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Amphetamine and cocaine induce drug-specific activation of the c-*fos* gene in striosome–matrix compartments and limbic subdivisions of the striatum

(immediate-early gene/proto-oncogene/dopamine/dopamine receptors/basal ganglia)

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ABSTRACT **Amphetamine and cocaine are stimulant drugs that act on central monoaminergic neurons to produce both acute psychomotor activation and long-lasting behavioral effects including addiction and psychosis. Here we report that single doses of these drugs induce rapid expression of the nuclear proto-oncogene c-*fos* in the forebrain and particularly in the striatum, an extrapyramidal structure implicated in addiction and in long-term drug-induced changes in motor function. The two drugs induce strikingly different patterns of c-*fos* expression in the striosome–matrix compartments and limbic subdivisions of the striatum, and their effects are pharmacologically distinct, although both are sensitive to dopamine receptor blockade. We propose that differential activation of immediate-early genes by psychostimulants may be an early step in drug-specific molecular cascades contributing to acute and long-lasting psychostimulant-induced changes in behavior.**

Dramatic changes in behavior occur after exposure to psychomotor stimulant drugs that affect dopaminergic and other monoaminergic systems in the brain. These changes can emerge acutely in response to a single exposure or a few exposures to drugs such as cocaine and amphetamine, and they can evolve into long-lasting behavioral alterations. The molecular mechanisms underlying these extended effects are not understood, but they are thought to involve dopamine receptors and transporters and to bring about modifications in the dopamine-containing nigrostriatal and mesolimbic fiber systems and their neuronal targets in the striatum (1–4).

In the experiments reported here, we explored the possibility, suggested by preliminary experiments (5–9), that psychomotor stimulants induce drug-specific molecular responses in striatal neurons by activating expression of immediate-early genes. The induction of several immediate-early genes, whose products influence the transcription of other genes (10), has been linked to stimulus conditions that lead to long-term changes in neuronal responsiveness (11–13). We injected rats with either amphetamine or cocaine and tested for induction of c-*fos*, a nuclear proto-oncogene whose protein product, the DNA-binding protein Fos, is expressed in a number of neural systems in response to extrinsic signals (14, 15). We reasoned that expression of Fos could serve as a sensitive indicator of differential gene activation by psychostimulants in a region of the brain that is directly implicated in the effects of these drugs.

MATERIALS AND METHODS

Drug-naive adult male Sprague–Dawley rats (250–300 g) were divided into groups receiving (*i*) no treatment or saline injections, (*ii*) injections of either amphetamine or cocaine alone, or (*iii*) injections of amphetamine or cocaine in combination with one of the following: the dopamine-depleting drug reserpine, the dopamine D1 receptor antagonist SCH 23390 (16), the dopamine D2 receptor antagonist YM-09151 (17), the serotonin antagonist metergoline (18), or the serotonin synthesis inhibitor *p*-chlorophenylalanine (18). All drugs were administered by intraperitoneal injection; doses and times are indicated in Table 1.

At the end of the post-treatment survival times, the rats were deeply anesthetized with Nembutal and perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer/0.9% NaCl, pH 7.4. Brains were post-fixed, blocked, and cut coronally on a Vibratome at 30–50 μm, or, rarely, on a sliding microtome at 20–30 μm after cryoprotection and freezing. Sections were carried through standard avidin–biotin immunohistochemical protocols for detection of Fos with one of three polyclonal antisera: Oncogene Science PC05, Cambridge Research Biochemicals OA-11-823, or Medac OPA 08/1. Control sections were incubated in the presence of the peptides to which the antibodies were raised (Cambridge Research Biochemicals, Cambridge, U.K. and Oncogene Science, Manhasset, NY, antisera) or without primary antiserum. Sections through the striatum were stained for Fos in sets permitting serial-section comparisons between the distribution of neurons expressing Fos-like immunoreactivity and the distribution of neurons expressing calbindin D_{28k} (28-kDa calbindin D)-like immunoreactivity, a marker (19) for the striosome/matrix organization of the striatum (20). For detection of calbindin D_{28k}, we used polyclonal antisera donated by K. G. Baimbridge (University of British Columbia) (diluted 1:2000) or by P. C. Emson (Institute of Animal Physiology, Barbaraham, U.K.) (diluted 1:4000). A few sections were counterstained for Nissl substance before being coverslipped.

