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Amphetamine alters Ras-guanine nucleotide-releasing factor expression in the rat striatum *in vivo*

Nikhil K. Parelkar^{a,b}, Qian Jiang^b, Xiang-Ping Chu^b, Ming-Lei Guo^b, Li-Min Mao^b, and John Q. Wang^{a,b,c,*}

^a Department of Pharmacology, School of Pharmacy, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, Missouri 64108, USA

^b Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, Missouri 64108, USA

^c Department of Anesthesiology, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, Missouri 64108, USA

Abstract

Ras-guanine nucleotide-releasing factors (Ras-GRFs) are densely expressed in neurons of the mammalian brain. As a Ras-specific activator predominantly concentrated at synaptic sites, Ras-GRFs activate the Ras-mitogen-activated protein kinase (Ras-MAPK) cascade in response to changing synaptic inputs, thereby modifying a variety of cellular and synaptic activities. While the Ras-MAPK cascade in the limbic reward circuit is well-known to be sensitive to dopamine inputs, the sensitivity of its upstream activator (Ras-GRFs) to dopamine remains to be investigated. In this study, the response of Ras-GRFs in their protein expression to dopamine stimulation was evaluated in the rat striatum *in vivo*. A single systemic injection of the psychostimulant amphetamine produced an increase in Ras-GRF1 protein levels in both the dorsal (caudoputamen) and ventral (nucleus accumbens) striatum. The increase in Ras-GRF1 proteins was dose-dependent. The reliable increase was seen 2.5 h after drug injection and returned to normal levels by 6 h. In contrast to Ras-GRF1, protein levels of Ras-GRF2 in the striatum were not altered by amphetamine. In addition to the striatum, the medial prefrontal cortex is another forebrain site where amphetamine induced a parallel increase in Ras-GRF1 but not Ras-GRF2. No significant change in Ras-GRF1/2 proteins was observed in the hippocampus. These data demonstrate that Ras-GRF1 is a susceptible and selective target of amphetamine in striatal and cortical neurons. Its protein expression is subject to the modulation by acute exposure of amphetamine.

Keywords

GEF; GRF; ERK; medial prefrontal cortex; hippocampus; nucleus accumbens

*Corresponding author: Dr. John Q. Wang, Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, Missouri 64108, tel: (816) 235-1786; fax: (816) 235-5574; wangjq@umkc.edu.

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1. Introduction

Mitogen-activated protein kinases (MAPKs) refer to a large family of serine/threonine protein kinases that function as a key signaling cascade to mediate cellular growth and differentiation in mammalian proliferative cells (Volmat and Pouyssegur, 2001). In postmitotic neurons of adult mammalian brain, MAPKs are also densely expressed. They are highly sensitive to diverse synaptic signals, and are vigorously involved in the transcription-dependent regulation of synaptic plasticity (Wang et al., 2007). The typical MAPK cascade involves a consecutive and sequential activation of four levels of signaling proteins: small GTPases (such as Ras), MAPK kinase kinases (Raf or MEKKs), MAPK kinases (MEKs), and MAPKs. The initial Ras proteins localize to the inner surface of the plasma membrane. They are activated when converted from the GDP-bound to the GTP-bound state. This conversion is catalyzed by a class of Ras-guanine nucleotide exchange factors (Ras-GEFs), including Ras-guanine nucleotide releasing factors (Ras-GRFs). Ras-GRFs are expressed in neurons, but not glial cells, in the central nervous system of adult animals (Zippel et al., 1997). The two subtypes of Ras-GRFs, Ras-GRF1/CDC25^{Mm} and Ras-GRF2 (Cen et al., 1992; Martegani et al., 1992; Shou et al., 1992), have been found to be predominantly enriched at synaptic sites (Sturani et al., 1997). These synaptic Ras-GRFs are sensitive to cytosolic Ca²⁺ signals. Through Ca²⁺/calmodulin binding to their N-terminal IQ motifs (Shou et al., 1992; Farnsworth et al., 1995; Fam et al., 1997), Ca²⁺ signals, derived from Ca²⁺ influx through either ligand or voltage-operated Ca²⁺ channels including NMDA receptors or from intracellular Ca²⁺ release following G-protein-coupled receptor activation, activates Ras-GRFs (Farnsworth et al., 1995). Active Ras-GRFs subsequently promote the activation of their specific effector Ras, which leads to the activation of the central signaling cascade, i.e., the MAPK cascade. Through regulating the strength and efficacy of excitatory synapses, activated MAPKs are involved in normal neural activities and the development of various enduring neuropsychiatric illnesses (reviewed in Sweatt, 2004; Thomas and Huganir, 2004; Wang et al., 2007).

