ERK2: a logical AND gate critical for drug-induced plasticity?

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Title:

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Truncated title:

ERK2 and drug-induced plasticity

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Summary

Drug addiction results in part from the distortion of dopamine-controlled plasticity. Extracellular signal-regulated kinase (ERK) plays an important role in the underlying molecular mechanisms. ERK is activated by drugs of abuse in a subset of neurons in reward-related brain regions. This activation, necessary for the expression of immediate early genes, depends on dopamine D_1 and glutamate receptors. Blockade of ERK activation prevents long-lasting behavioral changes, including psychomotor sensitization and conditioned place preference. It also interferes with drug craving and drug-associated memory reconsolidation. In contrast, ERK1 mutation enhances the effects of drugs. We suggest that the ERK2 pathway plays the role of a logical AND gate, permissive for plasticity, in neurons on which converge dopamine-mediated reward signals and glutamate-mediated contextual information.

Introduction

A variety of substances naturally produced by plants (cocaine, morphine, cannabinoids, nicotine) or synthesized by humans with various degrees of sophistication (ethanol, heroin, amphetamine) trigger a peculiar behavior of recurrent consumption, in humans as in experimental animals. This response can become compulsive and lead to a pathological state called addiction, with its well known deleterious consequences for health and society. Addiction is a chronic disease with a high risk of relapse even after a long time of withdrawal, revealing stable brain alterations. A chief aspect of addiction is related to the distortion of reward mechanisms dependent on dopamine (DA) [1-3]. Thus, drugs of abuse are the source of a major medical problem and, at the same time, the Ariane's thread that may lead neuroscientists through the maze of a major learning mechanism.

Drugs of abuse enhance extracellular DA levels in the forebrain, especially in the nucleus accumbens (NAcc) where they control corticostriatal glutamate transmission [1-3]. A critical question is the nature of the signaling mechanisms activated by DA in striatal and other target neurons that trigger the long-lasting alterations responsible for the behavioral effects of drugs of abuse. This question is not only theoretical but may have clinical implications, since deciphering these mechanisms may lead to novel strategies to modify, or even reverse, some of the seemingly irreversible consequences of addiction. Several signaling pathways have been implicated in the actions of DA. Here, we focus on the role of the extracellular signal-regulated kinase (ERK) pathway, one of the highly conserved mitogen-activated protein kinase (MAP-kinase) modules.

The ERK pathway

MAP-kinases modules comprise 3 classes of enzymes that act in a cascade of activatory phosphorylation: the upstream kinases, or MAP-kinase/ERK-kinase-kinases (MEKK), phosphorylate and activate the MAP-kinase/ERK-kinases (MEK). MEKs are dual specificity kinases, which trigger the activation MAP-kinases by phosphorylating a threonine and a tyrosine in their activation loop. Specificity in the signaling between these modules is achieved by protein-protein interactions and scaffolding molecules [4]. The ERK kinase family (ERK1-8) is characterized by a Thr-Glu-Tyr motif in the activation loop. Phosphorylation of both the Thr and Tyr residues in this motif is required for ERK activation.

This double phosphorylation is easily detected by specific antibodies, and in many studies is taken as a reliable index of the activity of ERKs. ERK1-2 is of paramount importance in the control of cell growth and differentiation, and is also involved in neuronal plasticity [5,6]. The ERK1/2 module is turned on by tyrosine kinase and G protein-coupled receptors through the generation of GTP-bound forms of small G proteins of the Ras family. These small G proteins activate MEKKs of the Raf family (mostly Raf-1 and B-Raf in the brain) [4], which phosphorylate and activate MEK1/2, which phosphorylate and activate ERK1/2. ERK1/2 phosphorylate numerous substrates at (Ser/Thr)-Pro sites in all cell compartments, including the nucleus where they play a key role in gene regulation. Among the other ERKs that have been studied in some details, ERK5 is also highly expressed in brain and is involved in cortical progenitor differentiation [7]. Since the role(s) of ERK3-8 in adult brain are not yet characterized this review focuses on ERK1/2.

Drugs of abuse activate ERK1/2 in a subset of neurons in brain reward circuits

Activation of ERK1/2 in response to addictive drugs was first reported in the ventral tegmental area (VTA) after a 5-day exposure of rats to morphine or cocaine [8]. Subsequently, acute treatment of mice with cocaine was shown to induce a rapid and transient increase in ERK phosphorylation in the NAcc [9], an effect reproduced with other drugs of abuse including Δ9-tetrahydrocannabinol (THC), D-amphetamine, 3,4-methylene-dioxy-methamphetamine (MDMA), morphine, and nicotine [10-13]. Brain mapping after acute treatment with drugs of abuse and other psychoactive compounds revealed a common regional pattern of ERK1/2 activation, characteristic of abused drugs, including nucleus accumbens, lateral bed nucleus of stria terminalis, central amygdala and deep layers of the prefrontal cortex [12] (Table 1).

