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Cocaine Up-Regulates Fra-2 and σ-1 Receptor Gene and Protein Expression in Brain Regions Involved in Addiction and Reward

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Abstract

σ Receptors have recently been implicated in the actions of cocaine, and antagonists of these receptors prevent many acute and subchronic cocaine effects. An earlier study revealed that the immediate early gene fra-2 is up-regulated following cocaine administration, and this effect is prevented by the σ-1 receptor antagonist BD1063. In the present study, the effects of cocaine and BD1063 on the expression of six fos and jun genes were evaluated in mouse brains using cDNA microarrays. Several of these genes were altered by cocaine, but only the alteration in fra-2 was prevented by BD1063. The time courses of fra-2 and σ-1 receptor gene and protein expression in different brain regions were also determined. Cocaine up-regulated fra-2, which was followed by a later up-regulation of σ-1 receptors. The cocaine-induced up-regulation of fra-2 and σ–1 receptor genes and proteins were detected in whole brain, striatum and cortex, but not in cerebellum. All of these cocaine-induced effects were prevented by BD1063. The interaction between cocaine, fra-2 and σ–1 receptors involves brain regions that are established components of the neural circuit for reward, suggesting that they may contribute to the enduring changes that underlie the cellular basis of drug abuse.

Introduction

Cocaine is a highly addictive substance. Acute cocaine administration can cause locomotor hyperactivity, convulsions and even lethality (Matsumoto et al., 2003; Maurice et al., 2002) depending on its dosage. Furthermore, repeated cocaine exposure may cause sensitization of the behavioral responses to cocaine (Post and Rose, 1976).

Many neurotransmitter systems and ion channels are involved in the behavioral effects of cocaine. They include neurotransmission systems for dopamine (Chausmer et al., 2002; Sorg and Kalivas, 1993), glutamate (Brackett et al., 2000), serotonin (Muller et al., 2002), and GABA (Suzuki et al., 2000). Calcium channels play an important role in the locomotor-stimulating effects of cocaine (Han et al., 2002) and sodium and potassium channels mediate some toxic effects of the drug (Bauman and DiDomenico, 2002)

Recently, σ receptors have been implicated in the actions of cocaine and suggested to be a promising medication development target for cocaine abuse (Matsumoto et al., 2003; Maurice et al., 2002). Of the two established σ receptor subtypes, σ-1 and σ-2, the σ-1 subtype appears to have the predominant role in modulating the actions of cocaine. Cocaine has preferential affinity for the σ-1 subtype, at concentrations that are physiologically relevant (Matsumoto et al., 2003). σ-1 Receptors are widely distributed in brain and peripheral tissues and have been localized to plasma membrane as well as intracellular structures, including endoplasmic reticulum (McCann et al., 1994; Alonso et al., 2000). Stimulated dopamine release has been reported to be modulated by σ-1 receptors through the protein kinase C signaling pathway (Nuwayhid and Werling, 2003). In addition, cocaine-induced effects on acute and subchronic behavioral activity are attenuated by various σ-1 receptor antagonists and antisense oligodeoxynucleotides

(Matsumoto et al., 2003; Maurice et al., 2002). However, the mechanisms through which σ-1 receptor antagonists convey anti-cocaine actions have not yet been fully characterized.

In an earlier study combining behavioral pharmacological approaches with cDNA microarray analysis and RT-PCR confirmations, we discovered that fra-2, an immediate early gene (IEG) and member of the fos family of transcription factors, is up-regulated by cocaine and prevented by behavioral protective doses of BD1063, a σ-1 receptor antagonist (Matsumoto et al., 2003). In addition to our study, the acute administration of cocaine has been shown by others to induce the up-regulation of many IEGs and transcription factors, such as c-fos, fosB, ΔFosB, and fra-2 (Rosen et al., 1994; Zhang et al., 2002), and the gene expression appears to be regulated through dopamine receptors (Zhang et al., 2002). Several studies have also shown that σ–1 receptor ligands, such as E-5842 (4-[4-fluorophenyl]-1,2,3,6-tetra-hydo-1-[4-[1,-2,4-triazol-1-il]butyl]pyridine citrate) and EMD 57445 (panamesine) can induce IEGs, particularly c-fos (Guitart and Farre, 1998; Dahmen et al., 1996). These results indicate that the protective effect of σ receptor antagonists may involve modulation of IEG-induced transcription cascades.

The induction of IEG expression by drugs of abuse is hypothesized to be an initial step by which these drugs alter the expression of late genes to produce long-lasting changes in neuronal function. Since IEGs have been suggested to play an important role in drug addiction and our initial microarray studies and RT-PCR confirmations (Matsumoto et al., 2003) revealed that fra-2 is significantly induced by cocaine and prevented by pretreatment with the σ-1 receptor antagonist BD1063, the present study was undertaken to further examine the relationship between cocaine-induced IEGs and σ-1 receptors.

In the first part of this study, the expression of genes in the fos and related jun family were investigated to determine whether the anti-cocaine effects produced by BD1063 are specific to fra-2 or can be generalized to other members of this family of transcription factors. Numerous studies now show that members of the Fos family of IEGs form heterodimers with members of the Jun family of IEGs (Foletta et al., 1996). These heterodimers can then bind together to a specific DNA sequence known as the AP-1 site (Foletta et al., 1996; Hope, 1998). Earlier studies have shown that the σ-1 receptor gene has an AP-1 sequence in its promoter, suggesting that it may serve as a target gene for Fra-2 (Seth et al., 1997). The second part of this study focused on the time course of fra-2 and σ-1 receptor gene expression in different brain regions after acute cocaine administration, and the effect of BD1063 on the expression of these genes. Finally, the third part of this study characterized the time course of Fra-2 and σ-1 receptor protein expression in different brain regions after acute cocaine administration and the effect of BD1063.

