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Coordinate Expression of c-*fos* and *jun B* Is Induced in the Rat Striatum by Cocaine

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In cells in culture, specific stimuli induce selective patterns of immediate-early gene induction. In the present study, we tested for such selectivity of stimulated gene expression by monitoring the expression of *fos/jun* gene mRNAs in the striatum in rats treated *in vivo* with the indirect dopamine agonist cocaine. We found by Northern blot and *in situ* hybridization analysis that cocaine induces the coordinate expression of c-*fos* and *jun B* mRNAs in neurons of the rat's striatum. By contrast, another immediate-early gene of the leucine-zipper family, c-*jun*, was not induced in striatal neurons by cocaine at any time tested from 1 to 24 hr after treatment. With the same probe, we could detect the induction of c-*jun* mRNA (as well as that of c-*fos* and *jun B* mRNAs) in the hippocampus following administration of pentylenetetrazol. The induction of expression of c-*fos* and *jun B* was rapid and transient, with peak expression occurring at approximately 1 hr after cocaine administration, and the induction of the two genes was in similar striatal sites. These results establish that differential patterns of expression of *fos/jun* genes occur in striatal neurons following exposure to cocaine, a potent psychomotor stimulant. We suggest that these tissue-specific patterns of gene expression may contribute to the response specificity of striatal neurons to stimulation by monoamines including dopamine.

[Key words: striatum, dopamine, cocaine, immediate-early genes, c-*fos*, jun B]

Studies of transcription factor activation in cell culture have established a high degree of combinatorial complexity in the acute genomic response of cells to stimuli ranging from growth factors to membrane depolarization agents (Sheng and Greenberg, 1990). An example is the activation of genes of the AP-1 transcription factor complex, which is composed of multiple members of the *fos/jun* immediate-early gene family (Cochran et al., 1984; Greenberg and Ziff, 1984; Muller et al., 1984; Bohmann et al., 1987; Distel et al., 1987; Lee et al., 1987; Angel et al., 1988a; Chiu et al., 1988; Curran and Franza, 1988; Rauscher et al., 1988b; Vinson et al., 1989; Abate et al., 1990a,b). Members of this family share leucine-zipper dimerization domains with basic DNA binding motifs, and the protein products of these genes can act in combination by forming heterodimers and homodimers (Bohmann et al., 1987; Chiu et al., 1988; Halazometis et al., 1988; Kourazides and Ziff, 1988; Landschulz et al., 1988; Nakabeppu et al., 1988; Rauscher et al., 1988a,b; Sassone-Corsi et al., 1988; O'Shea et al., 1989; Vinson et al., 1989). These DNA binding proteins can regulate transcription negatively as well as positively (Bohmann et al., 1987; Chiu et al., 1988, 1989; Schutte et al., 1989; Lamph et al., 1990), have different kinetics on induction (Sonnenberg et al., 1989a; Kovary and Bravo, 1991), and can differ markedly in their responses to different signaling agents (Bartel et al., 1989; de Groot et al., 1991).

Investigations of immediate-early gene regulation in the CNS are just beginning to take account of such complexities. For example, in the hippocampal seizure model, probes for immediate-early genes of the *fos/jun* (leucine-zipper) family and members of the *NGFI*-A (zinc finger) family are coinduced by intense electrical or pharmacological stimulation (Dragunow and Robertson, 1988; Saffen et al., 1988; Cole et al., 1989; Sonnenberg et al., 1989a; Watson and Milbrandt, 1989). By contrast, tetanic stimulation leading to long-term potentiation (LTP) readily induces only a subset of these immediate-early genes (*NGFI*-A and, less consistently, *jun B*) (White and Gall, 1987; Wisden et al., 1990). These different responses may be implicated in the selective regulation of other genes. Following induction of LTP, elevated transcription of neuropeptide (Gall et al., 1990) and of NGF (Gall et al., 1991) has been reported as late responses to stimuli activating immediate-early genes. AP-1-like sites, binding sites for Fos/Jun dimers, have been identified in both proenkephalin (Sonnenberg et al., 1989b) and prodynorphin (Naranjo et al., 1991).

In the experiments reported here, we tested for differential *in vivo* activation of *fos/jun* genes in the striatum, the principal target of the dopamine-containing nigrostriatal tract. We and others have shown that indirect dopamine agonists, including the psychomotor drugs cocaine and amphetamine, induce rapid D1-like dopamine receptor-mediated increases of c-*fos* mRNA and Fos-like proteins in the striatum (Graybiel et al., 1990; Young et al., 1991). The patterns of induction are anatomically selective, suggesting that stimulant drugs induce Fos-like transcription factors in particular subsets of striatal neurons having

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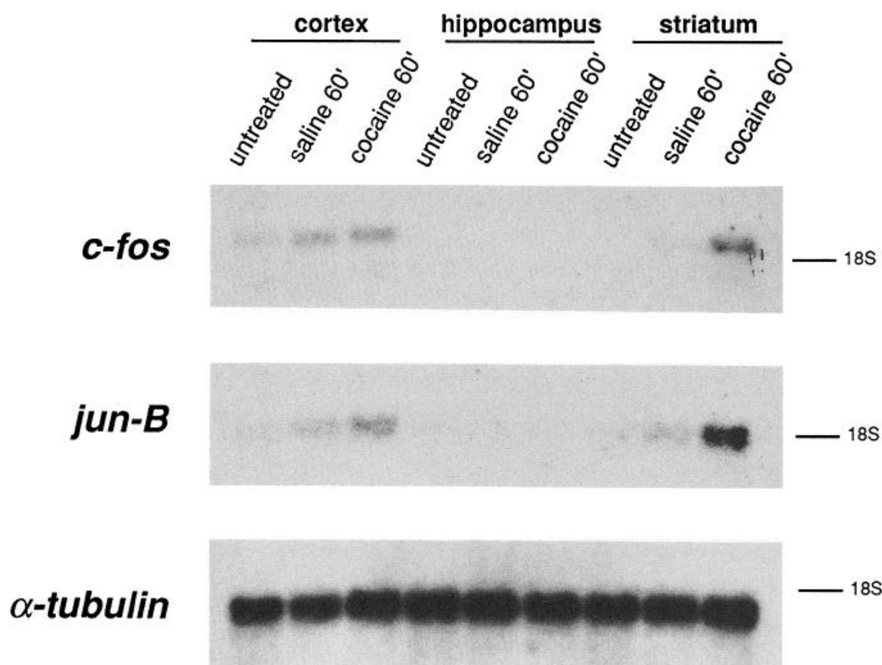


Figure 1. Northern blot analysis demonstrating induction of c-*fos* and *jun B* mRNAs in the rat striatum following *in vivo* exposure to cocaine (25 mg/kg, i.p.). Control rats were injected with saline. Five micrograms of RNA from each brain region were fractionated through agarose formaldehyde gels, blotted, and hybridized with the c-*fos* probe as described in Materials and Methods. The same blot was then stripped and rehybridized with the α-tubulin probe and then again with *jun B* probe. The position of the 18S ribosomal band is indicated.

specialized transmitter phenotypes and functions (Berretta et al., 1991a,b, 1992; Moratalla et al., 1991a,b, 1992; S. Beretta, H. A. Robertson, and A. M. Graybiel, unpublished observations).

