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Amphetamine elevates phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) in the rat forebrain via activating dopamine D1 and D2 receptors

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Abstract

Psychostimulants have an impact on protein synthesis, although underlying molecular mechanisms are unclear. Eukaryotic initiation factor 2 α -subunit (eIF2 α) is a key player in initiation of protein translation and is regulated by phosphorylation. While this factor is sensitive to changing synaptic input and is critical for synaptic plasticity, its sensitivity to stimulants is poorly understood. Here we systematically characterized responses of eIF2 α to a systemic administration of the stimulant amphetamine (AMPH) in dopamine responsive regions of adult rat brains. Intraperitoneal injection of AMPH at 5 mg/kg increased eIF2 α phosphorylation at serine 51 in the striatum. This increase was transient. In the medial prefrontal cortex (mPFC), AMPH induced a relatively delayed phosphorylation of the factor. Pretreatment with a dopamine D1 receptor antagonist SCH23390 blocked the AMPH-stimulated eIF2 α phosphorylation in both the striatum and mPFC. Similarly, a dopamine D2 receptor antagonist eticlopride reduced the effect of AMPH in the two regions. Two antagonists alone did not alter basal eIF2 α phosphorylation. AMPH and two antagonists did not change the amount of total eIF2 α proteins in both regions. These results demonstrate the sensitivity of eIF2 α to stimulant exposure. AMPH possesses the ability to stimulate eIF2 α phosphorylation in striatal and mPFC neurons *in vivo* in a D1 and D2 receptor-dependent manner.

Keywords

Initiation factor; protein synthesis; translation; caudate putamen; nucleus accumbens; dopamine receptor

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Conflict of interest

The authors declare that they have no conflict of interest.

1. Introduction

Protein biosynthesis is a tightly controlled process in which translation is an essential part. Four sequential steps are involved in the translation phase: activation, initiation, elongation, and termination. Among these steps, the initiation is the rate-limiting step and is subjected to the activity-dependent regulation (Costa-Mattioli et al., 2009). Eukaryotic initiation factors (eIFs) are a group of proteins required for the initiation of translation. These factors are subjected to the regulation by two independent ways. First, mTOR complex 1 regulates the eIF4F complex to control the initiation (Sonenberg and Hinnebusch, 2009; Laplante and Sabatini, 2012). Second, eIF2 α -subunit (eIF2 α) is regulated via a phosphorylation mechanism. That is, phosphorylation of eIF2 α at a specific residue (serine 51) prevents eIF2 α from forming a ternary complex with GTP and the initiator Met-tRNA, which results in suppression of general translation (Costa-Mattioli et al., 2009). However, paradoxically, phosphorylation of eIF2 α also selectively upregulates translation of a subset of mRNAs that contain upstream open reading frames in their 5' untranslated region (Dever, 2002; Hinnebusch, 2005; Jackson et al., 2010). Phosphorylation of eIF2 α at serine 51 is regulated by four serine/threonine protein kinases. These kinases are 1) protein kinase RNA-like endoplasmic reticulum kinase (PERK, also known as EIF2AK3) (Wek et al., 2006) which is activated in response to a variety of endoplasmic reticulum stresses (Harding et al., 1999), 2) double-stranded RNA-dependent protein kinase (PKR, or EIF2AK2), 3) general control non-derepressible-2 (GCN2, or EIF2AK4), and 4) heme-regulated inhibitor (HRI, or EIF2AK1) (Donnelly et al., 2013; Trinh and Klann, 2013). In contrast to eIF2 α kinases, protein phosphatase 1 dephosphorylates eIF2 α (Novoa et al., 2001). Both phosphorylation and dephosphorylation of eIF2 α have been implicated in modulating synaptic plasticity (Costa-Mattioli et al., 2009; Di Prisco et al., 2014). It is generally believed that phosphorylation of eIF2 α is an activity-regulated event and participates in the translational control of cellular signaling and synaptic plasticity related to various neurological and neuropsychiatric disorders (Costa-Mattioli et al., 2009; Jian et al., 2014).

