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Chronic Cocaine Treatment Suppresses Basal Expression of *zif268* in Rat Forebrain: *In Situ* Hybridization Studies[1]

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ABSTRACT

Transcription regulatory factors are rapidly induced in brain by a wide variety of stimuli and may be important in coordinating changes in gene expression underlying neuronal plasticity. In addition to the transient activation profile typically displayed by many of these genes, *zif268* exhibits prominent basal expression in the brain that is dependent on synaptic activity. Accordingly, *zif268* may play a key role in regulating neuronal gene expression induced by naturally occurring stimuli. Acute cocaine administration (15 mg/kg i.p.) produces a robust and transient induction of several transcription factors in the brain, including *zif268*. In the present study we report that, in contrast to the acute effects of a single dose, chronic cocaine treatment (15 mg/kg i.p., twice daily for a total of 10 injections), produces a widespread suppression of basal *zif268* mRNA levels in rat forebrain between 8 and 24 hr after the last cocaine injection. This reduction is not evident after a single injection of cocaine or comparable chronic treatment with a structural analog, procaine, that does not share cocaine's uptake inhibitor properties. The suppression of basal *zif268* expression may provide a mechanism for producing widespread effects of chronic cocaine administration on neuronal gene expression.

During the past few years, a variety of physiological and pharmacological stimuli that elicit a rapid and transient activation of several transcription factor genes in neuronal systems have been identified (Morgan and Curran, 1989; Sheng and Greenberg, 1990). By binding to DNA in a sequence-specific fashion, these transcription factors are thought to regulate changes in gene expression underlying stimulus-induced neuronal plasticity. The ability of cocaine and amphetamine to elicit marked increases in several transcription regulatory factors in striatum has focused attention on the role of these regulatory proteins in mediating the prominent long-term effects of these drugs (Graybiel *et al.*, 1990; Young *et al.*, 1991; Cole *et al.*, 1992).

In recent studies we have focused on characterizing the regulation in the brain of *zif268* [also named NGFI-A (Milbrandt, 1987), *krox*-24 (Chavrier *et al.*, 1988) or *egr*-1 (Sukhatme *et al.*, 1988)], a transcription factor that contains three tandemly repeated zinc finger sequences in its DNA binding domain (Milbrandt, 1987; Lemaire *et al.*, 1988; Christy *et al.*, 1988; Sukhatme *et al.*, 1988). Like c-*fos*, *zif268* is rapidly and transiently activated by seizure activity, most prominently in dentate granule cells of the hippocampus (Morgan *et al.*, 1987; Saffen *et al.*, 1988; Sukhatme *et al.*, 1988; Sonnenberg *et al.*, 1989; Cole *et al.*, 1990). However, interesting differences in activation of these transcription factors have also emerged. For example, brief tetanic stimulation of perforant path afferents to dentate granule cells that elicits long-term potentiation robustly activates *zif268* in these neurons, whereas c-*fos* is not induced by this type of synaptic stimulation (Cole *et al.*, 1989; Wisden *et al.*, 1990). Induction of *zif268*, like long-term potentiation, is blocked by N-methyl-D-aspartate receptor antagonists (Cole *et al.*, 1989; Worley *et al.*, 1990b), bolstering the hypothesis that this gene may, by orchestrating long-term changes in gene expression, play a key role in mediating neuronal plasticity.

In addition to transient stimulus-induced activation of *zif268*, this gene also displays prominent basal levels of expression in cortical neurons (Mack *et al.*, 1990; Mack and Mack, 1992; Worley *et al.*, 1990a, 1991). Monocular injection of tetrodotoxin which blocks retinal input to the cortex produces a marked, rapid reduction of *zif268* expression in the contralateral visual cortex (Worley *et al.*, 1991). These findings indicate that basal levels of *zif268* mRNA in the brain are driven by synaptic activity. Therefore, *zif268* may play a key role in coordinating programs of gene expression in cortical neurons that are normally maintained by physiological patterns of synaptic activity.

In this study we have investigated the effects of chronic cocaine treatment on regulation of *zif268* and report that this

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ABBREVIATIONS: SSC, sodium chloride-sodium citrate; SDS, sodium dodecyl sulfate; O.D. optical density.

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treatment produces a persistent, widespread suppression of *zif268* basal expression in the rat forebrain. These findings suggest that cocaine's effects on neuronal function may reflect, in part, its ability to disrupt physiological regulation of *zif268*.