In the regions listed above, the activation of ERK1/2 by drugs of abuse is blocked by a D_1 DA receptor (D_1-R) antagonist [9,11,12]. In the striatum, ERK1/2 activation in response to D-amphetamine occurs in a subset of GABAergic medium spiny neurons (MSNs) expressing D_1-R [14]. Selective stimulation of D_1-R in vivo has a weaker effect on ERK activation [15,16]. In fact ERK activation by drugs involves also glutamate since responses to cocaine, D-amphetamine or THC are prevented by NMDA antagonists [9,11,14] or, in the case of amphetamine, by group I metabotropic glutamate receptor (mGluR) [13]. These observations have important functional implications, since they imply that the ERK pathway behaves as a

coincidence detector of the activation of DA and glutamate pathways [14]. In striatal neurons in culture, D_1-R stimulation increases weakly ERK1/2 phosphorylation, whereas agonists of glutamate ionotropic and group I metabotropic receptors induce a stronger activation [17,18]. Activation of DA and glutamate receptors seems to potentiate each others [17,18]. Other receptors such as DA D_2 receptors [9] and cannabinoid CB_1 receptors (Corbillé et al in preparation) appear to play a role in the activation of ERK1/2 by drugs of abuse, although this role is likely to be indirect.

The precise mechanisms of activation of ERK1/2 in striatal neurons in response to drugs of abuse are still incompletely understood (Fig. 1). Several possible pathways coupling NMDA receptors activation to ERK1/2 phosphorylation through Ca^{2+} influx have been proposed in other systems (see [5,6]), but their role in MSNs remains to be clarified. On the other hand the regulation of ERK1/2 by D_1-R is better characterized and involves dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) [14] (Fig. 1). Although other links with D_1 receptors may exist, the regulation of protein phosphatases through DARPP-32 appears critical for the activation of ERK1/2 in the striatum.

Role of ERK in psychomotor sensitization and rewarding effects of drugs

The major tools used to address the role of ERK1/2 are inhibitors of MEK, either microinjected in specific brain regions (PD98059 and U0126) or, in the case of SL327 which crosses the blood brain barrier, administered intraperitoneally. The specificity of microinjected inhibitors is difficult to assess since their concentrations in the vicinity of the injection point is likely to exceed those recommended in vitro. On the other hand, the site of action of SL327 injected at the periphery cannot be determined. Therefore, both approaches have limitations and cautious interpretation of the results is advisable. This is further underscored by the fact that the spectrum of action of these compounds is not limited to the ERK1/2 pathway [7].

At a dose (30 mg/kg) which inhibited by >80% ERK phosphorylation in the striatum, SL327 did not alter mouse spontaneous activity or acute locomotor responses to cocaine or D-amphetamine [14,19]. Thus, ERK1/2 activation seems to play little role, if any, in the locomotor effects of the first injection of drug. By contrast, SL327 prevented the induction of locomotor sensitization by a single or repeated injections of cocaine or D-amphetamine

[14,19,20]. SL327 also prevented the acquisition of a conditioned locomotor response triggered by cocaine or D-amphetamine-paired context [19]. Importantly, blockade of ERK1/2 activation did not prevent the expression of previously acquired sensitization [19]. The site of action of SL327 may include the VTA since local inhibition of MEK abolished the initiation of sensitization to cocaine without affecting acute responses [21]. However, since the activation of ERK1/2 by cocaine is much less robust in the VTA than in other brain structures [12], ERK activation in the accumbens and/or cortex may also participate in the induction of psychomotor sensitization.

The induction of conditioned place preference (CPP), usually presented as an index of the "rewarding effects" of drugs, also requires ERK1/2 activation. Blockade of ERK1/2 activation, by systemic administration of SL327 and/or local injection of MEK inhibitors in the NAcc during the training sessions prevented the induction of conditioned place preference by cocaine, D-amphetamine, MDMA or THC [9-11,22].

Role of ERK1/2 **in drug-conditioned responses and in their "reconsolidation"**

Sensory stimuli by themselves unable to activate ERK1/2, can do so when they have been associated repeatedly with drugs. After acquisition of cocaine self-administration, the presentation of drug-associated cues increases ERK1/2 phosphorylation in central amygdala after one month of withdrawal [23]. In animals trained for CPP with cocaine or methamphetamine, exposure to the test apparatus is sufficient to activate ERK1/2 in the nucleus accumbens, caudate-putamen and prefrontal cortex [24-26]. Whether these activations of ERK1/2 by environmental cues are mediated by the same molecular pathways as those initially triggered by drugs, is not known.

Interestingly ERK1/2 seems to be important for the expression of drug conditioned responses. Local injection of MEK inhibitors in the NAcc abolished the expression of CPP to cocaine and methamphetamine [24,25]. Similarly, injection of U0126 in the central amygdala prevented cocaine seeking in rats previously trained to self-administer this drug [23]. These observations are very interesting since they suggest a more important role of ERK1/2 in the expression of the conditioned behavioral response than in the response to the initial drug injection.

In various learning models, including object recognition and fear conditioning, re-exposure to the sensory cues leads to memory reconsolidation. During this phase memories are sensitive to protein synthesis and MEK inhibitors [27,28]. Two studies have implicated ERK1/2 in the reconsolidation of CPP induced by cocaine or morphine [25,26]. Local injection of MEK inhibitors in the nucleus accumbens, before or immediately after retrieval of conditioned response disrupted cocaine CPP tested 1 or 14 days later [25]. Systemic injection of SL327, or of a protein synthesis inhibitor, erased previously acquired morphine or cocaine CPP [26]. However, SL327 was efficient only if administered just before a supplemental conditioning session with the drug, but not before a simple exposure to the conditioned environment. The same requirement for re-exposure to both drug and context was also observed in a study using protein synthesis inhibitors to erase morphine-conditioned responses [29]. Additional work is required to determine whether these discrepancies are due to differences in the experimental protocols. The important common finding is that inhibition of the ERK1/2 pathway during the "reconsolidation" phase eliminates the conditioned response to drugs. Similar results have been obtained by inhibition of Zif268 (also known as Krox24 or NGFI-A) expression in the amygdala [30], where its expression is also SL327-sensitive [31]. These studies suggest that established conditioning, which contributes to the high rate of relapse in addicts, could be accessible to therapeutic agents. It will be important to determine whether a strong and prolonged conditioning is also liable to pharmacological erasure. As could be expected, our preliminary results indicate that following a prolonged conditioning procedure, CPP becomes more resistant to erasure by a MEK inhibitor. Therefore, further experiments are required to determine to which extent pharmacological treatments, combined or not with appropriate behavioral stimuli, can reverse strong conditioning.

