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Dynamic Regulation of *NGFI*-A (*zif*268, *egr*1) Gene Expression in the Striatum

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The expression of immediate-early genes of the *fos/jun* leucine zipper family can be regulated in striatal neurons by stimuli affecting the dopaminergic nigrostriatal system. The regulatory effects are gene specific, region specific, and striatal compartment specific. In the experiments reported here, we have explored the possibility that dopaminergic stimulation might also affect striatal expression of *NGFI*-A, a member of the zinc finger family of immediate-early genes. We treated healthy adult rats with amphetamine or cocaine and monitored the acute response of striatal neurons by *in situ* hybridization with oligonucleotide probes for *NGFI*-A mRNA. Both drugs evoked rapid, anatomically patterned increases in *NGFI*-A mRNA expression in the dorsal striatum (caudoputamen) and in the ventral striatum (nucleus accumbens, olfactory tubercle). The main response to each drug was in medium-sized neurons, known to be the projection neurons of the striatum. At every dose of amphetamine eliciting a response, the increased *NGFI*-A mRNA expression was preferentially concentrated in striosomes of the rostral caudoputamen, whereas cocaine at each dose given induced expression of *NGFI*-A mRNA in both striosomes and matrix at these striatal levels. The two indirect agonists evoked *NGFI*-A expression in both striatal compartments farther caudally, especially in the central and medial caudoputamen. Activation by both drugs was blocked by pretreatment with the D1-selective dopamine receptor antagonist SCH23390. These patterns of *NGFI*-A activation are remarkably similar to those found for Fos-like immunoreactivity following acute amphetamine and cocaine treatments, suggesting that coordinate activation of members of at least two immediate-early gene families occurs in the striatum following catecholaminergic stimulation. Such patterns of induction strongly support the view that the genomic responsiveness of the striosome and of the matrix compartments of the rostral striatum are distinct at the level of early-response gene expression. These findings raise the interesting possibility that some of the well-known effects of dopaminergic stimulation on neuropeptide and neurotransmitter expression in the striatum may reflect particular combinatorial patterns of immediate-early gene activation.

Striosomes and matrix are neurochemically distinct macroscopic compartments characteristic of the striatum of many mammalian forms including humans. In the past dozen years, a very large number of neurotransmitter-related differences between these two compartments have been identified in anatomical studies, and striosomes and matrix have been shown to have different patterns of input–output connections and different developmental histories (see Graybiel, 1990, for review). This evidence and the results of push–pull cannula experiments testing dopamine release in the striatum (Gauchy et al., 1987; Kemel et al., 1989) provide indirect support for the idea that the two compartments represent functionally distinct subsystems in the striatum, striosomes being especially linked to certain limbic regions and matrix to certain sensorimotor regions of the forebrain (Donoghue and Herkenham, 1986; Ragsdale and Graybiel, 1988; Gerfen, 1989; Graybiel, 1990). Direct evidence for functional differences between striosomes and matrix is still lacking, however, and in particular, physiological evidence on this question has been difficult to obtain because of the low levels of firing of most striatal neurons in acute *in vivo* and *in vitro* preparations (DeLong and Georgopoulos, 1981; Misgeld et al., 1984; Nisenbaum et al., 1988).

Because of these difficulties, we recently attempted to monitor functional activation of neurons in the two striatal compartments by eliciting activation of immediate-early genes in the striatum by dopamine agonist stimulation (Graybiel et al., 1990; Moratalla et al., 1990). We took advantage of earlier findings that a variety of stimuli can induce expression of immediate-early gene mRNAs and gene products in system-specific patterns in the brain and spinal cord (Dragunow and Robertson, 1987; Hunt et al., 1987; Morgan et al., 1987; Sagar et al., 1988; Robertson et al., 1989; for review, see Sheng and Greenberg, 1990). Our strategy was to treat rats with drugs that are known to act on the nigrostriatal system, and to test anatomically for acute changes in expression of Fos-like protein in the striatum. We chose as drugs amphetamine and cocaine, indirect dopamine agonists that increase dopamine levels in the striatum by releasing catecholamines (the main effect of amphetamine) or binding to the dopamine transporter (the main effect of cocaine). These drugs induced strikingly different anatomical patterns of Fos-like immunoreactivity in the striosome and matrix compartments of the rostral caudoputamen. Amphetamine activated expression of Fos-like immunoreactivity that was most in-

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tense in the striosomal compartment, whereas cocaine induced apparently equivalent expression of Fos-like protein in striosomes and matrix, especially centrally and medially. The expression of Fos-like protein stimulated by each drug was blocked by the D1-like dopamine receptor–preferring antagonist SCH23390, indicating that the induction was receptor mediated. The induction by cocaine was abolished by pretreatment with reserpine, but the induction by amphetamine was reserpine resistent. These findings raised the possibility that the striosome and matrix systems of the rostral caudoputamen may be functionally activated through stimuli affecting different intracellular signaling systems related to biogenic amine receptors. Young et al. (1991) and Cole et al. (1990) have also demonstrated apparent D1 dopamine receptor selectivity of striatal c-*fos* induction by cocaine and amphetamine.

In the experiments reported here, we set out to test the generality of this compartment-selective pattern of acute genomic response by determining the effects of dopamine agonist treatments on striatal expression of *NGFI*-A (*zif*/268, *egr*1, and *krox*24), a member of the zinc finger family of immediate-early genes (Lau and Nathans, 1987; Milbrandt, 1987; Sukhatme et al., 1987, 1988; Christy et al., 1988; Lemaire et al., 1988; Waters et al., 1990). *NGFI*-A is highly responsive to neural stimulation and, in the hippocampal model of long-term potentiation (LTP), is more consistently induced by the high-frequency stimulation required for LTP there than are c-*fos* and other leucine zipper genes so far examined (Saffen et al., 1988; Cole et al., 1989; Wisden et al., 1990). We document here that amphetamine and cocaine rapidly increase the expression of *NGFI*-A mRNA in the caudoputamen and ventral striatum, and demonstrate that in the rostral caudoputamen the effects are striosome selective in response to amphetamine but not in response to cocaine over a large range of drug doses. Compartmental selectivity in the genomic response of striatal neurons to psychomotor stimulants thus holds for members of at least two families of immediate-early genes whose gene products have different DNA binding motifs and may act in different combinatorial patterns in response to different sets of intracellular signals.

Materials and Methods

All experiments were carried out on adult male Sprague–Dawley rats (230–350 gm) kept under a 12 hr light/12 hr dark cycle, with free access to food and water. On the day of the experiment, animals were brought to a quiet (low-stress) room, and drugs or saline were administered after at least 4 hr of light cycle. Drugs were dissolved in saline and were delivered by intraperitoneal injections at the following doses: *d*-amphetamine sulfate (Sigma), 0.5 mg/kg ($n = 2$), 2 mg/kg ($n = 3$), 5 mg/kg ($n = 3$), 10 mg/kg ($n = 3$), or 15 mg/kg ($n = 4$); cocaine hydrochloride (Sigma), 5 mg/kg ($n = 3$), 10 mg/kg ($n = 3$), 25 mg/kg ($n = 4$), or 50 mg/kg ($n = 4$); SCH23390 (Schering-Plough), 0.5 mg/kg given 30 min before 5 mg/kg amphetamine ($n = 4$) or 25 mg/kg cocaine ($n = 3$). The doses of amphetamine and cocaine are expressed as their respective salts. After treatment, animals were observed for behavioral effects. Both drugs increased locomotor activity and induced stereotyped behavior in a dose-dependent fashion, with increases in head movement, sniffing, and rearing at higher doses. The effects of cocaine were evident 5–10 min after administration, and those of amphetamine at 15–20 min or even later. SCH23390 completely blocked the behavioral effects of amphetamine and cocaine.

Tissue preparation. One hour following the last drug treatment, the rats were decapitated, and their brains were rapidly removed and placed in powdered dry ice until frozen. Transverse sections were cut at 10–15 μm on a cryostat, thaw mounted onto gelatin-coated slides, air dried, and stored at −70°C until use.

Probe labeling. A 45 base oligonucleotide probe complementary to amino acids 2–16 coded by *NGFI*-A (*zif*/268) protein was used. Its sequence was 5′-CCG-TTG-CTC-AGC-AGC-ATC-ATC-TCC-TCC-AGT-TTG-GGG-TAG-TTG-TCC-3′ (Bio-synthesis, Denton, TX). The probe was labeled at the 3′ end with ^{35}S-dATP (Du Pont/NEN) and terminal deoxynucleotidyl transferase (International Biotechnologies Incorporated) in a cobalt-containing buffer, and was purified in a NEN-sorb column (Du Pont/NEN) or Nunctrap push column (Stratagene), yielding a final radiolabeling of 0.4–1.2 × 10^6 cpm/μl. For *in situ* hybridization controls, an oligonucleotide complementary to the antisense probe cited above was prepared according to the same protocol.