Here we show that single doses of cocaine result in temporally and spatially similar increases in c-*fos* and *jun B* mRNA expression in the striatum, but that c-*jun* mRNA expression is not increased in parallel. Another *jun* family member, *jun D,* is expressed constitutively in the striatum. These findings suggest that the molecular signaling pathways stimulated by acute exposure to psychomotor stimulants lead to gene-specific patterns of expression of genes of the leucine-zipper family of immediate-early genes. We suggest that the differential actions of the protein products of these genes may help to account for some of the selectivity in the neuronal response to psychomotor stimulants not only acutely, but also in the longer term.

Materials and Methods

Animals and drug injections. Naive male Sprague–Dawley rats (250–350 gm; Charles River) were kept under 12 hr light/dark cycle with free access to food and water in the MIT animal facility for several days before being used, and were brought before injection to a quiet experimental room for at least 2 hr. Cocaine hydrochloride (Sigma) dissolved in saline was administered intraperitoneally (25 mg/kg) to rats at selected time points 45 min to 24 hr before death, as described below. The dose was chosen on the basis of a dose–response study indicating 25 mg/kg as a dose level at which consistent, strong induction of *NGFI*-A mRNA (Moratalla et al., 1991a, 1992) and Fos-like immunoreactivity (Graybiel et al., 1990; R. Moratalla and A. M. Graybiel, unpublished observations) could be detected. During the posttreatment interval, the animals were observed behaviorally and maintained under low-stress conditions.

Tissue preparation. For *in situ* hybridization analysis, rats were killed by decapitation at 45 min or 1 hr after cocaine treatment, and their brains were rapidly removed from the skull, frozen in powdered dry ice, wrapped in Parafilm, and stored at −70°C for at least 2 d. Transverse sections were cut at 15 μm on a cryostat, thaw mounted onto gelatin-coated microscope slides, dried under a cool air stream, and kept at −20°C until hybridization.

For Northern blot analysis, rats were decapitated at 45 min or 1, 2, 6, or 24 hr after cocaine treatment. The brains were removed, and samples from the striatum, cerebral cortex and hippocampus were quickly dissected out and frozen in liquid nitrogen under conditions designed to be RNase-free.

Hybridization probes. Oligonucleotide probes were carefully chosen to avoid sequences with high homology between members of the same gene family and at the same time to select sequences conserved across species, particularly for the *jun* members, for which only murine cDNA sequences were available. Probes were purchased from Bio-Synthesis Inc., Denton, TX. The probe sequences were as follows: c-*fos,* 48 bases long (5′-GAC-GCG-GGA-GGA-TGA-CGC-CTC-GTA-GTC-CGC-GTT-GAA-ACC-CGA-GAA-CAT-3′), complementary to the base sequence encoding amino acids 2–17 of the rat mRNA (Curran et al., 1987); *jun B,* 36 bases long (5′-GCC-CAG-GGA-CAC-GTT-GGG-GGG-CGT-CAC-GTG-GTT-CAT-3′), complementary to the base sequence encoding amino acids 146–157 of the mouse mRNA (Ryder et al., 1988); c-*jun,* 45 bases long (5′-GAC-CGG-CTG-TGC-CGC-GGA-GGT-GAC-ACT-GGG-AAG-CGT-GTT-CTG-GCT-3′), complementary to the base sequence of the mouse mRNA encoding amino acids 123–137 (Lamph et al., 1988); *jun D,* 36 bases long (5′-TGA-GGT-GGC-CGC-GGT-GGC-CGC-ACC-CAG-CTG-GCT-TTG-3′), complementary to the base sequence encoding amino acids 150–161 (Ryder et al., 1989); *fra*-1, 45 bases long (5′-GCT-ACC-CGC-CCC-ACT-CGT-GCT-GCT-GGT-ACC-ACC-TGT-GTC-CTT-CTT-3′), complementary to the base sequence encoding amino acids 181–195 (Cohen and Currant, 1988); *fra*-2, 45 bases long (5′-AGT-CCG-AAC-GCT-CTG-GAG-GCT-GCT-GGT-TGG-GCT-CCG-ACG-TTC-3′), complementary to the base sequence encoding amino acids 197–211 (Nishima et al., 1990); and *fos B,* 42 bases long (5′-ACT-GTG-TGT-AAA-GAG-AGA-AGC-CGT-CAG-GGG-GGG-TGC-GTC-3′), complementary to the base sequence encoding amino acids 271–284 (Zerial et al., 1989). Oligonucleotides complementary to c-*fos, jun B,* and c-*jun* probes were used as sense control probes to determine the specificity of the signal (see below). In addition, for Northern blotting, the *jun B* probe was the 1.9 kilobase (kb) EcoRI fragment of ATCC 63025 (Ryder et al., 1988); the c-*jun* probe was either the oligonucleotide or the 2.6 kb EcoRI fragment of ATCC 63026 (Ryder and Nathans, 1988); and the mouse c-*fos* probe was derived from the 1.5 kb EcoRI-SstI fragment of pc-*fos*-3 (Curran et al., 1983). The rat α-tubulin probe was derived from the 1.6 kb PstI fragment of the cDNA (Lemischka et al., 1981).

In situ *hybridization.* All oligonucleotide probes for *in situ* hybridization were labeled at the 3′ end with 100 μCi of ^{35}S-dATP [1500 Ci/mmol; DuPont/New England Nuclear (NEN)]. Eighty nanograms of oligonucleotide and 30 IU of terminal deoxynucleotidyl transferase (International Biotechnologies, Inc.) were reacted with the ^{35}S-dATP in a cobalt-containing buffer at 37°C for 30 min in a total volume of 25 μl,

and the reaction was stopped by adding 45 μl of STE buffer (0.1 M NaCl; 10 mM Tris HCl, pH 8; 1 mM EDTA, pH 8). Each sample was purified through a Nuctrap push column (Stratagene, Palo Alto, CA), yielding a specific activity of 0.6–3.0 × 10^8 cpm/μg.

Hybridizations were carried out following the procedures described by Baldino et al. (1989) and Young et al. (1986), with small modifications. Briefly, serial sections were fixed at room temperature for 5 min in 4% paraformaldehyde in 10 mM phosphate buffer with 120 mM NaCl and 2.7 mM KCl (PBS), pH 7.4, rinsed three times in PBS for 5 min, placed for 10 min in double-strength (2×) SSC solution (0.15 M NaCl, 15 mM sodium citrate), and defatted in a graded series of ethanols (50%, 70%, 95%, and 100%) for 3 min each followed by 10 min in chloroform. After being air dried, sections were hybridized overnight at 37°C in a humid chamber with 1–1.5 × 10^6 cpm of one of the labeled probes described above in 100 μl of the following hybridization solution: 50% (v/v) deionized formamide [Bethesda Research Labs (BRL)], 4× SSC; Denhardt's solution (Sigma) (bovine serum albumin, Ficoll, and polyvinylpyrrolidone, 0.02% each), 0.5 mg/ml of salmon sperm DNA (Sigma), 0.25 mg/ml yeast tRNA (BRL), 10% (v/v) dextran sulfate (Oncor Inc., Gaithersburg, MD), 100 mM dithiothreitol (DTT).

