Published in final edited form as:
Synapse. 2009 January ; 63(1): 42–53. doi:10.1002/syn.20577.

Cocaine activates Homer1 immediate early gene transcription in the mesocorticolimbic circuit: differential regulation by dopamine and glutamate signaling

M. Behnam Ghasemzadeh[1], **Lindsay K. Windham**[2], **Russell W. Lake**[2], **Christopher J. Acker**[1], and **Peter W. Kalivas**[2]

[1]Department of Biomedical Sciences, Marquette University, 561 N. 15th Street, SC 446 Milwaukee, WI 53233;

[2]Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, BSB 403, Charleston, SC 29425.

Abstract

Homer proteins are intracellular scaffolding proteins that, amongst glutamate receptors, selectively bind to group1 metabotropic glutamate receptors and regulate their trafficking and intracellular signaling. Homer proteins have been implicated in synaptic and behavioral plasticity, including drug-seeking behavior after cocaine treatment. *Homer1* gene activation leads to transcription of a variant mRNA (*Homer1a*) which functions as an immediate early gene. Homer1a competes with the constitutive Homer proteins (Homer1b/c/d, Homer2a/b, Homer3) for binding to group1 metabotropic glutamate and IP3 receptors. Binding of Homer1a to these proteins disrupts their association with the intracellular signaling scaffold and modulates receptor function. In this study, using RT-PCR, activation of *Homer1a* mRNA transcription in response to acute and repeated administration of cocaine was characterized in prefrontal cortex, nucleus accumbens, and ventral tegmental area, three mesocorticolimbic nuclei of the rat brain. Moreover, the dopaminergic and glutamatergic regulation of *Homer1* gene activation by cocaine was investigated. Acute cocaine rapidly and transiently activated transcription of *Homer1a* mRNA in all three nuclei. However, repeated administration of cocaine was not effective in inducing the *Homer1a* mRNA transcription after various withdrawal times ranging from two hours to three weeks. The acute cocaine-mediated activation of *Homer1* gene was regulated by D1 but not D2 dopamine receptors. The blockade of AMPA or NMDA glutamate receptors did not prevent cocaine-mediated activation of *Homer1* gene in the three mesocorticolimbic nuclei. These data indicate that acute administration of cocaine transiently activates *Homer1* gene producing the immediate early gene *Homer1a* mRNA in the three mesocorticolimbic nuclei of the rat brain. Activation of *Homer1* gene may contribute to the cocaine-mediated synaptic and behavioral plasticity.

Keywords

Prefrontal cortex; nucleus accumbens; ventral tegmental area; rat

Correspondence: M. Behnam Ghasemzadeh Department of Biomedical Sciences Marquette University 561 N. 15th Street, SC 446 Milwaukee, WI 53233 Fax: 414-288-6564 behnam.ghasemzadeh@marquette.edu.

INTRODUCTION

The metabotropic glutamate receptors (mGluRs) play an important role in regulation of neuronal development, synaptic plasticity, learning and memory as well as regulating other receptors and ion channels (Bashir et al., 1993; Conquet et al., 1994; Conn and Pin, 1997; Kammermeier et al., 2000; Foa et al., 2001; Barker et al., 2006). The group1 metabotropic glutamate receptors (mGluR1 and 5) are $G_{q/11}$-protein-coupled and exert their physiological effects through activation of phospholipase C and hydrolysis of membrane phosphoinositide lipids. The trafficking, membrane localization, and function of the group1 mGluR receptors are modulated through their interaction with Homer proteins, a family of intracellular scaffolding proteins that bind to the intracellular C-terminus of the receptor. The Homer family of proteins is coded by three genes and all member proteins contain N-terminal EVH1 (Enabled/vasodilator-stimulated phosphoprotein homology 1) domain able to bind a proline-rich motif (PPXXFR) present on cellular signal transduction modules such as mGluR1a, mGluR5a/b, IP3 receptors, ryanodine receptors, and Shank proteins, a component of the postsynaptic PSD95-GKAP-NMDA receptor protein complex (Xiao et al., 1998; Tu et al., 1998, 1999; Naisbitt et al., 1999; Bottai et al., 2002). All three *Homer* genes code for constitutively expressed long-form Homer proteins (Homer1b/c/d, Homer2a/b, Homer3) that contain a C-terminal coiled coil domain and leucine zipper motifs (Kato et al., 1998; Sun et al., 1998; Tadokoro et al., 1999). These proteins form multimer protein complexes through C-terminal interactions and can tether group1 mGluRs, IP3 receptors, and ionotropic glutamate receptors through interactions with Shank proteins (Tu et al., 1999; Naisbitt et al., 1999). *Homer1* is unique among *Homer* genes because, in response to an increase in cellular activity, is transcribed into immediate early gene (IEG) products Homer1a and Ania3 (Brakeman et al., 1997; Kato et al., 1997; Berke et al., 1998). The *Homer1a* and *Ania3* mRNA and protein sequences are very similar except for a few residues at the C-terminal end (Berke et al., 1998; Bottai et al., 2002). These IEG products share the EVH1 domain with the long-forms but lack the C-terminal domains and, hence, can compete and disrupt the interaction between the long-forms and their target proteins including group1 mGluRs (Xiao et al., 2000; Kammermeier and Worley, 2007). Therefore, induction of IEG Homers may play an important role in the regulation of group1 mGluR intracellular signaling. Recently, a unique role for Homer proteins in behavioral sensitization to cocaine and drug seeking behavior has been described (Swanson et al., 2001; Ghasemzadeh et al., 2003; Szumlinski et al., 2004, 2006).

In this study, we have characterized the dynamics of *Homer1* gene activation and mRNA transcription in response to an acute or repeated administration of cocaine in three areas of the mesocorticolimbic circuitry of the brain, prefrontal cortex, nucleus accumbens, ventral tegmental area, involved in various aspects of drug response and drug seeking behaviors (Vanderschuren and Kalivas, 2000; Everitt and Wolf, 2002; Kalivas and Volkow, 2005; Robinson and Berridge, 2003; Vanderschuren and Everitt, 2005). Moreover, the regulation of cocaine-mediated *Homer1* gene activation by dopaminergic and glutamatergic receptor signaling was investigated.

MATERIALS AND METHODS

Animal Housing

Male Sprague-Dawley rats weighing 250-300 g were obtained from Harlan laboratories (Indianapolis, IN) and housed two per cage with water and food available *ad libitum*. All housing and experimental procedures were conducted in AAALAC approved facilities and were approved by the Institutional Animal Care and Use Committee. A 12 hour light/dark cycle was used, with lights on at 7 AM. Animals were acclimated to the housing facility for one week before the start of experiments. The drug treatments and tissue collection were performed during the light cycle.

Cocaine Treatment

For acute cocaine studies, rats were injected with cocaine (30 mg/kg, ip) dissolved in saline and killed at various time intervals (0, 1, 2, 6, 12, 24 hours) by rapid decapitation (Graybiel et al., 1990; Steiner and Gerfen, 1993). After cocaine administration, except for the time zero when the rats were immediately decapitated, animals were placed in their home cage and returned to the animal colony room for the duration of the withdrawal time.

For chronic cocaine studies, rats were treated with seven once daily administration of either saline (1 ml/kg) or cocaine (days 1 and 7: 15 mg/kg, ip; days 2-6: 30 mg/kg, ip) and were killed at various withdrawal times (2 hours, 1 day, 1 week, 3 weeks) after the last saline or cocaine injection by rapid decapitation. After each saline or cocaine administration, all rats were placed in their home cages in the animal colony room. The cocaine doses and injection regimen employed were based on prior studies indicating enduring molecular and behavioral plasticity in rodents (Boudreau and Wolf, 2005; Boudreau et al. 2007; Ghasemzadeh et al., 2003; Pierce et al., 1996; Szumlinski et al., 2004). The saline and cocaine treatments were scheduled such that both treatment groups were present at each withdrawal time for tissue collection. Rats were killed by rapid decapitation and brains were removed rapidly and coronal sections were prepared on ice. The prefrontal cortex, nucleus accumbens, and ventral tegmental area were dissected on an ice-cooled plate from 1-2 mm slices and immediately frozen on dry ice and kept at −80 °C.

Pharmacological treatments

For examining the dopaminergic and glutamatergic regulation of the cocaine effects, selective dopamine and glutamate receptor antagonists were employed. These antagonists were dissolved in saline and administered 20 minutes before a saline (1 ml/kg, ip) or cocaine (30 mg/kg, ip) injection. All animals were killed at 2 hours after the second injections. The control groups received a saline injection instead of the receptor antagonist or cocaine. The 2 hours time interval was determined based on the acute cocaine time course experiments in this study. The following pharmacological agents were used based on results from prior studies: SCH23390, a selective D1 dopamine receptor antagonist (1 mg/kg, ip); Sulpiride, a selective D2 dopamine receptor antagonist (20 mg/kg, ip); GYKI52466, a selective AMPA glutamate receptor antagonist (15 mg/kg, ip); MK801, a selective NMDA glutamate receptor antagonist (1 mg/kg, ip) (Kuroki et al., 1999; Taepavarapruk et al., 2000; Kalivas and Duffy, 1997; Ozaki et al., 1997; Svensson and Mathe, 2000; Pollack et al., 2005, Storvik et al.,

2006). Cocaine hydrochloride was a gift from NIDA/NIH. Saline was used as the vehicle for all drugs. All drugs were purchased from Tocris Cookson Inc. (Ballwin, MO).