Materials and Methods

Animals and drug treatments. Adult male Sprague-Dawley rats (175–200 g) (Harlan, Indianapolis, IN) were maintained in a light controlled (12-hr light/12-hr dark) room with access to food and water *ad libitum*. For chronic treatments, cocaine hydrochloride (15 mg/kg) or procaine hydrochloride (15 mg/kg) [calculated as free base] were dissolved in distilled water and administered i.p. twice daily (8:00 A.M. and 6:00 P.M.) for a total of 5 or 10 injections. After drug treatments, rats were sacrificed at various intervals after the last injection. Some rats were challenged with acute injections of vehicle or cocaine and then were sacrificed 30 min later. After sacrifice, brains were removed rapidly, placed on ice and cut sagittally along the midline. Hemi-brains from control and drug-treated rats were aligned, embedded together in Tissue-Tek and frozen rapidly in a plastic mold kept on an ethanol-dry ice slurry. This technique was used to ensure identical treatment of tissue from control and treated rats through the entire *in situ* hybridization procedure.

***In situ* hybridization.** *In situ* hybridization was performed as described by Cole *et al.* (1990). Briefly, 12 μm of frozen cryostat (Microm, Heidelberg, Germany) sections of tissue blocks containing brains from both drug-treated and control rats were thaw-mounted on gelatin-coated glass slides and stored at −20°C. For *in situ* hybridization, sections were allowed to thaw to room temperature, fixed in 4% w/v paraformaldehyde, acetylated with 0.5% w/v acetic anhydride in 0.9% w/v NaCl containing 100 mM triethanolamine (pH 8.0), dehydrated by immersion in a graded series of ethanol solutions and delipidated in chloroform. After a final immersion in alcohol, the sections were air dried. ^{35}S-labeled riboprobe was added to the hybridization buffer [50% formamide, 600 mM NaCl, 1 × Denhardts, 10 mM Tris (pH 7.4), 1 mM EDTA, 10 mM dithiothreitol, 0.2 μg/μl of *t*RNA and 10% dextran] to give a final concentration of 1×10^6 cpm/50 μl of buffer. An aliquot (50 μl) of this mixture was added to each slide and covered with silane-treated coverslips. Hybridization was carried out overnight in moist sealed chambers at 56°C. Coverslips were then removed under 2 × SSC (1 × SSC contains 150 mM NaCl and 15 mM sodium citrate, pH 7.0) at room temperature. Slides were then incubated in a solution of 2 × SSC (30 min, 35°C) containing 10 μg/ml RNAse A. The slides were then rinsed in 2 × SSC at room temperature for an additional 30 min, quickly dipped in water, 70% ethanol and 95% ethanol, air dried and exposed to X-ray film (Kodak XAR) for 3 days. To obtain quantitative results, the O.D. of film images defined as −log (transmittance/maximal transmittance) was determined with a LOATS densitometer using coronal sections taken at the level of the striatum (in the areas shown in bottom panel of fig. 3). Comparable areas of either the cortex or striatum in autoradiograms of sections containing aligned hemi-brains from control and treated rats were used. Sections were Nissl-stained to identify anatomical structures in conjunction with the atlas of Paxinos and Watson (1982).

RNA probe synthesis. To synthesize antisense riboprobe, mouse *zif268* or rat NGFI-A cloned in the Bluescript SK$^+$ plasmid (Stratagene, La Jolla, CA) was linearized with the restriction enzyme Eco RI and transcribed with T7 RNA polymerase. The sense riboprobe was synthesized from the same plasmid linearized with XhoI and transcribed with the T3 RNA polymerase. The transcription reaction was conducted at 37°C for 1 hr in the presence of 10 μCi of uridine 5′-(α[^{35}S] thio)triphosphate (New England Nuclear, Boston, MD) and the indicated RNA polymerase enzyme. Probes were purified from unincorporated nucleotides by size-exclusion chromatography with DNA grade Nick G-50 columns (Pharmacia Fine Chemicals, Inc., Piscataway, NJ). In some experiments, only the 3′ noncoding region of *zif268* was transcribed by using plasmid linearized with PstI. Experiments utilizing this probe yielded results similar to those obtained with full length *zif268* probes. The synthesis of labeled riboprobes was monitored routinely by thin-layer chromatography on polyethyleneimine strips. Levels of radioactivity were determined on a Beckman Series LS5000CE counter.