Molecular targets of ERK

The evidence summarized above strongly supports the role of ERK1/2 in the acquisition of long lasting behavioral effects of drugs of abuse, and, possibly, in their expression. It is clearly of great interest to identify the substrates of ERK1/2 which are important in either case. Phosphorylated ERK1/2 is detected in both dendrites and perikarya of striatal neurons and rapidly accumulates in the nucleus. The rapid time scale of these responses [24,25] suggests that ERK influences conditioned responses via mechanisms other than transcription. A possible target could be the voltage-dependent K^+ channel, Kv4.2, which is an important

ERK1/2 substrate in the hippocampus [6]. Beyond these very rapid effects, ERK1/2 signaling plays a major role in regulating activity-related spine dynamics [32,33], glutamate receptors insertion at synapses [34,35], and dendritic protein synthesis [34,35]. All these effects of ERK1/2 have been identified in the hippocampus and examining their possible role in other brain regions more directly related to addiction is an interesting area for future research.

Drug administration triggers a complex transcriptional program in the striatum that depends on the combined stimulation of D_1 and NMDA receptors [36]. Gene expression is thought to be essential for the long-term behavioral changes induced by drugs. Gene transcription is markedly inhibited by pharmacological inhibition of MEK [9-11,20,37-39]. ERK regulates gene expression by controlling directly and indirectly the phosphorylation of transcription factors and chromatin components. The effects of ERK1/2 on transcription in the striatum have been examined recently [37,38] (Fig. 2). The mitogen- and stress-activated kinase 1 (MSK1) plays a critical role in the phosphorylation of cAMP-responsive element binding protein (CREB) and histone H3, and in transcription of many, but not all, of the studied genes [38]. Remarkably MSK1-null mice had a partially decreased psychomotor sensitization in response to cocaine, while CPP was slightly enhanced, in agreement with the role of CREB and dynorphin in the aversive effects of drugs. Thus, MSK1-independent genes, including Zif268, are critical for the rewarding effects of cocaine. There is direct support for the critical role of Zif268: CPP to cocaine was prevented in Zif268 knockout mice [37] and antisense oligonucleotides injected in the amygdala altered drug-conditioned memory reconsolidation [30]. Since Zif268 is itself a transcription factor, the identification of its targets will be important for characterizing long term effects of drugs of abuse. In this respect it is interesting to note that p35, the regulatory subunit of Cdk5, a protein important in the effects of cocaine (see [40]), is induced by ERK1/2 through Zif268 [41]. Interestingly glucocorticoids, which play an essential role in the regulation of drug responses by stress, up-regulate the ERK/Zif268 pathway [42].

Opposing roles of ERK1 and ERK2

Although they have 90% sequence identity, ERK 1 and ERK2 appear to have distinct, even sometimes opposite functional effects [43,44]. Thus, ERK1 can inhibit ERK2 signaling, possibly by competition for phosphorylation by MEK. Drug-induced phosphorylation of ERK2 appears much stronger than ERK1 (e.g. [14]). In ERK1 knockout mice, which are

viable and fertile, CPP evoked by morphine or cocaine and psychomotor sensitization to cocaine were enhanced [20,44]. Cocaine-induced gene transcription was also increased [20]. Assuming that the numerous effects of MEK inhibitors reported above are due to the inhibition of ERK1 and ERK2, these results indicate that ERK2 is the isoform important for the behavioral effects of drugs of abuse.

Conclusion: possible functional role of ERK2 in the effects of drugs of abuse and beyond

The evidence summarized above underlines the key role of the ERK2 pathway in long-lasting effects of drugs of abuse. In striatal MSNs its activation requires both dopamine and glutamate inputs, suggesting that it functions as a logical AND gate (Fig. 3). This gate will detect the coincidence of environmental cues (glutamate) and positive reward prediction error signals (dopamine). Evidence suggests that when activated the ERK2 pathway puts the neuron in a plasticity-permissive state, both at the level of dendrites and by regulating nuclear transcription (see Fig. 3). Although, it is likely that evolution has selected several molecular devices with similar properties to ensure an optimal signal processing in neuronal systems critical for survival, the ERK2 pathway is possibly the first to be clearly identified as such. Even though better characterized in the striatum, the same model might apply to other regions involved in the effects of drugs of abuse including amygdala and prefrontal cortex. Future work will test these hypotheses and determine how much of these mechanisms uncovered in the context of drugs of abuse is also relevant for learning controlled by natural rewards.

Acknowledgements

Work in the laboratory of J.A. Girault related to this review was supported by Inserm, *Agence Nationale de la Recherche* (ANR), Schlumberger Foundation for Education and Research, Bettencourt-Schueller Foundation, *Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie* (MILDT) and *Université Pierre et Marie Curie* (UPMC).