A number of recent studies have demonstrated that dopamine stimulation with psychostimulants activates MAPKs in the forebrain *in vivo*. Acute injection of the psychostimulant cocaine increased phosphorylation of extracellular signal-regulated kinases (ERKs), a subclass of MAPKs, in the striatum (Valjent et al., 2000; 2005; 2006; Zhang et al., 2004; Jenab et al., 2005). Acute injection of the psychostimulant amphetamine also increased ERK phosphorylation in the striatum (Choe et al., 2002; Choe and Wang, 2002; Valjent et al., 2004; 2005; 2006). The amphetamine-stimulated ERK phosphorylation requires the activation of group I metabotropic glutamate receptors and Ca²⁺/calmodulin-dependent protein kinases (CaMKs) since the inhibitors selective for these receptors or CaMKs blocked ERK responses to amphetamine (Choe et al., 2002; Choe and Wang, 2002). Together, these data indicate that the Ras-GRF-dependent MAPK cascade in striatal neurons is sensitive to psychostimulants. The drug-regulated MAPK activity likely plays a critical role in plastic changes in the limbic reward circuit essential for the addictive properties of drugs of abuse (Valjent et al., 2000; Wang et al., 2007). However, to date, no attempt has been made to unravel the influence of amphetamine over Ras-GRF expression in the forebrain.

This study was then designed to investigate the possible regulation of Ras-GRF expression in striatal neurons by dopamine inputs *in vivo*. A single systemic injection of amphetamine was given to adult rats. Alterations in basal levels of both Ras-GRF1 and Ras-GRF2 protein abundance in the striatum, including both the dorsal striatum/caudate putamen (CPu) and the ventral striatum/nucleus accumbens (NAc), and other forebrain structures were examined after drug injection.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats weighting 200–225 g (Charles River, New York, NY) were individually housed in clear plastic cages in a controlled environment at a constant temperature of 23°C and humidity of 50 ± 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. Rats were allowed 6–7 days of habituation to the animal colony before any treatment began. 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.

2.2. Systemic administration of drugs

Rats were randomly divided into different groups ($n = 3–5$ per group). All drugs were prepared freshly on the day of experiment. D-Amphetamine sulfate was purchased from Sigma-Aldrich (St. Louis, MO). Doses of amphetamine were calculated as the salt. A single dose of amphetamine at 1.25 or 5 mg/kg was given to animals in home cages through intraperitoneal (i.p.) injection. We selected these two doses of amphetamine because they stimulate motor activity to variable levels. The low dose of 1.25 mg/kg induces a small increase in locomotion whereas the high dose of 5 mg/kg usually induces both strong locomotion and stereotypy (Wang and McGinty, 1995). Age-matched animals received an i.p. injection of saline (1 ml/kg), which served as control.

2.3. Western blot

Western blot was carried out as described previously (Liu et al., 2006; 2009). Briefly, rats were anesthetized with Equithesin (9 ml/kg, i.p.) and decapitated 2.5 h after amphetamine injection or at the survival time indicated. Brains were removed rapidly. The brain areas of interest, including the medial prefrontal cortex, hippocampus, dorsal striatum and ventral striatum, were separately removed into a 1.5 ml microtube containing ice-cold sample buffer (20 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 10 mM NaF, 2 mM Na₃VO₄, 1 mM EDTA, 1 mM EGTA, 5 μM microcystin-LR, and 0.5 mM phenylmethylsulfonyl fluoride). The sample was homogenized by sonication. The homogenate was centrifuged at 700 g for 10 min at 4°C. The supernatant was again centrifuged at 10,000 g at 4°C for 30 min to generate the pellet (P2) which was resuspended in ice-cold sample buffer. Protein concentrations were determined. The equal amount of protein (20 μg/20 μl/lane) was separated on SDS NuPAGE Novex 4–12% gels (Invitrogen, Carlsbad, CA). Proteins were transferred to polyvinylidene fluoride membrane (Millipore, Bedford, MA) and blocked in blocking buffer (5% nonfat dry milk in PBS and 0.1% Tween 20) for 1 h. The blots were washed and incubated in the blocking buffer containing a primary rabbit antibody against Ras-GRF1 (Santa Cruz Biotechnology, Santa Cruz, CA), Ras-GRF2 (Santa Cruz Biotechnology), or actin (Santa Cruz Biotechnology) usually at 1:1000 overnight at 4°C. This was followed by 1 h incubation in a goat horseradish peroxidase-linked secondary antibody against rabbit (Jackson Immunoresearch Laboratory, West Grove, PA) at 1:5000. Immunoblots were developed with the enhanced chemiluminescence reagents (ECL; Amersham Pharmacia Biotech, Piscataway, NJ), and captured into Kodak Image Station 2000R. Kaleidoscope-prestained standards (Bio-Rad, Hercules, CA) and MagicMark XP Western protein standards (Invitrogen) were used for protein size determination. The density of immunoblots was measured using the Kodak 1D Image Analysis software.