Northern blots. Total cellular RNA was isolated from the rat forebrain by using the acid guanidinium-phenol extraction procedure of Chomczynski and Sacchi (1987). After extraction, RNA was measured spectrophotometrically and equal amounts were loaded onto a 1% agarose-formaldehyde gel containing 10 μg of ethidium bromide. RNA was separated by electrophoresis through the gel, transferred to nitrocellulose membranes overnight and probed as described by Linzer and Nathans (1983). Briefly, filters were prehybridized in a sealed bag containing 50% V/V formamide, 4 × SSC, 4 × Denhardt's solution, 0.1% W/V SDS, 0.1% W/V sodium pyrophosphate and 100 μg/ml of sheared salmon sperm DNA. ^{32}P-labeled DNA probe was prepared by the random primer method (Feinberg and Vogelstein, 1983) by using purified NGFI-A cDNA inserts as substrate. ^{32}P-labeled probe was added to a final concentration of 2×10^6 cpm/ml and filters were hybridized overnight at 42°C. Filters were washed in 2 changes of 2 × SSC with 0.1% W/V SDS at room temperature, and then in 2 changes of 0.1 × SSC with 0.5% SDS at 50°C, air dried and exposed to X-ray film (Kodak XAR) at −70°C with an intensifying screen for 3 days.

Statistics. To determine the effect of a given treatment, each treated animal was paired with a separate control animal. Either the right or left hemi-brain from the treated animal was then embedded with the complementary hemi-brain from the paired control animal. O.D. values were determined for each pair of hemi-brains and then analyzed for potential group differences using paired two-tailed *t* tests. For the purpose of displaying these data in graphic form, values were calculated as a percentage of their respective controls. Data shown in graphs are the mean ± S.E.M.

Results

In initial experiments, we characterized the acute effects of cocaine administration on *zif268* mRNA levels using *in situ* hybridization. Cocaine (15 mg/kg) produced a marked increase in *zif268* mRNA levels that was particularly prominent in the caudate-putamen and overlying cortex (fig. 1). Assessment of the time course of this response revealed that the increase in *zif268* mRNA peaked between 30 min and 1 hr and promptly returned to near control levels within 2 hr. We also examined whether a similar activation of *zif268* is produced by lower doses and found that a small but detectable increase was elicited by 5 mg/kg of cocaine (data not shown).

To assess the effect of chronic cocaine treatment on *zif268* gene expression, animals were administered cocaine (15 mg/kg i.p.) twice daily for a total of 10 injections. This paradigm was based on similar protocols used to examine neuronal effects of chronic cocaine treatment (Henry *et al.*, 1989; Nestler *et al.*, 1990). *In situ* hybridization studies were performed to compare the effect on *zif268* gene expression of a single injection of saline or cocaine in naive and chronically treated rats. Eight hours after the last injection of the chronic cocaine treatment paradigm, rats were challenged with either 5 mg/kg (data not shown) or 15 mg/kg of cocaine and sacrificed 30 min after this injection. The rise in *zif268* mRNA elicited in the cortex and striatum by the challenge injection of cocaine in chronically treated rats appeared blunted (fig. 2D) compared to the response produced in naive rats (fig. 2C). However, these effects were difficult to evaluate as a decrease in the basal *zif268* mRNA levels was observed in both the cortex and striatum of rats that had received chronic cocaine treatment (fig. 2B). To ensure the specificity of the probe used in the *in situ* studies,

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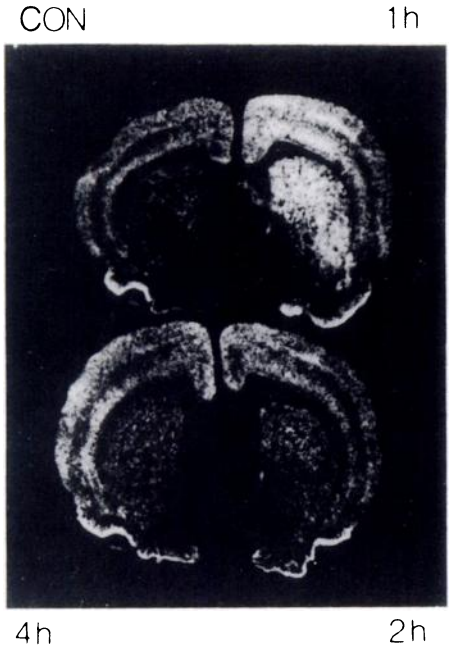

Fig. 1. Time course of *zif268* activation after acute cocaine injections. *In situ* autoradiogram of a coronal section taken at the level of the striatum displays marked transient increase in *zif268* mRNA levels after cocaine. Rats were sacrificed at the indicated times after a single injection of cocaine (15 mg/kg i.p.). Hemi-brains from rats were aligned, embedded together and processed with ^{35}S-labeled antisense *zif268* riboprobe. CON, control.

we checked that the sense RNA probe does not produce a hybridization signal (fig. 2, lower panel).