References

1. Hyman SE, Malenka RC, Nestler EJ: **Neural Mechanisms of Addiction: The Role of Reward-Related Learning and Memory**. *Annu Rev Neurosci* 2006.
2. Everitt BJ, Robbins TW: **Neural systems of reinforcement for drug addiction: from actions to habits to compulsion**. *Nat Neurosci* 2005, **8**:1481-1489.
3. Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D: **Dopamine and drug addiction: the nucleus accumbens shell connection**. *Neuropharmacology* 2004, **47 Suppl 1**:227-241.
4. Kolch W: **Coordinating ERK/MAPK signalling through scaffolds and inhibitors**. *Nat Rev Mol Cell Biol* 2005, **6**:827-837.
5. Thomas GM, Huganir RL: **MAPK cascade signalling and synaptic plasticity**. *Nat Rev Neurosci* 2004, **5**:173-183.
6. Sweatt JD: **Mitogen-activated protein kinases in synaptic plasticity and memory**. *Curr Opin Neurobiol* 2004, **14**:311-317.
7. Nishimoto S, Nishida E: **MAPK signalling: ERK5 versus ERK1/2**. *EMBO Rep* 2006, **7**:782-786.
8. Berhow MT, Hiroi N, Nestler EJ: **Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine**. *J Neurosci* 1996, **16**:4707-4715.
9. Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J: **Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties**. *J Neurosci* 2000, **20**:8701-8709.
10. Salzmann J, Marie-Claire C, Le Guen S, Roques BP, Noble F: **Importance of ERK activation in behavioral and biochemical effects induced by MDMA in mice**. *Br J Pharmacol* 2003, **140**:831-838.
11. Valjent E, Pages C, Rogard M, Besson MJ, Maldonado R, Caboche J: **Delta 9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depends on dopaminergic transmission**. *Eur J Neurosci* 2001, **14**:342-352.
12. Valjent E, Pages C, Herve D, Girault JA, Caboche J: **Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain**. *Eur J Neurosci* 2004, **19**:1826-1836.

13. Choe ES, Chung KT, Mao L, Wang JQ: **Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors**. *Neuropsychopharmacology* 2002, **27**:565-575.

• 14. Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, et al.: **Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum**. *Proc Natl Acad Sci U S A* 2005, **102**:491-496.

This paper shows that stimulation of D_1 and glutamate receptors is required for the activation of the ERK pathway by amphetamine. Using biochemical and immunofluorescence approaches in a variety of mutant mice it provides evidence that inhibition of protein phosphatase-1 by DARPP-32 is required for the activation of ERK by several drugs of abuse in the stritaum, but not in the prefrontal cortex. The regulation of ERK involves the tyrosine phosphatase STEP and effects upstream from ERK.

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

16. Kim DS, Palmiter RD, Cummins A, Gerfen CR: **Reversal of supersensitive striatal dopamine D1 receptor signaling and extracellular signal-regulated kinase activity in dopamine-deficient mice**. *Neuroscience* 2006, **137**:1381-1388.

• 17. Mao L, Yang L, Tang Q, Samdani S, Zhang G, Wang JQ: **The scaffold protein Homer1b/c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascades in neurons**. *J Neurosci* 2005, **25**:2741-2752.

This paper investigates the regulation of ERK by mGluR5 in striatal neurons in culture. It provides evidence for Ca^{2+}-dependent and -independent mechanisms, and shows the unexpected and important role of the scaffolding protein homer, although its precise mechanism is not identified.

18. Voulalas PJ, Holtzclaw L, Wolstenholme J, Russell JT, Hyman SE: **Metabotropic glutamate receptors and dopamine receptors cooperate to enhance extracellular signal-regulated kinase phosphorylation in striatal neurons**. *J Neurosci* 2005, **25**:3763-3773.

19. Valjent E, Corvol JC, Trzaskos JM, Girault JA, Herve D: **Role of the ERK pathway in psychostimulant-induced locomotor sensitization**. *BMC Neurosci* 2006, **7**:20.

• 20. Ferguson SM, Fasano S, Yang P, Brambilla R, Robinson TE: **Knockout of ERK1 Enhances Cocaine-Evoked Immediate Early Gene Expression and Behavioral Plasticity**. *Neuropsychopharmacology* 2006.

This paper shows that long term behavioral effects of cocaine and cFos induction are enhanced in ERK1 null mice. Together with the results of the Brambilla lab (Refs. 42, 43) this provides strong evidence for a negative role of ERK1 and designates ERK2 as the major isoform involved in the effects of drugs.

21. Pierce RC, Pierce-Bancroft AF, Prasad BM: **Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the Ras/Mitogen-activated protein kinase signal transduction cascade**. *J Neurosci* 1999, **19**:8685-8695.

22. Gerdjikov TV, Ross GM, Beninger RJ: **Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats**. *Behav Neurosci* 2004, **118**:740-750.

•• 23. Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y: **Central amygdala ERK signaling pathway is critical to incubation of cocaine craving**. *Nat Neurosci* 2005, **8**:212-219.

This paper provides strong evidence in vivo in the rat that ERK in the central amygdala is involved in the incubation and expression of cocaine craving.

24. Mizoguchi H, Yamada K, Mizuno M, Mizuno T, Nitta A, Noda Y, Nabeshima T: **Regulations of methamphetamine reward by extracellular signal-regulated kinase 1/2/ets-like gene-1 signaling pathway via the activation of dopamine receptors**. *Mol Pharmacol* 2004, **65**:1293-1301.

•• 25. Miller CA, Marshall JF: **Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory**. *Neuron* 2005, **47**:873-884.