Semiquantitative RT-PCR

Tissue homogenization, RNA extraction, and RT-PCR methods used in this study have been previously described in detail (Leonard et al., 1993; Somogyi et al., 1995; Ghasemzadeh et al., 1999). The PCR primer sequences were as follows: sense 5′-CTGTGCCTGAGTGTCTGAGAG-3′, antisense 5′-CCATGTCTCCTGCAATCGCTG-3′; GenBank accession number U92079. An RT-PCR calibration experiment was done for *Homer1a* mRNA transcript to establish the experimental conditions for the linear amplification of the mRNA transcripts as described previously (Ghasemzadeh et al., 1999). The following PCR steps were used for 28 cycles: denatration 94 °C, annealing 63 °C, extension 72 °C.

Data Analysis

Data for experiment two (repeated cocaine administration studies) were analyzed using a two-way ANOVA with drug treatment and withdrawal time as main factors. For rest of the experiments, the differences between groups were determined using one-way ANOVA, and *post hoc* comparisons between treatment groups were conducted with Fisher's PLSD. Statistical significance was set at $p < 0.05$. The data presented in Table 1 were subjected to the Levene's test of homogeneity of variance and, due to the lack of homogeneity in data obtained for dopamine antagonists, Welch's ANOVA and Scheffe's *post hoc* tests were used for comparing means of groups.

RESULTS

The purpose of this study was to characterize the dynamics of *Homer1* gene activation and its pharmacological regulation in three nuclei of the rat brain mesocorticolimbic circuit after acute and repeated exposure to cocaine. The tissue level of *Homer1a* mRNA transcripts in prefrontal cortex, nucleus accumbens, and ventral tegmental area were measured by RT-PCR. These nuclei were selected because of their functional roles in behavioral responses to psychostimulants and drug seeking behaviors (Vanderschuren and Kalivas, 2000; Everitt and Wolf, 2002; Robinson and Berridge, 2003; Kalivas and Volkow, 2005; Vanderschuren and Everitt, 2005). The RT-PCR technique has been characterized and validated in previous studies (Somogyi et al., 1995; Ghasemzadeh et al., 1999). The RT-PCR technique used cannot distinguish between *Homer1a* and *Ania3* mRNA transcripts since the mRNA sequences of *Homer1a* and *Ania3* are almost identical (Berke et al., 1998; Bottai et al., 2002). Therefore, the amplified and measured mRNAs is referred to as *Homer1a* mRNA transcript. A PCR calibration curve was constructed for the *Homer1a* mRNA to confirm the linear amplification of the target transcript under the conditions employed in this study (Figure 1).

Experiment one: Effect of acute cocaine administration on Homer1 gene activation

Rats were injected with cocaine (30 mg/kg, ip) and killed at various time intervals (0, 1, 2, 6, 12, 24 hours). Figure 2 shows that the level of *Homer1a* mRNA significantly changed in all

three brain regions in a time-dependent manner (PFC, $F(5,33) = 6.82$, $p < 0.001$; NA, $F(5,31) = 10.29$, $p < 0.001$; VTA $F(5,35) = 13.81$, $P < 0.001$). In these regions, the *Homer1a* mRNA was significantly increased by one hour post injection and remained elevated for the first two hours after cocaine administration and returned to control level by six hours. The magnitude of mRNA increase was highest in VTA, followed by NA and PFC. The mRNA levels for the long-forms of Homer proteins were not measured in this study because their mRNA and protein levels do not change after acute increase in cellular activity or cocaine administration (Bottai et al., 2002; Zhang et al., 2007).

Experiment two: Effect of repeated cocaine administration on Homer1 gene activation

Rats were treated with a repeated cocaine administration regimen that has been shown to produce long-lasting molecular and behavioral plasticity (Pierce et al., 1996; Ghasemzadeh, 1999; 2003; Boudreau and Wolf, 2005). This regimen involved 7 once daily administration of cocaine (2 days × 15 mg/kg; 5 days × 30 mg/kg, ip) followed by various times of withdrawal from drug treatment (2 hours, 1 day, 1 week, 3 weeks). A separate group of animals received an identical repeated saline treatment for each of the withdrawal times. All animals were killed after the designated withdrawal times. As shown in Figure 3, a two way analysis of variance yielded no significant changes in the *Homer1a* mRNA level at any of the withdrawal times examined (PFC, treatment $F(1,55) = 0.052$, $p = 0.82$, time $F(3,55) = 0.069$, $p = 0.98$, treatment × time $F(3,55) = 0.074$, $p = 0.97$; NA, treatment $F(1,48) = 0.957$, $p = 0.33$, time $F(3,48) = 0.037$, $p = 0.99$, treatment × time $F(3,48) = 0.045$, $p = 0.99$; VTA, treatment $F(1,56) = 0.134$, $p = 0.72$, time $F(3,56) = 0.639$, $p = 0.59$, treatment × time $F(3,56) = 0.676$, $p = 0.57$).

Experiment three: Dopaminergic regulation of Homer1 gene activation after acute cocaine

Table 1 shows that the blockade of either D1 (SCH23390, 1 mg/kg, ip) or D2 (Sulpiride, 20 mg/kg, ip) dopamine receptors, in the absence of cocaine, did not alter the basal *Homer1a* mRNA levels in any brain region examined (PFC, $F(2,12) = 1.11$, $p = 0.36$; NA, $F(2,4.3) = 0.40$, $p = 0.69$; VTA, $F(2,11) = 0.53$, $p = 0.60$). The drug doses were selected based on prior studies indicating their effectiveness in modulation of dopamine release, basal glutamate levels in nucleus accumbens, dopamine-mediated locomotor activity, and modulation of *fos* and *jun* immediate early gene mRNA transcription in the brain (Kuroki et al., 1999; Taepavarapruk et al., 2000; Kalivas and Duffy, 1997; Ozaki et al., 1997). An analysis of variance showed that pretreatment with dopamine receptor antagonists had a significant effect on cocaine mediated increase in *Homer1a* mRNA in all three structures examined (PFC, $F(4,45) = 2.65$, $p < 0.05$; NA, $F(4,47) = 3.48$, $p < 0.05$; VTA, $F(4,40) = 2.81$, $p < 0.05$). Post hoc analysis using Fisher's PLSD indicated that the blockade of D1 receptors prevented the cocaine-mediated *Homer1a* mRNA induction in PFC and NA but partially reversed the cocaine effect in VTA (Figure 4). While the blockade of D2 receptors partially reversed the cocaine-mediated *Homer1a* mRNA induction in prefrontal cortex, it was without effect in nucleus accumbens and ventral tegmental area. In addition, the blockade of both D1 and D2 dopamine receptors prevented the cocaine-mediated *Homer1a* mRNA induction in prefrontal cortex, partially reversed the effect in nucleus accumbens, but was not effective in ventral tegmental area.

Experiment four: Glutamatergic regulation of Homer1 gene activation after acute cocaine

Table 1 shows that the blockade of either AMPA (GYKI52466, 15 mg/kg, ip) or NMDA (MK801, 1 mg/kg, ip) glutamate receptors, in the absence of cocaine, did not alter the *Homer1a* mRNA levels in any brain region examined (PFC, $F(2,9) = 1.68$, $p = 0.24$; NA, $F(2,9) = 0.14$, $p = 0.87$; VTA, $F(2,11) = 2.44$, $p = 0.13$). The drug doses were selected based on prior studies showing their effectiveness in modulation of learning, behavioral sensitization, and cocaine-mediated gene expression (Svensson and Mathe, 2000; Pollack et al., 2005, Storvik et al., 2006). An analysis of variance showed that pretreatment with glutamate receptors had a significant effect on cocaine mediated increase in *Homer1a* mRNA in the three nuclei examined (PFC, $F(3,43) = 2.85$, $p < 0.05$; NA, $F(3,42) = 2.90$, $p < 0.05$; VTA, $F(3,33) = 2.89$, $p < 0.05$). Surprisingly, post hoc analysis using Fisher's PLSD indicated that the blockade of NMDA receptors was not effective in preventing the cocaine-mediated increase in *Homer1a* mRNA in any of the three brain regions examined (Figure 5). Moreover, the blockade of the AMPA receptors in nucleus accumbens only partially reversed the cocaine-mediated increase in *Homer1a* mRNA, while it was not effective in prefrontal cortex and ventral tegmental area.

DISCUSSION

Homer proteins regulate several key physiological processes including axonal guidance, spine growth, synaptic plasticity, ion channels and intracellular signaling. In addition, Homer proteins play an important role in the regulation of group1 mGluR signaling and several reports have implicated Homer proteins in the regulation of cocaine-mediated synaptic and behavioral plasticity, and drug seeking behavior (Ghasemzadeh et al., 2003; Szumlinski et al., 2004, 2006). In this study, we investigated the effects of acute and chronic cocaine administrations on *Homer1* gene activity and *Homer1a* mRNA expression in three nuclei of the mesocorticolimbic circuit of the rat brain and its regulation by dopaminergic and glutamatergic signaling.