Methods

Subjects

Male, Swiss Webster mice (24-28g) were acquired from Harlan Sprague Dawley (Dublin, VA) and housed in the University of Oklahoma Health Sciences Center animal facility for at least three days before being used. Room temperature was maintained at 21° C. Lights were on from 0630 to 1830. All procedures regarding the use and handling of animals were conducted as approved by the IACUC serving the University of Oklahoma Health Sciences Center.

Drug Application

Cocaine hydrochloride (Sigma, St. Louis, MO) was injected at a dosage of 10 mg/kg (1 mg/ml solution, i.p.). BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine, Tocris, Ballwin, MO) was injected at a dosage of 30 mg/kg (3 mg/ml solution, i.p.). Saline (10 ml/kg, i.p.) was used as the control.

Locomotor Activity Measurement

Horizontal locomotor activity was measured using an automated activity monitoring system (San Diego Instruments, San Diego, CA) as described in earlier studies (Matsumoto et al., 2001, 2002). Mice were individually adapted to testing chambers for 15 min, after which they received two injections separated by a 15 min interval of one of the following combinations of treatments: saline+cocaine, BD1063+cocaine, saline+BD1063, saline+saline, or without treatment. Locomotor activity recording started immediately after the second injection and continued for 20 min for the microarray studies. This testing

duration was selected because it corresponded to the time at which the locomotor stimulatory effects of cocaine were maximal in earlier time course measurements (Post and Rose, 1976). For the time course studies in the second part of the investigation, locomotor activity was recorded for 10, 20, 40, or 80 min.

Brain Tissue Sampling

Immediately after the locomotor recordings, brain tissues were collected whole for the microarray studies or dissected into different regions (left half brain, right cortex, right cerebellum, right hippocampus, and right striatum) for the time course study in different brain regions. The brain samples were immediately frozen in liquid nitrogen and stored in a freezer at -80°C before RNA and protein isolation.

Microarrays

Total RNA was isolated using the Atlas Pure Total RNA Labeling System (Clontech, Palo Alto, CA) from each brain: saline+saline (n=3), saline+cocaine (n=3), BD1063+cocaine (n=3), BD1063+saline (n=3). Probes were made from total RNA using a reverse transcription reaction and labeled with [α^{32}P]dATP. The extracted and labeled cDNA probes from each sample were hybridized overnight to 1.2K cDNA microarrays (Atlas Arrays, Clontech, Palo Alto, CA). Following a high stringency wash, the resulting data was quantified using a Storm 820 phosphorimager (Molecular Dynamics, Piscatawy, NJ) and the expression levels of individual genes from each of our 12 samples were quantified from the cDNA microarrays as optical density readings with background subtraction using Array Vision software (Imaging Research Inc., Ontario, Canada). Of the

1176 genes spotted on the membranes, six fos and jun family genes were represented on the microarray: fra-2, c-fos, fosB, c-jun, junB and junD. Statistical analysis was performed using analysis of variance and Bonferroni's post-hoc analysis.

RT-PCR

After the behavioral recordings for 10, 20, 40, and 80 min, brain tissues (half brain, striatum, cortex, hippocampus, cerebellum) were immediately sampled (n=4 for each data point). The expression of fra-2 and σ-1 receptor genes was determined in the different treatment groups and brain regions.

Total RNA was prepared from each brain sample using Trizol reagents (Gibco, Invitrogen Life Technologies, Carlsbad, CA) following the standard protocol for RNA extraction. First strand cDNA synthesis was performed using Superscipt II RNase H Reverse Transcriptase and Oligo$(dT)_{12\text{-}18}$ (Gibco, Invitrogen Life Technologies, Carlsbad, CA). PCR reactions were performed as suggested by the manufacturer (Gibco, Invitrogen Life Technologies, Carlsbad, CA). The oligos that were used as primers for each of our targeted genes and their PCR reaction conditions are listed in Table 1. The oligos were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA) using a standard desalting purification method.

The following cycling parameters were used to perform the PCR amplification: denature for 45 s at 94^{o}C, anneal as indicated in Table 1 (the specific temperature was determined based on the Tm indicated on the oligo synthesis report and adjusted based on initial PCR results), extend for 1 min at 72^{o}C for the number of cycles indicated in Table 1. The number of cycles for each reaction was selected to allow linear amplification of the

cDNA under study. β–actin was used as the housekeeping gene for normalization. Amplified products were analyzed by electrophoresis on 1.5% agarose gel and stained with ethidium bromide (0.5 μg/ml). The gel was then photographed using a UV transilluminator to visualize the ethidium bromide-stained bands. The RT-PCR reactions were repeated a minimum of three times to validate the results. The expression levels of the individual genes were assessed by Scion image software (Frederick, MD) and expressed in optical density units. The data from each of the assays was subject to analysis of variance and Bonferroni's multiple comparisons post-hoc tests to determine whether the differences between the experimental groups were statistically significant.