In situ *hybridization.* Selected sets of sections were fixed at room temperature for 5 min in 4% paraformaldehyde with 10 mM phosphate-buffered saline (PBS), pH 7.4, and were then rinsed in PBS three times for 5 min each; placed in double-strength (2×) saline–sodium citrate (SSC) buffer (0.15 M NaCl, 15 mM sodium citrate) for 10 min; dehydrated by sequential immersion in 50%, 70%, 95%, and 100% ethanols (3 min each); delipidated in chloroform for 10 min; and dried under a cool stream of air. Sections were hybridized overnight at 37°C in a humid chamber with 1 × 10^6 cpm of labeled oligonucleotide per 100 μl of the hybridization buffer containing 50% deionized formamide, 4 × SSC, 1 × Denhardt's solution (0.02% bovine serum albumin, 0.02% ficoll, 0.02% polyvinylpyrrolidone), 500 μg/ml salmon sperm DNA, 250 μg/ml yeast tRNA, 10% w/v dextran sulfate, 100 mM dithiothreitol. After incubation, slides were rinsed twice in 2× SSC and washed at room temperature, unless otherwise indicated, as follows: 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, 30 min in 0.1× SSC, and distilled water for 10 sec. All solutions contained 1% sodium thiosulfate and were continuously shaken. Once dried, sections were apposed to Hyperfilm βmax (Amersham) for 3–8 weeks. After the films were developed, selected slides were dipped in NTB2 photographic emulsion (Kodak) applied at a 1:1 ratio with 0.1% Dreft detergent in dH_2O, dried, and stored at 4°C for 6–8 weeks in light-tight boxes containing desiccant. After development, sections were stained for Nissl substance with cresylecht violet.

Opiate receptor autoradiography. Frozen sections adjacent to those chosen for *in situ* hybridization were brought to room temperature, preincubated for 5 min at 4°C in 50 mM Tris-HCl buffer in 100 μM NaCl, and incubated for 60 min at 4°C in the same buffer containing 2.5 nM ^{3}H-naloxone (Du Pont/NEN; 30.5 Ci/mmol). After incubation, sections were washed three times in 50 mM PBS for 20 sec at 4°C, briefly rinsed in distilled water, dried, and apposed to tritium-sensitive film (LKB Ultrofilm) for 6 weeks. Nonspecific binding was determined by the addition of unlabeled naloxone, 10^{-6} M (Sigma), to the incubation solution.

Results

Dose-dependent increases in NGFI-*A mRNA expression occur following acute amphetamine treatment*

Treatment with amphetamine produced a dose-dependent increase in *NGFI*-A mRNA expression in the striatum, both in its dorsal part, the caudoputamen, and in its ventral part (including the nucleus accumbens and the olfactory tubercle) (Fig. 1*A–D*). Low doses of amphetamine (0.5 mg/kg) did not produce a detectable increase in *NGFI*-A in the striatum. The hybridization signal in these brains was similar to that in brains from control rats treated with saline (see Fig. 7*C*). There was considerable signal in the cerebral cortex and strong signal in the piriform cortex. *NGFI*-A mRNA transcripts were also detectable in the striatum, but at much lower levels (cf Schlingensiepen et al., 1991). This control level of hybridization signal appeared to be homogeneously distributed within the caudoputamen and nucleus accumbens; there was slightly more signal in the olfactory tubercle than elsewhere in the striatum. Only one rat out of the three treated with 2 mg/kg exhibited a weak increase in the hybridization signal in the striatum. At 5 mg/kg, amphetamine always induced a strong and reliable increase in *NGFI*-A mRNA expression, as it did at higher doses.

In the rostral caudoputamen (Figs. 1*A–D*, 2*A*), the *in situ* radiolabeling induced by amphetamine at each dose level had a highly heterogeneous distribution in which small (~100–300

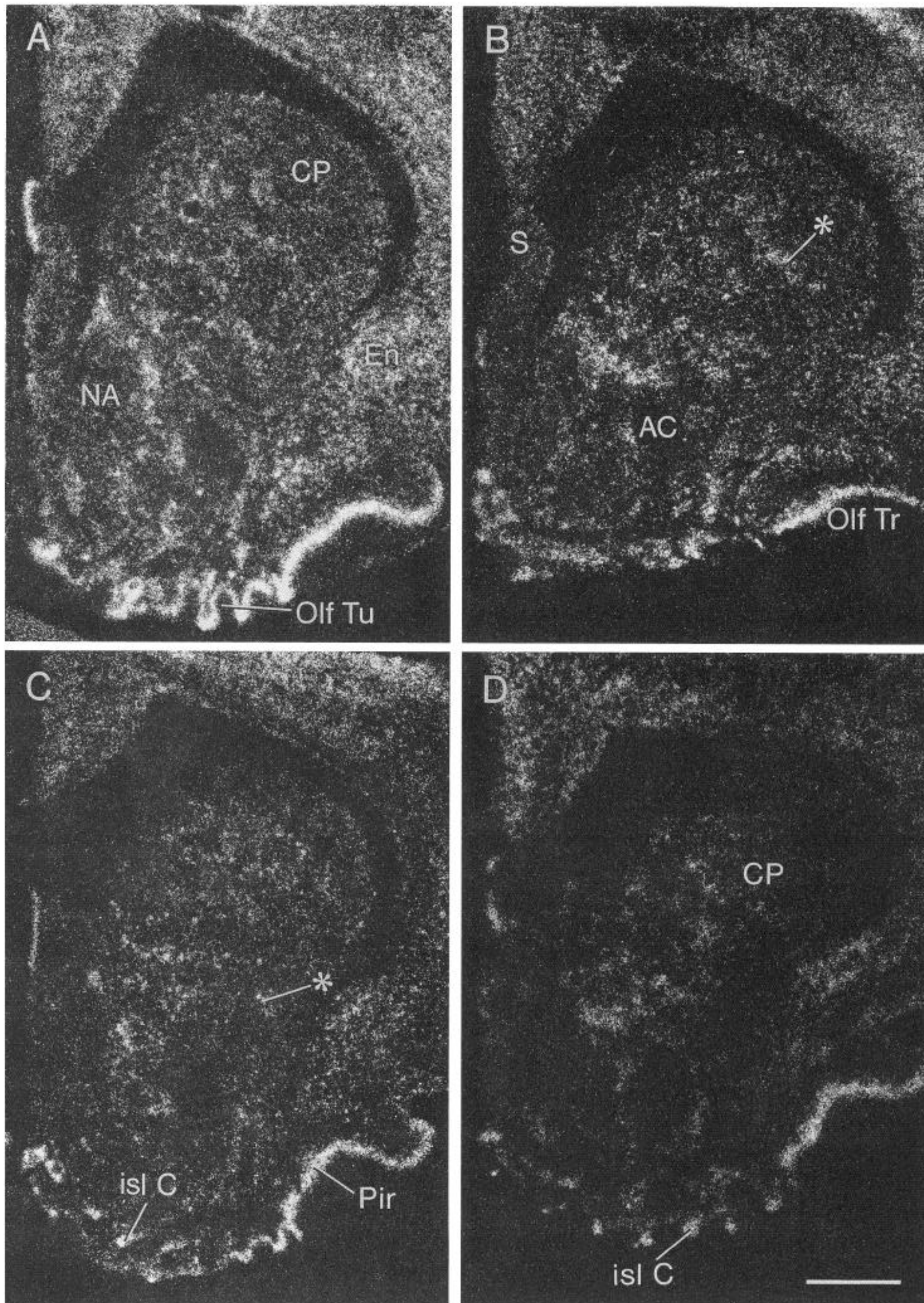


Figure 1. *In situ* autoradiograms of approximately matched striatal levels from adult rats, illustrating the highly heterogeneous pattern of increase in *NGFI*-A mRNA expression induced by amphetamine in the rostral caudoputamen at each of four different drug concentrations: 15 mg/kg (*A*), 10 mg/kg (*B*), 5 mg/kg (*C*), and 2 mg/kg (*D*). *Asterisks* in *B* and *C* indicate examples of patches of heightened labeling. Compare with Figure 2. Survival times for all animals were 1 hr. *AC*, anterior commissure; *CP*, caudoputamen; *isl C*, island of Calleja; *En*, endopiriform nucleus; *NA*, nucleus accumbens septi; *Olf Tr*, olfactory tract; *Olf Tu*, olfactory tubercle; *Pir*, piriform cortex; *S*, septum. Scale bar, 1 mm.

μm wide) patches of heightened labeling appeared scattered through the nucleus. This was true at each dose level eliciting a detectable response, including not only the lowest (2.0 mg/kg, Fig. 1*D*) but also the highest (15 mg/kg, Fig. 1*A*). *NGFI*-A mRNA expression also appeared in the tissue surrounding the patches, but in the rostral caudoputamen this signal was much weaker than that in the patches, even in rats treated with the highest doses of amphetamine. The patches of transcript expression were especially prominent in the ventral one-half to two-thirds of the caudoputamen. The patchiness of the *NGFI*-A mRNA expression gradually diminished at more caudal levels of the caudoputamen (Fig. 2*A–D*), apparently due to a rise in labeling around the patches (see below). With this bicompartment labeling, the total intensity of the signal was correspondingly augmented.