In situ hybridization with an ^{35}S-labeled oligonucleotide probe for c-*fos* was performed on 15-μm cryostat sections from fresh-frozen brains as described by Baldino *et al.* (21) with minor modifications. The c-*fos* probe sequence was 5′-GCA GCG GGA TGA GGC CTC GTA GTC CGC GTT GAA ACC CGA GAA CAT-3′ (Bio-Synthesis, Denton, TX). Control rats and rats injected with cocaine (1 hr survival) were used in the *in situ* hybridization experiments.

RESULTS

Amphetamine and cocaine induced widespread expression of Fos-like immunoreactivity in neurons of the dorsal or "sensorimotor" striatum (caudoputamen) and ventral or "limbic" striatum (nucleus accumbens and olfactory tubercle) (Fig. 1). Dorsomedial and ventrolateral limbic cortex, other limbic

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Table 1. Drug treatments for immunohistochemistry

Treatment	Doses i.p., mg/kg	No. of animals per treatment with indicated survival times after last injection*		
		≤1 hr	1.5–2 hr	3 (or 6) hr
Amphetamine (A) only	5 (or 10)	3	16 (2)	2 (1)
SCH 23390 + A	0.5 + 5		13	
YM 90151 + A	0.5 + 5		3	
PCPA + A	200 + 5		2	
Reserpine + A	10 + 5		15	
Chronic reserpine + A	1 + 5		2	
Cocaine (C) only	25 (or 50)	4	13 (2)	1 (1)
SCH 23390 + C	0.5 + 25		13	
YM 90151 + C	0.5 + 25		2	
Metergoline + C	1 (or 5) + 5		1 (1)	
Reserpine + C	10 + 25		13	
Chronic reserpine + C	1 + 25		2	

Doses in mg i.p. per kg of body weight and schedule of drug treatments. The standard series were: amphetamine with 2-hr survival (A2), cocaine with 2-hr survival (C2), and reserpine 18-hr pretreatment and amphetamine or cocaine treatment 2 hr before perfusion (R18A2, R18C2). For combined-drug treatments, times before final amphetamine or cocaine injections were 30 min each for SCH 23390, YM-90151, and metergoline; 16–18 hr for reserpine (or, for chronic treatment, twice a day for 5 days); and once a day for 3 days for *p*-chlorophenylalanine (PCPA). In control experiments, SCH 23390, YM-90151, PCPA, metergoline, and reserpine were also given individually at the doses indicated for the combined-drug experiments.

*Numbers in parentheses indicate animals given alternate doses shown in parentheses in lefthand column.

regions such as the septum, and parts of nonlimbic cortex and subcortex were among other sites exhibiting prominent c-*fos* induction. The immunostaining was nuclear and in the striatum was principally (if not exclusively) in the medium-sized neurons that make up 90% or more of all striatal neurons and are the output neurons of the striatum. Few Fos-positive striatal neurons were seen in untreated or saline-treated controls (Fig. 2*C*). Nuclear immunostaining was absent in control sections incubated in the presence of Fos peptide sequences, indicating specificity of the staining for Fos, Fos-related antigens, or other nuclear antigens having similar peptide sequences.

Fos-like immunoreactivity was detectable 30–45 min after intraperitoneal injection of either drug, became pronounced by 2 hr (standard A2 and C2 series, see Table 1 and Fig. 1), and had declined by 6 hr. *In situ* hybridization experiments carried out on cocaine-treated rats indicated that the psychostimulant activation of Fos was associated with increased expression of c-*fos* mRNA and therefore could reflect transcriptional activation of the c-*fos* gene. There was a strong