Western blots

At 20, 80, 160, 320 min and 24 h after the drug treatments, brain tissues (half brain, striatum, cortex, hippocampus, cerebellum) were immediately sampled (n=3-9 for each data point). The protein levels for Fra-2 and σ-1 receptors were measured in the different treatment groups and brain regions.

Pulverized samples were homogenized in T-PER Tissue Protein Extraction Reagent (Pierce Biotechnology, Rockford, IL) using a ratio of ~1g of tissue to 20 ml T-PER Reagent. The samples were centrifuged at 10,000 rpm for 5 min, and the supernatants collected. For small brain regions, like the striatum and hippocampus, tissues from three mice were pooled for protein extraction. Protein concentration was determined using a Bio-Rad protein assay kit and each sample normalized to its total protein concentration.

Boiled protein samples (40-60 μg) and molecular weight standards (Precision Plus Protein Standards, Bio-Rad, Hercules, CA, 5μl) were resolved on 12% or 15% SDS-

polyacrylamide gels, electrophoresed, and transferred onto nitrocellulose membranes. The membranes were then blocked with BSA for Fra-2 or non-fat dry milk for σ-1 receptors. The membranes were incubated with the following primary antibodies: Fra-2 (Santa Cruz Biotechnology, Santa Cruz, CA, Q20, sc-604; 1:1000), σ-1 receptors (Aves Labs, Tigard, OR, 1:1000), β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, I19, sc-1616; 1:5000). Incubation with the following horseradish peroxidase-conjugated secondary antibodies followed: antirabbit IgG for Fra-2, antigoat IgG for β-actin (Santa Cruz Biotechnology, Santa Cruz, CA), and antichicken IgY for σ-1 receptors (Aves Labs, Tigard, OR). NIH/3T3 nuclear extracts (Santa Cruz Biotechnology, Santa Cruz, CA) was used as a positive control for the Fra-2 antibody and depleted antibodies against σ-1 receptor antigens (Aves Labs, Tigard, OR) was used as a negative control for the σ-1 receptor antibody. Immunoreactivity was visualized using enhanced chemiluminescence. Western blots for each protein was repeated at least three times.

The optical density reading of each band was quantified using Quantity One software (Bio-Rad Laboratories, Hercules, CA) with background subtraction. The data from each experimental band was normalized to β-actin, then subject to analysis of variance. If there was an overall significant effect, Bonferroni's pairwise comparisons were used for post-hoc evaluations.

Results

Microarrays

The locomotor behaviors of the animals that were used for the microarrays (not shown) were consistent with the pattern previously reported in which cocaine produced locomotor stimulatory effects that were attenuated by BD1063 (Matsumoto et al., 2001, 2003). Analysis of variance confirmed a statistically significant difference in the locomotor scores among the treatment groups ($F[3,8]=16.96$, $P<0.001$).

Analysis of variance also indicated that there were significant differences among the experimental groups in the gene expression patterns of junD ($F[3,8]=6.73$, $P<0.05$), c-fos ($F[3,5]=6.35$, $P<0.05$), and fra-2 ($F[3,6]=37.02$, $P<0.001$). The expression of genes in the fos and jun families for each experimental group is shown in Fig. 1. Post-hoc analysis confirmed that cocaine significantly increased the expression of junD ($P<0.05$), c-fos ($P<0.05$), and fra-2 ($P<0.001$). Moreover, among these cocaine-induced IEGs that were up-regulated, post-hoc analysis confirmed that only fra-2 was prevented by pretreatment with the σ-1 receptor antagonist BD1063 ($P<0.001$).

Time Course of Locomotor Activity

Locomotor activity of mice increased following an acute cocaine administration, and this hyperlocomotion effect was completely prevented by pretreatment with BD1063 (Fig. 2). Fig. 2A presents locomotor activities counted in each 5 min period following treatment. Locomotor activity increased within the first 5 min after the cocaine injection, and the hyperlocomotion lasted for about 40 min with a peak at about 20 min. One-way analysis of variance confirmed that within 40 min, the differences between the treatment

groups were statistically significant ($p<0.05$ – $p<0.0001$). Fig. 2B shows the total locomotor activities counted during the 40 min period. One-way analysis of variance showed that the differences between the treatment groups were significant ($F[4,15]=4.45$, $p<0.05$). The difference between the saline control group and the cocaine group also reached significance ($p<0.05$).

Time Course Effects of Cocaine and BD1063 on Gene Expression in the Whole Brain

Fig. 3 shows the time course of fra-2 gene expression in the different groups after injection. The cocaine injection caused an up-regulation of the fra-2 gene during the 10-80 min post-administration period. Similar to the behavioral measurement, pretreatment with BD1063 diminished the cocaine-induced up-regulation of the fra-2 gene. One-way analysis of variance showed a significant difference at 20 ($p<0.05$), 40 ($p<0.05$), and 80 ($p<0.01$) min.

Fig. 4 shows the time course of σ-1 receptor gene expression in the different groups after injection. While expression levels of the σ-1 receptor gene remained constant during the post-administration period from 10-80 min in the control groups, the cocaine injection caused an up-regulation of the gene, which increased with time gradually. Similar to the behavioral results and fra-2 gene expression, pretreatment with BD1063 diminished the cocaine effect. One-way analysis of variance showed a significant difference only at 80 min ($p<0.01$).