2.4. Behavioral assessments

Motor activity was evaluated with an infrared photo-cell-based, automated Opto-Varimex-Micro apparatus (Columbus Instruments, Columbus, OH) in a sound-attenuated room as

described previously (Liu et al., 2006; Mao et al., 2009). Briefly, rats in standard transparent rectangular rodent cage ($42 \times 24 \times 20$ cm high) were habituated to the environment for 2 h. Three sensor pairs positioned in x, y (horizontal), and z (vertical, above the animal's normal height) directions were assigned to each cage to provide measurements about horizontal and vertical activities. Motor activity was recorded at 5 min intervals before and after drug injection. Status about infrared beam interruptions by presence of animals was transferred from all sensors to a computer with operating VersaMax software. Stereotypy was detected using computer-generated stereotypy time recorded by VersaMax monitors, which refers to the total time that stereotypic behaviors (repetitive breaks of a given beam or beams with an interval less than 1 s) were observed.

2.5. Statistics

The results are presented as mean $\pm$ S.E.M., and were evaluated using a one-way analysis of variance followed by a Bonferroni (Dunn) comparison of groups using least squares-adjusted means or two-tailed unpaired Student's t-test. Probability levels of < 0.05 were considered statistically significant.

3. Results

3.1. Dose-dependent effects of amphetamine on Ras-GRF expression in the striatum

To determine whether amphetamine regulates Ras-GRF expression in the rat striatum, we monitored the effect of acute systemic injection of amphetamine on protein levels of two closely-related isoforms of Ras-GRF (Ras-GRF1 and Ras-GRF2) in the two striatal structures (CPu and NAc) *in vivo*. We first detected responses of Ras-GRF1 in the CPu and NAc to amphetamine. In Western blot with a selective Ras-GRF1 antibody previously validated (Zhang et al., 2007), a single immunoreactive band in a molecular weight predicted for Ras-GRF1 (140 kDa) was exhibited in rats treated with saline (Fig. 1A). A single injection of amphetamine at 1.25 or 5 mg/kg (i.p., 2.5 h prior to protein collection) caused a dose-dependent increase in Ras-GRF1 protein levels in the CPu (Fig. 1A). At a lower dose (1.25 mg/kg), amphetamine did not seem to increase Ras-GRF1 to a statistically significant level ($111.1\% \pm 6.8\%$ of saline, $P > 0.05$). At a higher dose (5 mg/kg), amphetamine induced a moderate and significant increase in Ras-GRF1 levels ($128.0 \pm 3.3\%$ of saline, $F_{(2,11)} = 6.005$; $P < 0.05$). In the NAc, similar results were obtained. As shown in Fig. 1B, the lower dose of amphetamine did not significantly alter basal levels of Ras-GRF1 ($106.0 \pm 3.2\%$ of saline, $P > 0.05$) whereas the higher dose of the drug significantly elevated Ras-GRF1 protein levels ($127.0 \pm 1.3\%$ of saline, $F_{(2,11)} = 15.38$; $P < 0.05$). These data demonstrate a stimulative effect of amphetamine on Ras-GRF1 expression in the entire striatal region.

We next targeted Ras-GRF2 and tested the possible effect of amphetamine on its expression in the CPu and NAc. Ras-GRF2 proteins are known to migrate into two bands around 135 kDa in immunoblot analysis with a pre-validated antibody (Zhang et al., 2007). This was shown again in the immunoblot using CPu samples from saline-treated rats (Fig. 2A). Interestingly, unlike Ras-GRF1, Ras-GRF2 in the striatum was not responsive to amphetamine. In the CPu, amphetamine at 1.25 or 5 mg/kg induced insignificant changes in Ras-GRF2 protein expression ($84.0 \pm 12.5\%$ of saline at 1.25 mg/kg, $P > 0.05$; $94.1 \pm 8.4\%$ of saline at 5 mg/kg, $P > 0.05$; $F_{(2,10)} = 0.438$) (Fig. 2A). Similarly in the NAc, two doses of amphetamine were ineffective in affecting Ras-GRF2 abundance ($107 \pm 10.2\%$ of saline at 1.25 mg/kg, $P > 0.05$; $95 \pm 5.7\%$ of saline at 5 mg/kg, $P > 0.05$; $F_{(2,12)} = 0.431$) (Fig. 2B). The insensitivity of Ras-GRF2 to amphetamine indicates a selective effect of acute amphetamine exposure on Ras-GRF1 expression in striatal neurons.

Motor responses to acute amphetamine injection are worthy of description in terms of their correlation with the biochemical effect of amphetamine on Ras-GRF expression. Amphetamine at two doses caused typical motor responses (Wang and McGinty, 1995). At a lower dose (1.25 mg/kg), amphetamine induced a low to moderate increase in locomotor activity. At a higher dose (5 mg/kg), amphetamine induced an increase in both locomotor and stereotypical activities.