After hybridization, sections were brought to room temperature and rinsed twice in 2× SSC and then for 1 hr in 2× SSC, 1 hr in 1× SSC, 30 min in 0.5× SSC, 30 min in 0.5× SSC at 37°C, 30 min in 0.2× SSC, and 30 min in 0.1× SSC at room temperature before a final dipping in dH_2O for 10 sec. The temperature and salt concentration of the posthybridization solution with the highest stringency were below the melting temperatures of the oligodeoxynucleotides used. All posthybridization solutions contained 1% sodium thiosulfate, and the rinses were carried out on a shaker device.

The slides were dried and exposed to Hyperfilm βMax (Amersham) for 3–5 weeks before being developed. Selected slides were dipped in nuclear track emulsion NTB2 (Kodak) diluted 1:1 with 0.1% Dreft dissolved in dH_2O. Sections were dried and stored with desiccant at 4°C for 6–8 weeks, and were developed in Kodak D19. The sections were stained through the emulsion with cresylecht violet.

To check for cross-hybridization between the oligonucleotide probes used, we carried out a dot blot experiment. Samples containing 0.1, 0.01, and 0.001 ng of denatured full-length cDNAs of *c-fos, jun B,* and *c-jun* were applied to a nitrocellulose membrane and were hybridized with *c-fos, jun B,* and *c-jun* labeled oligonucleotide probes under the same conditions as for *in situ* hybridization. The dot blots were processed by film autoradiography as described above.

RNA isolation and Northern blots. RNA was isolated from dissected brain tissue by the sarkosyl/guanidinium isothiocyanate/cesium chloride procedure as described in Ausubel et al. (1989), with minor modifications. RNA was fractionated through 1% agarose formaldehyde gels and blotted to nitrocellulose or GeneScreenPlus membranes (NEN). Hybridizations to GeneScreenPlus membranes were performed according to manufacturer's instructions. Hybridizations to nitrocellulose were carried out overnight in (5×) SSPE (150 mM sodium chloride, 10 mM sodium phosphate monobasic, 1 mM EDTA, pH 7.4), 1× Denhardt's, 100 μg/ml single-stranded DNA, 10% dextran sulfate, 50% formamide at 42°C. Prehybridization was carried out in the same solution without probe for 1–12 hr. The most stringent wash of filters for double-stranded DNA probes was at 0.1× SSPE, 0.1% SDS for 30 min at 65°C. For reuse, blots were stripped of probe by incubating at 100°C for 10 min in 10 mM Tris, 1 mM EDTA, pH 8.0. Hybridizations using oligonucleotides as probes (*c-jun*) were carried out according to the protocol of Henderson et al. (1991) with the hybridization and final wash temperature of 55°C. Double-stranded DNA probes were labeled by random priming as described by Feinberg and Vogelstein (1983) and were purified on Elutip columns (Schleicher and Schuell). Oligonucleotide probes (*c-jun, jun B, jun D, fos B, fra-1,* and *fra-2*) were labeled by homopolymer tailing with terminal transferase. Briefly, 1 pmol of oligonucleotide was incubated in a 25 μl reaction with 100 μCi of ^{32}P-dATP and terminal transferase (10 U) in TdT buffer (BRL) for 1 hr at 37°C. Unincorporated nucleotides were removed by passing the reaction mixture over a Sephadex G50 column. Hybridization probes were used at a concentration at least 1 × 10^6 cpm/ml.

Results

Coordinate induction of c-fos *and* jun B *by cocaine*

To study the influence of acute cocaine treatment on the expression of *c-fos* and *jun B* in the striatum, we first carried out a Northern blot analysis of RNA isolated from the caudoputamen 60 min after a single intraperitoneal injection of cocaine (25 mg/kg). We analyzed neocortical and hippocampal samples as controls for the specificity of the striatal responses. Autoradiograms of nitrocellulose blots respectively hybridized with *c-fos* and *jun B* radiolabeled probes are shown in Figure 1. Cocaine induced the expression of the 2.2 kb *c-fos* and 2.1 kb *jun B* mRNAs in the striatum within 60 min of stimulation. For both genes, there was a lower level of induction in the neocortex and little or no expression in the hippocampus. The level of expression of *c-fos* in the neocortex in the saline-treated animals varied between experiments. Tissue from untreated rats showed little or no augmented expression of these proto-oncogenes.

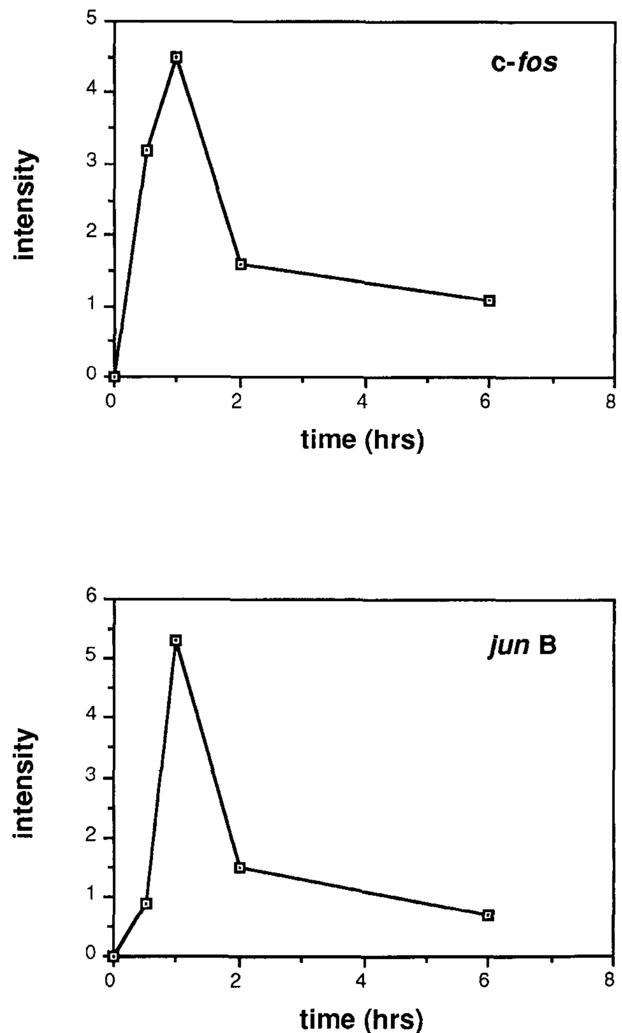


Figure 2. Time course of induction of *c-fos* and *jun B* mRNAs by cocaine in the rat striatum. Rats were injected with cocaine (25 mg/kg, i.p.), and striatal RNA was isolated at indicated time points and then was analyzed by Northern blotting as described in Materials and Methods. Intensity of expression was measured on a Molecular Dynamics phosphorimager and is given in arbitrary units.