Regional Differences of the Cocaine-Induced Up-Regulation of Fra-2 and σ-1 Receptor Genes in the Brain

Cocaine up-regulated fra-2 gene expression in the striatum, cortex, and hippocampus (Fig. 5A,B,C), but not in the cerebellum (Fig. 5D). One-way analysis of variance revealed that in the striatum and hippocampus, the differences between the treatment groups were significant at all time points ($p<0.05$ – $p<0.001$). In the cortex, the difference between the treatment groups reached statistical significance only at the 20 min time point ($p<0.05$), although the gene expression levels in the cocaine groups were consistently higher at all time points. Similar to earlier studies, pretreatment with BD1063 diminished the cocaine effect.

Cocaine-induced up-regulation of the σ-1 receptor gene was observed in the striatum and cortex, but not in the cerebellum and hippocampus (Fig. 6). One-way analysis of variance showed that the differences between the treatment groups reached statistical significance in the striatum and cortex only at the 80 min time point ($p<0.05$). Similar to above, pretreatment with BD1063 diminished the cocaine effect.

Cocaine-Induced Up-Regulation of Fra-2 and σ-1 Receptor Protein Expression in the Brain

Cocaine up-regulated Fra-2 protein levels in the whole brain, cortex, striatum, and hippocampus (Fig. 7A,B,C,D), but not in the cerebellum (Fig. 7E). One-way analysis of variance revealed that in the striatum, the differences between the treatment groups were significant at 160 and 320 min ($p<0.05$). In the hippocampus, the differences between the treatment groups were significant at 80 and 160 min ($p<0.05$-0.01). In the cortex, the

differences between the treatment groups were significant at 20, 80, 160 and 320 min ($p<0.05 – p<0.001$). In the half brain, the differences between the treatment groups did not reach statistical significance, although the protein levels in the cocaine groups were consistently higher at the 80, 160 and 320 min time points. Similar to earlier studies, pretreatment with BD1063 diminished the effects of cocaine.

Cocaine-induced up-regulation of the σ-1 receptor protein was observed in the half brain, cortex, and striatum (Fig. 8A,B,C), but not in the hippocampus and cerebellum (Fig. 8D,E). One-way analysis of variance showed that the differences between the treatment groups reached statistical significance at the 320 min time point ($p<0.05$-0.01). Similar to above, pretreatment with BD1063 diminished the effects of cocaine.

Discussion

Cocaine administration in mice caused both locomotor hyperactivity and up-regulation of fra-2 and σ-1 receptor gene and protein expression in the brain, but with different time courses. The time course of the cocaine-induced locomotor hyperactivity reflects the elimination curve of cocaine in the body (Lau et al., 1991). In contrast, the up-regulation of the fra-2 gene may mediate immediate as well as enduring effects of cocaine on the body, such as addiction and sensitization to cocaine. The up-regulation of the σ–1 receptor gene on the other hand, with its induction delayed until after much of the cocaine has been eliminated from the body, most likely represents transcriptional regulation by another early gene such as fra-2.

Similar to earlier studies, the results demonstrate that cocaine can induce a number of genes in the fos and jun families of transcription factors (Young et al., 1991; Hope, 1998). However, fra-2 is the most sensitive gene of those evaluated to σ–1 receptor-mediated influences since it was the only one of the six genes evaluated in the microarray analysis whose cocaine-induced expression could be prevented by BD1063, a σ-1 receptor antagonist. The ability of cocaine to stimulate fra-2 expression through a σ-1 mediated mechanism most likely involves the activation of intracellular calcium as an intervening step. σ Receptor agonists have been reported to increase intracellular calcium, which can be prevented by σ-1 receptor antagonists (Su and Hayashi, 2001). Since calcium can activate the expression of Fra-2, it is conceivable that the σ agonist actions of cocaine result in elevations in intracellular calcium and subsequent activation of Fra-2.

The cocaine-induced up-regulation of fra-2 is robust enough to be detected when the entire brain is evaluated, with the up-regulation of fra-2 occurring within minutes of stimulation and persisting for at least 80 min. The onset of fra-2 up-regulation by cocaine precedes the up-regulation of σ-1 receptor gene expression, suggesting that the σ-1 receptor gene may be a target for fra-2. Since the promoter of the σ-1 receptor gene contains an AP-1 sequence to which fra-2 may bind to activate transcription (Seth et al., 1997), the interaction between fra-2 and σ–1 receptor genes could start a cascade of further changes with enduring effects on central nervous system function. Similar to that proposed for other IEGs (He and Rosenfeld, 1991; Sheng et al., 1990; Goelet et al., 1986; Morgan and Curran, 1989), this interaction could thus serve as a bridge between the acute effects of cocaine and the long lasting changes that result following cocaine exposure.

The regional analysis of fra-2 and σ-1 receptor gene expression following cocaine administration further supports the relevance of this interaction for the cellular basis of drug abuse. Fra-2 and σ-1 receptor gene expression were found to be up-regulated by cocaine in the striatum and cortex, which contain the terminal fields of dopaminergic neurons that are involved in the neural circuit for reward (Porrino et al., 2002; Mazei et al., 2002). The striatal tissue used in the present study included both the nucleus accumbens in the ventral striatum, with its considerable role in reward (Sellings and Clarke, 2003; Lu et al., 2003; Panikkar, 1999), and the dorsal striatum with its established role in locomotor effects (Sellings and Clarke, 2003; Lu et al., 2003; Panikkar, 1999). Similar to when whole brain was analyzed, the up-regulation of fra-2 by cocaine preceded the up-regulation of σ-1 receptor gene expression in both of these brain regions. In contrast, there was no change in fra-2 or σ-1 receptor gene expression in the cerebellum, a brain region that is

rich in σ-1 receptors but does not have an established role in drug abuse (Bouchard and Quirion, 1997). This pattern of regional expression of fra-2 and σ-1 receptor genes in response to cocaine administration suggests that the changes are related to the addictive effects of cocaine and not simply a generalized response to σ-1 receptor activation.