This paper shows that exposure of rats previously conditioned for cocaine CPP to the conditioned cues activates the ERK pathway in the accumbens core. Moreover, using microinjections of MEK inhibitors it provides evidence that blocking this pathway prevents the expression of CPP 1 or 14 days later. This work suggests that drug-induced conditioning undergoes a reconsolidation process, activated by contextual cues, similar to other types of memories. Other studies (see Refs. 26, 29) have found similar results using inhibitors of MEK or protein synthesis inhibitors injected at the periphery. An interesting difference is that in the latter studies, exposure to the context alone was not sufficient for the pharmacological

disruption of the CPP, it also required re-exposure to the drug. This could be due to a lack of specificity of microinjected drugs or to differences in the experimental protocols.

• 26. Valjent E, Corbille AG, Bertran-Gonzalez J, Herve D, Girault JA: **Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference**. *Proc Natl Acad Sci U S A* 2006, **103**:2932-2937.

See discussion of Ref. 25.

27. Kelly A, Laroche S, Davis S: **Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory**. *J Neurosci* 2003, **23**:5354-5360.

28. Duvarci S, Nader K, LeDoux JE: **Activation of extracellular signal-regulated kinase-mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning**. *Eur J Neurosci* 2005, **21**:283-289.

• 29. Milekic MH, Brown SD, Castellini C, Alberini CM: **Persistent disruption of an established morphine conditioned place preference**. *J Neurosci* 2006, **26**:3010-3020.

See discussion of Ref. 25.

•• 30. Lee JL, Di Ciano P, Thomas KL, Everitt BJ: **Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior**. *Neuron* 2005, **47**:795-801.

This paper shows that infusion of Zif268 antisense oligodeoxynucleotides into the basolateral amygdala, prior to reexposition to conditioned stimulus (CS)-cocaine association, abolishes the acquired conditioned reinforcing properties of the drug-associated stimulus. This result is particularly interesting since it implicates one of the downstream effectors of the ERK pathway, in an operant conditioned behavioral response closer to addiction than CPP.

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

32. Wu GY, Deisseroth K, Tsien RW: **Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology**. *Nat Neurosci* 2001, **4**:151-158.

33. Goldin M, Segal M: **Protein kinase C and ERK involvement in dendritic spine plasticity in cultured rodent hippocampal neurons**. *Eur J Neurosci* 2003, **17**:2529-2539.

34. Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R: **Ras and Rap control AMPA receptor trafficking during synaptic plasticity**. *Cell* 2002, **110**:443-455.

35. Kelleher RJ, 3rd, Govindarajan A, Jung HY, Kang H, Tonegawa S: **Translational control by MAPK signaling in long-term synaptic plasticity and memory**. *Cell* 2004, **116**:467-479.

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

37. Valjent E, Aubier B, Corbille AG, Brami-Cherrier K, Caboche J, Topilko P, Girault JA, Herve D: **Plasticity-associated gene Krox24/Zif268 is required for long-lasting behavioral effects of cocaine**. *J Neurosci* 2006, **26**:4956-4960.

• 38. Brami-Cherrier K, Valjent E, Herve D, Darragh J, Corvol JC, Pages C, Arthur SJ, Girault JA, Caboche J: **Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice**. *J Neurosci* 2005, **25**:11444-11454.

This paper identifies the central role of MSK1 in nuclear responses to cocaine in the striatum. At the same time it shows that these responses are not necessary for CPP and points to the important role of MSK1-independent genes such as Zif268.

39. Ferguson SM, Robinson TE: **Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade**. *J Neurochem* 2004, **91**:337-348.

40. Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P: **DARPP-32: an integrator of neurotransmission**. *Annu Rev Pharmacol Toxicol* 2004, **44**:269-296.

41. Harada T, Morooka T, Ogawa S, Nishida E: **ERK induces p35, a neuron-specific activator of Cdk5, through induction of Egr1**. *Nat Cell Biol* 2001, **3**:453-459.

• 42. Revest JM, Di Blasi F, Kitchener P, Rouge-Pont F, Desmedt A, Turiault M, Tronche F, Piazza PV: **The MAPK pathway and Egr-1 mediate stress-related behavioral effects of glucocorticoids**. *Nat Neurosci* 2005, **8**:664-672.

Although this paper is primarily dealing with the hippocampus, it has interesting implications in the context of the effects of drugs of abuse. It provides a possible link between the well established role of stress in addiction and the ERK pathway, by showing that glucocorticoid receptor upregulates the levels of several proteins in the ERK pathway and the induction of Egr1/Zif268.

• 43. Vantaggiato C, Formentini I, Bondanza A, Bonini C, Naldini L, Brambilla R: **ERK1 and ERK2 mitogen-activated protein kinases affect Ras-dependent cell signaling differentially**. *J Biol* 2006, **5**:14.

Using non-neuronal cell systems this paper shows that ERK1 can exert effects opposite to those of ERK2 and that the equilibrium between the two isoforms probably controls the functional output of the ERK pathway. These results, which expand those reported in Ref. 43, are key for understanding the phenotype of ERK1 knockout mice (Refs. 20, 43) and are the basis for identifying the prominent role of ERK2.

44. Mazzucchelli C, Vantaggiato C, Ciamei A, Fasano S, Pakhotin P, Krezel W, Welzl H, Wolfer DP, Pages G, Valverde O, et al.: **Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory**. *Neuron* 2002, **34**:807-820.

45. Vanhoutte P, Barnier JV, Guibert B, Pagès C, Besson MJ, Hipskind RA, Caboche J: **Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices**. *Mol.Cell Biol.* 1999, **19**:136-146.