This characteristic topography of *NGFI*-A mRNA expression in response to acute amphetamine treatment was visible in all rats in which a response was detected. The main change in striatal labeling evident with the increasing doses of amphetamine was an increase in intensity and, with this, clearer definition of patches of heightened labeling in the rostral caudoputamen and of labeling in the medial division ("shell") of the nucleus accumbens. Labeling in the dorsolateral caudoputamen tended to be weaker than that elsewhere even at the highest drug doses. The 5 mg/kg dose was chosen for further detailed study of the mRNA expression after amphetamine because it

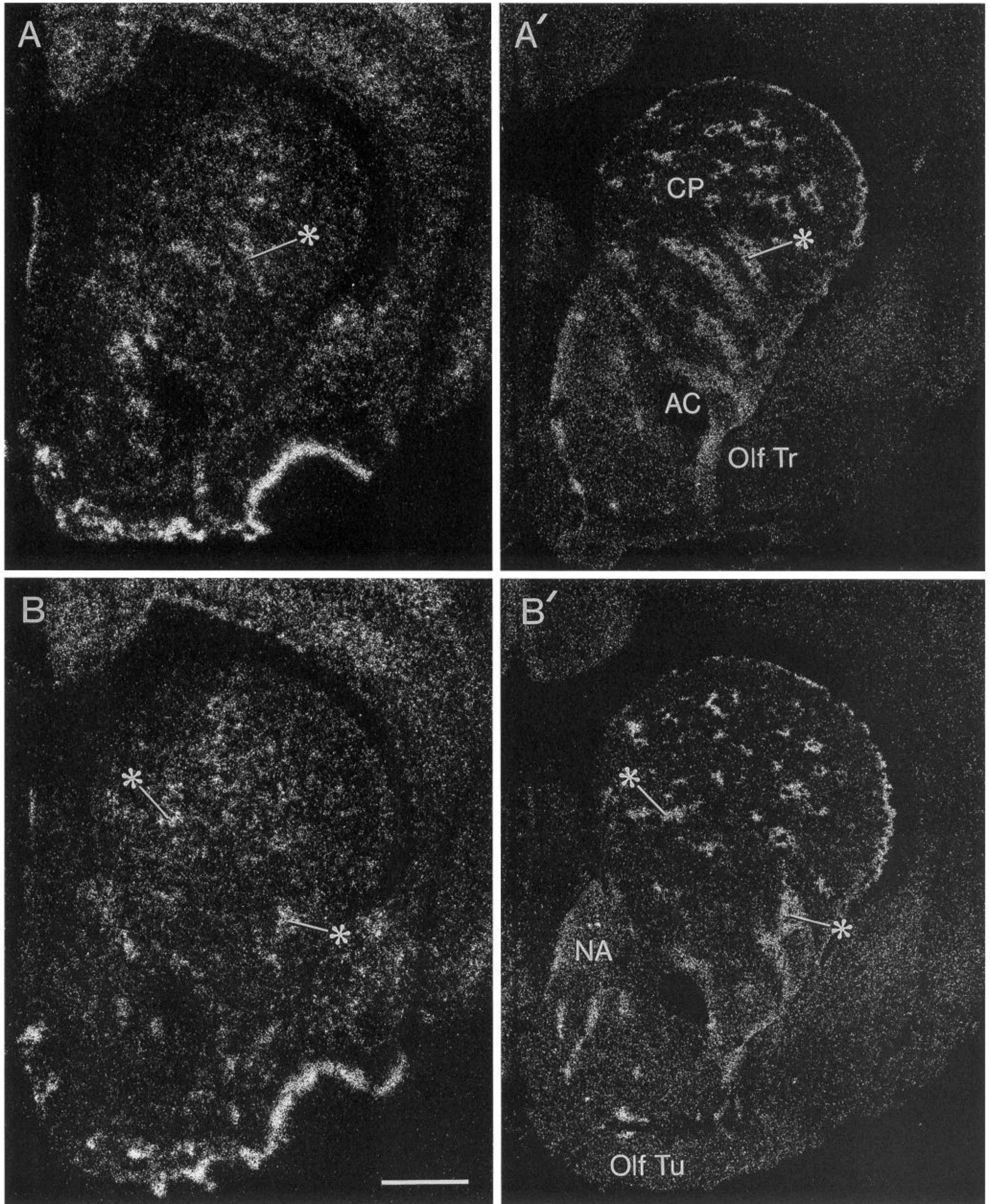
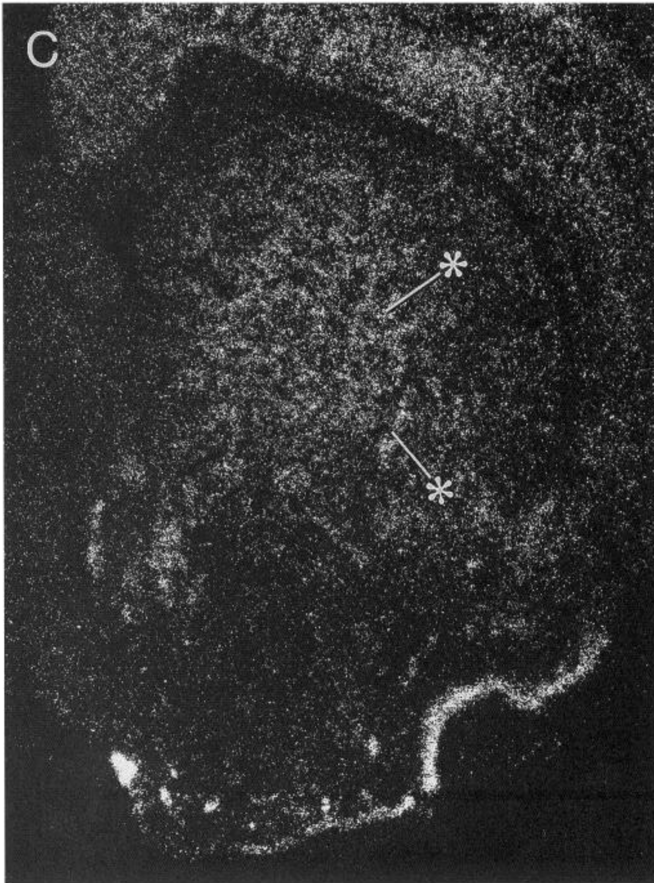