The gene expression pattern in the hippocampus in response to cocaine also deserves comment. In the hippocampus, cocaine up-regulated fra-2, but not σ-1 receptor, gene expression. The hippocampus has a well established role in learning and memory (Nestler, 2002), which could have important implications for an animal's ability to learn the association between the administration of cocaine and its related environmental cues and rewarding effects. The up-regulation of fra-2 is expected to alter the transcription of other genes that are involved in this learning process. Since σ-1 receptors have been implicated in earlier studies in learning and memory processes (Maurice et al., 2001), it is possible that the expression of the σ-1 receptor gene may be up-regulated but with a long delay (>80 min). It is even more likely that following acute cocaine administration, other genes, such as glutamate receptors which have an established role in learning and memory (Nestler, 2002), may serve as targets for fra-2 in the hippocampus.

Time dependent increases in the expression of Fra-2 and σ-1 receptor proteins were also observed following cocaine administration. The changes in protein levels followed the changes seen in mRNA, suggesting that the cocaine-induced alterations in gene expression were being translated into protein. Similar to the pattern seen in gene expression, cocaine caused an earlier up-regulation of Fra-2 protein levels in half brain, striatum, cortex and hippocampus, which was followed by a later up-regulation of σ-1

receptor protein levels in half brain, striatum and cortex. This temporal and regional pattern of cocaine-induced protein expression was significantly attenuated by the σ-1 receptor antagonist BD1063, suggesting that some of the behavioral protective effects of σ-1 receptor antagonists involve modulation of IEG-induced transcriptional cascades.

In summary, cocaine produces many behavioral changes that can be prevented by σ-1 receptor antagonists (Matsumoto et al., 2001, 2002; Maurice et al., 2002). Cocaine also induces the expression of numerous IEGs (Rosen et al., 1994; Zhang et al., 2002) that can trigger many long-term alterations in brain function. The present data demonstrate that fra-2 is one of these early genes that can be stimulated by cocaine, and that it appears to activate the transcription of the σ-1 receptor gene. These transcriptional changes appear to be translated to protein as seen by the delayed increases in Fra-2 and σ-1 receptor protein levels following cocaine administration. The interaction between cocaine, fra-2 and σ-1 receptor gene and protein expression involves brain regions that are established components of the neural circuit for reward. Together, the data suggest a novel mechanism involved in the cellular basis for addiction. Further studies are needed to determine whether these correlational changes reflect causative relationships.

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Footnotes

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b) Person to receive reprint requests: Rae R. Matsumoto, Ph.D., University of Mississippi, Department of Pharmacology, 303 Faser Hall, University, MS 38677, USA

Legends for Figures

Fig. 1: Effects of cocaine and BD1063 on the expression of fos and jun family genes, showing an increase in fra-2, c-fos and junD by cocaine, and prevention by BD1063 for fra-2. Mice were injected with saline or BD1063 (30 mg/kg, i.p.) followed 15 min later with either saline or cocaine (10 mg/kg, i.p.). Brain tissues were collected 20 min after the second injection. Total RNA was processed on cDNA microarrays and average expression levels in each treatment group (n=3 per group) are summarized. *p<0.05, **p<0.01, ***p<0.001 compared to naïve; +++P<0.001 compared to Sal-Coc.

Fig. 2: Effects of cocaine and BD1063 on locomotor activity of mice, showing an acute cocaine-induced hyperlocomotion being prevented by BD1063. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). Naïve mice received no injections. (A) Locomotor activity in 5 min periods (n=4-16 per point). (B) Cumulative locomotor activity during the 40 min period (n=4 mice per group) following the second injection. *p<0.05; **p<0.01; ***p<0.001.

Fig. 3: Effects of cocaine and BD1063 on fra-2 gene expression in whole brain, showing an up-regulation 10-80 min following cocaine injection being blocked by BD1063 administration. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). Naïve mice received no injections. (A) Example gels from the various experimental groups. (B)

Averaged measurements of the gene expressions (in optical density) are summarized. *p<0.05; **p<0.01; ***p<0.001.

Fig. 4: Effects of cocaine and BD1063 on σ-1 receptor gene expression in whole brain, showing a delayed up-regulation within 80 min following an acute cocaine injection being blocked by BD1063 administration. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). Naïve mice received no injections. (A) Example gels from the various experimental groups. (B) Averaged measurements of the gene expressions (in optical density) are summarized. *p<0.05; **p<0.01; ***p<0.001.

Fig. 5: Fra-2 gene expression in different brain regions, showing cocaine-induced fra-2 gene up-regulation in striatum, cortex, and hippocampus. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). Naïve mice received no injections. (A) Striatum; (B) Cortex; (C) Hippocampus; (D) Cerebellum. *p<0.05; **p<0.01; ***p<0.001.

Fig. 6: σ-1 Receptor gene expression in different brain regions, showing cocaine-induced σ-1 receptor gene up-regulation in striatum and cortex. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). Naïve mice received no injections. (A) Striatum; (B) Cortex; (C) Hippocampus; (D) Cerebellum. *p<0.05.

