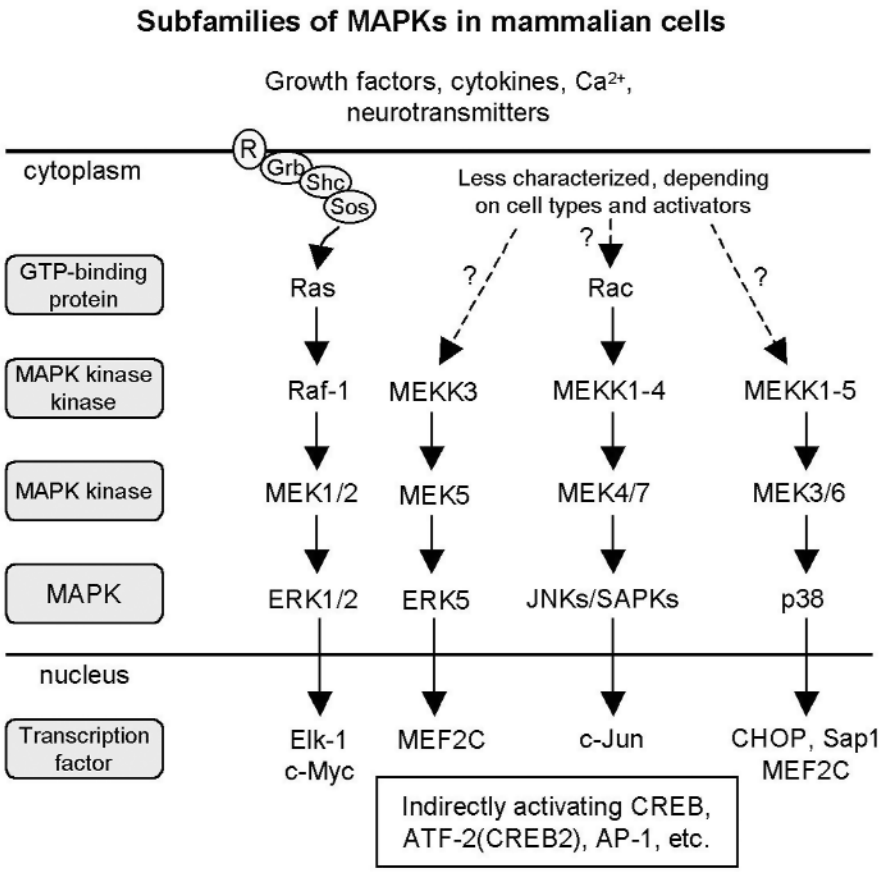

Fig. 1. Three subfamilies of mitogen-activated protein kinases (MAPKs) and their signaling cascades. MAPK cascades respond to a variety of stimuli and then transduce those extracellular signals to the nucleus through their distinct pathways for activating their direct transcription factor targets or indirect targets, such as cAMP response element-binding protein (CREB), activating transcription factor-2 (ATF-2; also CREB2), or activator protein-1 (AP-1). Extracellular signal-regulated protein kinase 5 (ERK5) has a separate pathway. The broken lines indicate less characterized routes. Other abbreviations: CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein (also GADD153, growth arrest, and DNA damage-inducible protein 153); JNKs, c-Jun N-terminal kinases; MEF2C, myocyte enhancer factor 2C; MEK, MAPK kinase; MEKK, MAPK kinase kinase; SAPKs, stress-activated protein kinases; Sos, Son of sevenless.

kinases in this subfamily, such as human ERK3, ERK4, ERK5 or big MAPK 1 (BMK1), and ERK7 *(12)*. As the prototypic MAPKs, ERK1/2 are activated via Thr202 and Tyr204 phosphorylation by diverse stimuli (mitogens, cytokines, ions, neurotransmitters, etc.) via a Ras/Raf/MEK1/2 pathway (Fig. 1). Phosphorylated ERK1/2 (pERK1/2) can translocate into the nucleus where they induce gene expression by activating specific transcription factors either directly or indirectly *(13)*. Up- and downstream-signaling links for ERK3/4/7 are poorly understood, and ERK5 is activated via a cascade distinguishable from the classical pathway that activates ERK1/2 (Fig. 1).