The psychostimulant amphetamine (AMPH) impacts gene expression (mRNAs or proteins) in neurons. These inducible genomic responses were mainly investigated in the striatum and medial prefrontal cortex (mPFC), two dopamine responsive areas in limbic reward circuits critical for addictive properties of drugs of abuse. Early data show that AMPH stimulated or inhibited different sets of gene expression in these regions (Gerfen et al., 1990; Graybiel et al., 1990; Ruskin and Marshall, 1994; Wang and McGinty, 1995b). These alterations in either up- or downregulation of gene expression are thought to contribute to long-term adaptations in the limbic reward system, leading to enduring drug seeking behavior (Nestler, 2001; McClung and Nestler, 2008). As a key factor regulating protein translation, eIF2 α may be sensitive to stimulants and may play a role in regulating the effect of drugs on gene expression at the posttranscriptional phase. However, to date it has not been investigated whether AMPH has an impact on phosphorylation and/or expression of eIF2 α in the dopamine-innervated areas.

In this study, we set forth to investigate whether dopamine stimulation has any impact on phosphorylation and expression of eIF2 α in adult rat brains *in vivo*. The effect of a single systemic injection of AMPH on phosphorylated and total amounts of eIF2 α proteins was

examined in the striatum and mPFC. We first carried out a study to examine the effect of AMPH at a lower and a higher dose. We then conducted a time-course study to define the temporal property of AMPH in altering eIF2 α . Finally, we evaluated the role of dopamine D1 receptors (D1Rs) and D2 receptors (D2Rs) in mediating the effect of AMPH by testing the effect of a D1R or D2R antagonist on the AMPH-stimulated eIF2 α phosphorylation.

2. Results

2.1. AMPH enhances eIF2 α phosphorylation in the striatum

We first carried out a study to investigate the effect of AMPH on eIF2 α phosphorylation and expression in the striatum and mPFC. Rats received a single dose of AMPH at 0.5 or 5 mg/kg (i.p.) and were sacrificed 20 min after AMPH administration. The striatum and mPFC were removed for Western blot analysis of changes in p-eIF2 α and total eIF2 α proteins in these regions. We found that AMPH dose-dependently increased phosphorylation of eIF2 α in the striatum. While AMPH at a lower dose (0.5 mg/kg) did not significantly alter p-eIF2 α levels, the stimulant at a higher dose (5 mg/kg) markedly elevated p-eIF2 α (Fig. 1A). In the mPFC, AMPH at either dose induced an insignificant change in p-eIF2 α signals (Fig. 1B). AMPH at both doses showed no effect on a total cellular amount of eIF2 α proteins in the striatum and mPFC. These data demonstrate that acute administration of AMPH at 5 mg/kg enhances eIF2 α phosphorylation in the striatum. Since total eIF2 α levels were not changed, increased p-eIF2 α signals reflect an increased phosphorylation rate of the protein in response to AMPH.

2.2. Effects of AMPH on eIF2 α phosphorylation: a time-course study

We next conducted a time-course study to profile temporal properties of the AMPH effect on eIF2 α phosphorylation and expression in the striatum and mPFC. To this end, rats were subjected to a single injection of saline or AMPH. They were then sacrificed at different time points (1, 3, or 6 h) after drug administration for harvesting brain tissue for Western blot analysis. In the striatum, as shown in Fig. 2A and 2B, AMPH enhanced p-eIF2 α levels 1 h after drug injection, similar to the above observation at 20 min. At 3 h, AMPH still increased p-eIF2 α to a significant level ($P < 0.05$). However, at 6 h, p-eIF2 α levels were not altered in AMPH-treated rats as compared to saline-treated rats. At all time points surveyed, total eIF2 α levels showed an insignificant change between AMPH- and saline-treated groups (Fig. 2A and 2C). Clearly, AMPH induces a time-limited and reversible increase in eIF2 α phosphorylation in striatal neurons.

In the same rats, we examined the time-dependent effect of AMPH in the mPFC. Remarkably, AMPH at 1 h significantly elevated p-eIF2 α levels (Fig. 3A and 3B). At 3 h, p-eIF2 α remained at a higher level in AMPH-treated than saline-treated group. At 6 h, p-eIF2 α was no longer increased in AMPH-treated rats relative to saline-treated rats. Total eIF2 α levels were not altered by AMPH at all three time points (Fig. 3A and 3C). These results indicate that AMPH induces a relatively delayed and reversible increase in eIF2 α phosphorylation in mPFC neurons.