Acute and repeated cocaine administration and Homer1 gene activity

An acute injection of cocaine produced a transient *Homer1* gene activation leading to an increase in *Homer1a* mRNA, the IEG short-form product, in all three brain regions examined. This is in agreement with previous reports showing that acute administration of the psychostimulants methylphenidate and cocaine or binge administration of cocaine produce a significant induction of IEG Homers (Brakeman et al., 1997; Yuferov et al., 2003; Yano and Steiner, 2005). This observation is also in agreement with cocaine regulation of other IEGs in the brain. For example, *fos*, *Arc*, and *zif268* demonstrate a similar transient increase in mRNA and protein expression after an increase in cellular activity (Hope et al., 1992; Steiner and Gerfen, 1993; Berke et al., 1998; Lyford et al., 1995; Yano and Steiner, 2005). *Homer1a* mRNA has an apparent slower transcription time course compared to most other IEGs, reaching a peak mRNA expression level between one to two hours after cocaine administration. By contrast, the time course of activation of other IEGs such as *fos*, *Arc*, and *zif268* is shorter and the mRNA induction is maximal at one hour after cellular activity with a decline in the mRNA levels by two hours (Bottai et al., 2002; Sato et al., 2001; Moratalla et al., 1992; Fosnaugh et al., 1995). The slower rate of *Homer1a* mRNA induction can be

explained by the large difference in the size of *Homer1* gene (> 100 kb) compared to most other IEGs (< 5 kb) (Bottai et al., 2002). The delay in the appearance of *Homer1a* mRNA is also reflected in the delay of the Homer1a protein peak level observed in striatum and nucleus accumbens three hours after an acute cocaine administration (Zhang et al., 2007). However, it is important to note that there is no difference between *Homer1* and other IEGs in the initiation of gene activation and primary RNA transcription (Bottai et al., 2002).

The acute cocaine-mediated increase in the *Homer1* gene activity is brain region selective. While frontal and prefrontal cortex, striatum, and nucleus accumbens displayed an increase in Homer1a expression, some more caudal brain regions including hippocampus, amygdala, and cerebellum were not affected (Zhang et al., 2007). In our study, acute cocaine administration significantly increased *Homer1a* mRNA levels in VTA, although, the elevated level of *Homer1a* mRNA was still lower than elevated levels in prefrontal cortex and nucleus accumbens. None the less, we were able to detect and quantify both the basal and stimulated *Homer1a* mRNA levels in VTA after RT-PCR amplification. In contrast to our observation, Zhang et al. (2007) reported an absence of Homer1a protein induction in VTA two hours after an acute cocaine administration. The Homer1a protein expression levels are low in the brain and its detection requires prolong exposure for optical signal measurement (Zhang et al., 2007). Moreover, Zhang et al., (2007) used a lower cocaine dose (20 mg/kg) and showed a weak increasing trend in Homer1a protein in VTA. The differences in detection techniques and cocaine dose may have contributed to the different results in detecting the acute cocaine effect in VTA. In contrast to *Homer1a* mRNA and proteins, the level of the constitutive *Homer* mRNA and proteins do not change after an increase in cellular activity or acute cocaine administration (Bottai et al., 2002; Zhang et al., 2007). Fourgeaud et al. (2004) reported an increase in Homer1b/c protein in nucleus accumbens at 24 hours after an acute cocaine administration. However, we were not able to replicate this finding in our laboratory (Saline (1 ml/kg, ip), 100 ± 10.4, n=8; Cocaine (30 mg/kg, ip), 94.5 ± 6.9, n=8).

Repeated daily administration of cocaine leads to the development of long-lasting synaptic, molecular, and behavioral plasticity in the mesocorticolimbic circuit (Vanderschuren and Kalivas, 2000; Everitt and Wolf, 2002; Kalivas and Volkow, 2005; Robinson and Berridge, 2003; Vanderschuren and Everitt, 2005; Boudreau and Wolf, 2005). Repeated daily treatment with cocaine did not lead to a sustained *Homer1* gene activation and *Homer1a* mRNA induction after withdrawal times ranging from 2 hours to three weeks in any of the brain regions examined. The lack of gene activation by an acute cocaine injection given after repeated daily administration of cocaine has also been reported for other IEGs in the brain (Hope et al., 1992, 2006; Steiner and Gerfen, 1993; Yano and Steiner, 2005). This observation, termed desensitization of IEG induction, has been documented in several IEGs using various methods of increasing cellular activity (Hope et al., 1992, 2006; Steiner and Gerfen, 1993; Morattala et al., 1996). In contrast to a lack of change in *Homer1a* mRNA, and presumably protein, Homer1b/c protein is decreased in nucleus accumbens after three weeks of withdrawal from repeated cocaine administration (Swanson et al., 2001; Ary and Szumlinsky, 2007). The decrease in Homer1b/c protein level in nucleus accumbens has been

implicated in the behavioral and biochemical plasticity after repeated administration of cocaine (Ghasemzadeh et al, 2003; Szumlinski et al., 2004, 2006).

The dopaminergic regulation of Homer1 gene activity

Dopaminergic signaling regulates activation of *fos* and other IEGs in the brain. In general, blockade of D1 dopamine receptor inhibits the cocaine and amphetamine induced IEG mRNA expression (Young et al., 1991; Bhat et al., 1992; Berretta et al., 1992; Simpson and Morris, 1995; Ozaki et al., 1997). In contrast, D2 dopamine receptor antagonists are generally ineffective in blocking the induction of IEGs by psychostimulants (Young et al., 1991; Simpson and Morris, 1995; Ozaki et al., 1997). Similarly, our data indicate that D1 dopamine signaling mediates cocaine-induced *Homer1a* mRNA induction in all three brain regions. The basal level of *Homer1* gene activity was not regulated by dopaminergic signaling since the blockade of either D1 or D2 dopamine receptors by SCH23390 and sulpiride, respectively, did not significantly alter the *Homer1a* mRNA levels in any of the brain areas examined (Young et al., 1991; Deutch and Duman, 1996; Semba et al., 1996; Robertson et al., 1994; Watanabe et al., 1998; Zhang et al., 2007). Although sulpiride administration at a high dose (100 mg/kg, ip) increases expression of *c-fos* mRNA or protein in nucleus accumbens shell (Deutch and Duman, 1996; Semba et al., 1996; Watanabe et al., 1998), the dose selected in this study (20 mg/kg, ip) does not affect *Homer1a* or *c-fos* mRNA induction in the brain (Fiore et al., 2003; Robertson et al., 1994; Deutch and Duman, 1996). However, recent studies suggest that there is heterogeneity in the effects of D2 dopamine receptor antagonists on basal *Homer1a* mRNA expression in nucleus accumbens and dorsal striatum. While eticlopride and sulpiride are without effect, haloperidol increases the *Homer1a* mRNA or protein levels in nucleus accumbens and dorsal striatum (Zhang et al., 2007; Fiore et al., 2003; Tomasetti et al., 2007; Ambesi-impiombato et al., 2007). The blockade of D1 dopamine receptor with SCH23390 inhibited the induction of *Homer1a* mRNA by acute cocaine administration in prefrontal cortex and nucleus accumbens and partially reversed the cocaine effect in ventral tegmental area. Similarly, the blockade of D1 dopamine receptor inhibited the induction of *Homer1a* mRNA in striatum by acute methylphenidate administration (Yano et al., 2006). Akin to other IEGs, blockade of the D2 dopamine receptors did not affect the cocaine induction of *Homer1a* mRNA in nucleus accumbens and ventral tegmental area, however, it was able to partially reverse the *Homer1a* mRNA induction in prefrontal cortex. Zhang et al. (2007) reported similar D1 and D2 dopamine receptor regulation of Homer1a protein expression in striatum and nucleus accumbens after acute cocaine administration. Recent in vitro studies indicate that the D1 dopamine receptor regulation of Homer1a induction is mediated through activation of protein kinase A and Ca^{2+}/calmodulin-dependent protein kinases, but not protein kinase C, and that their effects may involve CREB transcription factor (Zhang et al., 2007). These observations indicate that *Homer1* gene activity, similar to the other IEGs, is influenced by dopaminergic signaling in the three nuclei of the mesocorticolimbic circuit of the rat brain.