At least 2 additional subfamilies of MAPKs have recently been identified: the c-Jun N-terminal kinases/stress-activated protein kinases (JNKs/SAPKs) and p38 MAPKs *(14,15)* (*see* Table 1). There are at least 10 different

Table 1
Members of the MAPK Family *(15)*[a]

MAPK subtypes	P-site motifs[b]
ERKs	
ERK1 (p44 MAPK)	TEY
ERK2 (p42 MAPK)	TEY
ERK3	SEG
ERK4	TEY
ERK5 (BMK1)	TEY
ERK7	TEY
JNKs	
JNK1 (SAPK1γ)	TPY
JNK2 (SAPK1α)	TPY
JNK3 (SAPK1β)	TPY
p38	
p38α (SAPK2a)	TGY
p38β (SAPK2b)	TGY
p38γ (SAPK3, ERK6)	TGY
p38δ (SAPK4)	TGY

[a] *See* Fig. 1 and text for abbreviations.

[b] Contains serine, threonine, and tyrosine residues in the activation loop for phosphorylation.

mammalian JNK proteins produced by three genes: JNK1, JNK2, and JNK3. There are four isoforms of p38: p38α, p38β, p38γ (ERK6 or SAPK3), and p38δ (SAPK4). Although all three subfamilies of MAPKs are regulated by threonine and tyrosine phosphorylation, they are heterogeneous in the sequence around the activating phosphorylation sites, upstream kinases and activators, downstream substrates, and thus physiological roles (Fig. 1). Multiple distinct MAPK cascades are advantageous in terms of orchestrating the intracellular signaling networks, which determine precise intracellular responses to diverse changes in extracellular environments.

Glutamate Regulation of the Ras-MAPK Pathway

Because high levels of ionotropic and metabotropic glutamate receptors exist in striatal region (for a review, *see* ref. *16*), glutamate's roles in regulating MAPK cascades were extensively investigated in recent years (1998–2003). One of the best-illustrated MAPK subfamilies in striatal neurons in response to glutamate stimulation is ERK1/2. In addition to growth factors, cytokines, dopamine, and Ca^{2+}, increasing evidence from both in vivo and in vitro studies indicates that glutamate is among neurotransmitters that induce positive ERK1/2 responses. In rat striatal neuronal cultures or striatal slices, glutamate increased ERK1/2 phosphorylation *(17–19).* Electrical or chemical stimulation of the corticostriatal pathway in vivo also induced the same ERK1/2 response *(20,21).* The induced ERK1/2 phosphorylation observed in vivo and in vitro is rapid and transient, and occurs in small- to medium-sized neurons (i.e., striatonigral and striatopallidal projection neurons), but not glia. Active pERK1/2 is present in the nuclear envelop at a high level as opposed to a low level in the cytoplasm and neural processes, indicating a prime role in regulating gene expression. Noticeably, basal phosphorylation of ERK1/2 is almost absent under unstimulated conditions despite an existence of a high level of ERK1/2. This indicates that ERK1/2 is tonically inactive in maintaining normal cellular activity in adult striatal neurons.

All three subtypes of ionotropic receptors are believed to possess the ability to phosphorylate ERK1/2 upon activation. Early studies during the years 1996 to 1997 tie NMDA-receptor activity to ERK1/2 cascades in hippocampal and cortical neurons *(22–24).* Recently, pharmacological activation of NMDA receptors strongly increased ERK1/2 phosphorylation in striatal neurons, which was sensitive to the selective NMDA receptor antagonists *(17,25–27).* Studies regarding AMPA receptors are somewhat inconsistent. While AMPA did not appear to activate ERK1/2 in cultured rat hippocampal neurons *(28)* and striatal slices *(26),* AMPA increased ERK1/2 phosphorylation in cultured mouse or rat striatal neurons *(17,22,29).* AMPA receptor-mediated responses undergo rapid desensitization *(30),* which may

partially explain the failure to see changes in ERK activity in some studies. Indeed, when an AMPA receptor-specific desensitization inhibitor cyclothiazide was co-incubated, AMPA evoked a larger, detectable increase in ERK1/2 phosphorylation *(29).* Two reports have described an invariable increase in ERK1/2 phosphorylation in striatal neurons after kainate application *(17,26).* This defines all three subtypes of ionotropic glutamate receptors as independent surface mediators transducing extracellular signals to MAPK/ERK activation.