2.3. Effects of D1R and D2R antagonists on AMPH-stimulated eIF2 α phosphorylation

To determine the receptor that mediates the effect of AMPH, we carried out a pharmacological study. AMPH is well known to stimulate dopamine release in dopamine responsive regions (Butcher et al., 1988; Moghaddam et al., 1990). We thus evaluated the role of D1Rs and D2Rs in mediating the AMPH-stimulated eIF2 α phosphorylation. The D1R antagonist SCH23390 (0.5 mg/kg, i.p.) or the D2R antagonist eticlopride (0.5 mg/kg, i.p.) was administered 20 min prior to AMPH (5 mg/kg, i.p.). Rats were then sacrificed 1 h after AMPH injection. SCH23390 completely blocked the phosphorylation response of eIF2 α to AMPH in the striatum (Fig. 4A). Similarly, in the presence of eticlopride, AMPH produced a significantly less increase in eIF2 α phosphorylation. In the mPFC, similar results were observed. Pretreatment with SCH23390 or eticlopride reduced the AMPH-stimulated eIF2 α phosphorylation (Fig. 4B). There was no significant change in total eIF2 α proteins following all drug treatments. These results indicate that activation of both D1Rs and D2Rs is required for phosphorylation responses of eIF2 α to AMPH in striatal and mPFC neurons.

To determine the effect of D1R and D2R antagonists on basal eIF2 α phosphorylation, we injected rats with SCH23390 and eticlopride at 0.5 mg/kg (i.p.) and sacrificed rats 1 h after agent injection. Both antagonists had no significant impact on basal levels of p-eIF2 α in the striatum (Fig. 5A). Similar results were observed in the mPFC (Fig. 5B). The effectiveness of these agents after a systemic injection was confirmed by the observations from the same samples that SCH23390 reduced AMPA glutamate receptor GluA1 phosphorylation at serine 845 (S845), whereas eticlopride elevated it in the striatum (Fig. 5C), a known fact that D1Rs and D2Rs stimulate and inhibit S845 phosphorylation in striatal neurons, respectively (Snyder et al., 2000; Hakansson et al., 2006). These data suggest that D1Rs and D2Rs play an insignificant role in the regulation of basal eIF2 α phosphorylation in striatal and mPFC neurons.

3. Discussion

In this study, we investigated the regulation of eIF2 α phosphorylation and expression by dopamine in adult rat brains *in vivo*. We found that an acute systemic injection of AMPH at a higher but not lower dose increased eIF2 α phosphorylation in the striatum. AMPH also induced a transient elevation of eIF2 α phosphorylation in the striatum and mPFC. Blockade of D1Rs or D2Rs did not alter basal eIF2 α phosphorylation. However, both D1R and D2R antagonists significantly blocked the AMPH-stimulated eIF2 α phosphorylation. These results indicate that AMPH possesses the ability to upregulate eIF2 α phosphorylation in dopamine responsive regions via a mechanism involving D1R and D2R activation.

While we attempted to examine the effect of AMPH on both phosphorylation and expression of eIF2 α , we observed significant changes only in phosphorylation status but not expression of the factor. This indicates that the stimulant preferentially affects the phosphorylation rate of eIF2 α . The effect of AMPH in the striatum was clearly seen at a higher dose (5 mg/kg) but not a lower dose (0.5 mg/kg). In addition, AMPH enhanced eIF2 α phosphorylation in a time-related manner. In the striatum, an increase in eIF2 α phosphorylation was seen as early as 20 min after AMPH injection. The increase was reversible since p-eIF2 α levels returned to normal levels at 6 h. In the mPFC, notably, a relatively delayed increase in eIF2 α

phosphorylation occurred as a significant increase in p-eIF2 α started at a later time point (1 h). The reason for this slower onset is unclear. It may reflect that additional circuits and transmitters may be involved in processing eIF2 α responses to AMPH in this region.

The role of D1Rs and D2Rs in linking AMPH to eIF2 α is noteworthy. Both D1R and D2R antagonists blocked the AMPH effect, indicating that both receptors are critical for the event. In the striatum, D1Rs and D2Rs are enriched and are prominently segregated into two phenotypes of medium spiny output neurons, i.e., D1Rs in striatonigral neurons and D2Rs in striatopallidal neurons (Gerfen et al., 1990; Aubert et al., 2000; Bertran-Gonzalez et al., 2010). It is likely that AMPH activates D1Rs to phosphorylate eIF2 α in D1R-bearing striatonigral neurons, although this cell type-specific response needs to be proven experimentally. With regard to D2Rs, mechanism(s) underlying their roles are unclear. Presumably, an indirect pathway may enact. D2Rs inhibit local acetylcholine from intrinsic cholinergic interneurons (DeBoer and Abercrombie, 1996; Ding et al., 2000). Blocking D2Rs thus enhances acetylcholine release. Released acetylcholine can activate muscarinic M4 receptors to suppress the eIF2 α response to AMPH. M4 receptors are a major subtype of muscarinic receptors expressed in the striatum and are predominantly coexpressed with D1Rs in striatonigral neurons (Ince et al., 1997; Santiago and Potter, 2001). Activation of Gi/o-coupled M4 receptors is known to antagonize Gs-coupled D1Rs in many activity-dependent events, including the AMPH-stimulated immediate early gene expression in the striatum and behavior (Chou et al., 1992; Bernard et al., 1993; Morelli et al., 1993; Wang and McGinty, 1996a; 1996b; 1997). Apparently, a D1R/D2R synergy is implicated in modulating eIF2 α , which is consistent with the model that the D1R/D2R synergy mediates dopamine control of direct and indirect pathways and determines basal ganglia outflow and behavior (Onn et al., 2000).