Figure 2 shows the time course of expression of *c-fos* and *jun B* evoked in the striatum in response to cocaine. Both genes were transiently induced and showed a maximum expression at approximately 60 min after injection. Two hours after injection, expression of *c-fos* and *jun B* mRNAs had returned to levels only slightly elevated above baseline. Thus, *c-fos* and *jun B* are transiently induced with kinetics similar to those that have been

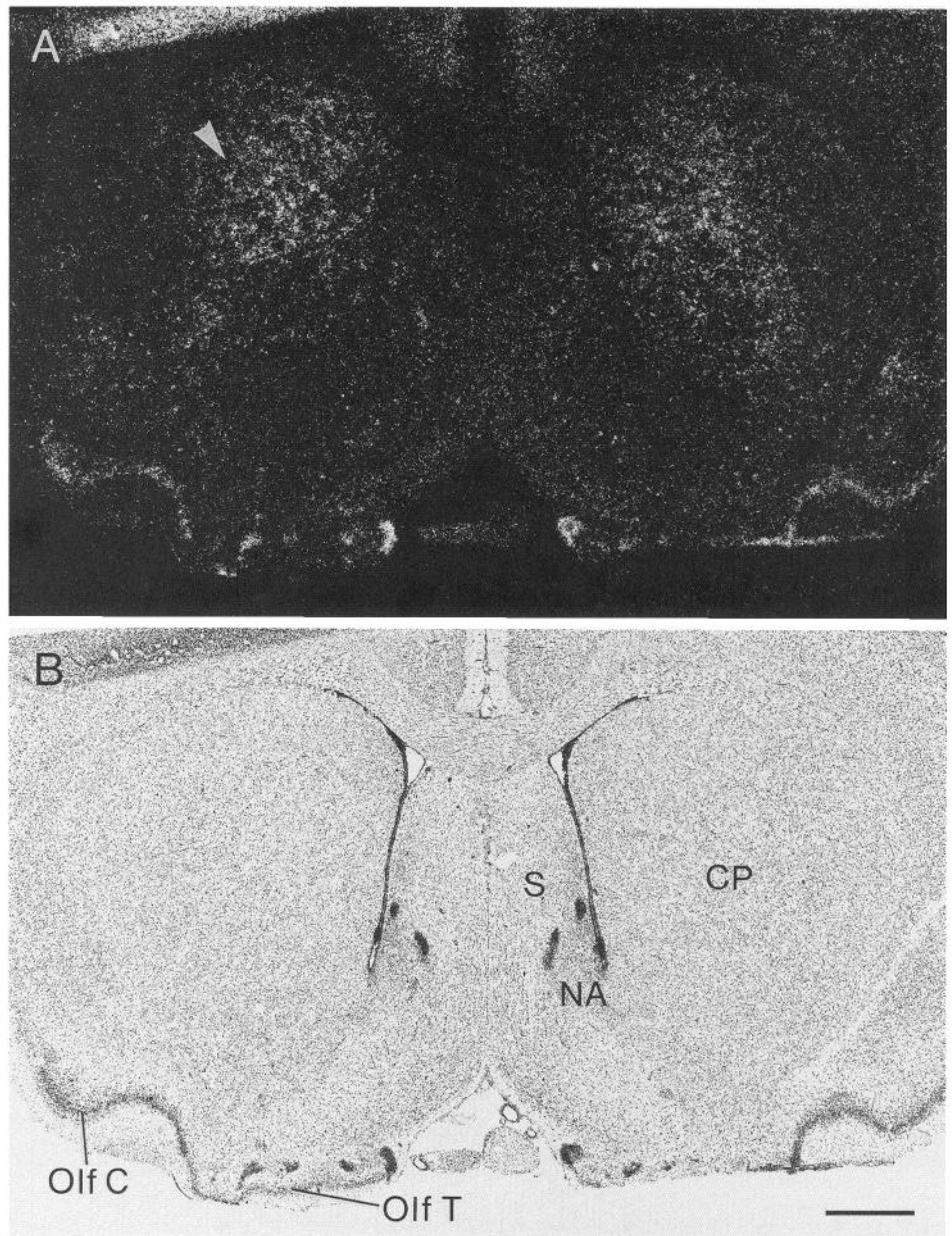


Figure 3. *A, In situ* hybridization autoradiogram illustrating the pattern of expression of c-*fos* mRNA in the striatum of a rat treated with 25 mg/kg intraperitoneal cocaine (45 min survival time). Radiolabeling appears *white. White flap at top left* is a tissue fold. *B,* Pattern of Nissl staining of the section shown in *A*. Note (see *arrowhead* in *A*) that there is an intense focus of mRNA labeling in a funnel-shaped region in the middle and medial caudoputamen (*CP*), and hybridization signal appears also in the olfactory cortex (*Olf C*) and in the olfactory tubercle (*Olf T*). *NA,* nucleus accumbens; *S,* septum. Scale bar, 1 mm.

found for cultured cells (Bartel et al., 1989). The peak of expression of the two genes is 15–30 min later than seen in cultured cells, a difference that could reflect tissue differences as well as greater time required for access of cocaine to the responding cells. The absolute levels of *jun B* expression appeared to be higher than those of c-*fos* expression both by Northern blot analysis and by *in situ* hybridization (see below).

Sites of c-fos *and* jun B *induction in the striatum following cocaine administration*

To determine the distribution of the induced c-*fos* and *jun B* expression in the striatum, we carried out *in situ* hybridization experiments with ^{35}S-labeled oligonucleotide probes (Figs. 3–6). As shown in Figure 3*A,* intense activation of c-*fos* mRNA was evoked in the striatum by 45 min after a single intraperitoneal injection of cocaine (25 mg/kg). The *in situ* hybridization signal was characteristically strongest in the middle and dorsomedial caudoputamen, was much weaker in the lateral caudoputamen, and was intermediate to weak in the nucleus accumbens. Expression of c-*fos* mRNA was pronounced in the olfactory tubercle and was also strong in the piriform cortex. Very little c-*fos* mRNA was detectable in the caudoputamen in saline-treated controls (Fig. 5*A′*), although signal was present in the olfactory cortex. No elevated signal was visible in sections hybridized with sense c-*fos* probe (Fig. 6*B*).

Emulsion-coated sections were prepared for *in situ* hybridization autoradiography with the c-*fos* probe to identify the cellular localization of the induced mRNA within the striatum. A representative section is shown in Figure 4. Medium-sized neurons resembling medium-sized projection neurons were labeled. There was no evidence for labeling of glial cells or giant striatal neurons.

Hybridization autoradiograms for *jun B* mRNA in the same brains (Fig. 5*B*) demonstrated that acute cocaine treatment induced *jun B* mRNA in the striatum in a pattern very similar to that of c-*fos* mRNA. In the saline-treated controls, only very

low levels of expression of the *jun B* mRNA were present in the striatum except in the olfactory tubercle, which, like the olfactory cortex, showed particularly marked expression of *jun B* mRNA (Fig. 5*B'*). Sense controls (Fig. 6*C*) showed no signal above background other than weak labeling in the olfactory cortex. The c-*fos* and *jun B* oligonucleotide probes were gene specific and did not cross-hybridize to c-*fos* or *jun B* cDNAs on dot blots under the hybridization conditions used for the *in situ* hybridization (see sequences in Materials and Methods, and data not shown).