G protein-coupled receptors (GPCR) activate multiple signaling pathways, including MAPK cascades *(31).* As a member of the GPCR family that is densely expressed in striatal neurons, mGluRs have a potential to regulate the MAPK pathway. A first study carried out by Choe and Wang (2001) shows that intra-caudate injection of a group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) upregulated ERK1/2 phosphorylation in vivo *(32),* similar to the results observed in cortical *(33,34)* and hippocampal *(28)* neurons or glia, or in CHO cells expressing transfected group I mGluRs *(35).* Group I mGluRs also mediate striatal ERK activation induced by ischemic insults *(36).* In a long-term striatal culture in which mGluRs are well developed, DHPG reliably elevated pERK1/2 levels (Wang et al., unpublished observations). Thus, group I mGluRs exert a facilitatory influence on the MAPK/ERK system. There are currently no available data regarding the influence of group II and III mGluRs on striatal MAPK cascades, although a few reports described an increased ERK phosphorylation after group II and/or III stimulation in cerebellar *(35),* glial *(37),* or CHO cells *(38,39).* Apparently the mGluR connection to the MAPK pathway remains a novel and intriguing area to be explored in the future.

Besides ERK, glutamate may affect the other two MAPK subfamilies (JNKs and p38). NMDA activated JNK and p38 kinases in hippocampal *(40)* and cerebellar *(41,42)* neurons. Quinolinic and kainic acid-induced excitotoxicity in the cortex and the hippocampus was accompanied by an increased JNKs and p38 phosphorylation *(43,44).* Glutamate or NMDA also activated JNK in cultured striatal neurons (18,19,45, but 26). Thus, like ERKs, JNK and p38 subfamilies can positively respond to the signals from ionotropic types of glutamate receptors.

NMDA Receptor Signaling to the Ras-MAPK Pathway

Several studies have made attempts to dissect signaling steps from NMDA receptors to the Ras-MAPK in striatal neurons in details (*see* Fig. 2). The Ca^{2+}-permeable NMDA is believed to initiate its activation of ERK1/2 via Ca^{2+} influx because NMDA no longer induces ERK1/2 activation in the Ca^{2+}-free medium, and low concentrations of extracellular Ca^{2+} impair NMDA activation of ERK1/2 *(17,27).* Efforts to identify the Ca^{2+}-dependent kinase that relays Ca^{2+} signals reveal the importance of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a major Ca^{2+}-sensitive kinase in the postsynaptic NMDA receptor complex. Inhibition of calmodulin with W-7 or CaMKII with KN62 or KN93 prevents NMDA-induced ERK1/2 phosphorylation in striatal neurons *(18,27).* It is reasonable to assume that NMDA-receptor stimulation results in a sizable increase in CaMKII phosphorylation. However, it has been difficult to experimentally confirm such a change owing to a high level of constitutively active pCaMKII. Perhaps a subtle change in CaMKII phosphorylation only occurs at the localized, affected NMDA synapses. Another proposed Ca^{2+}-dependent kinase which is also localized at NMDA synapses that might be involved in NMDA-evoked ERK1/2 phosphorylation is PKC. However, in striatal neurons, two reports consistently observed an insignificant effect of the PKC inhibitors Ro-31-8220 and Gö6983 on ERK1/2 phosphorylation induced by glutamate and NMDA *(17,46),* distinctive to a partial blockade of ERK1/2 activation by the PKC inhibitors in hippocampal neurons *(28).* Thus, PKC activation, if there is, is not a necessary link in the NMDA/ERK cascade in striatal neurons.