3.2. Time-dependent effects of amphetamine on Ras-GRF expression in the striatum

A time-course study was carried out to characterize the temporal property of an increase in Ras-GRF1 proteins in rats treated with acute injection of saline or amphetamine. An effective dose of amphetamine (5 mg/kg) validated above was used in this study. The three time points (1, 2.5, and 6 h after drug injection) was chosen based on our early study in which acute injection of cocaine induced an increase in striatal Ras-GRF1 expression at 2 h, while this increase did not show at 1 h and returned to the normal level by 6 h after injection (Zhang et al., 2007a). Similar to cocaine, amphetamine at an early time point (1 h after injection) did not alter basal levels of Ras-GRF1 in the CPu ($105 \pm 3.6\%$ of saline, $P > 0.05$) (Fig. 3A). Only at 2.5 h, amphetamine significantly increased Ras-GRF1 in the CPu. This effect is reversible because the elevated Ras-GRF1 returned to the normal level 6 h after amphetamine injection ($107 \pm 0.7\%$, $P > 0.05$) (Fig. 3A). Similar results were seen in the NAc. A reliable increase in Ras-GRF1 expression was induced at 2.5 h, which became insignificant as compared to saline at 6 h (Fig. 3B). These data reveal a relatively delayed and transient nature of Ras-GRF1 induction in striatal neurons in response to acute amphetamine administration.

Ras-GRF2 expression was insensitive to amphetamine when detected at 2.5 h after drug injection (see above). To determine if this is still the case at an earlier or later time point, we examined the effect of amphetamine at 1 and 6 h. No significant change in Ras-GRF2 protein levels was observed at the 1-h time point in both the CPu (Fig. 3C) and NAc (Fig. 3D). Neither was reliable change in Ras-GRF2 levels shown at 6 h in the two regions (Fig. 3C and 3D). Apparently, Ras-GRF2 was consistently unresponsive to amphetamine at all time points surveyed.

3.3. Effects of amphetamine on Ras-GRF expression in other forebrain regions

In addition to the striatum, other forebrain regions are involved in processing drug action. The medial prefrontal cortex and hippocampus are among the important structures well documented to be the central targets of psychoactive drugs. We therefore examined the effect of amphetamine (1.25 or 5 mg/kg, 2.5 h prior to protein collection) on Ras-GRF expression in those two areas. As shown in Fig. 4A, a single dose of amphetamine induced a dose-dependent increase in Ras-GRF1 protein expression in the medial prefrontal cortex. Like the results seen in the striatum, a lower dose of amphetamine (1.25 mg/kg) did not affect basal Ras-GRF1 levels in the prefrontal cortex ($95 \pm 3.2\%$ of saline, $P > 0.05$). Amphetamine at a higher dose (5 mg/kg) was effective in inducing an increase in Ras-GRF1 proteins ($127 \pm 4.3\%$ of saline, $F_{(2,12)} = 18.58$; $P < 0.05$). In contrast to Ras-GRF1, Ras-GRF2 was not altered in its abundance after amphetamine injection at either 1.25 or 5 mg/kg ($88 \pm 4.7\%$ of saline at 1.25 mg/kg, $P > 0.05$; $96 \pm 4.7\%$ of saline at 5 mg/kg, $P > 0.05$; $F_{(2,12)} = 0.572$) (Fig. 4B). These data demonstrate that the medial prefrontal cortex is another limbic site where acute amphetamine administration can induce a selective increase in Ras-GRF1 expression.

Similar experiments were carried out using protein extracts from the hippocampus. We found that amphetamine at the two doses did not alter Ras-GRF1 expression in this region ($102 \pm 2.8\%$ of saline at 1.25 mg/kg, $P > 0.05$; $106 \pm 3.3\%$ of saline at 5 mg/kg, $P > 0.05$; $F_{(2,12)} = 0.768$) (Fig. 5A). Neither did amphetamine affect Ras-GRF2 expression ($93 \pm 11\%$ of saline

at 1.25 mg/kg, $P > 0.05$; $95 \pm 2.8\%$ of saline at 5 mg/kg, $P > 0.05$; $F_{(2,12)} = 0.78$) (Fig. 5B). Thus, amphetamine is not able to affect Ras-GRF1/2 expression in the hippocampus.

3.4. Effects of amphetamine on motor activity

Motor responses to acute amphetamine injection are worthy description in terms of its correlation with the biochemical effect of amphetamine on Ras-GRF expression. Amphetamine at two doses caused typical motor responses (Wang and McGinty, 1995). At a lower dose (1.25 mg/kg), amphetamine induced a low to moderate increase in ambulatory (horizontal) and stereotypy activity (Fig. 6). At a higher dose (5 mg/kg), amphetamine induced a stronger locomotor and stereotypy responses (Fig. 6).