Lack of induction of c-jun *by cocaine*

Because c-*jun* is often coordinately regulated with *jun B* (Lamph et al., 1988; Ryder and Nathans, 1988), we tested whether this gene is also induced by cocaine in the striatum. From Figures 5*C* and 6*D*, it is clear that no induction was found at a 1 hr time point by *in situ* hybridization autoradiography. A similarly negative result was obtained by Northern blot analysis (Fig. 7). Little or no induction of c-*jun* could be detected in the striatum at 1, 6, 12, or 24 hr after injection of cocaine (Fig. 7). Neither technique demonstrated induction of c-*jun* in the hippocampus or cerebral cortex (data not shown).

As controls for detection of c-*jun* mRNA, we carried out two experiments. First, for the Northern blot analysis, RNA was extracted from Balb/c-3T3 cells (clone A31) after treatment with serum and cycloheximide for 1 hr. As expected, this treatment induced 3.2 and 2.7 kb c-*jun* mRNAs (Fig. 7). To test the efficacy of our c-*jun* probe for *in situ* hybridization, we used it, along with probes for c-*fos* and *jun B,* to assay for induction of the three genes in the hippocampus following treatment with pentylenetetrazol (Metrazol, 50 mg/kg, 1 hr survival time). This seizure-inducing stimulation is known to lead to rapid induction of all three mRNAs (Saffen et al., 1988), and this pattern of coinduction of c-*fos, jun B,* and c-*jun* was evident in our experiments (Fig. 8). There was marked expression of the three mRNAs in the dentate gyrus and moderate expression in the CA fields. Coexpression was not, however, evident everywhere in these brains. For example, only with the c-*fos* mRNA probe was strong activation apparent in the cortex and thalamus (Fig. 8*A*).

Expression of other fos/jun *family genes in the striatum in response to cocaine*

We tested for induction of other *fos/jun* family genes in the striatum of cocaine-treated animals by carrying out Northern blot analysis with oligonucleotide probes. *Jun D* showed constitutive levels of expression at all time points examined (Fig. 7). Thus, all three *jun* family genes show different patterns of expression in the striatum. We found that *fra*-1 and *fra*-2 were expressed at low levels in the striatum, and that their expression did not change appreciably after cocaine injection (data not shown). *Fos B* mRNA expression was undetectable by Northern blot of striatal RNA at any time before or after cocaine administration. Thus, striatal expression of the *fos* family members also exhibits heterogeneity in responsiveness to cocaine.

Discussion

We have found that in the rat's striatum, *in vivo* exposure to cocaine rapidly and transiently induces c-*fos* and *jun B,* members of the leucine-zipper AP-1 transcription factor family. The parallel kinetics of induction of the two genes, and the similarity in the anatomical distributions of their mRNAs, suggest that their regulation is coordinate. By contrast, another member of this immediate-early gene transcription factor family, c-*jun,* was not induced in the striatum following cocaine treatment, and a third, *jun D,* was constitutively expressed. This evidence for a selective pattern of *fos/jun* gene induction, taken together with previous reports (Graybiel et al., 1990; Young et al., 1991), strongly suggests that the genomic response to cocaine is not only brain region specific but also gene specific.

With a polyclonal antiserum raised against a conserved c-Fos peptide, Young et al. (1991) observed multiple immunoreactive proteins by Western blot analysis of striatal nuclei. In their study, the expression of a 55 kDa and of a 35 kDa protein was markedly enhanced by cocaine administration. Proteins of 35, 41, and 44 kDa also showed significant basal levels of expression. The relationships between these protein bands and the known *fos* gene family members were not determined in this study. However, the 55 kDa protein is presumably c-Fos, and its induction would be consistent with our observations on the induction of the c-*fos* mRNA by cocaine.

Studies on the regulation of c-*fos,* c-*jun,* and *jun B* by growth factors in fibroblasts and PC12 cells *in vitro* indicate that these genes can be coinduced, but that the coinduction is specific for particular stimulus conditions (Lamph et al., 1988; Ryder and Nathans, 1988; Ryder et al., 1988; Bartel et al., 1989; Mechta et al., 1989; Auwerx et al., 1990). The pattern of c-*fos,* c-*jun,* and *jun B* expression that we have observed in the striatum in

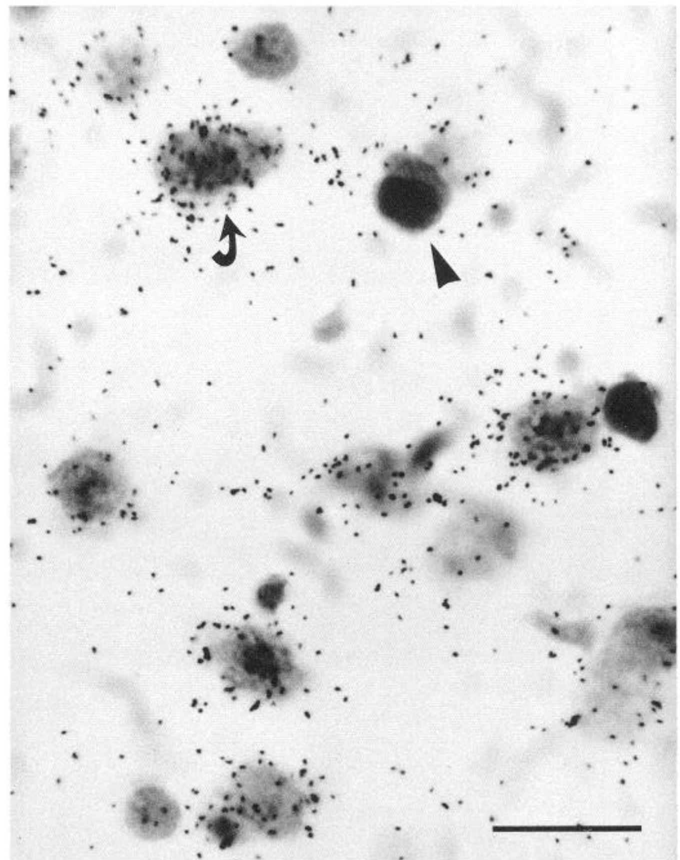
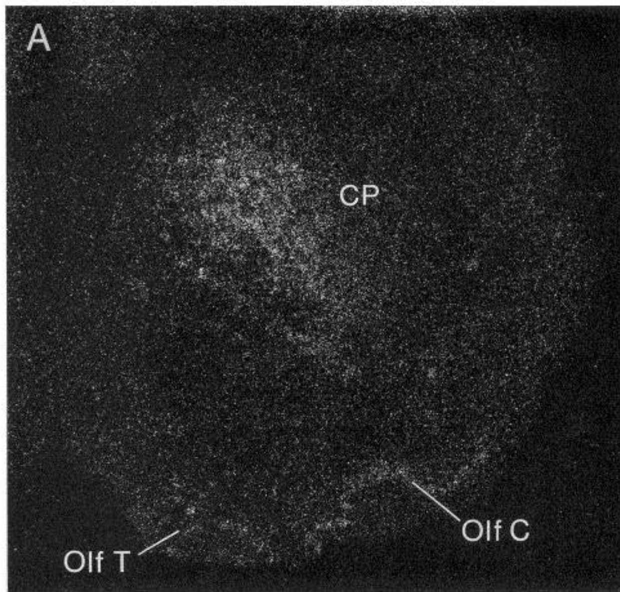