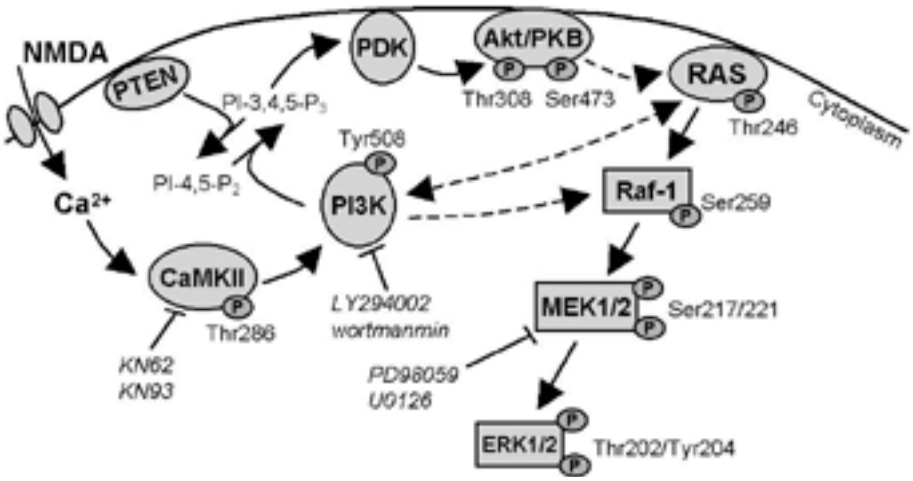

Fig. 2. A schematic diagram illustrating characterized signaling cascades transducing NMDA receptor signals to Ras-MAPKs. Ca^{2+} influx through NMDA receptors activates CaMKII, which in turn activates PI3-kinase. Active PI3-kinase triggers activation of Ras or Raf-1 through its protein kinase activity. Alternatively, PI3-kinase increases the lipid product of PI-3,4,5-P_3 (PIP_3) through its lipid kinase activity, which may activate Ras through a PDK/Akt-dependent pathway. The broken lines indicate less characterized pathways. Abbreviations: CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; ERK1/2, extracellular signal-regulated protein kinase 1 and 2; MEK1/2, mitogen-activated protein kinase kinase 1 and 2; PDK, 3-phosphoinositide-dependent kinase; PI3-kinase, phosphoinositide 3-kinase; PKB, protein kinase B; PTEN, phosphatase and tensin homolog deleted on chromosome 10.

A novel finding in further screening of responsible protein kinases is the discovery of a significant contribution of phosphatidylinositol (phosphoinositide) 3-kinase (PI3-kinase) to NMDA activation of ERK. This is demonstrated by a finding that the PI3-kinase inhibitors (LY294002 and wortmannin) totally blocked ERK1/2 activation induced by NMDA and the Ca^{2+} ionophore ionomycin in striatal neurons *(27,46).* The PI3-kinase family contains 3 distinct subclasses: IA/IB, II, and III. The 3 class IA isoforms (α, β, and δ) are composed of heterodimeric complexes comprising a 110-kDa catalytic subunit (p110) and a 50–85 kDa regulatory subunit (typically p85), and class IB (isoform γ) lacks an N-terminal binding site for the aforementioned regulatory subunits *(47).* All IA isoforms (at least their regulatory subunits) are expressed in neurons *(48)* whereas IB p110γ appears to be deficit in nervous tissue *(49).* Thus, it seems likely that class IA PI3-kinases are involved in NMDA activation of ERK1/2, although the precise IA isoforms remain to be identified. With regard to the relationship between PI3-kinase and CaMKII, we found that the CaMKII inhibitor blocked NMDA-induced PI3-kinase (p85α) phosphorylation, whereas the PI3-kinase inhibitor did not alter CaMKIIα phosphorylation under normal and NMDA-stimulated conditions *(46).* These data, together with recent evidence showing a high affinity calmodulin target sequence in PI3-kinase *(50,51),* are consistent with a model in which NMDA/Ca^{2+} activates the Ras-MAPK cascade by first inducing CaMKII phosphorylation followed by PI3-kinase phosphorylation.