4. Discussion

To define the dopamine-dependent regulation of Ras-GFR expression in striatal neurons, we monitored alterations of Ras-GRF1 and Ras-GRF2 protein levels in the striatum after acute injection of amphetamine *in vivo*. We found that amphetamine induced a dose- and time-dependent increase in Ras-GRF1 protein abundance in the striatum. In contrast, amphetamine did not alter Ras-GRF2 protein expression in the same region. Amphetamine also increased Ras-GRF1, but not Ras-GRF2, in the medial prefrontal cortex. No change in the two isoforms was observed in the hippocampus. These results suggest that the Ras-GRF1 rather than the Ras-GRF2 is a sensitive and selective target of amphetamine in striatal and cortical neurons. Dopamine stimulation with amphetamine readily regulates its protein expression in a dose-, time-, and region-specific manner. It should be pointed out that we have not examined the effect of vehicle (saline) injection alone on Ras-GRF protein expression in the striatum or other brain regions. Such an effect, if there is any, could be minimal and might not affect the comparison between saline and amphetamine injected through same procedures.

Another psychostimulant, cocaine, has been shown to increase Ras-GRF1 proteins in the rat striatum, while cocaine had no effect on Ras-GRF2 in the same region (Zhang et al., 2007a). Like cocaine, amphetamine in this study was found to affect Ras-GRF1, but not Ras-GRF2. The reason for this different susceptibility of two Ras-GRF isoforms to cocaine and amphetamine is unclear. It is known that both Ras-GRF1 and Ras-GRF2 share many similarities in expression profiles and physiology. They both are present predominantly in the mammalian brain, where they are expressed in neuronal cells, but not in glia (Zippel et al., 1997). They are sensitive to Ca^{2+} signals (Farnsworth et al., 1995). Biochemically, both of them contain two GEF domains, a C-terminal cell division cycle 25 (CDC25) domain that activates the Ras/ERK cascade, and a more N-terminal dbl homology (DH) domain that activates a Rac/p38 cascade (Shou et al., 1992; Fam et al., 1997; Buchsbaum et al., 2002). However, differences in their roles in regulating synaptic plasticity are noted in a recent report. In hippocampal neurons, Ras-GRF1 was found to preferentially contribute to long-term depression whereas Ras-GRF2 to long-term potentiation, which is consistent with the observation that Ras-GRF1 mediates p38 activation via NR2B-containing NMDA receptors whereas Ras-GRF2 mediates ERK activation via NR2A-containing NMDA receptors (Li et al., 2006). Psychostimulants are known to inhibit basal excitability of spiny neurons and alter synaptic plasticity, which is deemed to be a cardinal feature of synaptic plasticity relevant to addictive properties of drugs of abuse (Hyman et al., 2006; Kalivas and Hu, 2006; Mao et al., 2009). The selective effect of psychostimulants on Ras-GRF1 may therefore be involved in triggering a Ras-GRF1-dependent mechanism important for long-lasting adaptations of excitatory synapses to drug exposure, while detailed steps of this scenario remain elusive.

The Ras-MAPK cascade downstream to Ras-GRFs is readily activated in response to acute psychostimulant exposure (see Introduction). This activation is characterized by a rapid and transient temporal property as evidenced by the finding that MAPK/ERK phosphorylation was

increased as early as 5 min after an injection of cocaine (20 mg/kg) and the elevated MAPK/ERK phosphorylation returned to the normal level by 1 h (Valjent et al., 2000). Such rapid and transient activation of MAPK/ERK was also consistently seen in striatal neurons in response to many other different types of extracellular stimuli (Yang et al., 2004; 2006; Mao et al., 2004; 2005). However, in this study, we did not observe an increase in Ras-GRF1 expression at the early time point (1 h after drug injection). A reliable increase in Ras-GRF1 expression was observed only at 2.5 h. Thus, the increase in Ras-GRF1 abundance is less likely to serve as an essential step leading to the rapid MAPK/ERK activation. Instead, the relatively delayed induction of Ras-GRF1 may link to some gene expression induced at the same time window in response to acute psychostimulant exposure. In fact, the kinetics of Ras-GRF1 induction that occurred at 2.5 h and returned to the normal level at 6 h following amphetamine injection seems to closely parallel the time-course of induction of immediate early genes, such as c-Fos, in the striatum in response to acute cocaine stimulation (Young et al., 1991; Zhang et al., 2007b). This immediate early gene is an inducible transcription factor that is involved in facilitating relatively late gene expression, such as prodynorphin gene expression, in response to dopamine stimulation (Wang et al., 1994; Wang and McGinty, 1995; Wang et al., 1995; Wang and McGinty, 1996). Thus, the upregulated Ras-GRF1 expression in striatal neurons may be an important component of signaling pathways underlying late gene expression. Given that Ras-GRF1 is an upstream activator of Ras-MAPK cascades, upregulated Ras-GRF1 expression may enhance the sensitivity of the Ras-MAPK pathway to subsequent stimulation. It is interesting to investigate responses of Ras-GRF1 and Ras-MAPKs to chronic stimulant exposure and possible roles of Ras-GRF1 and Ras-MAPKs in synaptic and behavioral plasticity in response to chronic stimulant administration. Future studies will need to elucidate whether and how Ras-GRF1 interacts with its downstream signaling proteins in promoting late gene expression and in regulating synaptic and behavioral adaptations.