In the mPFC, D1Rs are present in pyramidal spiny output neurons, a major population of neurons in the region (de Almeida et al., 2008; Bergson et al., 1995). Thus, AMPH may activate D1Rs to phosphorylate eIF2 α . As to D2Rs, D2R agonists negatively modulate basal and NMDA-mediated increases in acetylcholine release in the prefrontal cortex (Brooks et al., 2007). Therefore, like the striatum, blockade of D2Rs results in more acetylcholine release and activates muscarinic M4 receptors localized on mPFC perikarya (Levey et al., 1991; Volpicelli and Levey, 2004). This in turn likely suppresses the eIF2 α response to AMPH. It should be pointed out that AMPH could alter many other neurotransmitters which may be involved in the D2R-dependent regulation of eIF2 α .

An important finding in this study is that AMPH stimulated eIF2 α phosphorylation. A noticeable feature of AMPH and other stimulants in producing the long-term effect is its dependence on new protein synthesis (Karler et al., 1993; Shimosato and Saito, 1993; Luo et al., 2011; Contreras et al., 2012). However, at present, little is known about the detailed translational program underlying the enduring effect of AMPH. It is thought that stimulants enhance expression of some proteins, while they at the same time inhibit expression of other proteins. This enhanced synthesis of a set of 'addiction proteins' together with inhibited synthesis of another set of 'addiction proteins' acts jointly to construct the translational program critical for drug addiction. In searching for a regulator at the translational level, eIF2 α becomes an attractive target. This factor can be activity-dependently regulated. Its

function is characterized by inhibiting general mRNA translation but also initiating the translation of specific mRNAs that contain open reading frames in their 5' untranslated regions (Dever, 2002; Hinnebusch, 2005; Jackson et al., 2010). Moreover, the eIF2 α -mediated inhibition of general translation may reduce the competition of mRNAs for the translational machinery, leading to favoring translation of a selective set of mRNAs presumably associated with AMPH action. In this study, we for the first time observed that eIF2 α is regulated by AMPH. While a change in its phosphorylation state does not necessarily mean a functional role, it will be intriguing to investigate whether eIF2 α , as a regulatable factor in protein translation, participates in the drug-related protein translation and thereby contributes to synaptic plasticity and drug seeking behavior. Of note, a recent study shows that direct stimulation of eIF2 α phosphorylation with an eIF2 α phosphatase inhibitor Sal003 induced synaptic plasticity in the form of long-term depression in hippocampal synapses by activating the translation of specific mRNAs rather than by blocking general translation (Di Prisco et al., 2014). Future studies will identify proteins that are down- or upregulated in their synthesis through an eIF2 α -dependent translational control in response to AMPH or other stimulants.

Jian et al. (2014) reported that eIF2 α was dephosphorylated in the rat basolateral amygdala, which mediated reconsolidation of cocaine memory. A recent study found that a low dose of cocaine (5 mg/kg, i.p.) selectively lowered eIF2 α phosphorylation in the ventral tegmental area (VTA) of adolescent (5 weeks old) but not adult (3–5 months old) mice, while cocaine at 5 and 10 mg/kg (i.p.) did not alter eIF2 α phosphorylation in the nucleus accumbens of adolescent and adult mice, respectively (Huang et al., 2016). The cocaine-induced dephosphorylation of eIF2 α is linked to long-term potentiation in VTA dopaminergic neurons and behavior (Huang et al., 2016). Thus, in addition to phosphorylation, dephosphorylation of eIF2 α plays a critical role in controlling translational program related to drug memory. It appears that, in an experience-dependent mental disorder such as drug addiction, adaptive changes in eIF2 α phosphorylation and function may contribute to the drug-induced synaptic and behavioral plasticity. Moreover, eIF2 α may achieve its effects by affecting translation of particular genes rather than general protein translation (Jiang et al., 2010) and may play a role in a brain site-, circuit-, drug-, and disease state-specific manner. In sum, studies on eIF2 α -regulated protein synthesis in relation to stimulant action are just emerging. More studies at the translational level are needed in the future to illustrate molecular mechanisms underlying enduring drug seeking behavior.