Little is known about how PI3-kinase activates the Ras-MAPK pathway. PI3-kinase is a dual specificity kinase (i.e., possessing both lipid and protein phosphorylation capacities). Through its lipid kinase activity, PI3-kinase phosphorylates a phosphatidylinositol 4,5-

diphosphate (PIP_2; also PI-4,5-P_2) at the 3-position to a phosphatidylinositol 3,4,5-triphosphate (PIP_3; PI-3,4,5-P_3). The lipid product PIP_3 can then lead to activation of Akt kinase, a serine/threonine kinase also referred to as protein kinase B (PKB) or Rac, through the dual effects of phosphoinositide-dependent kinases (PDKs) and PIP_3 recruitment of Akt to membrane-bound PDKs *(52).* Since NMDA induced a robust PI3-kinase-dependent increase in Akt phosphorylation at Ser473 *(27),* a process known to be required for full activation of the kinase *(53),* the PDK/Akt pathway may have a potential to serve as a downstream target of PI3-kinase to bridge PI3-kinase to the Ras-MAPK system. The fact that the MEK inhibitors did not alter NMDA activation of Akt kinase *(27)* and that the PI3-kinase mutant that lacks lipid kinase activity, lost its ability to mediate Ras activation *(54)* seem to support the role of lipid PDK/Akt kinases. However, more convincing evidence favoring or against the contribution of PDK/Akt needs to be obtained with antagonistic tools towards these kinases (such as antisense oligonucleotides, RNA interference, dominant negative mutation, knockout mice, or pharmacological inhibitors that are currently lacking).

As compared to lipid kinase activity, protein–protein interactions catalyzed by protein kinase activity emerging from PI3-kinase seem more likely to be responsible for its linkage to Ras-MAPK *(55).* Even though the immediate downstream target protein for PI3-kinase is unknown, there is some evidence showing that PI3-kinase, through its protein kinase activity, regulates Ras, Raf, or MEK in some cell types *(55–57).* The engineered PI3-kinase that has protein kinase activity but lacks lipid kinase activity appears to activate MEK through protein phosphorylation *(55).* On the other hand, PI3-kinase is under the regulation by Ras as GTP-Ras can bind to the p110 catalytic subunit to enhance its catalytic activity *(47,58).* Ras can also activate PI3-kinase *(59),* which then mediates some of the biological actions of Ras *(60,61).* Future studies need to determine the specific site(s) in the Ras/Raf/MEK cascade that PI3-kinase interacts with and define whether PI3-kinase lies upstream or downstream of Ras in NMDA-receptor signaling.

Classical growth factor tyrosine kinase receptor-associated signal cascades involve PI3 kinase activation. It is very interesting to learn whether NMDA activates PI3-kinase through a growth factor receptor-dependent mechanism. One scenario is that NMDA intracellularly enhances responses of the epidermal growth factor (EGF) receptor to its ligand, which leads to increased PI3-kinase activity. To evaluate this mechanism, the EGF receptor inhibitors were found not to affect NMDA activation of PI3-kinase and ERK1/2 *(22,46),* indicating an EGF ligand-binding independent pathway that transduces NMDA receptor signals to PI3-kinase. Although EGF receptors are able to activate PI3-kinase, active PI3-kinase by this mechanism is proposed to be a parallel cascade to Ras activation via a signaling mechanism involving the Grb/Shc/Sos complex *(62).* Like the receptor tyrosine kinase, Src-like nonreceptor tyrosine kinases are defective in processing NMDA activation of ERK1/2 *(22,46).*

A tumor suppressor on human chromosome 10 that was cloned in 1997 and is now known as phosphatase and tensin homolog deleted on chromosome 10 (PTEN; also MMAC1 and TEP1) *(63,64)* may be a powerful negative regulator of MAPK cascades in both cancer cells and neurons. PTEN encodes a phosphatase which, like PI3-kinase, is a dual-specificity protein phosphatase *(65)* and a PIP_3 lipid phosphatase *(66)* with a high level of expression in adult rodent forebrain *(67).* Since PTEN and PI3-kinase have opposing effects on PIP_3 levels (Fig. 2), PTEN may counterbalance PI3-kinase-mediated cellular activities, including activation of the MAPK pathway *(68).* To date, in striatal neurons, no data are available regarding putative negative roles of PTEN in regulating NMDA/PI3-kinase signaling to the MAPK pathway.