Figure 4. Photomicrograph of emulsion autoradiogram from a cocaine-treated rat (25 mg/kg, i.p.; 45 min survival time), illustrating c-*fos* mRNA *in situ* hybridization signal (*black dots* in autoradiogram) associated with medium-sized neurons of caudoputamen (example at *curved arrow*). Labeling of glial cells (example at *arrowhead*) was not observed. Scale bar, 20 μm.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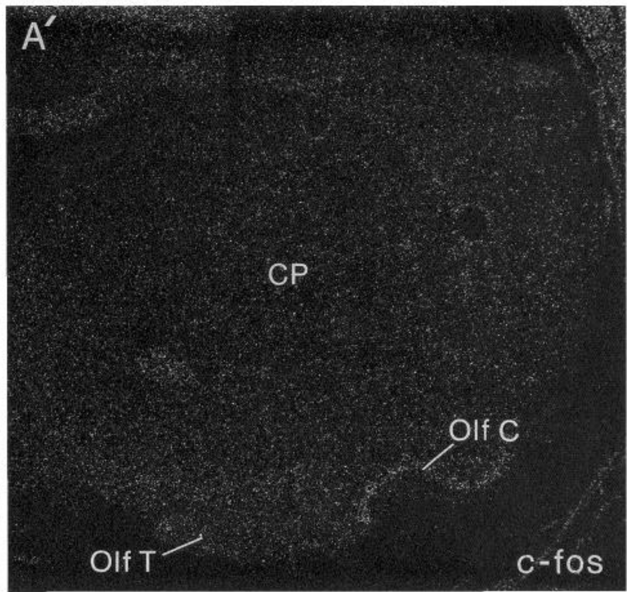
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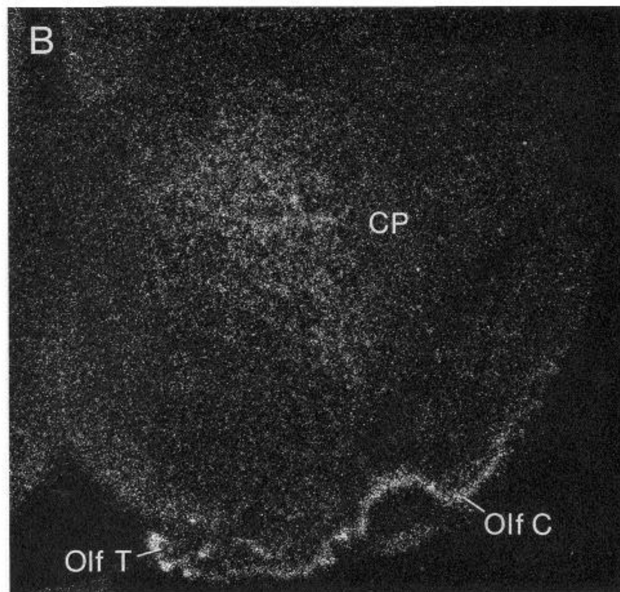
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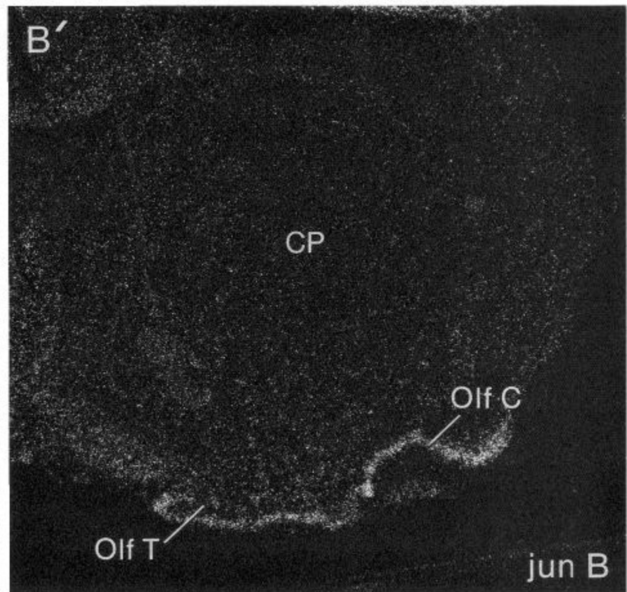
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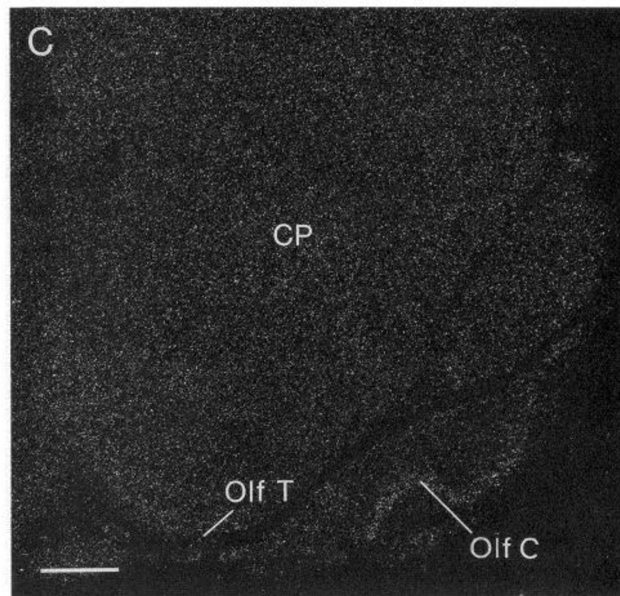
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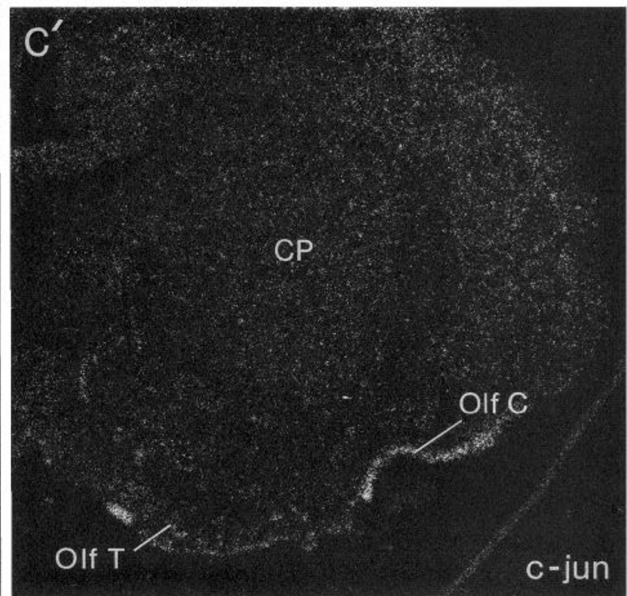
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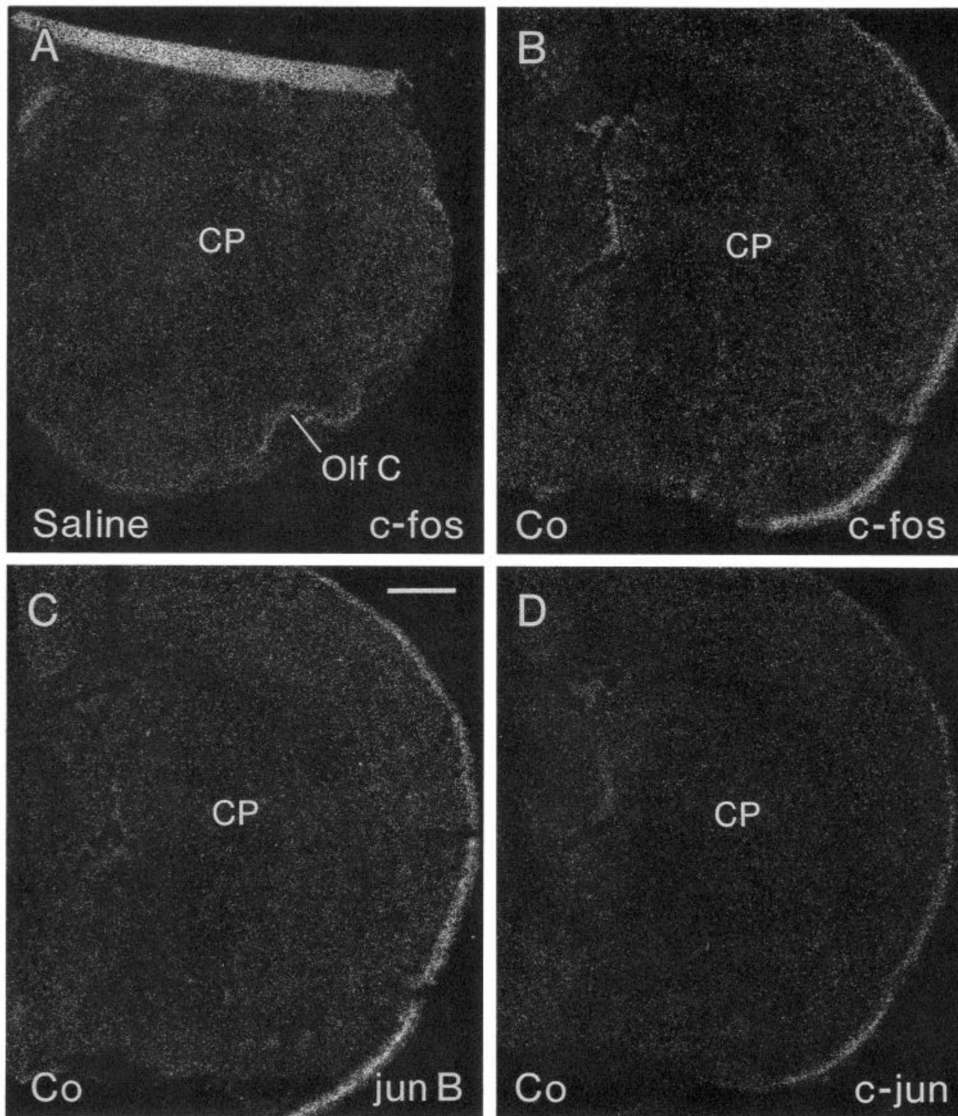