To investigate further the effect of cocaine on basal *zif268* expression, we monitored mRNA levels in chronically treated rats at various times after the last cocaine injection (figs. 3 and 4). These experiments revealed that, after the slight rise in *zif268* mRNA levels detected at 30 min, *zif268* mRNA fall below those found in naive control rats between 4 and 8 hr after the last injection of the chronic cocaine treatment paradigm. To assess in a quantitative manner the changes observed, the O.D. of autoradiographic images was determined. As hemi-brain sections from control and chronically treated animals were always processed together, the O.D. of the image from the treated animal was compared to the value obtained from the adjacent control section present on the same slide. In brain sections from animals sacrificed 8 hr after cessation of chronic cocaine treatment, the O.D. of the hybridization signal showed a 35% decrease below those of controls when measured in the cortex ($P < .01$) and 20% in the striatum ($P < .01$). Similar significant reductions were detected 16 hr after the last cocaine injection. At 24 hr after termination of the chronic treatment, levels were still significantly reduced in the cortex (19%, $P < .05$) and striatum (14%, $P < .05$). Levels of *zif268* mRNA returned to control values by 36 and 48 hr after chronic cocaine treatment.

To assess the treatment parameters needed to elicit this suppression, we examined effects of altering the dose and duration of cocaine treatment on *zif268* expression. A similar suppression of *zif268* mRNA levels was not observed at 8 hr (figs. 4 and 5, C and D) or 24 hr (data not shown) after a single cocaine injection (15 mg/kg). Chronic treatment with a lower dose of cocaine (3 mg/kg) did not reduce *zif268* mRNA levels (fig. 5, G and H). Shorter treatment (15 mg/kg i.p. twice daily

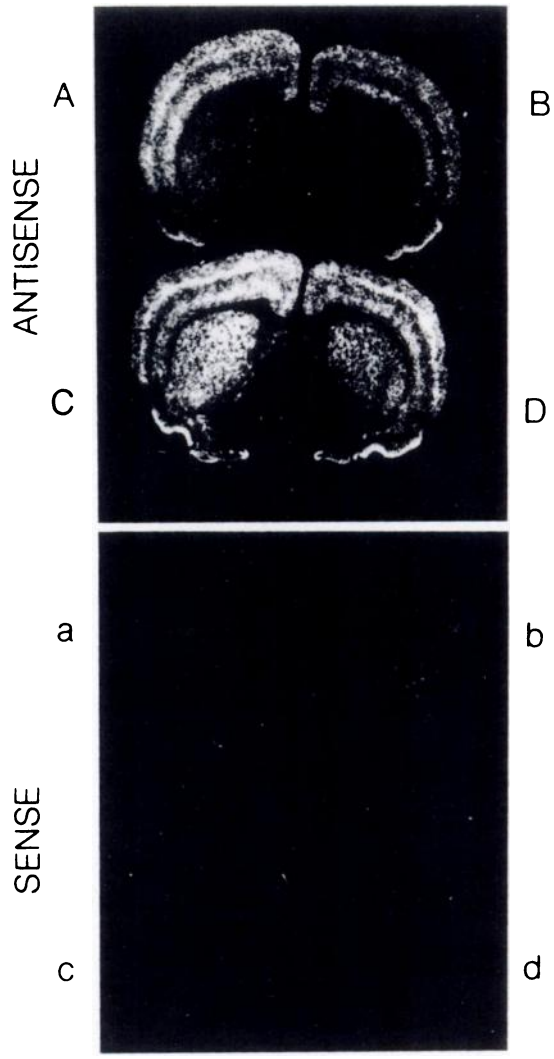

Fig. 2. Responsiveness of *zif268* mRNA after chronic cocaine treatment. Upper panel: cocaine (15 mg/kg) was administered to rats twice daily for a total of 10 injections. Eight hours after the last injection, rats were challenged with an acute injection of cocaine (15 mg/kg). For comparisons, naive rats were injected with a single injection of cocaine (15 mg/kg). *In situ* autoradiograms show the hemi-brains of rats treated as follows: naive control (A), chronic cocaine and vehicle challenge 8 hr later (B), naive rat given cocaine challenge (C) and chronic cocaine followed by cocaine challenge 8 hr later (D). Rats were sacrificed 30 min after the challenge injections. The acute *zif268* mRNA response to a challenge injection is blunted after chronic cocaine treatment. However, a decrease in the basal expression of *zif268* mRNA is also observed (B) when compared to control (A). Lower panel, total lack of hybridization of the sense *zif268* riboprobe to an adjacent section.