The medial prefrontal cortex is a central site targeted by psychostimulants in addition to the striatum. Acute injection of cocaine or amphetamine increases extracellular dopamine levels and stimulates expression of c-Fos and other immediate early genes in the prefrontal cortex (Moghaddam and Bunney, 1989; Moghaddam et al., 1990; Sorg and Kalivas, 1993; Engber et al., 1998; Ostrander et al., 2003). In this study, we found that amphetamine elevated Ras-GRF1 protein levels in this region as in the case in the striatum. Unlike the prefrontal cortex and the striatum, amphetamine did not induce any change in Ras-GRF1 in the hippocampus. Thus, the effect of amphetamine on Ras-GRF1 is region-specific. Altered Ras-GRF1 expression in both the prefrontal cortex and the striatum is thought to play an important role in the drug action.

In sum, we investigated alterations in Ras-GRF protein expression in the rat forebrain in response to a single injection of psychostimulant. We found that acute injection of AMPH significantly altered Ras-GRF1 expression in the striatum in a dose- and time-dependent manner. In contrast, amphetamine had no effect on Ras-GRF2 protein expression in the same area. In the medial prefrontal cortex, amphetamine increased Ras-GRF1 expression, while it did not alter Ras-GRF2 levels. No significant change in the two isoforms was observed in the hippocampus. Together, our data support a notion that striatal and cortical Ras-GRF1 in abundance is subject to the modulation by amphetamine. Such sensitivity of Ras-GRF1 to amphetamine implies a potential functional role of Ras-GRF1 in controlling short- and/or long-term drug action.

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References

- Buchsbaum RJ, Connolly BA, Feig LA. Interaction of Rac exchange factors Tiam1 and Ras-GRF1 with a scaffold for the p38 mitogen-activated protein kinase cascade. *Mol Cell Biol* 2002;22:4073–4085. [PubMed: 12024021]
- Cen H, Papageorge AG, Zippel R, Lowy DR, Zhang K. Isolation of multiple mouse cDNAs with coding homology to *Saccharomyces cerevisiae* CDC25: identification of a region related to Bcr, Vav, Dbl and CDC24. *EMBO J* 1992;11:4007–4015. [PubMed: 1396590]
- 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. [PubMed: 12377393]
- Choe ES, Wang JQ. CaMKII regulates amphetamine-induced ERK1/2 phosphorylation in striatal neurons. *Neuroreport* 2002;13:1013–1016. [PubMed: 12060798]
- Engber TM, Koury EJ, Dennis SA, Miller MS, Contreras PC, Bhat RV. Differential patterns of regional c-Fos induction in the rat brain by amphetamine and the novel wakefulness-promoting agent modafinil. *Neurosci Lett* 1998;241:95–98. [PubMed: 9507929]
- Fam NP, Fan WT, Wang Z, Zhang LJ, Chen Z, Moran MF. Cloning and characterization of Ras-GRF2, a novel guanine nucleotide exchange factor for Ras. *Mol Cell Biol* 1997;17:1396–1406. [PubMed: 9032266]
- Farnsworth CL, Freshney NW, Rosen LB, Ghosh A, Greenberg ME, Feig LA. Calcium activation of Ras mediated by neuronal exchange factor Ras-GRF. *Nature* 1995;376:524–527. [PubMed: 7637786]
- Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* 2006;29:565–598. [PubMed: 16776597]
- Jenab S, Festa ED, Nazarian A, Wu HB, Sun WL, Hazim R, Russo SJ, Quinones-Jenab V. Cocaine induction of ERK proteins in dorsal striatum of Fischer rats. *Mol Brain Res* 2005;142:134–138. [PubMed: 16271798]
- Kalivas PW, Hu XT. Exciting inhibition in psychostimulant addiction. *Trends Neurosci* 2006;29:610–616. [PubMed: 16956674]
- Li S, Tian X, Hartley DM, Feig LA. Distinct roles for Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) and Ras-GRF2 in the induction of long-term potentiation and long-term depression. *J Neurosci* 2006;26:1721–1729. [PubMed: 16467520]
- Liu XY, Chu XP, Mao LM, Wang M, Lan HX, Li MH, Zhang GC, Parelkar NK, Fibuch EE, Haines M, Neve KA, Liu F, Xiong ZG, Wang JQ. Modulation of D2R-NR2B interactions in response to cocaine. *Neuron* 2006;52:897–909. [PubMed: 17145509]
- Liu XY, Mao LM, Zhang GC, Papasian CJ, Fibuch EE, Lan HX, Zhou HF, Xu M, Wang JQ. Activity-dependent modulation of limbic dopamine D3 receptors by CaMKII. *Neuron* 2009;61:425–438. [PubMed: 19217379]
- Mao L, Tang Q, Samdani S, Liu Z, Wang JQ. Regulation of MAPK/ERK phosphorylation via ionotropic glutamate receptors in cultured rat striatal neurons. *Eur J Neurosci* 2004;19:1207–1216. [PubMed: 15016079]
- Mao L, Wang W, Chu XP, Zhang GC, Liu XY, Yang YJ, Haines M, Papasian CJ, Fibuch EE, Buch S, Chen JG, Wang JQ. Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nat Neurosci* 2009;12:602–610. [PubMed: 19349975]
- 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. [PubMed: 15758184]
- Martegani E, Vanoni M, Zippel R, Coccetti P, Brambilla R, Ferrari C, Sturani E, Alberghina L. Cloning by functional complementation of a mouse cDNA encoding a homologue of CDC25, a *Saccharomyces cerevisiae* Ras activator. *EMBO J* 1992;11:2151–2157. [PubMed: 1376246]
- Moghaddam B, Bunney BS. Differential effect of cocaine on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens: comparison to amphetamine. *Synapse* 1989;4:156–161. [PubMed: 2781466]