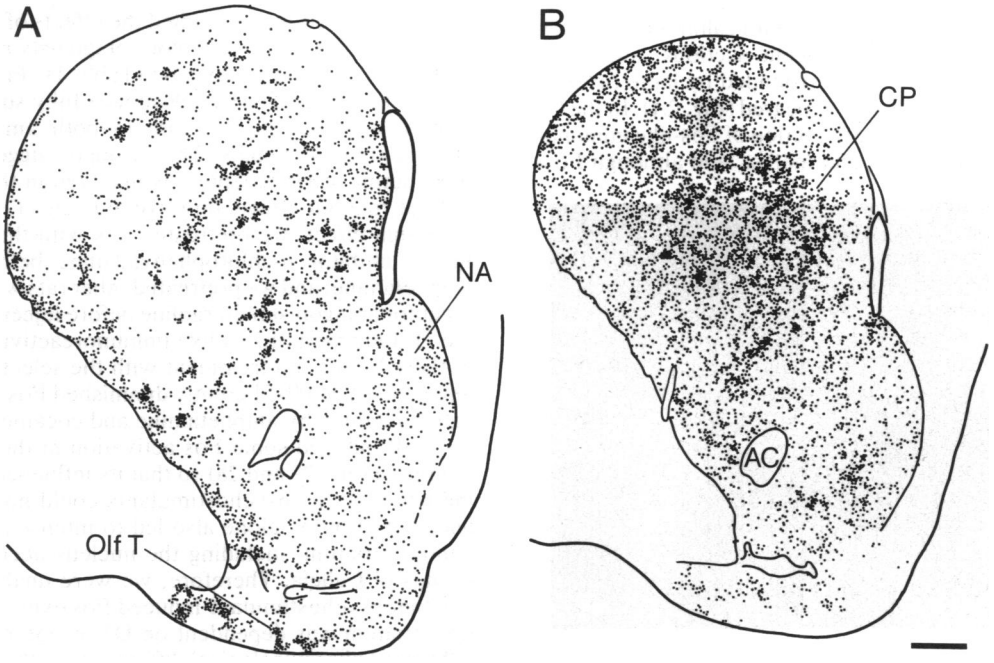

FIG. 1. Patterns of induction of nuclear Fos-like immunoreactivity in neurons of the caudoputamen (CP), nucleus accumbens (NA), and olfactory tubercle (Olf T) of rats given intraperitoneal injections of amphetamine (5 mg/kg of body weight) (*A*) and cocaine (25 mg/kg) (*B*) 2 hr before perfusion (standard A2 and C2 series of Table 1). Each black dot indicates one Fos-positive nucleus; no distinction is shown between darkly and weakly stained nuclei (compare with Fig. 2). AC, anterior commissure. (Bar = 0.5 mm.)

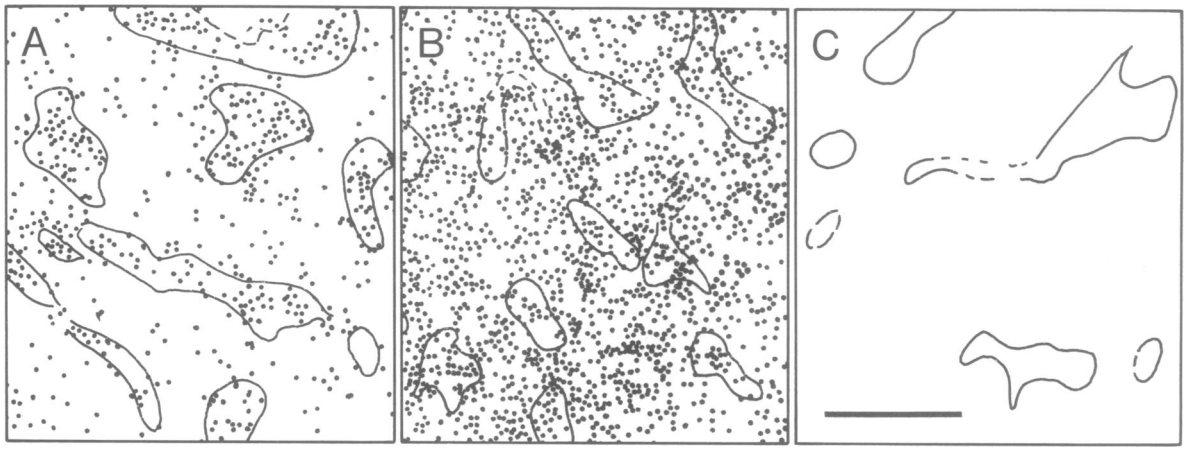

FIG. 2. Charts of zones in the anterior caudoputamen of amphetamine-treated (5 mg/kg of body weight for 2 hr) (*A*), cocaine-treated (25 mg/kg for 2 hr) (*B*), and control (untreated) (*C*) rats. The charts show the distribution of Fos-positive neurons (black dots) in relation to striosomal borders (solid and dotted forms) drawn from serial sections stained for calbindin D_{28k}. The zones illustrated are from sections at approximately matched levels. *B* is from the section shown in Fig. 1*B*. (Bar = 0.5 mm.)

hybridization signal in the striatum 1 hr after injection of cocaine (Fig. 3), whereas c-*fos* mRNA was undetectable in the striatum in untreated controls.