for a total of 5 injections) appeared to be insufficient to produce a reliable suppression of *zif268*, as only four of seven animals displayed a reduction at 24 hr. To assess whether this suppression may reflect cocaine's local anesthetic properties we examined, in parallel studies, the effect of chronic treatment with a structurally similar local anesthetic, procaine (15 mg/kg i.p.; Pitts and Marwah, 1986). This treatment (5 or 10 injections) did not affect the basal levels of *zif268* mRNA (figs. 4 and 5, E and F).

To determine the anatomical areas affected by chronic cocaine treatment, *zif268* expression was examined in coronal sections taken at several forebrain levels from animals sacrificed 8 hr after the final injection in the chronic treatment

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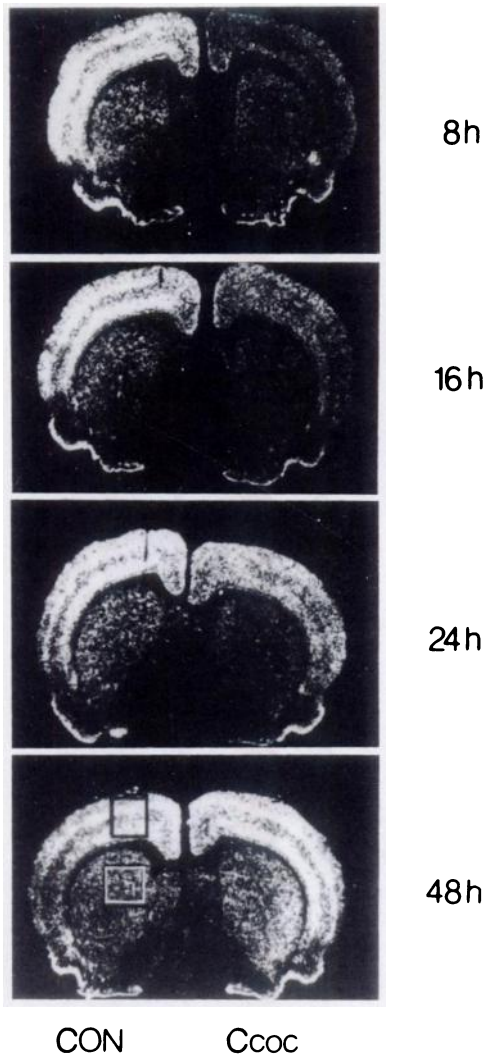

Fig. 3. Time course of the reduced basal expression of *zif268* mRNA after chronic cocaine (Ccoc) treatment. *In situ* autoradiograms of coronal sections of paired hemi-brains from control (CON) rats and rats sacrificed at the indicated time after (Ccoc) treatment using ^{35}S-labeled antisense *zif268* probe. Boxes represent areas of cortex and striatum on which O.D. measurements were performed.