- Moghaddam B, Roth RH, Bunney BS. Characterization of dopamine release in the rat medial prefrontal cortex as assessed by in vivo microdialysis: comparison to the striatum. *Neuroscience* 1990;36:669–676. [PubMed: 2234405]
- Ostrander MM, Richtand NM, Herman JP. Stress and amphetamine induce Fos expression in medial prefrontal cortex neurons containing glucocorticoid receptors. *Brain Res* 2003;990:209–214. [PubMed: 14568346]
- Shou C, Farnsworth CL, Neel BG, Feig LA. Molecular cloning of cDNAs encoding a guanine-nucleotide-releasing factor for Ras p21. *Nature* 1992;358:351–354. [PubMed: 1379346]
- Sorg BA, Kalivas PW. Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. 1993;53:695–703.
- Sturani E, Abbondio A, Branduardi P, Ferrari C, Zippel R, Martegani E, Vanoni M, Denis-Donini S. The Ras quinine nucleotide exchange factor CDC25Mm is present at synaptic junctions. *Exp Cell Res* 1997;235:117–123. [PubMed: 9281359]
- Sweatt JD. Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol* 2004;14:311–317. [PubMed: 15194111]
- Thomas GM, Huganir RL. MAPK cascade signaling and synaptic plasticity. *Nat Rev Neurosci* 2004;5:173–183. [PubMed: 14976517]
- 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. [PubMed: 11102476]
- 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. [PubMed: 16512905]
- Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induces distinct and specific patterns of ERK activation in mouse brain. *Eur J Neurosci* 2004;19:1826–1836. [PubMed: 15078556]
- Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JG, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc Natl Acad Sci USA* 2005;102:253–254. [PubMed: 15632217]
- Volmat V, Pouysseur J. Spatiotemporal regulation of the p42/p44 MAPK pathway. *Biol Cell* 2001;93:71–79. [PubMed: 11730325]
- Wang JQ, Daunais JB, McGinty JF. NMDA receptors mediate amphetamine-induced regulation of *zif/268* and preprodynorphin mRNA expression in rat striatum. *Synapse* 1994;18:343–353. [PubMed: 7886627]
- Wang JQ, Fibuch EE, Mao L. Regulation of mitogen-activated protein kinases by glutamate receptors. *J Neurochem* 2007;100:1–11. [PubMed: 17018022]
- Wang JQ, McGinty JF. Dose-dependent alteration in *zif/268* and preprodynorphin mRNA expression induced by amphetamine and methamphetamine in rat forebrain. *J Pharmacol Exp Ther* 1995;273:909–917.
- Wang JQ, McGinty JF. D₁ and D₂ receptor regulation of preproenkephalin and preprodynorphin mRNA expression in rat striatum following acute injection of amphetamine or methamphetamine. *Synapse* 1996;22:114–122. [PubMed: 8787127]
- Wang JQ, Smith AJW, McGinty JF. A single injection of amphetamine and methamphetamine induces dynamic alterations in *c-fos*, *zif/268* and preprodynorphin mRNA expression in rat forebrain. *Neuroscience* 1995;68:83–95. [PubMed: 7477938]
- Yang L, Mao L, Chen H, Catavsan M, Kozinn J, Arora A, Liu X, Wang JQ. A signaling mechanism from Gαq-protein-coupled glutamate receptors to gene expression: role of the c-Jun N-terminal kinase pathway. *J Neurosci* 2006;26:971–980. [PubMed: 16421317]
- Yang L, Mao L, Tang Q, Samdani S, Liu Z, Wang JQ. A novel Ca²⁺-independent signaling pathway to extracellular signal-regulated protein kinase by coactivation of NMDA receptors and metabotropic glutamate receptor 5 in neurons. *J Neurosci* 2004;24:10846–10857. [PubMed: 15574735]
- Young ST, Porrino LJ, Iadarola MJ. Cocaine induces striatal c-Fos-immunoreactive proteins via dopaminergic D1 receptors. *Proc Natl Acad Sci USA* 1991;88:1291–1295. [PubMed: 1825356]