AMPA Receptor Signaling to the Ras-MAPK Pathway

Ca^{2+}-permeable AMPA receptors transduce signals from the cell surface to MAPK cascades through a largely similar path aforementioned for NMDA receptors. According to a study conducted on cultured mouse striatal neurons (ref. *29;* also Tang and Wang, unpublished observations), Ca^{2+} influx through AMPA receptors activates CaMKII, which subsequently triggers activation of PI3-kinase. PI3-kinase then recruits a neuron-specific, Ca^{2+}/calmodulin-dependent guanine nucleotide exchange factor (GEF), such as Ras-GRF, or a nonreceptor protein tyrosine kinase, such as Src or PYK2, to Ras for Ras activation and the following activation of raf, MEK, and MAPK. A large number of recent studies in various cell lines demonstrating the role of Ras-GRF, Src, and PYK2 in Ca^{2+}/calmodulin-dependent activation of MAPK cascades (for a review, *see* refs. *69*,*70*) support the connecting role of these small lipid kinases from PI3-kinase to Ras.

The similarity in major signaling proteins, as well as in spatiotemporal and amplitude properties of ERK1/2 phosphorylation after NMDA and AMPA stimulation *(17),* suggests that the AMPA receptor may achieve its action via a subsequent activation of the closely located NMDA receptor or vice versa. However, the NMDA and AMPA receptor antagonists failed to block each other's phosphorylation of ERK1/2 *(17).* Thus, the two receptors at the receptor level, independently activate a similar Ca^{2+}-dependent pathway towards Ras-MAPK activation. It is unclear why the two different receptors share a largely common route to MAPKs, although it is still assumed that some discrepancies or cross-talk may exist between the two receptor-associated signaling cascades for the integration and accurate control of MAPK responses to glutamate stimulation.

mGluR Signaling to the Ras-MAPK Pathway

Intracaudate injection of a group I mGluR agonist increased ERK1/2 phosphorylation in vivo *(32).* This effect seems to involve the activation of CaMKII since the CaMKII inhibitor attenuated the ERK1/2 activation *(71).* In an in vitro ischemic model, group I mGluR-sensitive PKC is active in processing ERK activation in striatal neurons *(36).* Further signaling information concerning mGluR-sensitive route to the Ras-MAPK pathway is not available. Future studies need to evaluate the importance of several high-potential candidates in this signaling event, which include intracelluler Ca^{2+} release, PKC, PI3-kinase, and small adapting proteins (GRF, Src, PYK2, etc.).

A study in rat cortical astrocytes suggests a novel path from mGluR5 to Ras-MAPK activation *(72).* Upon activation, mGluR5 receptors are recruited to the EGF receptor to form a signaling complex activating Ras-ERK2. This process involves the activation of $G_{\alpha q}$ and Src tyrosine kinases, but is independent of the activation of phospholipase $C_{\beta 1}$, PKC, and Ca^{2+} that are traditional effectors in the mGluR5-initiated pathway.

Glutamate-Sensitive Ras-MAPK Pathways in Inducible Gene Expression

As an information super-highway between the surface membrane and the nucleus, Ras-MAPK cascades couple environmental changes to DNA transcription. In the nucleus, this action involves the MAPK-mediated activation (phosphorylation) of various transcription factors via a direct or indirect mechanism. Active transcription factors in turn bind to specific sites on target DNA promoters in a sequence-specific manner to facilitate transcription *(73,74).* For ERK1/2, a specific nuclear target is one of ternary complex factors (TCF), a sub-

group of the ETS domain transcription factor family. Conventionally, TCF dimerizes with serum response factors (SRF) to assemble multiprotein complexes binding to serum response element (SRE) on the promoter for the sake of regulating gene expression *(75–77).* Among the three members of TCFs, i.e. Elk-1 (p62TCF), SAP1, and SAP2/ERP/Net *78–81,* Elk-1 represents a sensitive substrate of ERK1/2 and is strongly and exclusively expressed in neuronal cells, including striatal neurons *(20).* Through rapid phosphorylation on Ser383 and Ser389 in its C-terminal region by ERK1/2, phosphorylated (p)Elk-1 increases its ability to form a ternary complex with SRF and SRE *(82,83)* and as a result, facilitates transcription of target DNAs.