Strikingly different distributions of Fos-immunoreactive neurons appeared in the striatum in the amphetamine-treated and cocaine-treated rats (Fig. 1). In A2 animals, there was a vividly patchy pattern of Fos expression in the rostral caudoputamen (Fig. 1*A*). By contrast, in the C2 animals, there was much more homogeneous expression of Fos-like immunoreactivity (Fig. 1*B*). To explore this difference, we compared patterns of Fos-like immunoreactivity in the A2 and C2 animals to the patterns of striatal calbindin D_{28k}-like immunoreactivity, a marker that demonstrates the patchwork of striosomes as calbindin-poor zones embedded in a larger calbindin-positive matrix. The striosomes and matrix are the major neurotransmitter-specific compartments of the caudoputamen, and have different input–output connections and dopaminergic characteristics (20, 22–25).

In the A2 animals, the clusters of neurons with Fos-immunoreactive nuclei were aligned with calbindin-poor striosomes observed in serially adjacent sections, and these Fos-positive cell clusters were surrounded by large fields in which fewer Fos-positive nuclei appeared (Fig. 2*A*). In the C2 animals, there was generalized induction of Fos-like immunoreactivity in both striosomes and matrix, and although Fos-positive cells were not fully uniform in their distribution, the Fos expression did not seem selective for either compartment (Fig. 2*B*). Striosome-selective expression of Fos was a prominent feature of A2 animals at anterior striatal levels. At progressively more posterior levels, and especially medially, Fos expression in matrix cells increased until patchiness at some levels was no longer apparent or occurred only laterally. At more posterior levels in the cocaine brains, the region of induction tended to become progressively focused within the medial and dorsal caudoputamen.

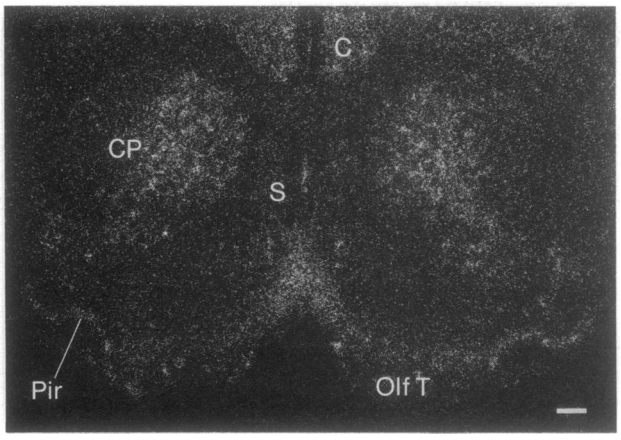

FIG. 3. *In situ* hybridization film autoradiogram documenting the presence of c-*fos* mRNA in the striatum of a rat treated with cocaine (25 mg/kg of body weight for 1 hr). CP, caudoputamen; Olf T, olfactory tubercle; Pir, piriform cortex; C, cingulate gyrus; S, septum. (Bar = 0.5 mm.)

The patterns of Fos expression induced by amphetamine and cocaine also differed in subregions of the ventral striatum. An example is shown in Fig. 1, where numerous Fos-positive neurons appear in the pyramidal layer of the olfactory tubercle in the A2 animal but not in the C2 animal. Cocaine typically elicited less expression of Fos in the core than in the shell of the nucleus accumbens, a pattern that was not characteristic of the amphetamine-treated brains.

To determine whether dopamine receptors were necessary for c-*fos* activation, we studied the effects of exposing rats to D1 and D2 dopamine receptor antagonists before treatment with amphetamine or cocaine (Table 1). Pretreatment with the D1 antagonist SCH 23390 almost fully suppressed induction of c-*fos* in the striatum in both amphetamine- and cocaine-treated animals. This strongly suggested that dopamine acting at D1 receptors is involved in the induction but did not rule out effects on serotonin sites (26). To test for a serotonin effect, we pretreated rats with the serotonin synthesis inhibitor *p*-chlorophenylalanine before injection of amphetamine, and we pretreated other rats with the serotonin receptor antagonist metergoline before injecting cocaine (Table 1). Induction of Fos-like immunoreactivity still occurred in these brains. Pretreatment with the selective D2 receptor antagonist YM-09151 greatly diminished Fos activation in the caudoputamen by amphetamine and cocaine, but YM-09151 by itself induced some Fos activation in the caudoputamen (compare refs. 27 and 28) so that its influence on subsequent induction by the psychostimulants could not be fully established. YM-09151 alone also led to intense activation in the ventral striatum, including the nucleus accumbens and the islands of Calleja. Therefore, we were unable to determine whether psychostimulant-induced Fos expression in the ventral striatum was dependent on D2 receptor stimulation.