The inability of the D1 and combined D1/D2 dopamine receptor blockade in VTA to prevent the cocaine induced *Homer1* gene activation may be attributed to the regulation of GABA and glutamate release through activation of presynaptic D1 and 5HT1D receptors (Cameron and Williams, 1993, 1994; Kalivas and Duffy, 1995). Administration of cocaine

leads to inhibition of dopamine and serotonin reuptake and an increase in their extracellular levels in VTA (Bradberry and Roth, 1989; Cameron and Williams, 1993, 1994). The modulation of GABA and glutamate neurotransmission in VTA by cocaine takes place via activation of presynatic D1 and 5-HTD1 receptors located on afferents to VTA (Cameron and Williams, 1993, 1994; Kalivas and Duffy, 1995). The overall effect of cocaine on enhancing extracellular glutamate and reducing GABA levels leads to an increase in neuronal activity and transcription of *Homer1a* mRNA in VTA. The blockade of D1 receptors by SCH23390 prevents the cocaine-mediated glutamate release without any effect on cocaine-mediated reduction in GABA and may be responsible for the partial reversal of cocaine effect in VTA (Kalivas and Duffy, 1995). The blockade of somatodendritic D2 dopamine autoreceptors will lead to further dopaminergic neuronal activity and dopamine release by reducing the inhibitory influence of the somatodendritic D2 dopamine autoreceptors and, hence, will not modulate the cocaine effect on *Homer1a* mRNA transcription (Cameron and Williams, 1994; Adell and Artigas, 2004). The combined blockade of D1 and D2 receptors in VTA was not effective in reducing the cocaine effect because the increase in neuronal activity mediated by the inhibition of D2 dopamine autoreceptors may counter balance the partial reversal effect of D1 receptor blockade.

The glutamatergic regulation of Homer1 gene activity

Our data suggest a lack of tonic effects of the ionotropic glutamate receptors on the basal *Homer1* gene activity and the expression levels of *Homer1a* mRNA in the brain regions examined. This is in agreement with the previous reports showing that blockade of AMPA/kainate or NMDA glutamate receptors does not alter the basal mRNA or protein levels of immediate early genes *fos* or *zif268* in prefrontal cortex, nucleus accumbens, dorsal striatum, and VTA (Wang et al., 1994; Dalia and Wallace, 1995; Keefe and Adams, 1998; Hussain et al., 2001; De Leonibus et al., 2002; Lee and Rajakumar, 2003). However, the effect of NMDA receptor blockade on IEG activity seems to be region and antagonist dependent. Whereas MK801 does not activate *c-fos* expression in prefrontal cortex, striatum, nucleus accumbens and ventral tegmental area, phencyclidine increases *c-fos* activity in prefrontal cortex, decreases in striatum and produce no effect in nucleus accumbens (Keefe and Adams, 1998; Hussain et al., 2001; De Leonibus et al. 2002; Lee and Rajakumar, 2003; Kargieman et al., 2007; Hansen et al., 2007). Similarly, a recent report has indicated that blockade of NMDA receptors by phencyclidine increased *Homer1a* mRNA in prefrontal cortex in a region and layer specific manner (Cochran et al., 2002). Moreover, acute cocaine-mediated increases in *Homer1a* mRNA does not seem to be under AMPA or NMDA glutamate receptor regulation in the three mesocorticolimbic nuclei examined; although, blockade of AMPA/kainate receptors partially reversed the acute cocaine-mediated *Homer1a* mRNA levels in nucleus accumbens (Figure 5). The lack of glutamatergic control over IEG induction is consistent with previous studies. The blockade of AMPA or NMDA receptors did not inhibit the acute amphetamine mediated increase in Fos protein expression in nucleus accumbens (Dalia and Wallace, 1995). The blockade of AMPA/kainate receptors inhibited the acute amphetamine mediated increase in *zif268* mRNA expression in striatum but was without effect in cortex (Wang et al., 1994). In cortical neuronal cell culture, AMPA receptors did not block the glutamate-mediated increase in *c-fos* and *zif268* mRNA expression; however, blockade of NMDA receptors

abolished the stimulated gene expression (Condorelli et al., 1994). Taken together, these observations indicate that glutamatergic regulation of the stimulated immediate early gene expression is heterogeneous across genes and brain structures. Therefore, plasticity in glutamate neurotransmission and signaling which plays an important role in a variety of physiological and pathological states may be able to differentially regulate the expression of immediate early genes across different brain structure, allowing selective regulation of down-stream genes in these areas. The lack of glutamatergic regulation of the IEG expression after acute cocaine administration may be due to cocaine-mediated increase in the extracellular dopamine in prefrontal cortex, nucleus accumbens, and ventral tegmental area. Stimulation of D1 dopamine receptors and activation of the related intracellular signaling is sufficient to activate the IEGs and, therefore, inhibition of glutamatergic signaling would not prevent cocaine-mediated IEG activation (Zhang et al., 2007; Simpson and Morris, 1995). Supporting a sufficient, but not necessary, regulation by glutamate, agonist stimulation of AMPA, NMDA, and mGluR receptors leads to activation of several IEGs (Vaccarino et al., 1992; Wang, 1998).

Consequences of Homer1 gene induction

An increase in synaptic activity or acute exposure to psychostimulants leads to activation of *Homer1* gene and an increase in expression of *Homer1a* mRNA and protein. The elevated cellular levels of Homer1a protein may lead to numerous synaptic, cellular, and behavioral alterations. These may include alterations in dendritic spine and axons, PSD95, AMPA and NMDA receptor clusters, surface expression and activity of AMPA, NMDA, and group1 mGluR receptors, disruption of memory formation (Tu et al., 1998; Ango et al., 2000, 2001, 2002; Ciruela et al., 1999, 2000; Shiraishi et al., 1999, 2003; Foa et al., 2001; Serge et al, 2002; Sala et al., 2001, 2003; Hennou et al., 2003; Roche et al., 1999; Tadokoro et al., 1999; Kammermeier and Worley, 2007; Klugmann et al., 2005). An increase in Homer1a protein may lead to modulation of intracellular calcium release, increased translocation of TRP calcium channels and calcium influx, and enhanced group1 mGluR-mediated N-type calcium and M-type potassium channel current inhibition (Tu et al., 1998; Yuan et al., 2003; Kim et al., 2006; Kammermeier et al., 2000; Kammermeier and Worley, 2007). An increased level of Homer1a protein may also decrease coupling of mGluR5 and ERK signaling and nuclear transcription factors activation in striatum and nucleus accumbens neurons (Mao et al., 2005).

Increased levels of Homer1a protein in neurons disrupts coupling between group1 mGluR receptors and the constitutively expressed Homer proteins (Tu et al., 1998, Xiao et al., 1998). This molecular uncoupling modulates mGluR-induced intracellular signaling and calcium release which may play a role in cellular and behavioral effects of acute and chronic psychostimulant treatments. Overexpression of Homer1a protein leads to blunted glutamate release in prefrontal cortex after an acute cocaine administration (Lominac et al., 2005). Transgenic mice overexpressing Homer1a protein in dorsal striatum exhibit an augmented behavioral response and a significantly higher level of Fos protein expression after an acute amphetamine administration. These animals suffer from a higher level of anxiety and defects in locomotor performance (Tappe and Kuner, 2006). A transient reduction in or knock out of

Homer1 proteins in nucleus accumbens leads to an augmented behavioral response to cocaine administration (Ghasemzadeh et al., 2003; Szumlinski et al., 2004, 2006).

Taken together, these data suggest that an increase in Homer1a protein followed by alterations of group1 mGluR signaling produces significant changes in multitude of signaling pathways leading to modification of cellular and behavioral responses. The molecular mechanisms by which Homer1a mediates these numerous and varied array of changes is not well understood and awaits future studies.

Acknowledgments

The authors wish to acknowledge National Institute on Drug Abuse for the generous gift of cocaine. This work was supported, in part, by National Institutes of Health grants DA14328 (MBG) and DA03906 (PWK).

Abbreviations

AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	N-methyl-D-aspartate
DA	dopamine
IEG	immediate early gene
PFC	prefrontal cortex
NA	nucleus accumbens
VTA	ventral tegmental area
mGluR	metabotropic glutamate receptor
IP3	inositol triphosphate
PSD95	postsynaptic density 95
CREB	cAMP response element-binding protein
RT-PCR	reverse transcription-polymerase chain reaction

References

Adell A, Artigas F. The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. Neurosci Biobehav Rev. 2004; 28:415–431. [PubMed: 15289006]

Ambesi-impiombato A, Panariello F, Dell'aversano C, Tomasetti C, Muscettola G, De Bartolomeis A. Differential expression of Homer1 gene by acute and chronic administration of antipsychotics and dopamine transporter inhibitors in the rat forebrain. Synapse. 2007; 61:429–439. [PubMed: 17372981]

Ango F, Pin JP, Tu JC, Xiao B, Worley PF, Bockaert J, Fagni L. Dendritic and axonal targeting of type 5 metabotropic glutamate receptor is regulated by homer1 proteins and neuronal excitation. J Neurosci. 2000; 20:8710–8716. [PubMed: 11102477]

Ango F, Prezeau L, Muller T, Tu JC, Xiao B, Worley PF, Pin JP, Bockaert J, Fagni L. Agonist-independent activation of metabotropic glutamate receptors by the intracellular protein homer. Nature. 2001; 411:962–965. [PubMed: 11418862]

Ango F, Robbe D, Tu JC, Xiao B, Worley PF, Pin JP, Bockaert J, Fagni L. Homer-dependent cell surface expression of metabotropic glutamate receptor type 5 in neurons. Mol Cell Neurosci. 2002; 20:323–329. [PubMed: 12093163]

Ary AW, Szumlinski KK. Regional differences in the effects of withdrawal from repeated cocaine upon Homer and glutamate receptor expression: a two-species comparison. Brain Res. 2007; 1184:295–305. [PubMed: 17950706]