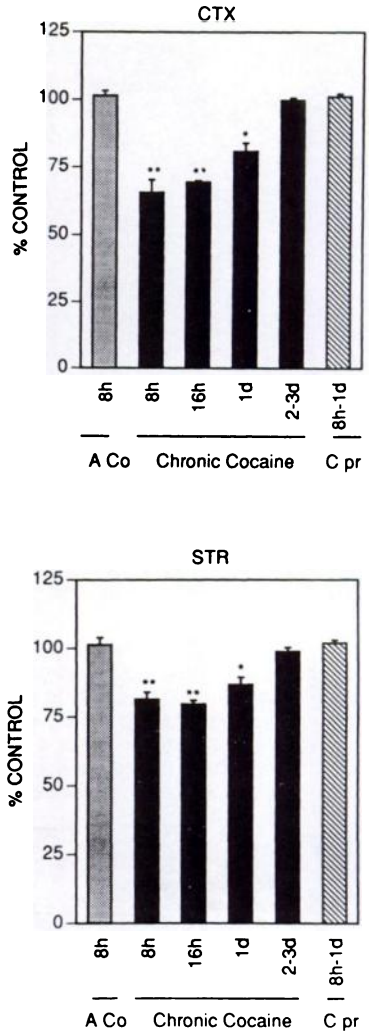

Fig. 4. Effect of cocaine treatment on *zif268* mRNA levels. The O.D. values of *in situ* autoradiograms in either cortex (CTX) or striatum (STR) were determined in sections from control and treated animals that were embedded together. The values of the treated groups are expressed as a percentage of the adjacent control. Bar graphs show the mean ± S.E.M. of these percentages. Acute cocaine (Aco), 15 mg/kg (n = 3); chronic cocaine, 8 hr (n = 8), 16 hr (n = 3), 1 day (n = 6) and 2 to 3 days (n = 4); and chronic procaine (Cpr), 8 hr to 1 day (n = 7) (as described under "Materials and Methods"). The time interval between the last injection and sacrificing the animals is indicated after the treatment group abbreviation. *P < .05; **P < .01 by paired two-tailed t tests. A significant reduction in O.D. is observed in the cortex and striatum after 8, 16 and 24 hr after chronic cocaine treatment, but not after Cpr (8 hr or 1 day) or an acute injection of cocaine.

paradigm. These *in situ* hybridization studies indicate that the suppression of *zif268* mRNA produced by chronic cocaine treatment is widespread and is evident throughout the cortex and caudate-putamen. However, there does appear to be regional heterogeneity as this treatment spares *zif268* mRNA levels in the olfactory tubercle and the pyramidal cell layer of the hippocampus (fig. 6).

To check the results obtained by *in situ* hybridization, we performed Northern blot analysis on RNA harvested from forebrain of rats treated either acutely or chronically with cocaine (fig. 7). For these studies, animals were sacrificed 30 min after a cocaine challenge injection (15 mg/kg). In naive animals, cocaine produces a robust increase in *zif268* mRNA (lanes 1 and 2). In animals treated chronically with cocaine (15 mg/kg i.p., 10 injections), the challenge injection was administered 8 hr after completion of the chronic treatment protocol. The rise in *zif268* mRNA observed in these animals is blunted (lanes 3 and 4) compared to that detected in naive rats challenged with cocaine. With this approach, a decrease in *zif268* mRNA levels is detected in animals sacrificed 8 hr after cessation of chronic cocaine treatment (lanes 1 and 3), but not 8 hr after a single injection of cocaine in a naive rat (lanes 1 and 5).

Discussion

The major finding of this study is that in contrast to the rapid and relatively short-lived activation of *zif268* induced by cocaine, repeated administration of this agent elicits a persistent suppression of basal *zif268* mRNA levels. The suppressive effects on this transcription factor gene are not limited to the striatum, an area in which the most prominent rise in *zif268*

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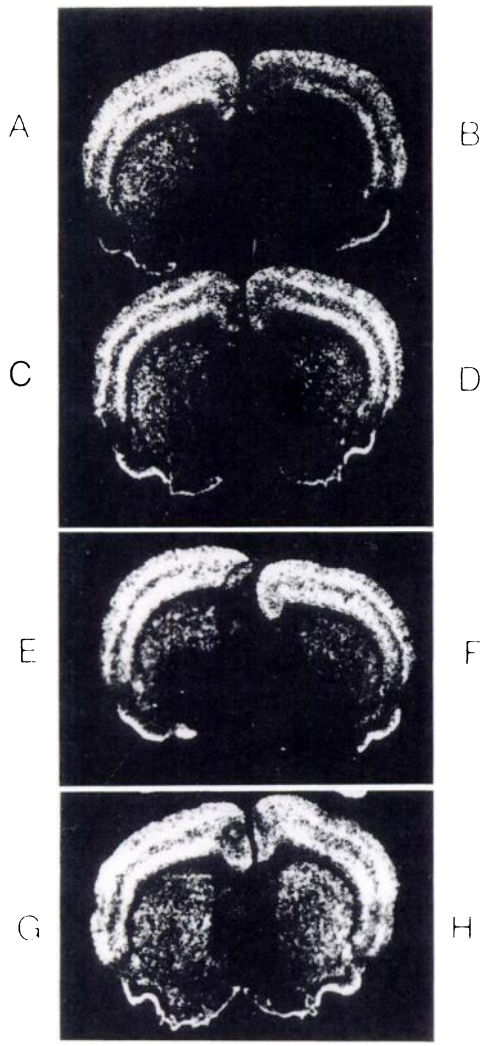

Fig. 5. Specificity of *zif268* suppression produced by chronic cocaine. In each pair shown, the left hemi-brain is taken from a control rat and the right hemi-brain from animals sacrificed 8 hr after the following treatments: B, chronic cocaine treatment (15 mg/kg); D, single injection of cocaine (15 mg/kg i.p.); F, chronic procaine treatment; and H, chronic cocaine treatment (3 mg/kg).