Figure 2. Evidence that the patches of amphetamine-activated *NGFI*-A mRNA expression in the rostral caudoputamen correspond to striosomes. *Left* (*A–D*), *In situ* autoradiograms illustrating *NGFI*-A mRNA expression induced by 5 mg/kg amphetamine 1 hr before brain processing. *Right* (*A′–D′*), Serially adjoining sections illustrating striosomes as zones of heightened ^{3}H-naloxone binding. *Asterisks* in *A–C* and *A′–C′* indicate examples of matching striosomal patches in the three pairs of sections. Note that the striosomal selectivity of the *NGFI*-A mRNA induction fades at progressively more caudal levels (*A–C*). *AC*, anterior commissure; *CP*, caudoputamen; *GP*, globus pallidus; *NA*, nucleus accumbens; *Olf Tr*, olfactory tract; *Olf Tu*, olfactory tubercle; *S*, septum. Scale bar, 1 mm.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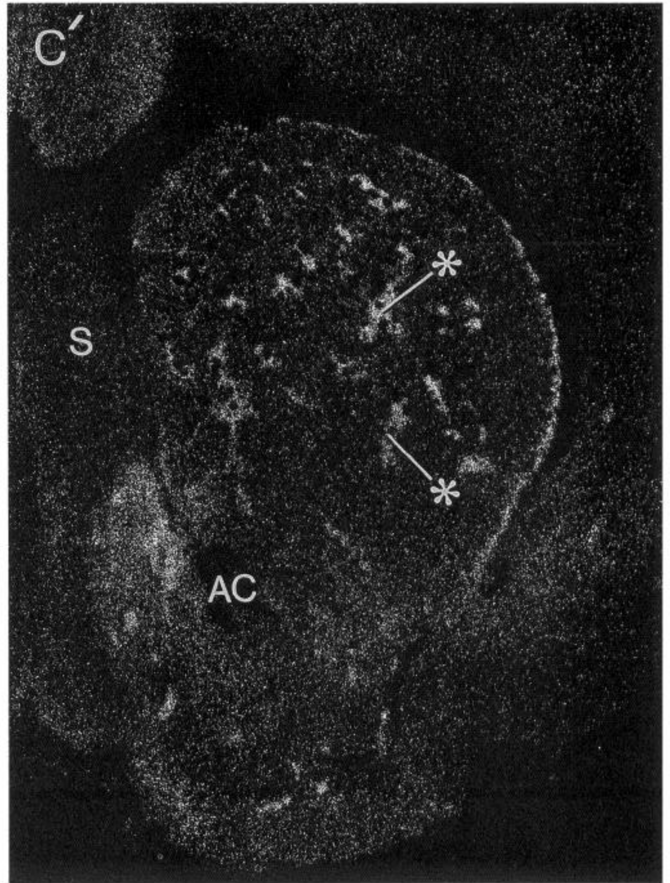
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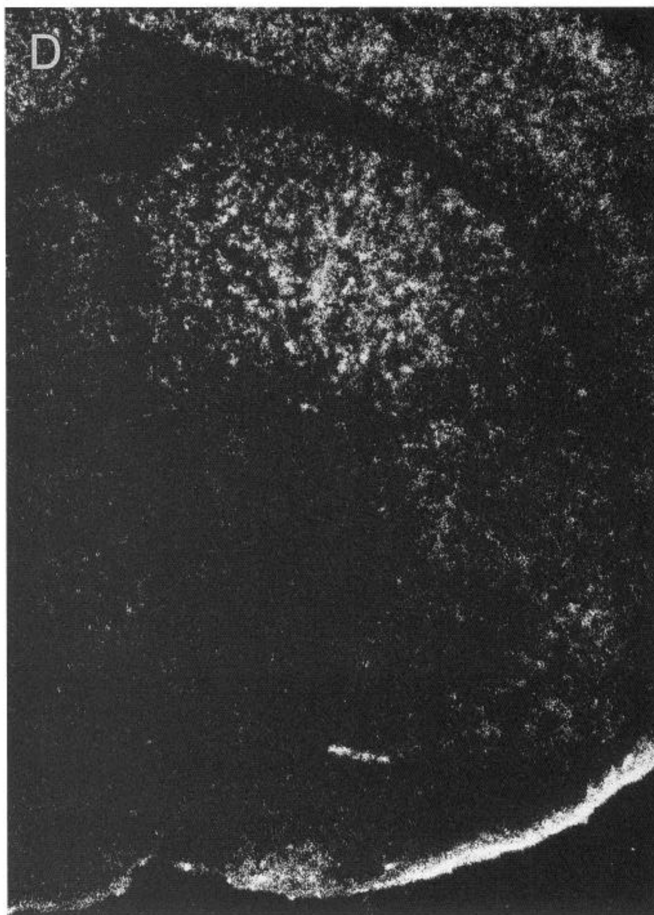
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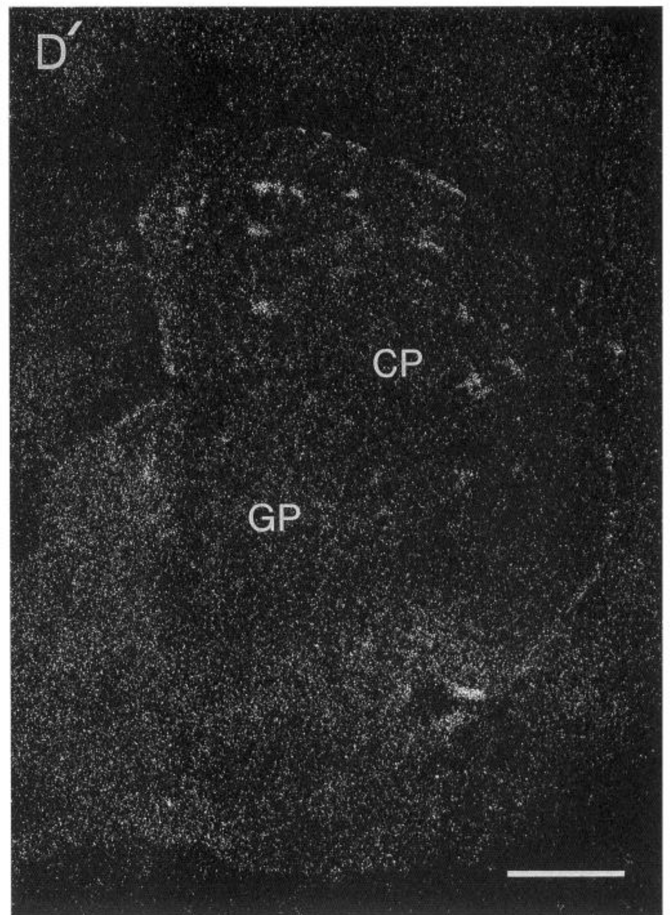
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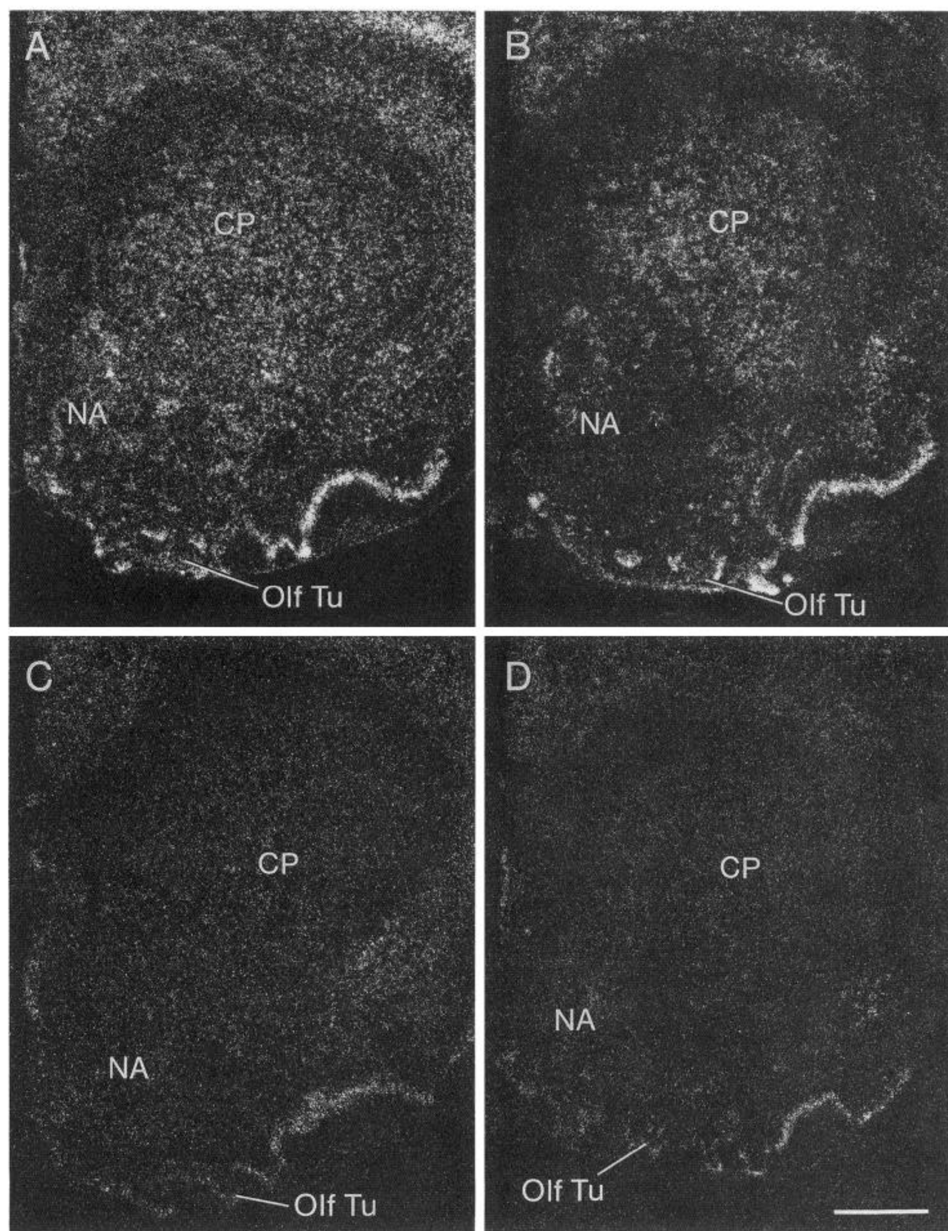


Figure 3. Dose–response series of *in situ* autoradiograms illustrating *NGFI*-A mRNA expression evoked in the rostral striatum by cocaine at 50 mg/kg (*A*), 25 mg/kg (*B*), 10 mg/kg (*C*), and 5 mg/kg (*D*). Note that the *NGFI*-A activation involves both striosomes and matrix. Note also that only at the highest doses is there intense activation in the nucleus accumbens septi. Survival times for all animals were 1 hr. *CP*, caudoputamen; *NA*, nucleus accumbens; *Olf Tu*, olfactory tubercle. Scale bar, 1 mm.

was the minimum dose that produced a reliable and reproducible signal after the treatment.

Striosomal predominance of heightened NGFI-*A mRNA in the rostral caudoputamen of amphetamine-treated rats*

To test whether the localized zones of enhanced *NGFI*-A mRNA expression in the rostral caudoputamen corresponded to striosomes, we compared the patterns of hybridization to the distribution of ^{3}H-naloxone binding in adjoining sections (Fig. 2). Patches of heightened μ-opioid receptor ligand binding labeled with ^{3}H-naloxone are known to represent striosomes (Pert et al., 1976; Herkenham and Pert, 1981). As shown in Figure 2, virtually every patch of high *NGFI*-A mRNA expression corresponded to a patch of heightened ^{3}H-naloxone binding. Slight changes in the shapes of the patches of hybridization signal and ligand binding occurred from section to section, but these were systematic and in accord with the three-dimensional continuity of the patches as striosomal labyrinths.

Three details of the serial-section comparisons shown in Figure 2 are worth emphasizing. First, the relative dimensions of the two sets of patches tended to be similar. For example, just as the ^{3}H-naloxone–labeled striosomes are larger and less angular in the ventral than in the dorsal caudoputamen, so are the corresponding matches of *NGFI*-A mRNA hybridization. Second, not all of the ^{3}H-naloxone–labeled striosomes had *NGFI*-A mRNA patches at the 5 mg/kg dose, but as shown in Figure 1, with higher doses of amphetamine there was increased vividness of the *NGFI*-A mRNA patches, as if more of the striosomal system were recruited. Third, even at middle and caudal levels of the caudoputamen, where there was increasingly strong induction of *NGFI*-A in the extrastriosomal matrix, slightly higher levels of hybridization in patches corresponding to ^{3}H-naloxone patches often could be detected nonetheless. Thus, *NGFI*-A mRNA was expressed in large parts of the striosomal system even at caudal levels, but at these levels, transcript expression in the matrix was nearly or fully as strong.