- Zhang GC, Hoffmann J, Parelkar NK, Liu XY, Mao LM, Fibuch EE, Wang JQ. Cocaine increases Ras-guanine nucleotide-releasing factor 1 protein expression in the rat striatum *in vivo*. *Neurosci Lett* 2007a;427:117–121. [PubMed: 17931779]
- Zhang GC, Mao LM, Liu XY, Parelkar NK, Arora A, Yang L, Haines M, Fibuch EE, Wang JQ. *In vivo* regulation of Homer1a expression in the striatum by cocaine. *Mol Pharmacol* 2007b;71:1148–1158. [PubMed: 17234898]
- Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, Zhang J, Xu M. Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J Neurosci* 2004;24:3344–3354. [PubMed: 15056714]
- Zippel R, Gnesutta N, Matus-Leibovitch N, Mancinelli E, Saya D, Vogel Z, Sturani E. Ras-GRF, the activator of Ras, is expressed preferentially in mature neurons of the central nervous system. *Mol Brain Res* 1997;48:140–144. [PubMed: 9379834]

Fig. 1.

Amphetamine increases Ras-GRF1 expression in the rat striatum in a dose-dependent manner. (A) Amphetamine increased Ras-GRF1 expression in the CPu. (B) Amphetamine increased Ras-GRF1 expression in the NAc. Note that amphetamine at a higher dose (5 mg/kg), but not a lower dose (1.25 mg/kg), showed a significant effect on Ras-GRF1 protein expression. Representative immunoblots were shown above the quantification of immunoblot results. A single dose of amphetamine (1.25 or 5 mg/kg) or saline (1 ml/kg) was injected i.p. Rats were sacrificed 2.5 h after injection. The quantified data are expressed as means $\pm$ S.E.M. ($n = 4-5$ per group). * $P < 0.05$ versus saline.

Fig. 2. Effects of amphetamine on Ras-GRF2 expression in the rat CPu (A) and NAc (B). Representative immunoblots were shown above the quantification of immunoblot results. A single dose of amphetamine (1.25 or 5 mg/kg) or saline (1 ml/kg) was injected i.p. Rats were sacrificed 2.5 h after injection. The quantified data are expressed as means $\pm$ S.E.M. ($n = 4-5$ per group).

Fig. 3.

Amphetamine increases Ras-GRF1 expression in the rat striatum in a time-dependent manner. (A and B) Time-dependent effects of amphetamine on Ras-GRF1 protein levels in the CPu (A) and NAc (B). (C and D) Effects of amphetamine on Ras-GRF2 protein levels in the CPu (C) and NAc (D) detected at different time points. Note that the amphetamine-stimulated Ras-GRF1 expression peaked at 2.5 h and returned to the normal level within 6 h. A single dose of amphetamine (5 mg/kg) or saline (1 ml/kg) was injected i.p. Rats were sacrificed at different time points (1, 2.5, or 6 h) after drug injection. The quantified data are expressed as means $\pm$ S.E.M. ($n = 4-5$ per group). * $P < 0.05$ versus saline at the same time point.

Fig. 4.

Amphetamine increases Ras-GRF1 expression in the rat medial prefrontal cortex in a dose-dependent manner. (A) Amphetamine increased Ras-GRF1 expression in the medial prefrontal cortex. Representative immunoblots were shown above the quantification of immunoblot results. Note that amphetamine at a higher dose (5 mg/kg), but not a lower dose (1.25 mg/kg), showed a significant effect on Ras-GRF1 protein expression. (B) Effects of amphetamine on Ras-GRF2 protein levels in the medial prefrontal cortex. A single dose of amphetamine (1.25 or 5 mg/kg) or saline (1 ml/kg) was injected i.p. Rats were sacrificed 2.5 h after injection. The quantified data are expressed as means $\pm$ S.E.M. ($n = 5$ per group). * $P < 0.05$ versus saline.

Fig. 5. Effects of amphetamine on Ras-GRF1 and Ras-GRF2 expression in the rat hippocampus. amphetamine had no effect on Ras-GRF1 (A) and Ras-GRF2 (B) in this region. A single dose of amphetamine (1.25 or 5 mg/kg) or saline (1 ml/kg) was injected i.p. Rats were sacrificed 2.5 h after injection. The quantified data are expressed as means $\pm$ S.E.M. ($n = 5$ per group).

Fig. 6.

Effects of acute injection of amphetamine on motor activity in rats. A single dose of amphetamine (1.25 or 5 mg/kg) or saline (1 ml/kg) was injected i.p. The data are expressed as means $\pm$ S.E.M. ($n = 3-5$ per group).