46. Choe ES, Wang JQ: **CaMKII regulates amphetamine-induced ERK1/2 phosphorylation in striatal neurons**. *Neuroreport* 2002, **13**:1013-1016.

47. Illario M, Cavallo AL, Bayer KU, Di Matola T, Fenzi G, Rossi G, Vitale M: **Calcium/calmodulin-dependent protein kinase II binds to Raf-1 and modulates integrin-stimulated ERK activation**. *J Biol Chem* 2003, **278**:45101-45108.

48. Krapivinsky G, Krapivinsky L, Manasian Y, Ivanov A, Tyzio R, Pellegrino C, Ben-Ari Y, Clapham DE, Medina I: **The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1**. *Neuron* 2003, **40**:775-784.

49. Crossthwaite AJ, Valli H, Williams RJ: **Inhibiting Src family tyrosine kinase activity blocks glutamate signalling to ERK1/2 and Akt/PKB but not JNK in cultured striatal neurones**. *J Neurochem* 2004, **88**:1127-1139.

50. Braithwaite SP, Paul S, Nairn AC, Lombroso PJ: **Synaptic plasticity: one STEP at a time**. *Trends Neurosci* 2006, **29**:452-458.

51. Heffron D, Mandell JW: **Differential localization of MAPK-activated protein kinases RSK1 and MSK1 in mouse brain**. *Brain Res Mol Brain Res* 2005, **136**:134-141.

52. Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, et al.: **Chromatin remodeling is a key**

mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 2005, **48**:303-314.

53. Schultz W: **Behavioral theories and the neurophysiology of reward**. *Annu Rev Psychol* 2006, **57**:87-115.

54. Ferrell JE, Jr.: **Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability**. *Curr Opin Cell Biol* 2002, **14**:140-148.

Table 1: Effects of drugs of abuse on ERK1/2 phosphorylation in mouse brain

Brain regions	Cocaine	Nicotine	Morphine	THC
Dorsal striatum	++	++	-	++
N. accumbens core	++	+	+	+
N. accumbens shell	++	++	++	++
Ventral Pallidum	-	-	-	-
Globus Pallidus	-	-	-	-
Lateral BNST	++	++	++	++
Amygdala: - Central	++	++	++	++
- Basolateral	++	++	-	-
- Lateral	-	-	-	-
Hippocampus (CA1-3)	++	+	-	++
Cingular cortex (deep layers)	++	++	-	-
Prefrontal cortex (deep layers)	++	++	++	++
Lateral Septum	-	+	+	+
VTA	-	-	+	-
Substantia nigra	-	-	-	-

The number of P-ERK1/2-positive cells was measured 10 min after the injection of cocaine (20 mg/kg) or 20 min after the injection of nicotine (0.4 mg/kg), morphine (5 mg/kg), or THC (1mg/kg). Significant increases, as compared to vehicle administration: + (<3-fold) and ++ (≥3-fold). Drugs of abuse were the only substances to activate ERK1/2 in all the brain regions indicated in bold and italics. These increases were prevented in SCH23390-pretreated mice. The NAcc was the only region in which P-ERK1/2 was not increased by non-addictive drugs. Data from Ref. [12]. BNST: bed nucleus stria terminalis.

Figure Legends

Figure 1: Signaling pathways regulating ERK1/2 activation in striatal neurons. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) seems required for ERK1/2 activation in striatal slices [45] and in vivo [46]. Although CaMKII participates in the activation of Raf-1 [47], its relationship with the ERK pathway in the striatum is not known. Another possible link is Ras-GRF, a guanine-nucleotide exchange factor activated by Ca^{2+}/calmodulin binding, and associated to NR2B [48]. Src family tyrosine kinases appear important for activation of ERK1/2 by NMDA receptors in striatal neurons in culture [49], but there is no evidence for their role in vivo (our unpublished observations). In striatal neurons in culture, mGluR5 activate ERK1/2 through Ca^{2+}-dependent and independent mechanisms, the latter involving the scaffolding protein homer [17]. D_1 receptor is mostly coupled to type 5 adenylyl cyclase (AC5) through an heterotrimeric G protein containing the α_{olf}, $\gamma 7$, and presumably $\beta 1$ subunits (Golf). Activated cAMP-dependent protein kinase (PKA) phophorylates DARPP-32 on Thr-34, turning it into a potent inhibitor of protein phosphatase-1 (PP-1) [40]. The inhibition of PP-1 by DARPP-32 is essential for ERK1/2 activation in response to drugs of abuse in the striatum, since this activation was selectively prevented in the striatum of DARPP-32-null and Thr-34-Ala knock-in mutant mice [14]. PP-1 is not directly active on ERK1/2, but regulates striatal-enriched phosphatase (STEP), which dephosphorylates the tyrosine residue of ERK1/2 activation loop [14,50]. DARPP-32 is also active upstream from ERK since phosphorylation of MEK1/2 was impaired in DARPP-32 mutant mice [14], at level(s) which remain(s) to be identified. Additional links between D_1 receptors and the ERK1/2 pathway are possible. Note that in all studies ERK2 is more strongly activated than ERK1, and that ERK1 inhibits ERK2 signaling [43,44] (see text). P: phosphate.