Barker GR, Bashir ZI, Brown MW, Warburton EC. A temporally distinct role for group I and II metabotropic glutamate receptors in the object recognition memory. Learn Mem. 2006; 13:178–186. [PubMed: 16585793]

Bashir ZI, Bortolotto ZA, Davies CH, Berretta N, Irving AJ, Seal AJ, Henley JM, Jane DE, Watkins JC, Collingridge GL. Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature. 1993; 363:347–350. [PubMed: 8388549]

Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR. A complex program of striatal gene expression induced by dopaminergic stimulation. J Neurosci. 1998; 18:5301–5310. [PubMed: 9651213]

Berretta S, Robertson HA, Graybiel AM. Dopamine and glutamate agonist stimulate neuron-specific expression of Fos-like protein in the striatum. J Neurophysiol. 1992; 68:767–777. [PubMed: 1359024]

Bhat RV, Cole AJ, Baraban JM. Role of monoamine systems in activation of zif268 by cocaine. J Psychiatry Neurosci. 1992; 17:94–102. [PubMed: 1356432]

Bottai D, Guzowski JF, Schwarz MK, Kang SH, Xiao B, Lanahan A, Worley PF, Seeburg PH. Synaptic activity-induced conversion of intronic to exonic sequence in homer 1 immediate early gene expression. J Neurosci. 2002; 22:167–175. [PubMed: 11756499]

Boudreau AC, Wolf ME. Behavioural sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci. 2005; 25:9144–9151. [PubMed: 16207873]

Boudreau AC, Reimers JM, Milovanovic, Wolf ME. Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. J neurosci. 2007; 27:10621–10635. [PubMed: 17898233]

Bradberry CW, Roth RH. Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by in vivo microdialysis. Neurosci Lett. 1989; 103:97–102. [PubMed: 2779859]

Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL, Worley PF. Homer: a protein that selectively binds metabotropic glutamate receptors. Nature. 1997; 386:284–288. [PubMed: 9069287]

Cameron DL, Williams JT. Dopamine D1 receptors facilitate transmitter release. Nature. 1993; 366:344–347. [PubMed: 8247128]

Cameron DL, Williams JT. Cocaine inhibits GABA release in the VTA through endogenous 5-HT. J Neurosci. 1994; 14:6763–6767. [PubMed: 7965077]

Ciruela F, Soloviev MM, McIlhinney RA. Co-expression of metabotropic glutamate receptor type 1alpha with homer-1a/Vesl-1S increases the cell surface expression of the receptor. Biochem J. 1999; 341:795–803. [PubMed: 10417346]

Ciruela F, Soloviev MM, Chan WY, McIlhinney RA. Homer-1c/Vesl-1L modulates the cell surface targeting of metabotropic glutamate receptor type 1alpha: evidence for an anchoring function. Mol Cell Neurosci. 2000; 15:36–50. [PubMed: 10662504]

Cochran SM, Fujimura M, Morris BJ, Pratt JA. Acute and delayed effects of phencyclidine upon mRNA levels of markers of glutamatergic and GABAergic neurotransmitter function in the rat brain. Synapse. 2002; 46:206–214. [PubMed: 12325047]

Condorelli DF, Dell'Albani P, Amico C, Lukasiuk K, Kaczmarek L, Giuffrida-Stella AM. Glutamate receptor-driven activation of transcription factors in primary neuronal cultures. Neurochem Res. 1994; 19:489–499. [PubMed: 7520539]

Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol. 1997; 37:205–237. [PubMed: 9131252]

Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Conde F. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. Nature. 1994; 372:237–243. [PubMed: 7969468]

Dalia A, Wallace LJ. Amphetamine induction of c-fos in the nucleus accumbens is not inhibited by glutamate antagonists. Brain Res. 1995; 694:299–307. [PubMed: 8974658]

De Leonibus E, Mele A, Oliverio A, Pert A. Distinct pattern of c-FOS mRNA expression after systemic and intra-accumbens amphetamine and MK-801. Neuroscience. 2002; 115:67–78. [PubMed: 12401322]

Deutch AY, Duman RS. The effects of antipsychotic drugs on Fos protein expression in the prefrontal cortex: cellular localization and pharmacological characterization. Neuroscience. 1996; 70:377–389. [PubMed: 8848147]

Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. J Neurosci. 2002; 22:3312–3320. [PubMed: 11978805]

Fiore G, Cicale M, Magara S, Mondola R, Muscettola G, De Bartolomeis A. Antipsychotics with different D2 dopamine receptor potency affect differentially the postsynaptic density protein Homer at glutamatergic metabotropic synapse. Schizophrenia Research. 2003; 60:106.

Foa L, Rajan I, Haas K, Wu GY, Brakeman P, Worley P, Cline H. The scaffold protein, homer1b/c, regulates axon pathfinding in the central nervous system in vivo. Nat Neurosci. 2001; 4:499–506. [PubMed: 11319558]

Fosnaugh JS, Bhat RV, Yamagata K, Worley PF, Baraban JM. Activation of arc, a putative “effector” immediate early gene, by cocaine in rat brain. J Neurochem. 1995; 64:2377–2380. [PubMed: 7722525]

Fourgeaud L, Mato S, Bouchet D, Hemar A, Worley PF, Manzoni OJ. A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. J Neurosci. 2004; 24:6939–6945. [PubMed: 15295029]

Ghasemzadeh MB, Nelson LC, Lu X, Kalivas PW. Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. J Neurochem. 1999; 72:157–165. [PubMed: 9886066]

Ghasemzadeh MB, Permenter LK, Lake R, Worley PF, Kalivas PW. Homer1 Proteins and AMPA receptors modulate cocaine-induced behavioral plasticity. Eur J Neurosci. 2003; 18:1645–1651. [PubMed: 14511343]

Graybiel AM, Moratalla R, Robertson HA. Amphetamine and cocaine induce drug-specific activation of the *c-fos* gene in striosome-matrix compartment and limbic subdivisions of the striatum. Proc Natl Acad Sci USA. 1990; 87:6912–6916. [PubMed: 2118661]

Hansen HH, Andreasen JT, Weikop P, Mirza N, Scheel-Kruger J, Mikkelsen JD. The neuronal KCNQ channel opener retigabine inhibits locomotor activity and reduces forebrain excitatory responses to the psychostimulants cocaine, methylphenidate and phencyclidine. Eur J Pharmacology. 2007; 570:77–88.

Hennou S, Kato A, Schneider EM, Lundstrom K, Gahwiler Bh, Inokuchi K, Gerber U, Ehrengruber MU. Homer-1a/Vesl-1S enhances hippocampal synaptic transmission. Eur J Neurosci. 2003; 18:811–819. [PubMed: 12925007]

Hope B, Kosofsky B, Hyman SE, Nestler EJ. Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci. 1992; 89:5764–5768. [PubMed: 1631058]

Hope BT, Simmons DE, Mitchell TB, Kreuter JD, Mattson BJ. Cocaine-induced locomotor activity and Fos expression in nucleus accumbens are sensitized for 6 months after repeated cocaine administration outside the home cage. Eur J Neurosci. 2006; 24:867–875. [PubMed: 16930414]

Huang Q, Zhou D, Chase K, Gusella JF, Aronin N, DiFiglia M. Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. Proc Nat Acad Sci. 1992; 89:11988–11992. [PubMed: 1281547]

Hussain N, Flumerfelt BA, Rajakumar N. Glutamatergic regulation of haloperidol-induced c-fos expression in the rat striatum and nucleus accumbens. Neuroscience. 2001; 102:391–399. [PubMed: 11166125]

Kalivas PW, Duffy P. D1 receptors modulate glutamate transmission in the ventral tegmental area. J Neurosci. 1995; 15:5379–5388. [PubMed: 7623160]

Kalivas PW, Duffy P. Dopamine regulation of extracellular glutamate in the nucleus accumbens. Brain Res. 1997; 761:173–177. [PubMed: 9247082]

Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry. 2005; 162:1403–1413. [PubMed: 16055761]

Kammermeier PJ, Worley PF. Homer1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. Proc Natl Acad Sci. 2007; 104:6055–6060. [PubMed: 17389377]

Kammermeier PJ, Xiao B, Tu JC, Worley PF, Ikeda SR. Homer proteins regulate coupling of group I metabotropic glutamate receptors to N- type calcium and M-type potassium channels. J Neurosci. 2000; 20:7238–7245. [PubMed: 11007880]

Kargieman L, Santana N, Mengod G, Celada P, Artigas F. Antipsychotic drugs reverse the disruption in prefrontal cortex function produced by NMDA receptor blockade with phencyclidine. Proc Natl Acad Sci USA. 2007; 104:14843–14848. [PubMed: 17785415]

Kato A, Ozawa F, Saitoh Y, Hirai K, Inokuchi K. vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis. FEBS Lett. 1997; 412:183–189. [PubMed: 9257717]

Kato A, Ozawa F, Saitoh Y, Fukazawa Y, Sugiyama H, Inokuchi K. Novel members of the Vesl/Homer family of PDZ proteins that bind metabotropic glutamate receptors. J Biol Chem. 1998; 273:23969–23975. [PubMed: 9727012]

Keefe KA, Adams AC. Differential effects of N-methyl-D-aspartate receptor blockade on eticlopride-induced immediate early gene expression in the medial and lateral striatum. J pharm Exp Ther. 1998; 287:1076–1083.