4. Experimental procedures

4.1. Animals

We used male Wistar rats weighting 230–330 g (3–4 months old) (Charles River, New York, NY). These animals were individually housed at 23°C and humidity of 50 $\pm$ 10% with food and water available *ad libitum*. The animal room was on a 12/12 h light/dark cycle with lights on at 0700. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

4.2. Systemic drug injection and experimental arrangements

Rats received a single dose of AMPH. The drug was given via an intraperitoneal (i.p.) injection at a volume of ~0.5 ml. The dose of AMPH was calculated as the salt. In the first study, rats were injected with AMPH at two different doses (0.5 and 5 mg/kg) and were sacrificed 20 min after drug injection for Western blot analysis of proteins of interest in their responses to AMPH. The two doses of AMPH were chosen based on our previous work in which an i.p. administration of AMPH at 0.69 and 5.53 mg/kg produced litter and considerable behavioral responses in rats, respectively (Wang and McGinty, 1995a). To carry out a time-course study, rats were given a single dose of AMPH (5 mg/kg) and were sacrificed at different time points (1, 3, or 6 h) after AMPH injection. In a pharmacological study defining the role of D1Rs and D2Rs in mediating the effect of AMPH, rats were given an injection of the D1R antagonist SCH23390 (0.5 mg/kg) or D2R antagonist eticlopride (0.5 mg/kg) 20 min prior to an injection of AMPH at 5 mg/kg. Rats were then sacrificed 1 h after AMPH injection. The effectiveness of the two dopamine receptor antagonists following a systemic injection has been well demonstrated in previous behavioral and neurochemical studies (Wang and McGinty, 1995b; 1996a). Age-matched rats were given an injection of saline (~0.5 ml), which served as controls.

4.3. Western blot analysis

Western blot analysis was conducted according to our previous work (Mao et al., 2009; Van Dolah et al., 2011). Briefly, rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and sacrificed by decapitation. We quickly removed rat brains and cut brains into coronal slices (~1 mm). We then dissected brain regions on coronal slices laid on an ice-cold dissection plate using a 15-gauge blunt tissue punch. The striatum was dissected at the striatal level (0.7–1.7 mm anterior to bregma; Paxinos and Watson, 1997), which contained the caudate putamen and nucleus accumbens (both core and shell subregions). The mPFC was dissected at the level of the mPFC (2.7–3.7 mm anterior to bregma). The dissected mPFC included the anterior cingulate, prelimbic, and infralimbic cortices (Melendez et al., 2004). Brain tissue was lysed in RIPA (radioimmunoprecipitation assay) buffer containing 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, and 1 μ g/ml leupeptin. Protein concentrations were determined. Samples were stored at –80°C until use. To carry out immunoblots, proteins were separated on SDS NuPAGE 4–12% Bis-Tris gels (Invitrogen, Carlsbad, CA). Separated proteins were transferred to polyvinylidene fluoride membranes. These membranes were incubated with primary antibodies overnight at 4°C. After primary antibody incubation, membranes were incubated with a secondary antibody (1:2,000). Immunoblots were developed with the enhanced chemiluminescence reagent (GE Healthcare Life Sciences, Piscataway, NJ). Immunoblots on films were measured using NIH ImageJ gel analysis software. β -Actin was used as a loading control and normalization in Western blot analysis.

4.4. Antibodies and pharmacological agents

Primary antibodies used in this study include rabbit antibodies against phosphorylated eIF2 α (p-eIF2 α) at serine 51 (1:500, Cell Signaling Technology, Beverly, MA), phosphorylated

GluA1 at serine 845 (1:1000, pS845, PhosphoSolutions, Aurora, CO), GluA1 (1:1000, Millipore, Bedford, MA), or β -actin (1:5000, Sigma-Aldrich, St. Louis, MO), or a mouse antibody against eIF2 α (1:1000, Cell Signaling Technology). Pharmacological agents include D-amphetamine sulfate, R(+)-SCH23390 hydrochloride, and S-(–)-eticlopride hydrochloride which were purchased from Sigma-Aldrich (St. Louis, MO). AMPH and the two antagonists were dissolved in physiological saline. All agents were freshly prepared at the day of experiments.