Figure 6. Control experiments demonstrating specificity of gene induction patterns: *in situ* hybridization autoradiograms from saline-treated rat brain (*A*) and from cocaine-treated rat brains (*B–D*; 25 mg/kg, cocaine, i.p.; 1 hr survival time). Sections illustrated were processed with radiolabeled anti-sense probe for c-*fos* (*A*), and with radiolabeled sense probes for c-*fos* (*B*), *jun B* (*C*), and c-*jun* (*D*). Except for very weak signal sometimes present in the olfactory cortex (*Olf C*; see *A*), no labeling above background was present. *White rims* around some of the sections represent edge artifact; *white flap* in *A* is tissue fold. *CP*, caudoputamen. Scale bar, 1 mm.

response to cocaine is strikingly similar to that induced by membrane depolarization of PC12 cells (Bartel et al., 1989) and by cAMP in fibroblasts (Mechta et al., 1989). In PC12 cells, NGF and EGF can induce c-*fos*, *jun B*, and c-*jun* coordinately, but membrane depolarization induced by KCl or nicotine stimulates the induction of c-*fos* and *jun B* without coordinate induction of c-*jun* (Bartel et al., 1989). In fibroblasts, cAMP analogs induce c-*fos* and *jun B*, but inhibit c-*jun* (Mechta et al., 1989). In addition, previous work on PC12 cells has implicated calcium and voltage-gated calcium channels in the signaling pathways by which membrane depolarization induces c-*fos* (Morgan and Curran, 1986).

Thus, if striatal neurons are responding in a similar manner, plausible candidates for the second messengers mediating cocaine's induction of c-*fos* and *jun B* are intracellular calcium or cAMP, or a combination of the two. Induction via the cAMP/protein kinase A pathway would be consistent with the fact that the dopamine D1-like receptor antagonist SCH23390 blocks induction of Fos and Fos-related antigens by cocaine (Graybiel et al., 1990; Young et al., 1991; Berretta et al., 1992). Stimulation of D1-like receptors is known to generate a cAMP second signal (Stoof and Kebabian, 1981). In *Xenopus* oocytes, it has been reported that some D1-like receptors are positively linked to inositol phospholipid (inositol trisphosphate) messenger systems (Mahan et al., 1990). Thus, induction via a D1-like receptor–mediated increase in Ca^{2+} flux may also occur. These results also leave open the possibility that other receptor systems are involved, including 5-HT receptors, given that SCH23390 is known to block some 5-HT receptors in rodent brain (Bischoff et al., 1986). Responses mediated by cAMP and Ca^{2+} are known to be generated by some 5-HT receptor subtypes as well (Julius, 1991).

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Figure 5. *A–C*, Demonstration of differential expression of *fos/jun* genes induced in the striatum by cocaine (25 mg/kg, i.p.; 1 hr survival time). *In situ* hybridization autoradiograms illustrate selective induction of c-*fos* mRNA (*A*) and *jun B* mRNA (*B*) but not c-*jun* mRNA (*C*) in the caudoputamen following cocaine treatment. *A′–C′*, *In situ* hybridization autoradiograms from control rats treated with intraperitoneal saline 1 hr before tissue harvesting, prepared with the same probes for c-*fos* (*A′*), *jun B* (*B′*), and c-*jun* (*C′*) used in the cocaine experiments. Little signal is present following saline treatment except in the olfactory cortex and olfactory tubercle. Abbreviations are as in Figure 3. Scale bar, 1 mm.