Kim JY, Zeng W, Kiselyov K, Yuan JP, Dehoff MH, Mikoshiba K, Worley PF, Muallem S. Homer1 mediates store- and inositol 1,4,5-trisphosphate receptor-dependent translocation and retrieval of TRPC3 to the plasma membrane. J Biol Chem. 2006; 281:32540–32549. [PubMed: 16887806]

Klugmann M, Symes CW, Leichtlein CB, Klaussner BK, Dunning J, Fong D, Young D, During MJ. AAV-mediated hippocampal expression of short and long Homer1 proteins differentially affect cognition and seizure activity in adult rats. Mol Cell Neurosci. 2005; 28:347–360. [PubMed: 15691715]

Kuroki T, Meltzer HY, Ichikawa J. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J Pharmacol Exp Ther. 1999; 288:774–781. [PubMed: 9918588]

Lee J, Rajakumar N. Role of NR2B-containing N-methyl-D-aspartate receptors in haloperidol-induced c-fos expression in the striatum and nucleus accumbens. Neuroscience. 2003; 122:739–745. [PubMed: 14622917]

Leonard MW, Lim K-C, Engel JD. Expression of the chicken GATA factor family during early erythroid development and differentiation. Development. 1993; 119:519–531. [PubMed: 8287800]

Lominac KD, Oleson EB, Pava M, Klugmann M, Schwarz MK, Seeburg PH, During MJ, Worley PF, Kalivas PW, Szumlinski KK. Distinct roles for different homer1 isoforms in behaviors and associated prefrontal cortex function. J Neurosci. 2005; 25:11586–11594. [PubMed: 16354916]

Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron. 1995; 14:433–445. [PubMed: 7857651]

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]

Moratalla R, Robertson HA, Graybiel AM. Dynamic regulation of NGFI-A (zif268, egr1) gene expression in the striatum. J Neurosci. 1992; 12:2609–2622. [PubMed: 1613551]

Moratalla R, Elibol B, Vallejo M, Graybiel AM. Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron. 1996; 17:147–156. [PubMed: 8755486]

Naisbitt S, Kim E, Tu JC, Xiao B, Sala C, Valtschanoff J, Weinberg RJ, Worley PF, Sheng M. Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptors/PSD-95/GKAP complex and cortactin. Neuron. 1999; 23:569–582. [PubMed: 10433268]

Ozaki T, Katsumoto E, Yokotani N, Yamagami S. The comparative effects of haloperidol, (-)-sulpiride, and SCH23390 on c-fos and c-jun mRNA expressions, and AP-1 DNA binding activity. Eur Neuropsychopharmacol. 1997; 7:181–187. [PubMed: 9213076]

Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci. 1996; 16:1550–1560. [PubMed: 8778304]

Robertson GS, Matsumura H, Fibiger HC. Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharm Exp Ther. 1994; 271:1058–1066.

Robinson TE, Berridge KC. Addiction. Annu Rev Psychol. 2003; 54:25–53. [PubMed: 12185211]

Pollack AE, St Martin JL, MacPherson AT. Role of NMDA and AMPA glutamate receptors in the induction and the expression of dopamine-mediated sensitization in 6-hydroxydopamine-lesioned rats. Synapse. 2005; 56:45–53. [PubMed: 15700284]

Roche KW, Tu JC, Petralia RS, Xiao B, Wenthold RJ, Worley PF. Homer 1b regulates the trafficking of group I metabotropic glutamate receptors. J Biol Chem. 1999; 274:25953–25957. [PubMed: 10464340]

Sala C, Piech V, Wilson NR, Passafaro M, Liu G, Sheng M. Regulation of dendritic spine morphology and synaptic function by shank and homer. Neuron. 2001; 31:115–130. [PubMed: 11498055]

Sala C, Futai K, Yamamoto K, Worley PF, Hayashi Y, Sheng M. Inhibition of dendritic spine morphogenesis and synaptic transmission by activity- inducible protein homer1a. J Neurosci. 2003; 23:6327–6337. [PubMed: 12867517]

Sato M, Suzuki K, Nakanishi S. NMDA receptor stimulation and brain-derived neurotrophic factor upregulate homer1a mRNA via the mitogen-activated protein kinase cascade in cultured cerebellar granule cells. J Neurosci. 2001; 21:3797–3805. [PubMed: 11356868]

Semba J, Sakai M, Miyoshi R, Mataga N, Fukamauchi F, Kito S. Differential expression of c-fos mRNA in rat prefrontal cortex, striatum, N. accumbens and lateral septum after typical and atypical antipsychotics: an in situ hybridization study. Neurochem Int. 1996; 29:435–442. [PubMed: 8939453]

Serge A, Fourgeaud L, Hemar A, Choquet D. Receptor activation and homer differentially control the lateral mobility of metabotropic glutamate receptor 5 in the neuronal membrane. J Neurosci. 2002; 22:3910–3920. [PubMed: 12019310]

Shiraishi Y, Mizutani A, Bito H, Fujisawa K, Narumiya S, Mikoshiba K, Furuichi T. Cupidin, an isoform of Homer/Vesl, interacts with the actin cytoskeleton and activated rho family small GTPases and is expressed in developing mouse cerebellar granule cells. J Neurosci. 1999; 19:8389–8400. [PubMed: 10493740]

Shiraishi Y, Mizutani A, Yuasa S, Mikoshiba K, Furuichi T. Glutamate-induced declustering of post-synaptic adaptor protein Cupidun (Homer 2/vesl-2) in cultured cerebellar granule cells. J Neurochem. 2003; 87:364–376. [PubMed: 14511114]

Simpson CS, Morris BJ. Induction of c-fos and zif/268 gene expression in rat striatal neurons, following stimulation of D1-like dopamine receptors, involves protein kinase A and protein kinase C. Neuroscience. 1995; 68:97–106. [PubMed: 7477939]

Somogyi R, Wen X, Ma W, Barker JL. Developmental kinetics of GAD family mRNAs parallel neurogenesis in the rat spinal cord. J Neurosci. 1995; 15:2575–2591. [PubMed: 7722616]

Steiner H, Gerfen CR. Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. J Neurosci. 1993; 12:5066–5081. [PubMed: 7504719]

Storvik M, Tiikkainen P, van Iersel M, Wong G. Distinct gene expression profiles in adult rat brains after acute MK-801 and cocaine treatments. Eur Neuropsychopharmacol. 2006; 16:211–219. [PubMed: 16242920]

Sun J, Tadokoro S, Imanaka T, Murakami SD, Nakamura M, Kashiwada K, Ko J, Nishida W, Sobue K. Isolation of PSD-Zip45, a novel Homer/Vesl family protein containing leucine ipper motifs, from rat brain. FEBS Lett. 1998; 437:304–308. [PubMed: 9824313]

Svensson TH, Mathe JM. Atypical antipsychotic-like effect of AMPA receptor antagonists in the rat. Amino Acids. 2000; 19:221–226. [PubMed: 11026492]

Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW. Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for homer. J Neurosci. 2001; 21:9043–9052. [PubMed: 11698615]

Szumlinski KK, Abernathy KE, Oleson EB, Klugmann M, Lominac KD, He D, Ron D, During M, Kalivas PW. Homer isoforms differentially regulate cocaine-induced neuroplasticity. Neuropsychopharmacology. 2006; 31:768–777. [PubMed: 16160706]

Szumlinski KK, Dehoff MH, Kang SH, Frys KA, Lominac KD, Klugmann M, Rohrer J, Griffin III W, Toda S, Champtiaux NP, Berry T, Tu JC, Shealy SE, During MJ, Middaugh LD, Worley PF, Kalivas PW. Homer proteins regulate sensitivity to cocaine. Neuron. 2004; 43:401–413. [PubMed: 15294147]

Tadokoro S, Tachibana T, Imanaka T, Nishida W, Sobue K. Involvement of unique leucine-zipper motif of PSD-Zip45 (Homer 1c/vesl-1L) in group 1 metabotropic glutamate receptor clustering. Proc Natl Acad Sci. 1999; 96:13801–13806. [PubMed: 10570153]

Taepavarapruk P, Floresco SB, Phillips AG. Hyperlocomotion and increased dopamine efflux in the rat nucleus accumbens evoked by electrical simulation of the ventral subiculum: role of ionotropic glutamate and dopamine D1 receptors. Psychopharmacoloy (Berl). 2000; 151:242–251.