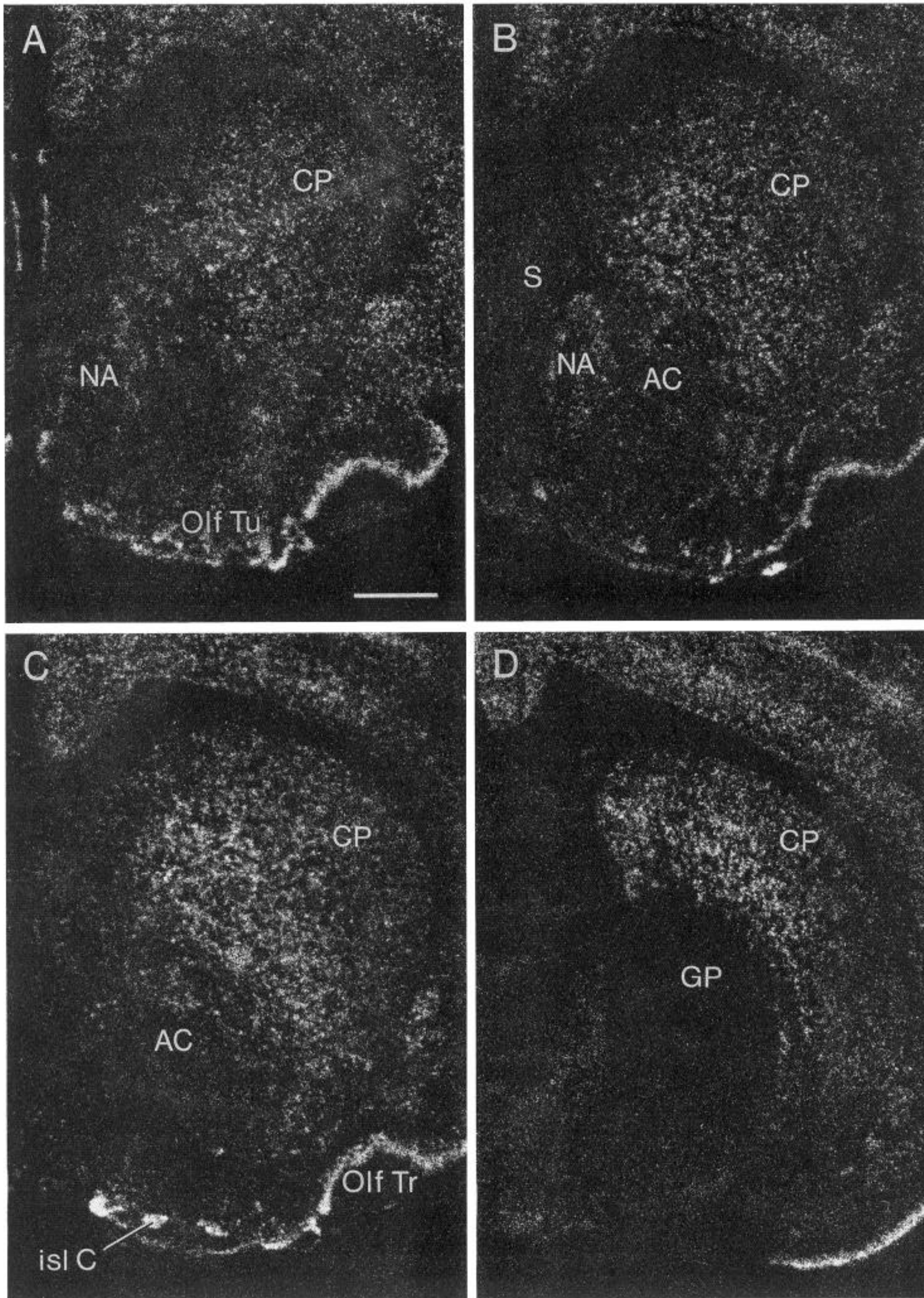
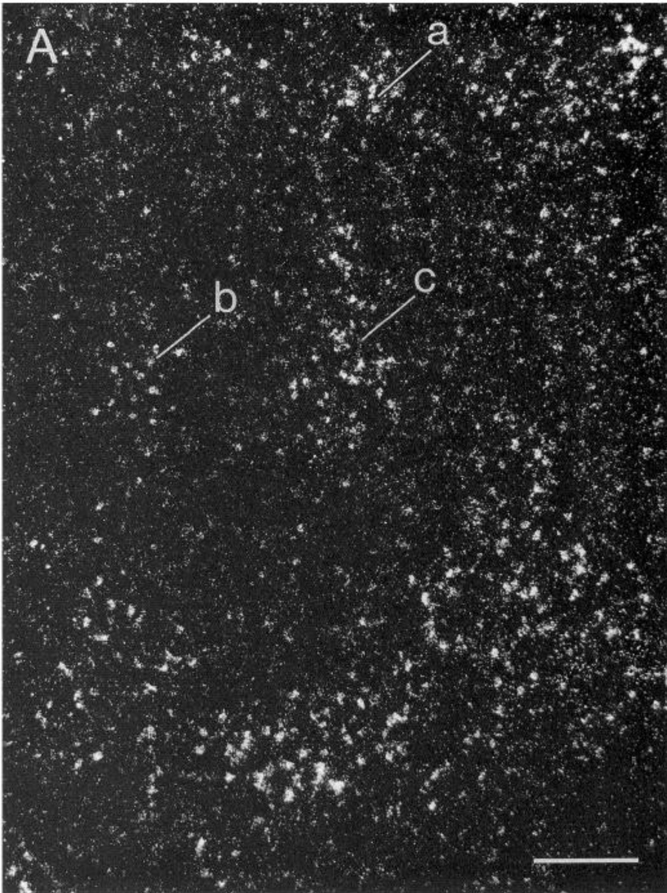


Figure 4. Rostral-to-caudal (*A–D*) series of *in situ* autoradiograms illustrating distribution of *NGFI*-A mRNA induced in striatum by 25 mg/kg cocaine 1 hr before brain processing. There is strong expression in the medial and central parts of the caudoputamen (*CP*), whereas there is much less activation in the dorsal and lateral rim of the caudoputamen. Note strong activation of *NGFI*-A mRNA expression in the islands of Calleja (*isl C*), but much weaker activation in the nucleus accumbens (*NA*), especially in its core subdivision. *AC,* anterior commissure; *GP,* globus pallidus; *Olf Tr,* olfactory tract; *Olf Tu,* olfactory tubercle; *S,* septum. Scale bar, 1 mm.

Cocaine-induced expression of NGFI-*A in striosome and matrix compartments and in the ventral striatum*

Acute treatment with cocaine induced widespread expression of *NGFI*-A mRNA in the caudoputamen without obvious compartmental heterogeneity at any anteroposterior level (Figs. 3, 4). This diffuse pattern of activation occurred even at the lowest doses given (5 and 10 mg/kg), after which there was only a weak elevation in hybridization signal relative to control levels (Fig. 3). There was, nevertheless, a marked change in the pattern of *NGFI*-A mRNA expression with increasing dose: the distribution of heightened mRNA signal became progressively more widespread both in the caudoputamen and in the nucleus accumbens (Fig. 3). In the caudoputamen, 25 mg/kg produced a pattern of induction that was typically most pronounced in a funnel-shaped zone in the central and medial part of the nucleus. Laterally and dorsally, expression was relatively weak. Farther caudally, the induction was mainly medial (Fig. 4). It was only at the highest dose level (50 mg/kg) that hybridization signal appeared in the dorsal and lateral periphery of the caudoputamen as well as in its centromedial part. Even so, the peripheral expression of *NGFI*-A was weaker than that in the central caudoputamen.