Fig. 7: Fra-2 protein level in different brain regions, showing cocaine-induced Fra-2 up-regulation in half brain, striatum, cortex, and hippocampus. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). (A) Half brain; (B) Cortex; (C) Striatum; (D) Hippocampus; (E) Cerebellum. *$p<0.05$; **$p<0.01$; ***$p<0.001$. Lane 1: Saline+Saline; Lane 2: Saline+Cocaine; Lane 3: BD1063+Cocaine; Lane 4: BD1063+Saline.

Fig. 8: σ-1 Receptor protein expression in different brain regions, showing cocaine-induced σ-1 receptor up-regulation in half brain, striatum and cortex. Mice were injected with saline (Sal) or BD1063 (BD, 30 mg/kg, i.p.) followed 15 min later with either saline (Sal) or cocaine (Coc, 10 mg/kg, i.p.). (A) Half brain; (B) Cortex; (C) Striatum; (D) Hippocampus; (E) Cerebellum. *$p<0.05$; **$p<0.01$; ***$p<0.001$. Lane 1: Saline+Saline; Lane 2: Saline+Cocaine; Lane 3: BD1063+Cocaine; Lane 4: BD1063+Saline.

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Table 1. PCR primers and reaction conditions

Gene		Primer		PCR condition	
Gene name	Gene code	Upper	Lower	Anneal	Cycles
σ-1 receptor	U79525	TGCACTCACATCCA CCCAGAAAGG	AGCTTTCCCATTC TAACCCCAACA	57°C 45 sec	32
fos-related antigen 2	AF 004927	AGGAGAAGGAG AAGCTAGAGTTCA	AGTTCAAGGAGT CTGATGACTGGT	57°C 45 sec	32
β–actin	X03672	CCTAAGGCCAAC CGTGAAAAG	TCTTCATGGTG CTAGGAGCCA	57°C 45 sec	28

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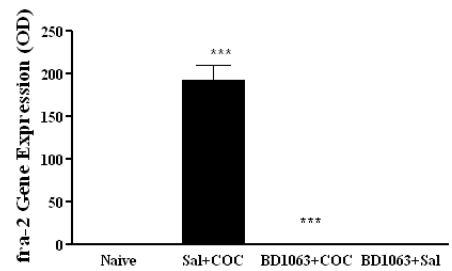

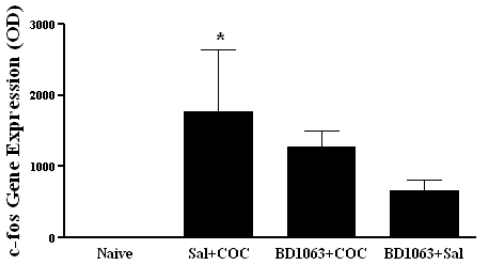

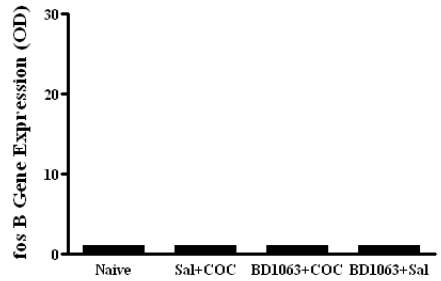

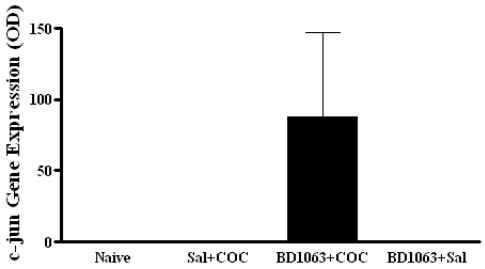

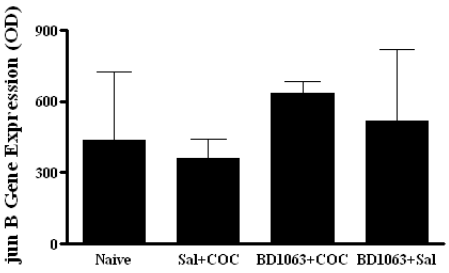

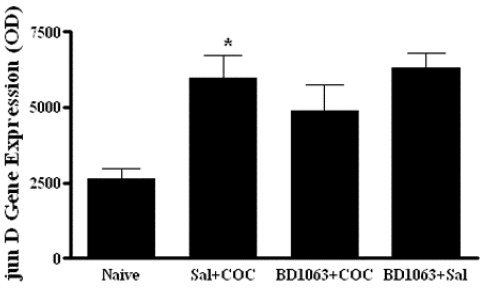

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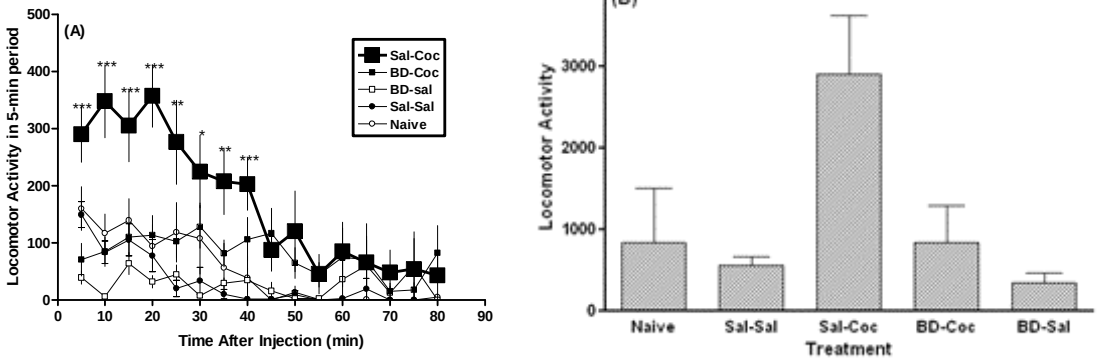