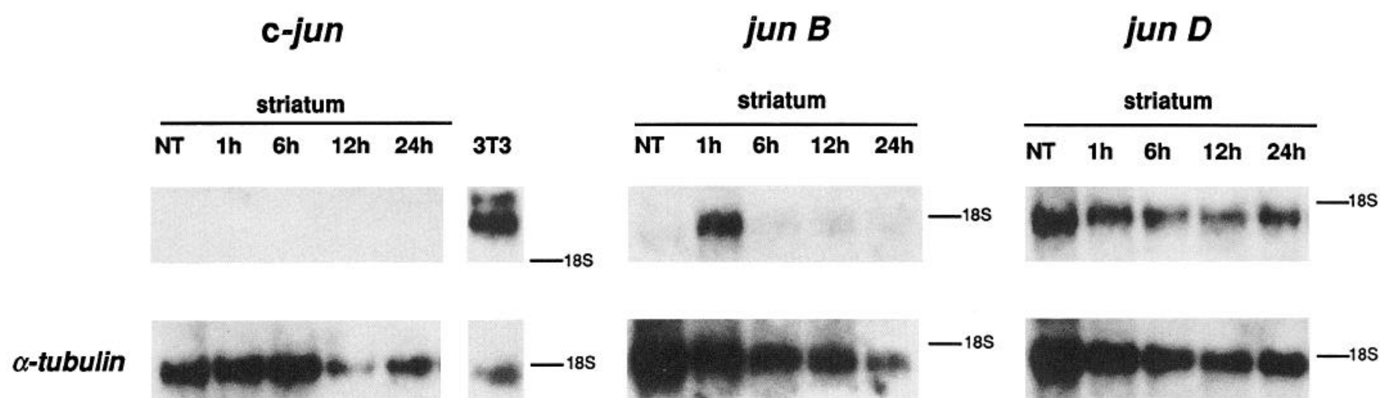

Figure 7. Northern blot analysis demonstrating different patterns of expression of *jun* family mRNAs in the rat striatum. Rats were injected intraperitoneally with cocaine (25 mg/kg) and were killed at indicated times after injection, or were untreated (*NT*). For each time point, 5 μg of RNA were fractionated through formaldehyde-agarose gels, blotted to nitrocellulose, and hybridized to c-*jun, jun B,* or *jun D* probes. In parallel, 5 μg of RNA from quiescent Balb/c-3T3 cells (clone A31) that were treated for 3 hr with 10% calf serum and 10 μg/ml cycloheximide were hybridized to the c-*jun* probe. After exposure to x-ray film, each blot was stripped and rehybridized to a probe for α-tubulin.

Figure 8. Demonstration of coordinate induction of *fos/jun* mRNAs in hippocampus in rats treated with metrazol 1 hr before death. *In situ* hybridization autoradiograms of sections through the hippocampus show coinduction of c-*fos* (*A*), *jun B* (*B*), and c-*jun* (*C*) mRNAs in the dentate gyrus (*dg*) and CA fields of the hippocampus (*CA*). *A′–C′* demonstrate lack of hybridization signal in hippocampus of saline-treated control rats (only right hippocampus is shown in each panel). The *in situ* hybridization was carried out with the same probes used for the cocaine experiments (Figs. 3–6). Scale bar, 1 mm.

A key question raised by our findings is whether the complexity of the immediate-early gene response to cocaine can be understood in terms of what is known about transcriptional control by elements upstream of these genes. The analysis of regulatory elements of c-*fos*, c-*jun*, and *jun B* is far from complete, but the information so far available suggests a plausible accounting for the divergent patterns we found. The c-*fos* promoter, the best studied of these promoters, contains elements that are responsive to platelet-derived growth factor (PDGF), serum, cAMP, phorbol esters, and calcium (Verma and Sassone-Corsi, 1987). The element at −65 upstream of the human c-*fos* gene is calcium- and cAMP-responsive (CaRE/CRE), and would therefore be a good candidate element for mediating the response to cocaine (Sheng et al., 1988, 1990; Berkowitz et al., 1989). The *jun B* promotor does not have a serum response element such as c-*fos* does or a 12-0-tetradecanoylphorbol 13-acetate (TPA)-responsive element (TRE or AP-1 site) like the one shown to be important in the mediation of c-*jun* induction (Angel et al., 1988b). However, the *jun B* promoter does contain a novel inverted repeat element that can mediate responses to protein kinase A and protein kinase C (de Groot et al., 1991). Elements of the *jun B* promoter that might respond to calcium have not been characterized.

The major regulatory element upstream of c-*jun* appears to be a TRE or AP-1-type site (Angel et al., 1988b). In some cells, the c-*jun* promotor is responsive to phorbol esters, but not to elevated levels of cAMP (Chiu et al., 1989; Auwerx et al., 1990). When the c-*jun* TRE is placed upstream of a thymidine kinase promoter chloramphenicol acetyl transferase gene fusion (TK CAT) construct and is cotransfected with protein kinase A, CAT expression is inhibited (de Groot et al., 1991). Moreover, the c-*jun* TRE can be inhibited by *jun B* expression (Chiu et al., 1989). The coordinate induction of c-*fos* and *jun B*, but not c-*jun*, by cocaine could, from this evidence, result from the generation of a cAMP and/or calcium-mediated second signal that activates c-*fos* expression through −65 CaRE/CRE element and *jun B* expression through the inverted repeat element. C-*jun* would not be expressed either because the protein kinase C pathway is not activated or because it is actively repressed by cAMP or *jun B*.

Members of the *fos*/*jun* family of transcription factor genes have been proposed to be couplers between short-term neural activity (seconds to minutes) and longer-term changes in neural signaling (hours/day) (Berridge, 1986; Goelet et al., 1986; Morgan and Curran, 1989; Dash et al., 1990). Cocaine is known to have extensive long-term effects, and we show here that even acute cocaine treatment induces significant levels of a c-*fos* and *jun B* out within striatal neurons. This complex would be likely to have positive and/or negative regulatory effects on genes containing AP-1-like regulatory elements. Moreover, the fact that c-Fos/Jun B complexes can interact with CREs suggests that the induction of these genes could further influence signaling through these elements (Zerial et al., 1989; Dwarki et al., 1990; Macgregor et al., 1990; Ryseck and Bravo, 1991). The target genes for such effects are unknown, but might include genes that code for molecules involved in neurotransmission (Sonnenberg et al., 1989b; Graybiel, 1990; Jiang et al., 1990; Nestler et al., 1990; Gerfen et al., 1991; Naranjo et al., 1991).

Our results raise the possibility that the c-Fos/Jun B transcription factor complex may play a physiological role in the response of striatal neurons to psychomotor stimulants. We have also shown that cocaine and amphetamine rapidly induce *NGFI-A* (*zif* 268, *egr* 1) in striatal neurons in anatomical patterns similar to those shown here for c-*fos* and *jun B* (Moratalla et al., 1991a,b, 1992). Together, these findings point to a highly selective induction of immediate-early genes by agents stimulating monoamine receptors in the striatum. These differential patterns of induction may help in identifying the molecular effects of dopamine and other monoamines on striatal neurons.

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