A dose-related increase in the distribution of *NGFI*-A mRNA expression also occurred in the nucleus accumbens (Fig. 3). At 25 mg/kg cocaine, labeling in the nucleus was mainly in the shell. At the highest dose level (50 mg/kg), hybridization was strong in both the shell and the core subdivisions of the nucleus.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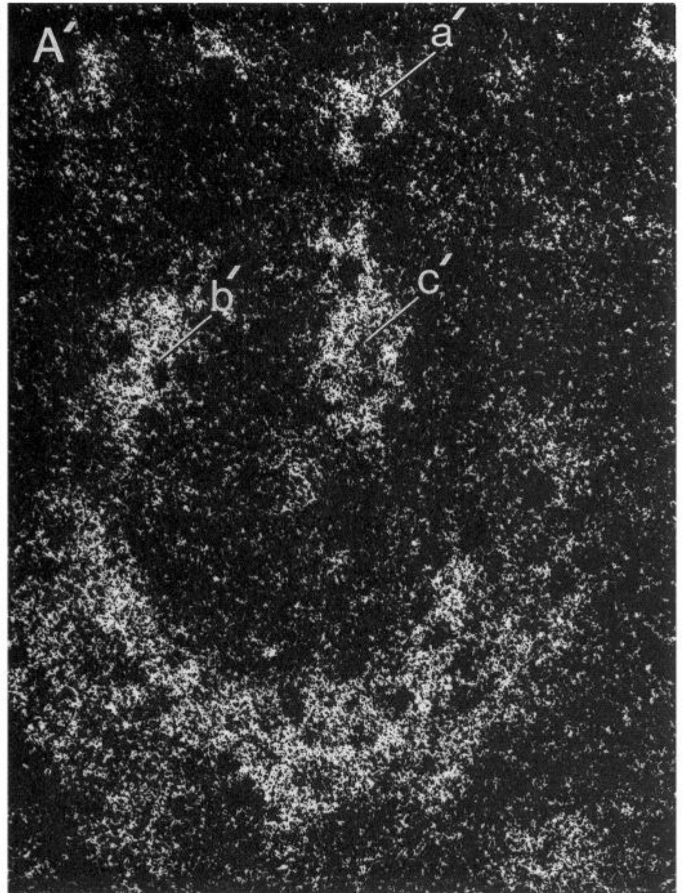
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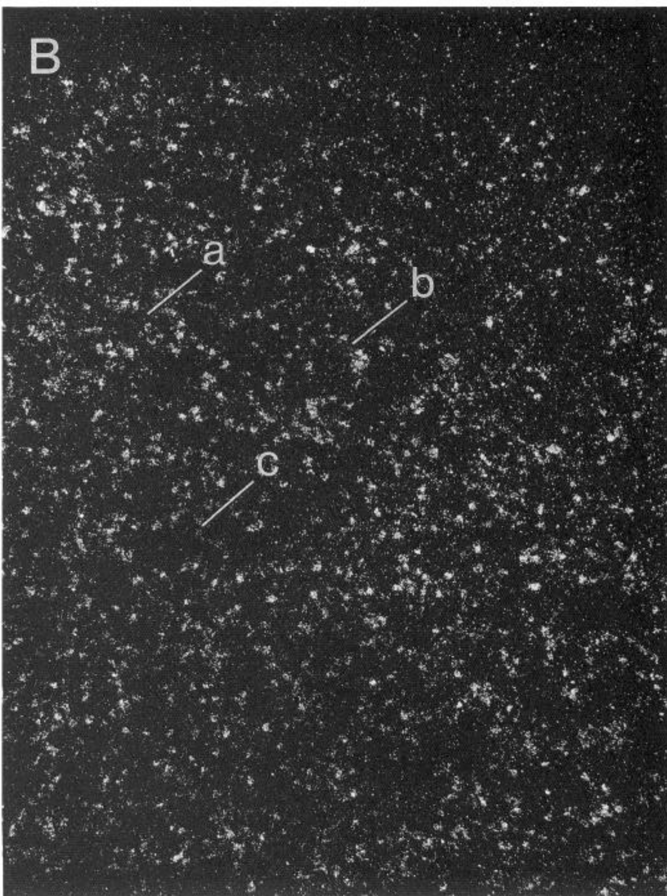
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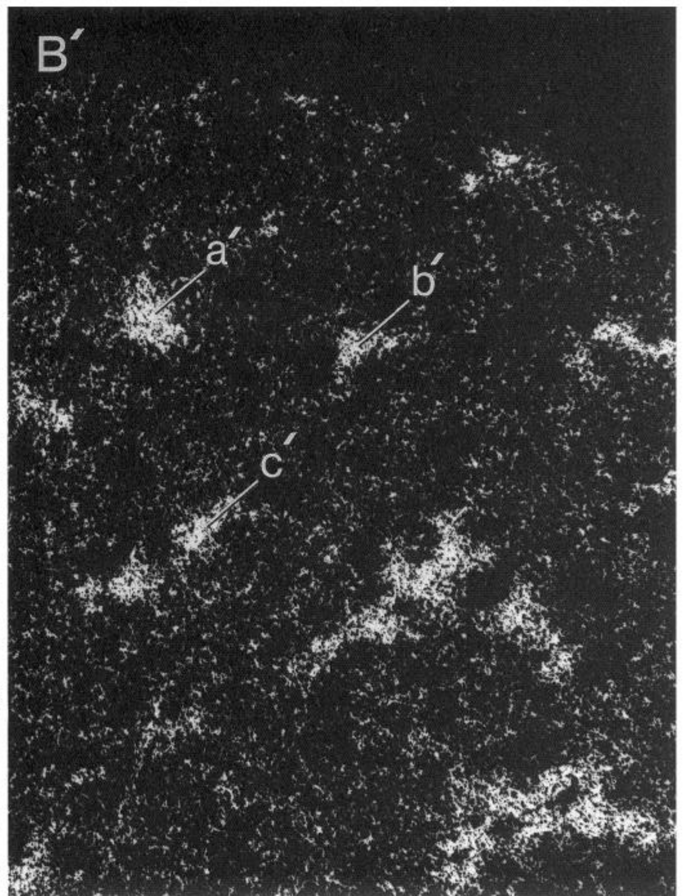
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To compare in detail the distributions of *NGFI*-A mRNA expression induced by cocaine with that induced by amphetamine, we prepared a series of rats treated, respectively, with 25 mg/kg cocaine or with 5 mg/kg amphetamine, the lowest dose levels at which consistent strong hybridization patterns appeared in our dose–response analysis. At these doses, the two drugs elicited comparable levels of sniffing and rearing, as reported previously (Scheel-Krüger et al., 1977). In the anterior caudoputamen, 25 mg/kg of cocaine produced a generalized induction of *NGFI*-A expression in the central zone of the nucleus, with weak expression laterally and dorsally (Fig. 4*A,B*). This pattern of induction was clearly different from that produced by 5 mg/kg of amphetamine (Fig. 3*A,B*), in which a greater breadth of the nucleus contained hybridization signal but the signal had a predominant striosomal distribution. Farther caudally in the caudoputamen, the patterns of induction by cocaine and amphetamine were both relatively diffuse (Figs. 2*C,D*; 4*C,D*), but their distributions were still not identical.

Cellular localization of NGFI-*A mRNA transcripts*

Figures 5 and 6 illustrate the localization of *NGFI*-A transcripts visible in emulsion-dipped sections. Confirming the results of the film autoradiography, cells expressing strong *NGFI*-A mRNA signal were clustered in striosomes in the rostral caudoputamen after amphetamine treatment (Fig. 5*A,A'*), but were about equally represented in striosomes and matrix in cocaine-treated animals (Fig. 5*B,B'*). Most of the labeled cells appeared to be the common medium-sized neurons of the striatum, judging from their appearance in sections counterstained for Nissl substance (Fig. 6).

Blockade of both amphetamine-induced and cocaine-induced expression of NGFI-*A mRNA in the striatum by SCH23390*

In rats pretreated with the dopamine D1 receptor–selective antagonist SCH23390 (0.5 mg/kg), subsequent administration of amphetamine (5 mg/kg, $n = 4$) or cocaine (25 mg/kg, $n = 3$) failed to induce detectable *NGFI*-A hybridization in the striatum (Fig. 7*A,B*). Indeed, even with prolonged autoradiographic exposure times, scarcely any signal was detectable in the striatum. In contrast to *NGFI*-A mRNA expression in the caudoputamen, expression of *NGFI*-A mRNA signal in the neocortex and piriform cortex was not abolished by pretreatment with SCH23390. It is not clear whether this cortical expression is constitutive or related to the stress of even gentle handling; Sharp et al. (1991) have found extreme sensitivity of c-*fos* in cerebral cortex (but not caudoputamen) to such external stimulation.

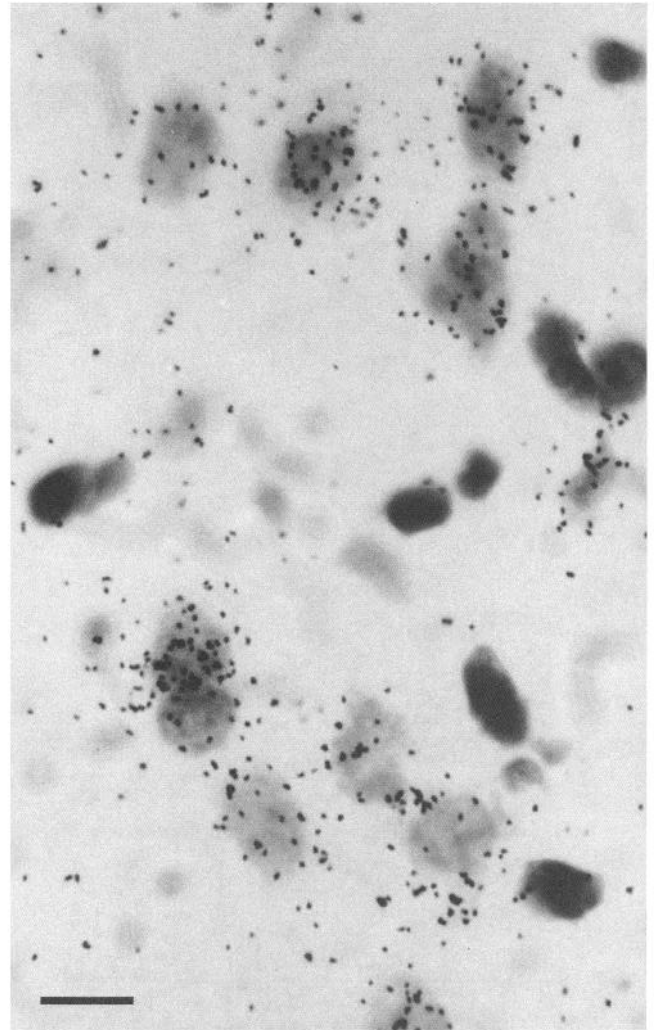

Figure 6. Bright-field photomicrograph demonstrating *in situ* autoradiographic labeling with *NGFI*-A mRNA probe of medium-sized neurons in the amphetamine-treated case shown in the dark-field photograph of Figure 5. The neurons are located at the top of the central patch (labeled c) illustrated in Figure 5*A*. Scale bar, 10 μm.