A great deal of evidence has shown the significant role of Ras-MAPK cascades in regulating gene expression in a variety of cell types in response to different stimuli. In striatal neurons, MAPK cascades are also revealed to process glutamatergic signals to inducible gene expression. Electrical stimulation of the glutamatergic corticostriatal pathway induced Elk-1 phosphorylation in the rat striatum in vivo that showed a strict spatiotemporal correspondence to concomitant induction of ERK and immediate early genes (c-*fos* and *zif/268*) *(20).* The MEK inhibitor that inhibited Elk-1 phosphorylation attenuated the c-*fos* induction *(21).* Thus, Elk-1 as a nuclear target of ERK controls immediate early gene expression in striatal neurons. In addition to Elk-1, another transcription factor, cAMP response element-binding protein (CREB), may also contribute to the ERK regulation of gene expression because a MEK-dependent hyperphosphorylation of CREB was also seen following the corticostriatal stimulation *(21).*

Similar results were reported in striatal cultures or slides *(18,27).* Attempts to unravel the role of MAPK cascades in the mGluR-regulated gene expression found that the group I mGluR agonist increased Elk-1 phosphorylation in striatal neurons both in vivo *(32,71)* and in vitro *(84).* Antisense oligonucleotides that selectively knocked down the increase in Elk-1 phosphorylation attenuated c-*fos* mRNA expression induced by the group I agonist *(84).* Apparently, Elk-1 is a key responsible transcription factor that is downstream to active MAPK cascades and processes group I mGluR-regulated gene expression at the transcription level.

Ras-MAPK Pathways in Striatal Neuronal Plasticity Related to Addiction

An important characteristic of drugs of abuse is their stimulative effects on gene expression in discrete brain structures, including the striatum. The genomic responses are critically involved in the formation of neuroplasticity related to addictive properties of drugs. Although Ras-MAPK pathways have been well implicated in several neurobiological processes, its functional roles in drug addiction are poorly understood. Emerging evidence shows that acute or chronic administration of a psychostimulant cocaine or amphetamine activated ERK throughout the striatum *(85–88).* The activation of ERK depends on not only dopamine D_1 receptors, but also on NMDA receptors and group I mGluRs, since the antagonists selective for these receptors effectively block the ERK phosphorylation *(85,86).* Moreover, activation of CaMKII is needed for amphetamine activation of ERK *(89).*

The activated ERK is believed to facilitate gene expression (such as c-*fos*) because: 1) the ERK activation kinetically corresponds to the Elk-1 and CREB induction; and 2) more importantly, the MEK inhibitor SL327 antagonizes cocaine-induced hyperphosphorylation of Elk-1 and CREB and c-*fos* expression *(85,86).* Behaviorally, systemic injection of the MEK inhibitor abolished the rewarding effects of cocaine in the place-conditioning paradigm *(85).* Microinjection of the MEK inhibitor PD98059 into the ventral tegmental area (VTA)

blocked behavioral sensitization to cocaine *(90).* Thus, the MAPK cascade is proposed to be involved in the prime burst of gene expression underlying long-term behavioral changes in response to drug exposure *(85).*

A recent focus of drug addiction study is the investigation of learning and memory mechanisms involved in compulsive drug use and relapse (for a review, *see* refs. *91–93*). Increasing evidence supports the existence of an "addiction memory" that is developed after chronic drug abuse. The formation of addiction memory could then participate in distinct phases of drug-seeking and drug-taking behaviors. Recently, MAPK cascades have been found to engage a set of molecular events in striatal neurons that are normally involved in associative learning and memory, such as long-term potentiation (LTP), long-term depression (LTD), and altered gene expression. For instance, the MAPK pathway inhibitors blocked glutamate-dependent LTP at rat corticostriatal synapses, a cellular substrate of learning and memory *(94).* Similarly, the MEK inhibitors reduced the LTD induced by the selective mGluR2/3 agonists LY354740 and L-CCG1 in mouse corticostriatal synapses *(95).* These results support a pivotal role of MAPK cascades in mediating glutamate-dependent long-term memory in striatal region which may lead to permanent changes in striatal neurons related to the development of drug addiction. Apparently, study of the MAPK's role in striatum-dependent learning and memory and drug addiction is in its infant stage *(96).* Further functional studies are necessary, along with the development of inhibitors for major members of MAPK family with demonstrated efficacy and selectivity in vivo *(97),* to address this issue.

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