Figure 2: Regulation of transcription by ERK in the striatum. The ternary complex components of the Ets family, including Elk1, are well-established targets of ERK, which regulate promoters with serum-responsive elements. Elk-1 is phosphorylated in the striatum in response to cocaine and THC in a SL327-sensitive fashion [9,11]. The precise role of Elk1 in the effects of drugs may be difficult to pin down because of the redundancy within the Ets family. ERK (in striatal neurons ERK2 is the functionally important enzyme, see text) can activate several nuclear protein kinases including p90 ribosomal protein S6 kinases (RSK1/2) and mitogen- and stress-activated kinases 1 and 2 (MSK1/2). These kinases have multiple substrates, including CREB, a transcription factor which regulates transcription at cAMP-

responsive elements. MSK1 is enriched in the striatum [51] where it is phosphorylated in a SL327-sensitive fashion in response to cocaine [38]. Cocaine-induced phosphorylation of CREB at Ser-133 and histone H3 at Ser-10 is severely impaired in MSK1 knockout mice [38]. The effect on CREB Ser-133 phosphorylation is particularly remarkable since this residue is a substrate for various protein kinases including RSK1/2, CaMKIV and PKA, the latter being considered, traditionally, as the major CREB kinase in the striatum. Histone H3 phosphorylation participates in chromatin remodeling induced by drugs of abuse that favor their effect on transcription [38,52]. The lack of MSK1 had markedly different consequences on the induction of various genes: cocaine induction of immediate early genes cFos and dynorphin was blocked in MSK1-KO mice, whereas induction of Egr-1/Zif268 was unaltered [38]. Pol: RNA polymerase II transcription complex. P: phosphate.

Figure 3: The ERK2 pathway as a plasticity-permissive logical AND gate. The ERK pathway is activated by drugs of abuse in a subset of neurons in regions important for their behavioral effects. Activation of this pathway is necessary for long-lasting effects of drugs. **A-** In striatal neurons (MSNs) ERK2 activation requires the concomitant stimulation of dopamine D_1 receptors and glutamate receptors of the NMDA subtype, and possibly other receptors including mGluR5 (see Fig. 1). Thus, the ERK2 pathway operates as a logical AND gate, which detects the coincident activation of glutamate inputs, presumably encoding contextual information related to environmental cues and internal state, and DA inputs, encoding the reward prediction error signal [53]. It satisfies a key requirement for successful plasticity underlying reward-controlled learning. **B-** In other systems the ERK2 pathway is a molecular switch, transforming graded inputs into all-or-none responses [54]. It may have a similar role in striatal neurons: when a certain level of activation is achieved, as a combined function of dopamine (DA) and glutamate (Glu) inputs, the threshold for ERK2 activation is reached and the neuron switches into a plasticity-permissive state that favors the establishment of long-lasting changes. This function may be implemented: 1) at the level of dendrites by facilitating back-propagation of action potentials, as proposed in the hippocampus [6] or by controlling local processes [6,32-35]; 2) in the nucleus by regulating the chromatin response and the induction of genes, such as Zif268, required for long term stabilization of synaptic changes (see Fig. 2). Reactivation of this pathway during the "reconsolidation" phase may depend on the repetition of the environmental stimuli (glutamate) and the DA signal generated by the acquired conditional value of the cues [2,53], if it reaches a sufficient level, or by the repeated chemical action of the drug.