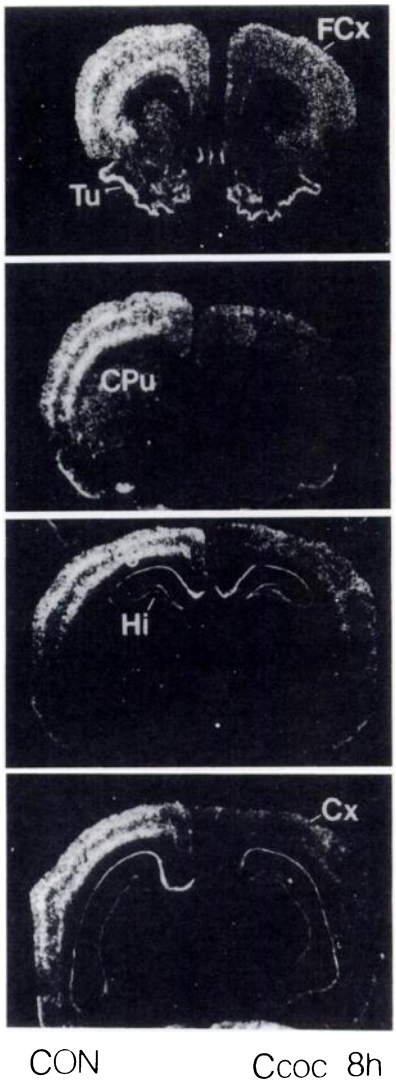

Fig. 6. *zif268* mRNA distribution after chronic cocaine (Ccoc) treatment. Ccoc treatment, rats were sacrificed 8 hr after the last cocaine injection and coronal sections of paired hemi-brains from control (CON) and treated rats (Ccoc) were hybridized with ^{35}S-labeled *zif268* probe. A widespread reduction in *zif268* is seen through the forebrain except in the hippocampus (Hi) and olfactory tubercle (Tu). FCx, frontal cortex; CPu, caudate putamen.

mRNA levels are observed with acute treatment. Instead, reductions are widespread throughout the neocortex.

Suppression of basal *zif268* mRNA levels is not produced by a single injection with cocaine, indicating that repeated cocaine treatment is required to disrupt basal expression of this transcription factor. Although at present the basis for this reduction is unclear, two general types of mechanisms need to be considered. In previous studies we have examined the regulation of the basal levels of *zif268* mRNA in the cortex and obtained evidence that these are dynamically regulated by physiological synaptic inputs (Worley *et al.*, 1991). For example, administration of a single high dose of MK-801, which would be expected to block N-methyl-D-aspartate receptor-mediated synaptic activity in the brain, causes a dramatic reduction in *zif268* basal mRNA levels within a few hours. These results indicate that ongoing synaptic activity is critical for maintaining basal expression of *zif268*. More discrete forms of sensory deprivation such as blockade of visual input with monocular injections of tetrodotoxin elicit a similar reduction that is restricted to the contralateral visual cortex (Worley *et al.*, 1991). As basal expression of *zif268* is sensitive to alterations in physiological afferent activity, it is plausible that these reductions produced by chronic cocaine reflect a suppression of the types of synaptic activity important for driving basal expression of *zif268*.

Alternatively, these changes in *zif268* may reflect alterations secondary to repeated activation of *zif268* and other immediate early genes that occurs with each cocaine treatment. Previous studies indicate that, in addition to regulation of target genes, these transcription factors exert negative feedback on their own expression (Sassone-Corsi *et al.*, 1988; Gius *et al.*, 1990). Comparable reductions in basal levels of the transcription factor c-*fos* have been observed after repeated electroconvulsive treatment, a stimulus that also elicits a robust activation of transcription factor genes (Daval *et al.*, 1989; Winston *et al.*, 1990; Cole *et al.*, 1990). In preliminary studies we have observed similar suppression of *zif268* basal mRNA levels with repeated electroconvulsive treatment, indicating that the suppression

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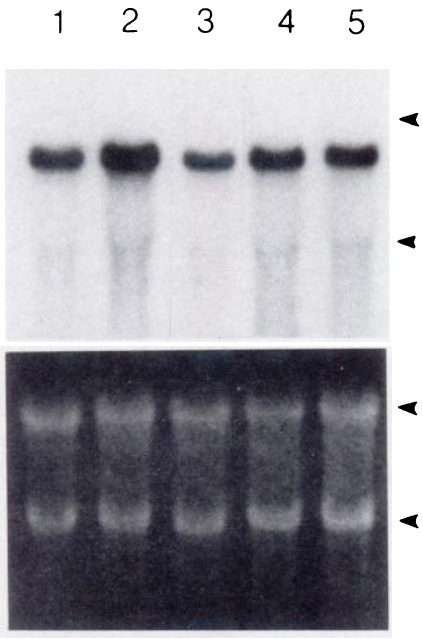