Tappe A, Kuner R. Regulation of motor performance and striatal function by synaptic scaffolding proteins of the homer1 family. Proc Natl Acad Sci. 2006; 103:774–779. [PubMed: 16407107]

Tomasetti C, Dell'aversano C, Iasevoli F, De Bartolomeis A. Homer splice variants modulation within cortico-subcortical regions by dopamine D2 antagonists, a partial agonist, and an indirect agonist: implication for glutamatergic postsynaptic density in antipsychotics action. Neuroscience. 2007; 150:144–158. [PubMed: 17916411]

Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M, Linden DJ, Worley PF. Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. Neuron. 1998; 21:717–726. [PubMed: 9808459]

Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P, Doan A, Aakalu VK, Lanahan AA, Sheng M, Worley PF. Coupling of mGluR/Homer and PSD-95 complexes by the shank family of postsynaptic density proteins. Neuron. 1999; 23:583–592. [PubMed: 10433269]

Vaccarino FM, Hayward MD, Nestler EJ, Duman RS, Tallman JF. Differential induction of immediate early genes by excitatory amino acid receptor types in primary cultures of cortical and striatal neurons. Mol Brain Res. 1992; 12:233–241. [PubMed: 1347632]

Vanderschuren LJ, Kalivas PW. Alterations to dopaminrgic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl). 2000; 151:99–120. [PubMed: 10972458]

Vanderschuren LJ, Everitt BJ. Behavioral and neural mechanisms of compulsive drug seeking. Eur J Pharmacol. 2005; 526:77–88. [PubMed: 16310768]

Wang JO. Regulation of immediate early gene c-fos and zif/268 mRNA expression in rat striatum by metabotropic glutamate receptor. Mol Brain Res. 1998; 57:46–53. [PubMed: 9630506]

Wang JO, Daunais JB, McGinty JF. Role of kainate/AMPA receptors in induction of striatal zif/268 and preprodynorphin mRNA by a single injections of amphetamine. Mol. Brain Res. 1994; 27:118–126. [PubMed: 7877442]

Watanabe K, Hashimoto K, Nishimura T, Tsunashima K, Minabe Y. Expression of fos protein in rat brain following administration of a nicotinic acetylcholine receptor agonist epibatidine. Brain Res. 1998; 797:135–142. [PubMed: 9630573]

Xiao B, Tu JC, Worley PF. Homer: a link between neural activity and glutamate receptor function. Curr Opin Neurobiol. 2000; 10:370–374. [PubMed: 10851183]

Xiao B, Tu JC, Petralia RS, Yuan JP, Doan A, Breder CD, Ruggiero A, Lanahan AA, Wenthold RJ, Worley PF. Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. Neuron. 1998; 21:707–716. [PubMed: 9808458]

Yano M, Steiner H. Methylphenidate (Ritalin) induces homer1a and zif268 expression in specific corticostriatal circuits. Neuroscience. 2005; 132:855–865. [PubMed: 15837145]

Yano M, Beverley JA, Steiner H. Inhibition of methylphenidate-induced gene expression in the striatum by local blockade of D1 dopamine receptors: interhemispheric effects. Neuroscience. 2006; 140:699–709. [PubMed: 16549270]

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]

Yuan JP, Kiselyov K, Shin DM, Chen J, Shchevnikov N, Kang SH, Dehoff MH, Schwarz MK, Seeburg PH, Muallem S, Worley PF. Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. Cell. 2003; 114:777–789. [PubMed: 14505576]

Yuferov V, Kroslak T, Laforge KS, Zhou Y, Ho A, Kreek MJ. Differential gene expression in the rat caudate putamen after “binge” cocaine administration: advantage of triplicate microarray analysis. Synapse. 2003; 48:157–169. [PubMed: 12687634]

Zhang G, Mao L, Liu X, Parelkar NK, Arora A, Yang L, Hains M, Fibuch EE, Wang JQ. In vivo regulation of homer1a expression in the striatum by cocaine. Mol Pharmacol. 2007; 71:1148–1158. [PubMed: 17234898]

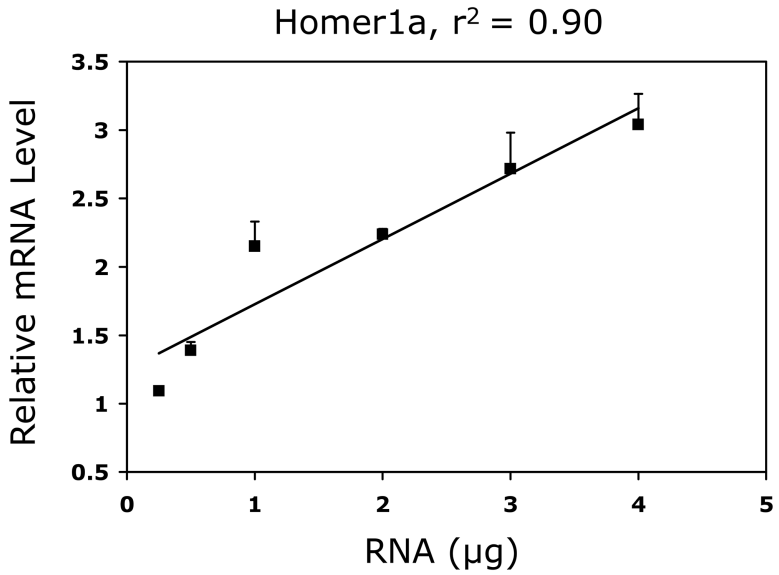

Figure 1. RT-PCR calibration curve for Homer1a mRNA transcript.

Data are presented as mean ± SEM (n=3). Total RNA from whole brain tissue was used to synthesize cDNA for these experiments. The RT and PCR conditions were identical to the conditions used for experimental samples.

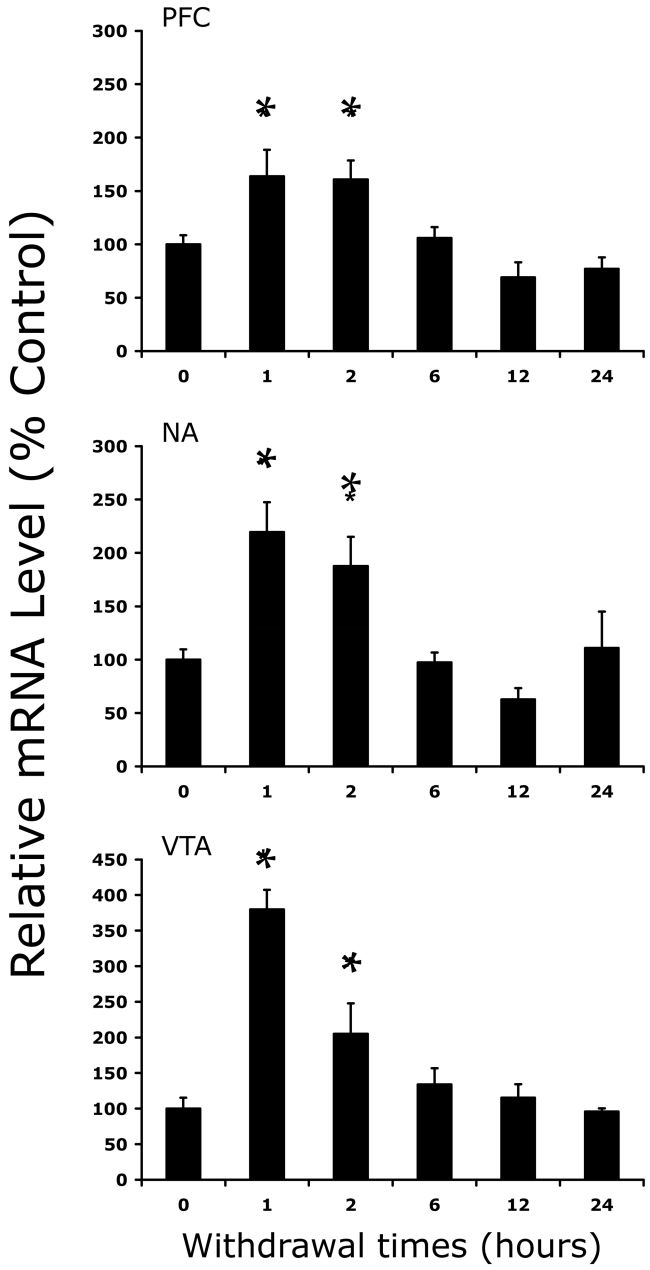

Figure 2. Effect of an acute cocaine administration on Homer1a mRNA transcription at various withdrawal times.

Cocaine (30 mg/kg, ip) was administered at time zero and animals were killed at the specified withdrawal times. Data are presented as mean ± SEM (n= 4-8 rats/time point) and were evaluated using one-way ANOVA followed by *post hoc* Fisher's PLSD test for multiple comparisons. * $p<0.05$ compared to time zero.