Discussion

Regulation of striatal NGFI-*A expression by biogenic amines*

The findings we report here demonstrate that the expression of *NGFI*-A mRNA in the caudoputamen and the ventral striatum can be regulated by biogenic amine activity. Expression of *NGFI*-A transcripts was markedly enhanced by stimulation with either of two indirect dopamine agonists, and this enhanced expression was blocked by prior administration of the dopamine receptor antagonist SCH23390. This pharmacological profile makes it likely that dopamine D1–like receptor activation was a principal inducing stimulus for the increase in *NGFI*-A expression. Our findings do not rule out a serotonergic component in the genomic response, however, for SCH23390 also is known to bind to serotonergic receptors in the rodent brain (Bischoff et al., 1986). Nor do our findings rule out the possibility that dopamine D1–like receptors other than the pharmacologically defined D1 subtype are blocked by SCH23390 and participate in the signaling pathway (Sokoloff et al., 1990; Sunahara et al., 1991; Van Tol et al., 1991), including particularly the newly

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Figure 5. Photomicrographs of *NGFI*-A mRNA *in situ* autoradiograms from emulsion-dipped sections (*A* and *B*, respectively) and from serially adjacent sections processed for ^{3}H-naloxone binding (*A'* and *B'*, respectively) illustrating disposition of *NGFI*-A mRNA–positive-striatal cells following 5 mg/kg amphetamine exposure (*A, A'*) and 25 mg/kg cocaine exposure (*B, B'*). Note that most of the *NGFI*-A mRNA–positive cells in the amphetamine case are in striosomes (*a–c* and *a'–c'* indicate three corresponding pairs of patches). By contrast, in the cocaine-treated case, labeled cells are in both striosomes and matrix (*a–c* and *a'–c'* indicate three examples of corresponding striosomal loci). Survival times, 1 hr. Scale bar, 200 μm.

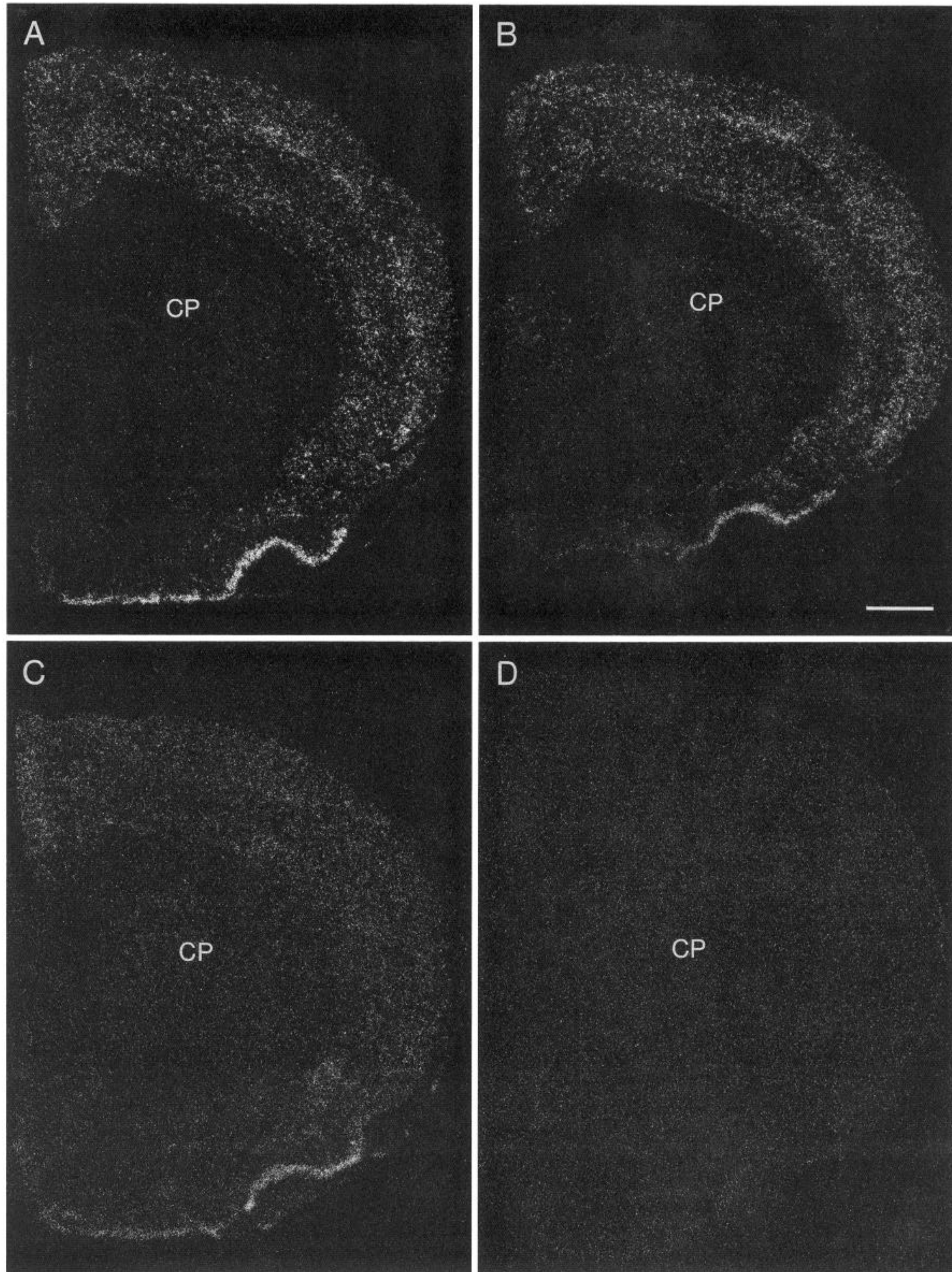

Figure 7. *In situ* autoradiograms from representative control experiments. *A* and *B,* In rats pretreated with the D1-selective dopamine receptor antagonist SCH23390 (0.5 mg/kg), there was blockade of the intrastriatal induction of *NGFI*-A mRNA expression both by amphetamine (5 mg/kg, *A*) and by cocaine (25 mg/kg, *B*). Note that expression of *NGFI*-A mRNA in the cerebral cortex was not blocked. *C,* Saline injection induced little if any *NGFI*-A mRNA expression in the striatum. *D,* No hybridization signal was seen when the sense probe for *NGFI*-A was used. The section illustrated is from the brain of a rat treated with amphetamine (5 mg/kg). *CP,* caudoputamen. Scale bar, 1 mm.

cloned D5 dopamine receptor, whose forebrain distribution is reported to resemble that of the D1 receptor (Sunahara et al., 1991). Only when agonist and antagonist drugs specific to cloned dopamine receptor subtypes become available will it be possible to identify fully the pharmacological specificity of the psychostimulant effects shown here. We did not test the effects of dopamine D2–selective antagonists. We and others have found that D2 receptor antagonists themselves can induce immediate-early gene induction in the caudoputamen (Dragunow et al., 1990; Graybiel et al., 1990; Miller, 1990), and Miller (1990) has shown that D2 agonist treatment can block the induction of c-*fos* mRNA produced by D2 antagonists.

Compartmental selectivity of the NGFI-*A response*

The most striking finding of our study was that the two indirect dopamine agonists we administered, amphetamine and cocaine, produced different patterns of *NGFI*-A expression in the caudoputamen and ventral striatum. Rostrally in the caudoputamen, amphetamine induced *NGFI*-A mRNA expression preferentially in the striosomal compartment of the striatum. This effect was not dose related, as the striosomal pattern was observed with the highest dose tested (15 mg/kg) that led to induce the gene as well as with the lowest dose (2 mg/kg). The pattern induced by cocaine, even at the lowest dose (5 mg/kg), was not compartment selective. The basis of these differences is not clear, but could involve the different mechanisms by which the two drugs increase extracellular biogenic amines. Amphetamine has the predominant effect of releasing biogenic amines (Moore, 1978; Zetterström et al., 1983), whereas cocaine principally blocks catecholamine reuptake (Taylor and Ho, 1978; Vickroy and Johnson, 1982; Kilty et al., 1991). These modes of action could produce contrasts in the synaptic concentrations of amine generated, the proportion of synaptic versus extrasynaptic concentrations of amine present, and the temporal pattern of drug-induced increases in amine. The effects could also reflect indirect effects on vascular supply, enhancing or diminishing the turnover of amine. As we gave the drugs intraperitoneally, the contrasting compartmental patterns of induction could reflect either differences in the responses to stimulation of receptors in the caudoputamen (e.g., depending on densities of high-affinity uptake sites and/or receptors in striosomes and matrix), or differences in responsiveness at sites projecting to the caudoputamen, or both (see below).