Fig. 7. Northern blot hybridization analysis of *zif268* mRNA after cocaine treatment. Total cellular RNA (15 μg), extracted from the forebrain of two rats per group, was separated by gel electrophoresis and transferred to nitrocellulose membranes. Top panel, autoradiogram obtained after blot hybridization to ^{32}P-labeled *zif268* DNA probes. Numbered lanes contain RNA from the following treatment groups: 1, control; 2, acute cocaine (15 mg/kg i.p., 30 min); 3, 8 hr after the last chronic cocaine injection; 4, 30 min after last chronic cocaine injection; and 5, 8 hr after an acute cocaine injection. Lower panel, ethidium bromide fluorescence of 18S and 28S RNA bands (arrows) after agarose gel electrophoresis, indicating all lanes contain equivalent amounts of RNA. Ethidium bromide was incorporated into a 1% agarose-formaldehyde gel before electrophoresis.

observed after chronic cocaine may be produced with repeated activation by other stimuli as well.

The widespread effects observed emphasize the pervasive effects of cocaine on neuronal activity. Many previous studies have demonstrated effects of cocaine on selected monoamine or neuropeptide transmitter systems (Kalivas *et al.*, 1988; Henry *et al.*, 1989; Smiley *et al.*, 1990; Peris *et al.*, 1990; Wahlestedt *et al.*, 1991). The *in situ* hybridization approach used in this study demonstrate that these effects do not appear to be restricted. As cocaine exerts effects on the three major monoamine systems (Koob and Bloom, 1988; Kuhar *et al.*, 1991), which project extensively throughout the brain, it does not seem unexpected that this drug could exert widespread effects. In previous studies examining induction of c-*fos* in the striatum, several groups have obtained evidence that activation of this gene, as well as other transcription factors, is mediated by dopamine receptor activation, specifically D_1 dopamine receptors (Graybiel *et al.*, 1990; Young *et al.*, 1991; Cole *et al.*, 1992; Bhat *et al.*, 1992). On the basis of these studies, it might be expected that cocaine's effects would be more restricted in the cortex to the few areas that receive substantial dopamine input. However, we have found that although cocaine's induction of *zif268* in striatum is totally blocked by SCH23390, a selective D_1 receptor antagonist, this agent does not block activation of *zif268* in the overlying cortex, suggesting that in these areas other monoamine systems may be involved (R. V. Bhat and J. M. Baraban, in preparation).

zif268 binds to DNA in a sequence-specific fashion (Christy *et al.*, 1988; Chavrier *et al.*, 1988). Each of the three zinc fingers contacts three DNA base pairs and specifies the specific sequence recognized (Pavletich and Pabo, 1991). Although a *zif268* binding site has been identified, little progress has been made in identifying specific target genes regulated by this transcription factor. Recent studies of the adenosine deaminase promoter provide evidence for an interaction of *zif268* and another zinc finger transcription factor referred to as Sp1 (Ackerman *et al.*, 1991). Although the sites recognized by these homologous factors is distinct, their binding sites overlap in this promoter and allow *zif268* to inhibit transcription mediated by Sp1. Thus, *zif268* may act as a repressor of transcription under certain conditions. The high basal level of *zif268* expression in cortical neurons and its responsiveness to synaptic activity suggest that it plays a key role in regulating changes in gene expression in response to physiological activity. Our results indicate that cocaine disrupts this physiological role of *zif268* and by interfering with its transcriptional effects may alter neuronal gene expression. Recent studies of neurochemical effects of chronic cocaine treatment have identified interesting examples of changes in expression of several proteins, such as members of the G-protein family or levels of neuropeptide transmitters (Terwilliger *et al.*, 1991; Wahlestedt *et al.*, 1991). Whereas these effects were detected in specific brain regions, our results suggest that cocaine may also induce more widespread effects on neuronal gene expression. Although the suppression in *zif268* is relatively short-lived, returning to control levels within 2 days, the effects on target genes may be much longer-lasting. Identification of target genes regulated by *zif268* will be useful in understanding the implication of these findings for neuronal function.

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