Figure 1

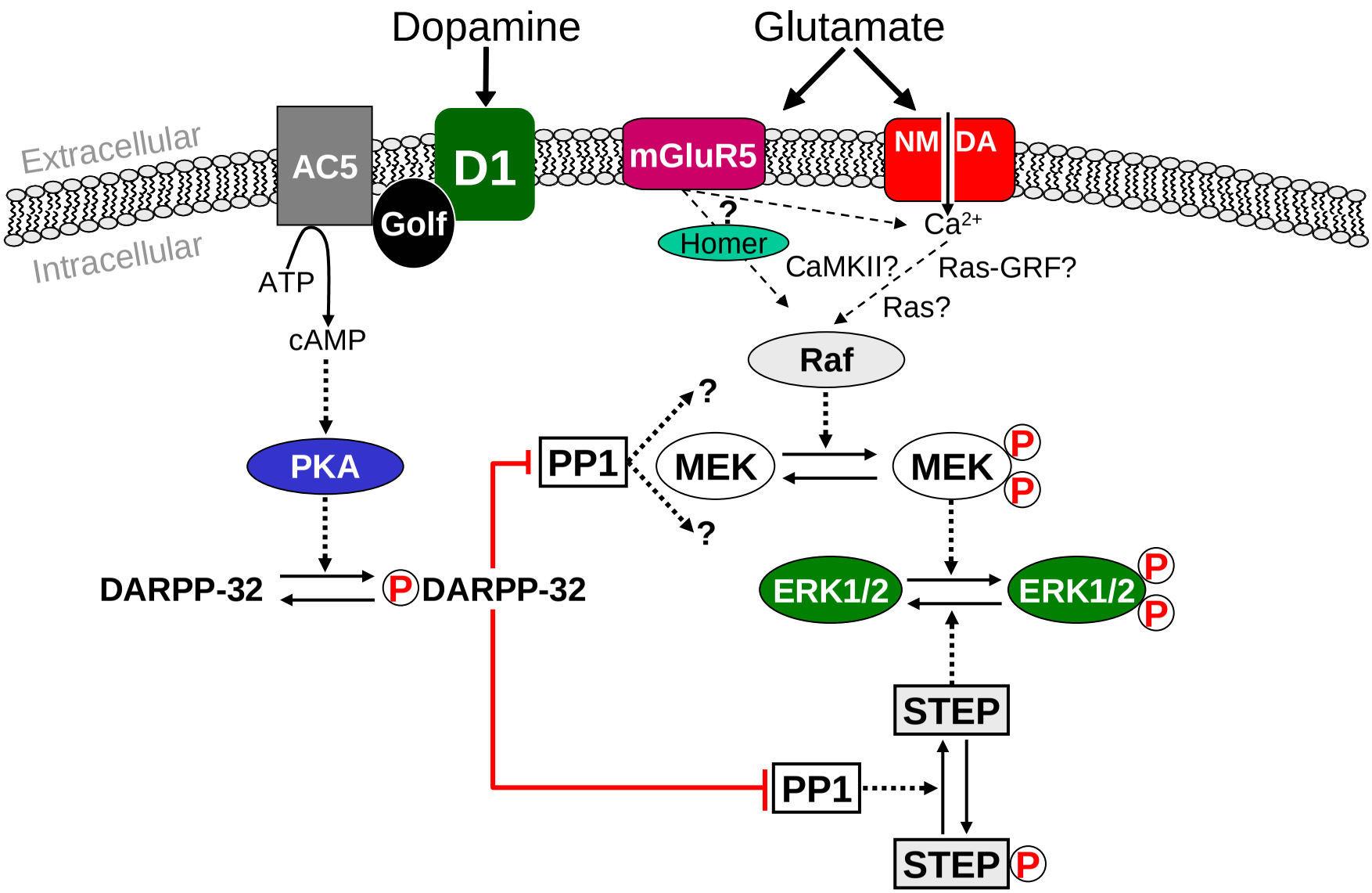

Figure 2

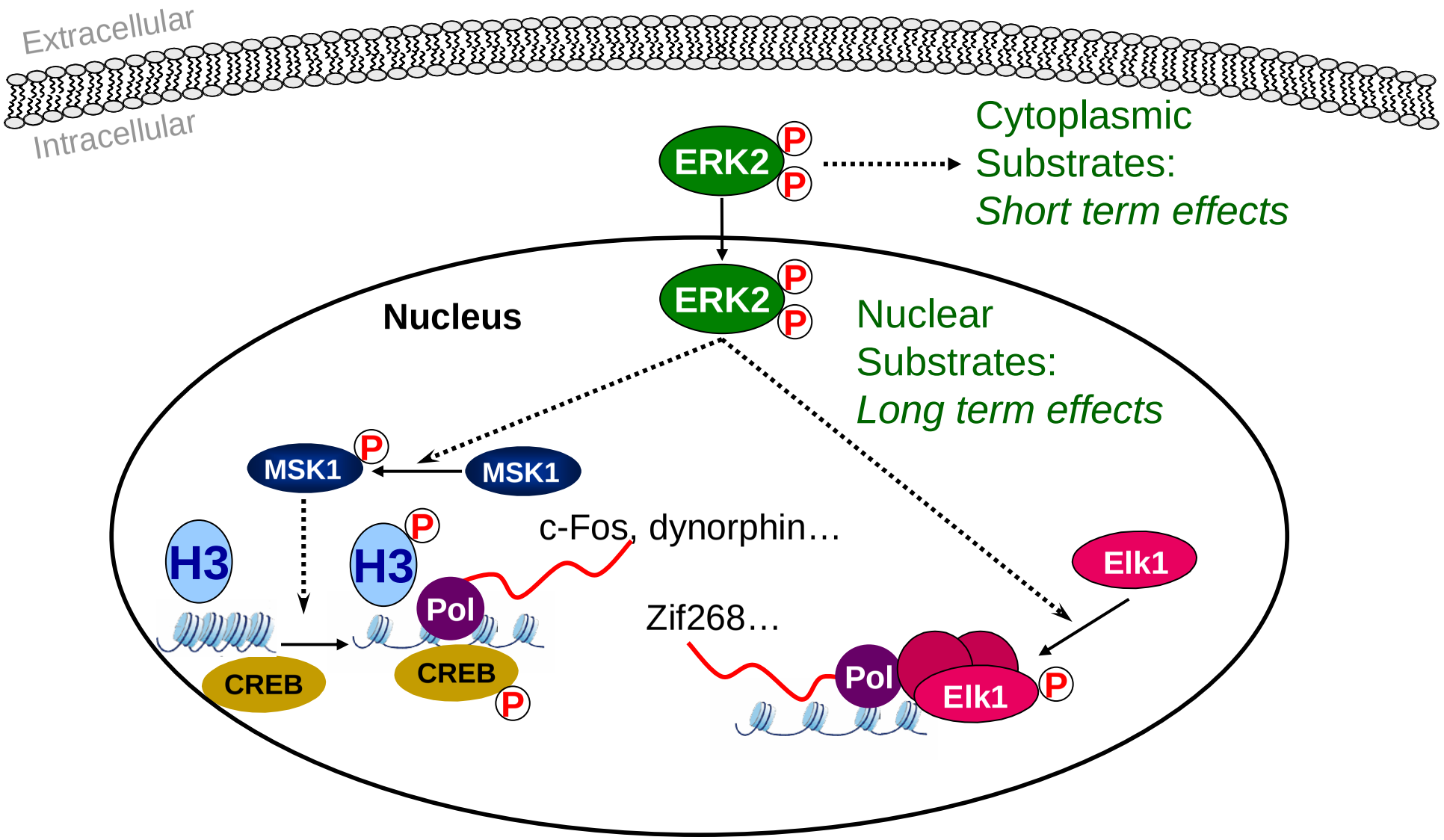

Figure 3