4.5. Statistics

Data in this study are presented as means $\pm$ SEM and were evaluated using one- or two-way analysis of variance (ANOVA), as appropriate, followed by a Bonferroni (Dunn) comparison of groups. Probability levels of < 0.05 were considered statistically significant.

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Highlights

Amphetamine increases eIF2 α phosphorylation in the rat striatum.

Amphetamine also increases eIF2 α phosphorylation in the prefrontal cortex.

The effect of amphetamine is time-dependent and reversible.

Both dopamine D1 and D2 receptor antagonists block the amphetamine effect.

Figure 1. Effects of AMPH at a lower and a higher dose on phosphorylation and expression of eIF2 α in the striatum and mPFC

A, Effects of AMPH on phosphorylation and expression of eIF2 α in the striatum. **B**, Effects of AMPH on phosphorylation and expression of eIF2 α in the mPFC. Note that AMPH only at a higher dose increased p-eIF2 α levels in the striatum. Representative immunoblots are shown to the left of the quantified data. Rats were given an i.p. injection of saline (Sal) or AMPH at 0.5 or 5 mg/kg and were sacrificed 20 min after drug injection for Western blot analysis of changes in p-eIF2 α and eIF2 α expression. Data are presented as means $\pm$ SEM ($n = 4-7$ per group). * $p < 0.05$ versus saline (one-way ANOVA followed by a post hoc test).

Figure 2. Effects of AMPH at different time points on phosphorylation and expression of eIF2α in the striatum

A, Representative immunoblots illustrating effects of AMPH at different times on phosphorylation and expression of eIF2α. **B** and **C**, Quantification of p-eIF2α (**B**) and eIF2α (**C**) immunoblots. Note that AMPH induced a transient and reversible increase in p-eIF2α levels. Rats were given an i.p. injection of saline or AMPH (5 mg/kg) and were sacrificed at different time points (1, 3 or 6 h) after drug injection. Data are presented as means ± SEM (n = 4–6 per group). *p < 0.05 versus saline (two-way ANOVA followed by a post hoc test).

Figure 3. Effects of AMPH at different time points on phosphorylation and expression of eIF2 α in the mPFC

A, Representative immunoblots illustrating effects of AMPH at different times on phosphorylation and expression of eIF2 α . **B** and **C**, Quantification of p-eIF2 α (**B**) and eIF2 α (**C**) immunoblots. Note that AMPH induced a reversible increase in p-eIF2 α levels. Rats were given an i.p. injection of saline or AMPH (5 mg/kg) and were sacrificed at different time points (1, 3 or 6 h) after drug injection. Data are presented as means $\pm$ SEM ($n = 4-6$ per group). * $p < 0.05$ versus saline (two-way ANOVA followed by a post hoc test).

Figure 4. Effects of D1R and D2R antagonists on the AMPH-stimulated eIF2α phosphorylation in the striatum and mPFC

A and B. Effects of SCH23390 (SCH) and eticlopride (Eti) on the AMPH-stimulated eIF2α phosphorylation in the striatum (A) and mPFC (B). Note that both SCH23390 and eticlopride blocked an increase in p-eIF2α proteins in response to AMPH. Representative immunoblots are shown to the left of the quantified data. Rats were given the first i.p. injection of saline, SCH23390 (0.5 mg/kg) or eticlopride (0.5 mg/kg) 20 min prior to the second i.p. injection of saline or AMPH (5 mg/kg) and were sacrificed 1 h after AMPH injection. Data are presented as means $\pm$ SEM ($n = 4$ per group). * $p < 0.05$ versus saline (Sal) + saline; + $p < 0.05$ versus saline + AMPH (one-way ANOVA followed by a post hoc test).

Figure 5. Effects of D1R and D2R antagonists on basal eIF2 α phosphorylation in the striatum and mPFC

A and B, Effects of SCH23390 (SCH) and eticlopride (Eti) on basal eIF2 α phosphorylation in the striatum (A) and mPFC (B). **C,** Effects of SCH23390 and eticlopride on GluA1 S845 phosphorylation in the striatum. Representative immunoblots are shown to the left of the quantified data. Rats were given an i.p. injection of saline (Sal), SCH23390 (0.5 mg/kg), or eticlopride (0.5 mg/kg) and were sacrificed 1 h after drug injection. Data are presented as means $\pm$ SEM ($n = 4$ per group). * $p < 0.05$ versus saline (one-way ANOVA followed by a post hoc test).