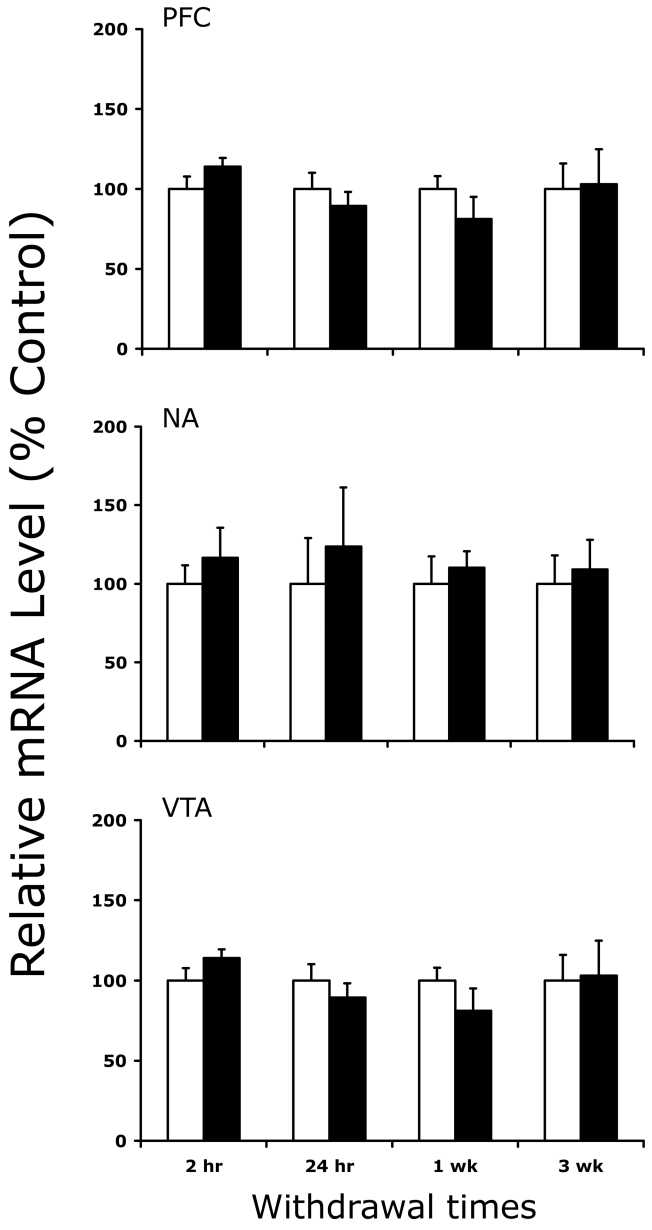

Figure 3. Effect of repeated cocaine administration on Homer1a mRNA transcription at various withdrawal times.

Rats were treated with cocaine for 7 days (2 × 15 mg/kg and 5 × 30 mg/kg, ip) followed by specified withdrawal times from the last cocaine injection. A separate group of repeated saline treated rats were paired with the corresponding repeated cocaine treatment group for each of the withdrawal times. Data are mean ± SEM (n= 7-8 rats/time point) and were analyzed using two-way ANOVA with treatment and withdrawal time as main factors. There was no statistical significance in main factors, or interactions. White bars, saline; black bars, cocaine.

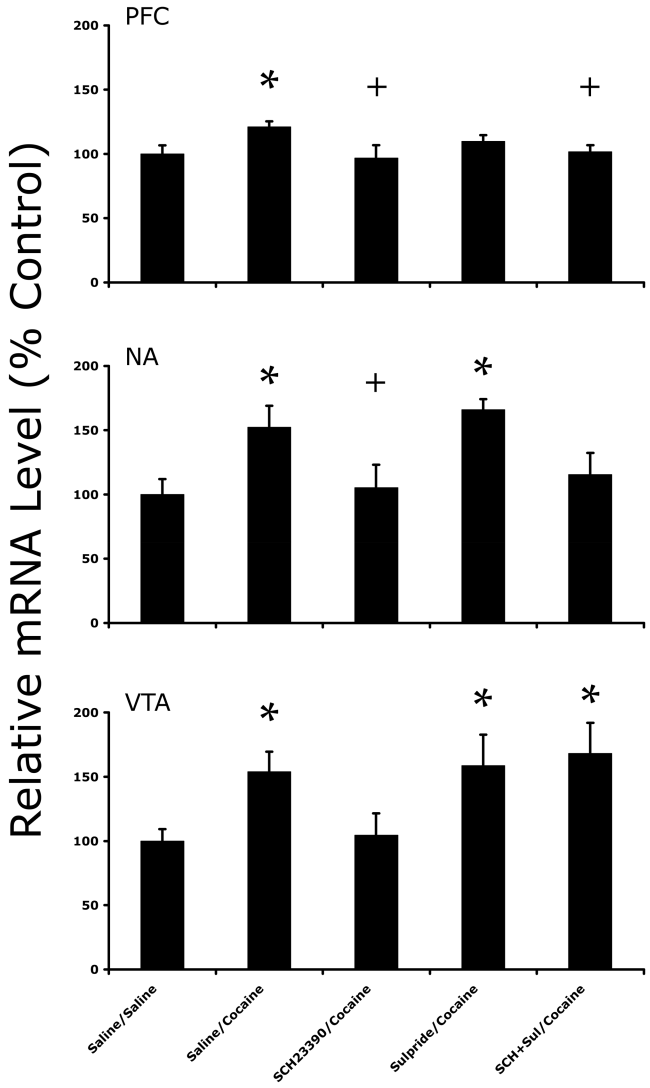

Figure 4. The dopaminergic regulation of cocaine stimulated Homer1a mRNA transcription.

Rats were treated with Saline (1 ml/kg, ip), SCH23390 (1 mg/kg, ip), Sulpiride (20 mg/kg, ip) or a cocktail of SCH23390 and Sulpiride followed 20 minutes later by a saline (1 ml/kg, ip) or an acute cocaine (30 mg/kg, ip) administration. All rats were killed at 2 hours after the second injection. The data are presented as mean ± SEM (n= 9-16 rats/treatment group) and were evaluated using one-way ANOVA followed by *post hoc* Fisher's PLSD test for multiple comparisons. * $p< 0.05$ compared to Saline/Saline, $^{+}$ $p< 0.05$ compared to Saline/Cocaine.

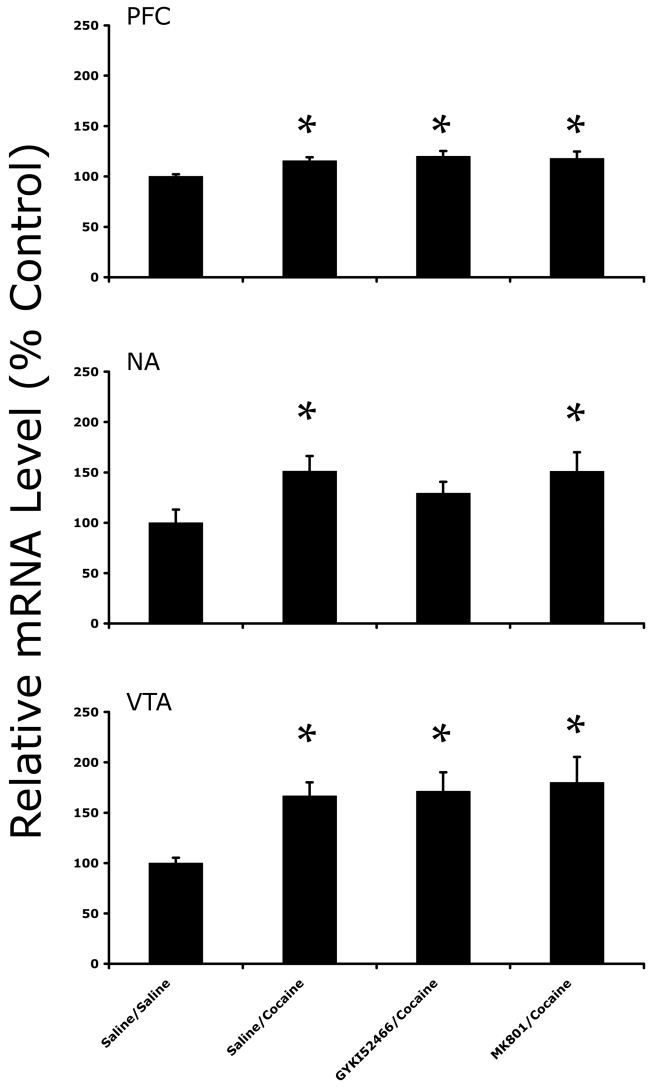

Figure 5. The glutamatergic regulation of cocaine stimulated Homer1a mRNA transcription.

Rats were treated with Saline (1 mg/kg, ip), GYKI52466 (15 mg/kg, ip) or MK801 (1 mg/kg, ip) followed 20 minutes later by a Saline (1 ml/kg, ip) or an acute cocaine (30 mg/kg, ip) administration. All rats were killed at 2 hours after the second injection. The data are presented as mean ± SEM (n= 10-15 rats/treatment group) and were evaluated using one-way ANOVA followed by *post hoc* Fisher's PLSD test for multiple comparisons. * $p < 0.05$ compared to Saline/Saline.

Table 1

Effect of dopamine or glutamate receptor blockade on Homer1 gene activity

	PFC	NA	VTA
A. Dopamine			
Saline/Saline	100 ± 5.5	100 ± 4.5	100 ± 7.3
SCH23390/Saline	89.4 ± 5.8	100.9 ± 21.0	99.6 ± 8.1
Sulpiride/Saline	93.1 ± 3.8	78.9 ± 21.2	114.2 ± 15.9
B. Glutamate			
Saline/Saline	100 ± 5.2	100 ± 4.6	100 ± 7.0
GYKI52466/Saline	102.9 ± 8.6	113.6 ± 49.6	91.7 ± 3.0
MK801/Saline	86.3 ± 6.2	116.2 ± 23	79.7 ± 8.2

Data are presented as mean ± SEM per cent of saline/saline value. There was no statistical significant difference between treatments in any brain regions, using one-way ANOVA comparison of treatment groups, n=3-5 per treatment.