The regional selectivity for striosomal *NGFI*-A mRNA activation that we observed in the amphetamine-treated animals is similar to the patterns we have observed in amphetamine-treated rats by immunohistochemistry with polyclonal antisera raised against peptide sequences in Fos (Graybiel et al., 1990). Following acute amphetamine at the same doses, Fos-like immunoreactivity is preferentially expressed in striosomes rostrally but in both striosomes and matrix caudally. Furthermore, the sharpness of the striosomal delineations with the Fos-like immunoreactivity is roughly similar to that seen with the *NGFI*-A probes. Interestingly, however, in ongoing *in situ* hybridization studies of c-*fos* and *jun*-B expression induced by amphetamine (R. Moratalla and A. M. Graybiel, unpublished observations), we have not observed as sharp a delineation of striosomes as evident with *NGFI*-A hybridization or Fos-like immunoreaction. This suggests that the *NGFI*-A mRNA response selectivity for the striosomal system may be particularly marked, and may be paralleled by some but not other members of the *fos/jun* family (Cohen and Curran, 1988; Cohen et al., 1989; Nishina et al., 1990). The differential compartmental expression of *NGFI*-A mRNA in the response of striatal neurons to amphetamine thus may be not only stimulus specific, but also gene specific. The D1-like receptor selectivity of the effects suggests that stimulation of CRE (cAMP responsive element) could be involved, and both *NGFI*-A and c-*fos* have at least one CRE in their regulatory regions (Tsai-Morris et al., 1988; Herschman, 1991). The *NGFI*-A gene is reported to have two CRE sites, whereas c-*fos* has only one. The *NGFI*-A promotor is also notable for having five serum response element (SRE) sites, as compared to one for the c-*fos* promotor (Tsai-Morris et al., 1988; Herschman, 1991). Conceivably, these differences could account for the gene selectivity we observe.

Molecular signaling pathways in striosome and matrix compartments of the striatum

The fact that *NGFI*-A mRNA and Fos-like transcription factors show similar drug-specific compartmental and regional patterns of enhanced expression in the striatum raises the possibility that there are functionally distinct molecular signaling pathways in striosomes and matrix. If so, understanding the signaling pathways leading to selective immediate-early gene responses may be key to determining the molecular specificity of the striosomal system. Finding out more about these signaling pathways could also have considerable practical significance, as drugs then could be designed to act selectively on these two neurochemical compartments of the striatum.

There are clues to what these signaling pathways might be, but no definitive evidence. Both compartments express the neurotransmitter substances characteristic of aminergic transmission, but to different degrees. In primates and cats, striosomes tend to have more D1-like but less D2-like dopamine receptor–selective ligand binding (Joyce et al., 1986; Besson et al., 1988), lower catecholamine transporter-related binding (Graybiel and Moratalla, 1989; Lowenstein et al., 1990), lower tyrosine hydroxylase–like immunoreactivity (Ferrante and Kowall, 1987; Graybiel et al., 1987), lower 5-HT–like immunoreactivity (Lavoie and Parent, 1990), and higher immunostaining for markers of the phosphoinositide second messenger system (Fotuhi et al., 1991) than nearby parts of the matrix.

These characteristics suggest that adenylate cyclase and/or phosphoinositide pathways could be differentially active in striosomes and matrix. Curiously, the compartmentalization of many of these substances is not as striking in the rat's caudoputamen except during development (Olson et al., 1972; Tennyson et al., 1972; Butcher and Hodge, 1976; Kent et al., 1982; van der Kooy, 1984; Foster et al., 1987; Voorn et al., 1988; Happe and Murrin, 1989; Loopuijt, 1989). Pharmacological treatments affecting dopamine and other biogenic amines can nevertheless induce highly compartmental changes in expression of neuropeptides in the caudoputamen of the rat (see below). Apparently, the anatomical markers so far available for localization studies do not adequately demonstrate differential actions of the biogenic amines on the striosomes and surrounding matrix in the rat. As we report here, these compartmental effects as viewed by immediate-early gene responses can be marked, particularly in the rostral part of the caudoputamen.

Differences in the connections of striosomes and matrix could also account for the different patterns of *NGFI*-A activation by amphetamine and cocaine. In the rostral caudoputamen of the rat, for example, where the striosomal activation of *NGFI*-A was most pronounced in response to amphetamine challenge,

striosomes receive a particularly dense projection from part of the prefrontal cortex, which itself receives a dopaminergic innervation (Donoghue and Herkenham, 1986; Gerfen, 1989; Sesack et al., 1989). Striosomes and matrix in this species also receive compartment-selective inputs from parts of the thalamus and amygdala that are linked indirectly to midbrain aminergic groups (Jones et al., 1988; Wallace et al., 1989; Cornwall et al., 1990; Gonzales and Chesselet, 1990; Kita and Kitai, 1990; Berendse, 1991). Finally, striosomes and matrix are innervated by different populations of midbrain dopamine-containing neurons in the rat, as in other species (Gerfen et al., 1987; Jiménez-Castellanos and Graybiel, 1987; Langer and Graybiel, 1989). Each of these afferent systems could be affected directly or indirectly by dopamine agonist treatments, so that the striosome-selective effects of amphetamine could themselves be indirect (e.g., via part of the prefrontal cortex or thalamus) rather than occurring as the result of a direct action of amphetamine on amine release in the caudoputamen.

Neuropeptide regulation as a possible consequence of NGFI-*A activation in the striatum*

Pronounced changes in peptide expression occur in the striatum following chronic treatments with dopamine agonists and antagonists, and also following lesions or stimulation of dopaminergic mesostriatal fiber systems (see Graybiel, 1990, for review; Gerfen et al., 1990, 1991; Jiang et al., 1990). Some of these changes result in a sharp increase in the detectability of peptide-positive patches—in some instances through a documented selective increase in neuropeptide expression in patches corresponding to striosomes. Such large, patchy upswings in neuropeptide expression in striosomes have been found for neurotensin-like immunoreactivity following extended reserpine pretreatment (Bean et al., 1989) and for dynorphin-like immunoreactivity (Sivam et al., 1987; Jiang et al., 1990; Gerfen et al., 1991) and prodynorphin mRNA hybridization (Gerfen et al., 1991) following chronic apomorphine treatment. The fact that alterations in mRNA expression are found suggests that changes in gene transcription or in mRNA stabilization are induced.

In the experiments we report here, expression of *NGFI*-A transcripts was weak or nil at the lowest doses of amphetamine and cocaine that we administered. Physiological and behavioral effects of these drugs are nevertheless known to occur at such doses (Randrup and Munkvad, 1974; Sharp et al., 1987). This difference makes it difficult to approach correlations between our findings and those on the physiology. The regional and neuronal specificity of the induction we see may nevertheless indicate functional specializations.

One interesting possibility is that the early responses to dopamine agonists seen by monitoring changes in expression of *NGFI*-A mRNA and other immediate-early genes could lead to a systematic series of longer-term changes in gene transcription in the striatum and that, in particular, these could include compartment-selective changes in neuropeptide and neuropeptide mRNA levels observed in the caudoputamen. A major difference in the paradigms eliciting the immediate-early gene and neuropeptide gene responses is the duration of the stimulus. We see greatly increased expression of *NGFI*-A mRNA within an hour of intraperitoneal injection of dopamine agonists, whereas the effects of dopamine agonist treatments on neuropeptide mRNA levels are found after prolonged (10–14 d) exposures. Interestingly, however, Grimes et al., (1990) have reported that after chronic exposure to the direct dopamine agonist apomorphine, Fos-like immunoreactivity is induced selectively in striosomes in less than 2 hr following the final treatment, and such chronic treatments are known to produce upregulation of dynorphin immunoreactivity and prodynorphin mRNA in patches in the caudoputamen that correspond to striosomes (Gerfen et al., 1991). The apparent parallel in distribution of the Fos and peptide responses to chronic apomorphine exposure suggests a strong correlation between these immediate-early gene and peptide gene responses and raises the possibility that the effects could reflect a common signaling pathway. A similar point has already been made in respect to parallel regulation of *fos/jun* family immediate-early genes and dynorphin in the spinal cord (Draisci and Iadarola, 1989; Naranjo et al., 1991; see also Gogas et al., 1991). The *NGFI*-A response in striosomes on exposure to amphetamine could be another early response in the signaling pathway leading to dynorphin regulation, given that—at least in the cat—striosomal neurons have enhanced expression of dynorphin relative to matrix neurons (Graybiel and Chesselet, 1984; Besson et al., 1990). Tachykinins could also be implicated. Tachykinin (substance P)-like peptides almost invariably coexist with dynorphin-like peptides in neurons of the caudoputamen (Besson et al., 1990, 1991; Reiner and Anderson, 1990). Kohno et al. (1984) suggest that there is a distinctly patchy distribution of neurons expressing substance P–like (tachykinin-like) immunoreactivity rostrally, but not caudally, in the rat's caudoputamen. This pattern is remarkably reminiscent of the *NGFI*-A mRNA activation patterns induced by amphetamine.

Cooperativity in transcriptional activation of striatal neurons

The evidence we report here, combined with findings from other recent studies (Cole et al., 1990; Graybiel et al., 1990; Young et al., 1991), demonstrates unequivocally that mRNA transcripts of at least two families of immediate-early genes can be activated in the striatum by dopamine agonists, and that the patterns of activation have highly similar anatomical, and therefore possibly functional, specificity. Molecular mapping studies suggest that the known genes of the leucine zipper and zinc finger families may have different constellations of upstream elements influencing their promotor sites (see, e.g., Tsai-Morris et al., 1988; Janssen-Timmen et al., 1989), and that their gene products can act in different combinatorial patterns. The particular impact of the present results is the suggestion that this combinatorial complexity at the molecular level may be played out in the basal ganglia so as to allow different patterns of positive or negative cooperativity of genes responding to stimuli ultimately affecting the same neurotransmitter system. If so, this highly differentiated immediate-early gene response may underlie some of the complexity evident in the eventual functional response of the basal ganglia to modulation by dopamine.

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