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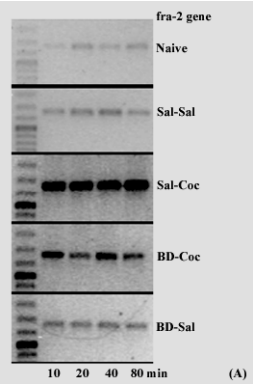

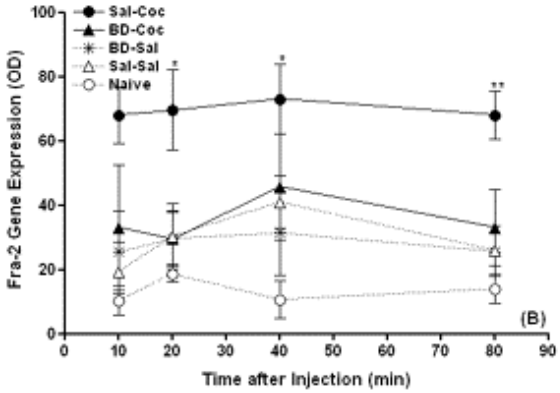

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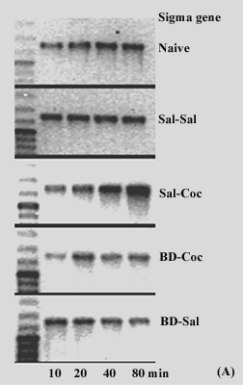

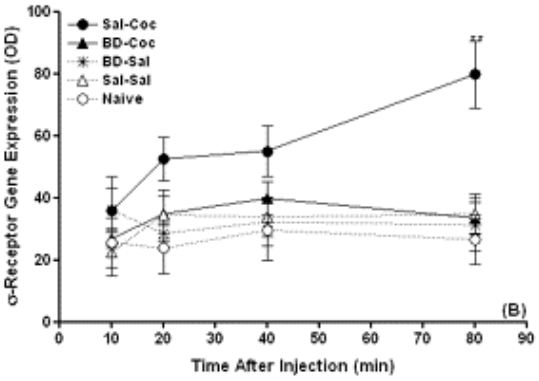

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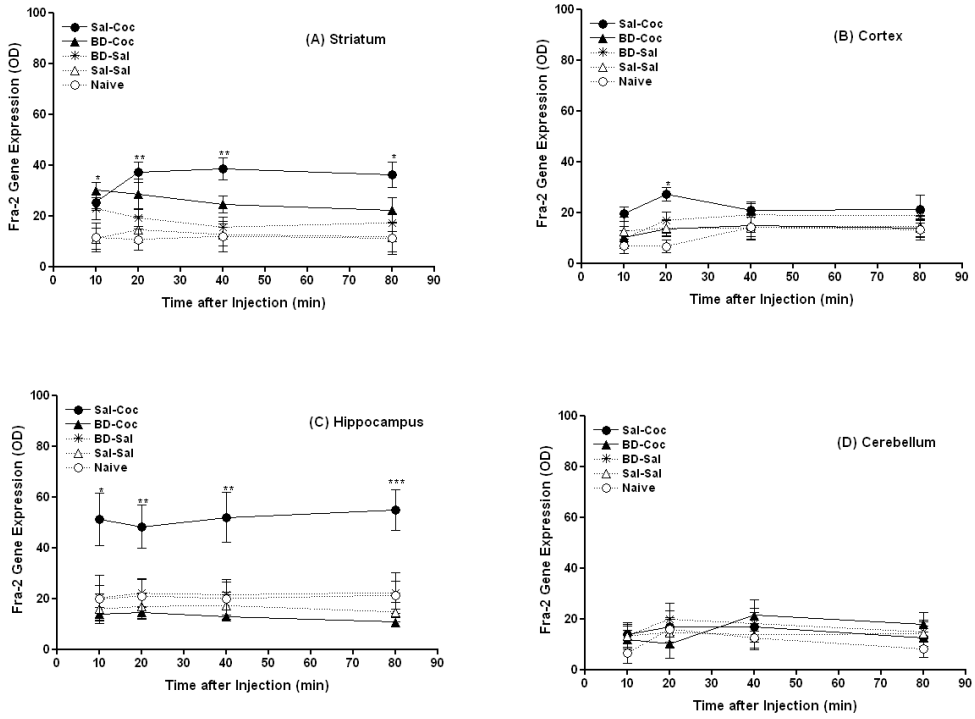

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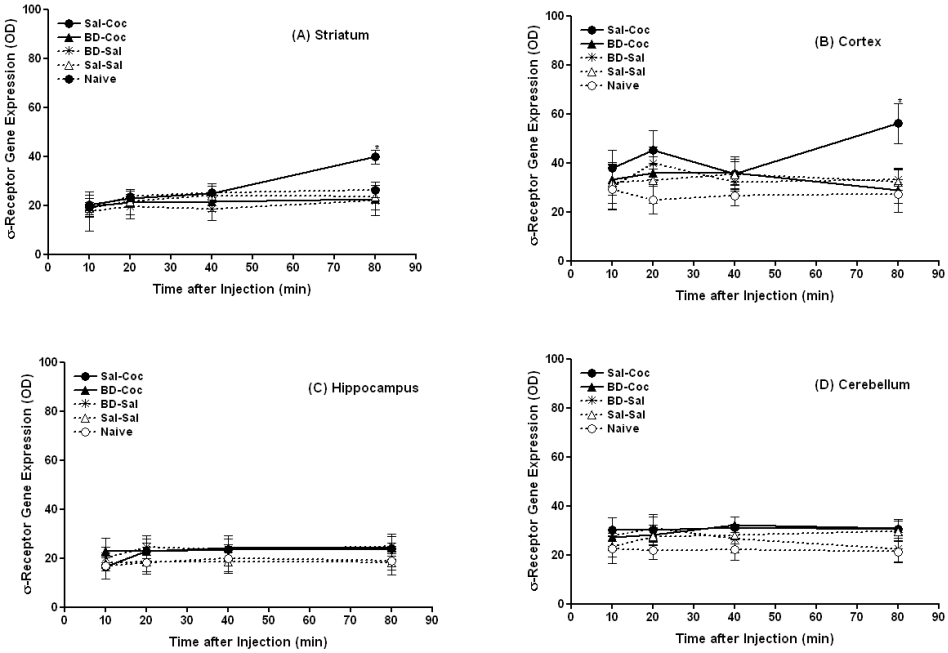

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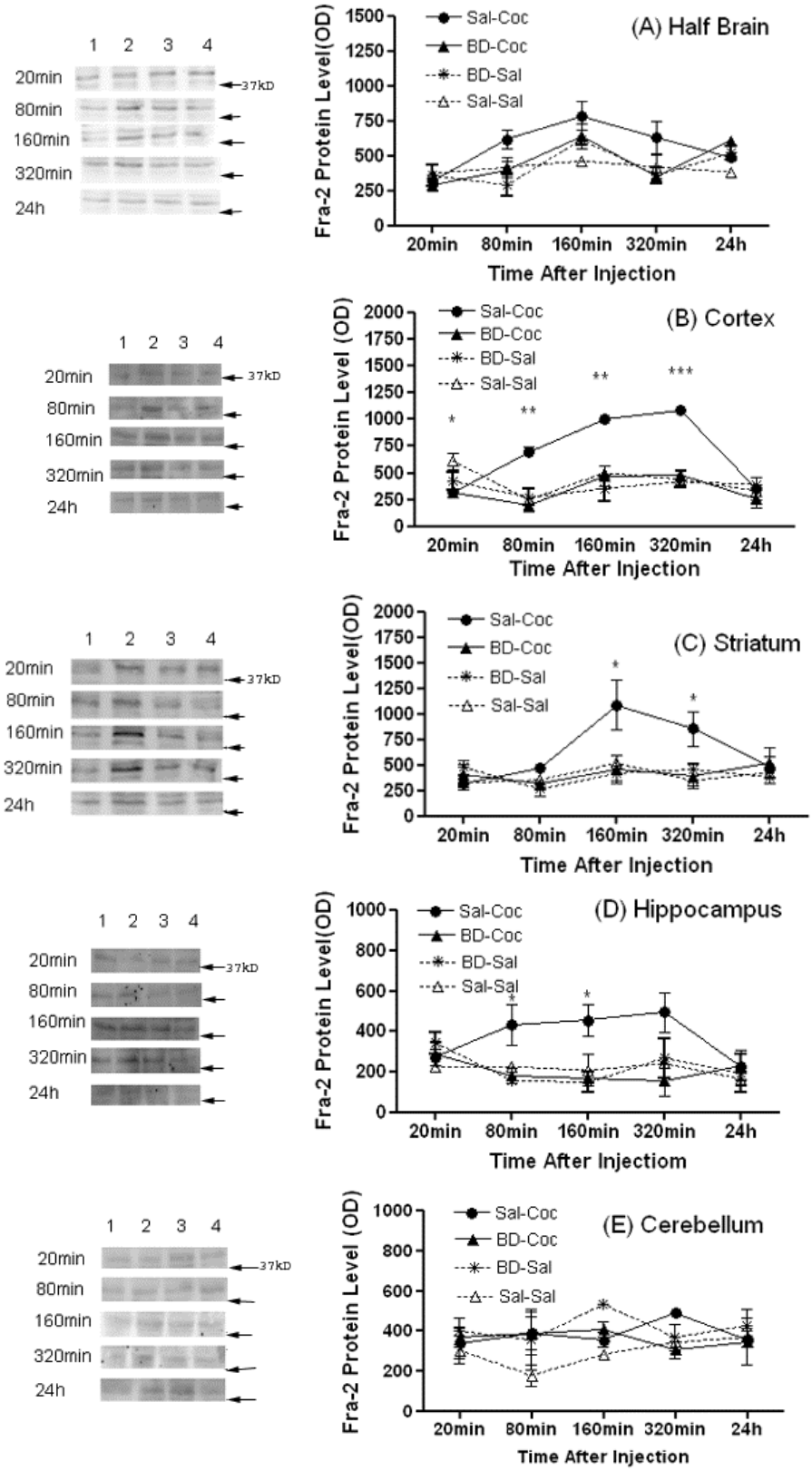

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