Marked pharmacological differences in the c-*fos* activation induced by amphetamine and cocaine were suggested by the effects of monoamine depletion by reserpine (Table 1). In the caudoputamen (Fig. 4), acute or chronic pretreatment with reserpine greatly suppressed subsequent c-*fos* induction by

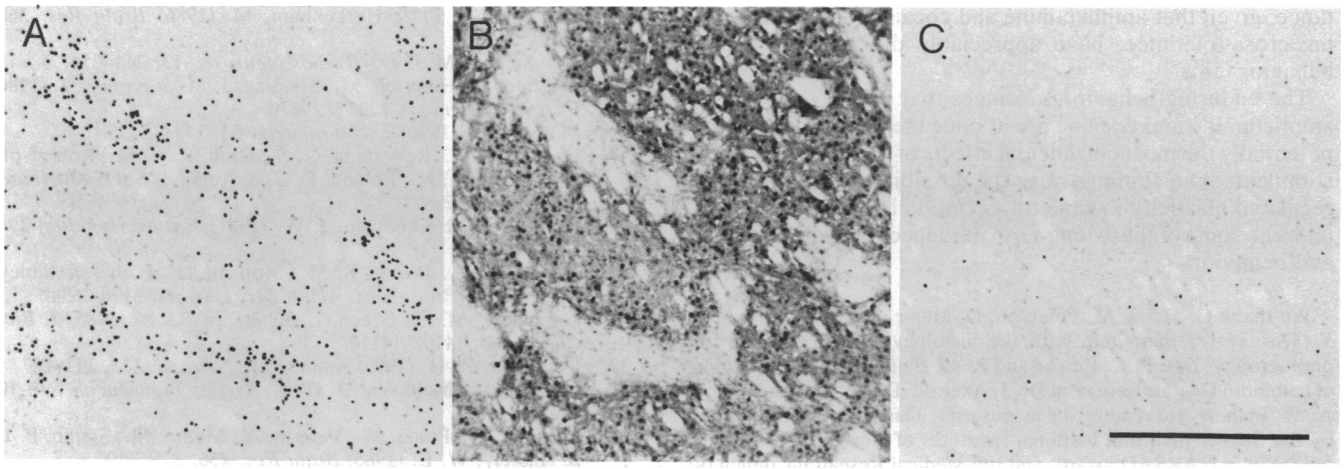

FIG. 4. Photographs showing contrasting effects of reserpine pretreatment on subsequent induction of Fos-like immunoreactivity in the caudoputamen by amphetamine (*A*) and cocaine (*C*). Reserpine (10 mg/kg of body weight) was injected 18 hr before perfusion, and amphetamine (5 mg/kg) and cocaine (25 mg/kg) were injected 2 hr before perfusion (R18A2 and R18C2 series, Table 1). (*A* and *B*) Matched zones from adjacent sections stained, respectively, for Fos-like immunoreactivity (*A*) and calbindin D_{28k} immunoreactivity (*B*). (Bar = 0.5 mm.)

cocaine (Fig. 4*C*). By contrast, amphetamine still evoked intense expression of Fos in acutely and chronically reserpine-pretreated rats (Fig. 4*A*). Striosomal patterning of Fos expression was even more pronounced in the reserpine- plus-amphetamine-treated rats than in many of the rats treated with amphetamine alone. In R18A2 animals, striosomes could be recognized throughout the caudoputamen as sites of especially strong immunostaining despite the presence of Fos-positive nuclei in the matrix (Fig. 4 *A* and *B*). Parallel effects were found for the ventral striatum in the chronically reserpine-treated animals: amphetamine still evoked activation of c-*fos* expression, but cocaine did not. Acute (18 hr) treatment with reserpine alone led to strong expression of Fos in the ventral striatum, and this pattern was seen also in the R18A2 and R18C2 animals.

DISCUSSION

The experiments described here establish that brief exposure to psychomotor stimulant drugs leads to rapid, drug-specific patterns of gene activation in neurons of the sensory-motor and limbic striatum. These findings have practical significance in suggesting that monitoring immediate-early genes may be a way to determine directly in *in vivo* experiments the central nervous system sites of action of psychostimulants. The observations also have a direct bearing on studies of the mechanisms underlying psychostimulant effects.

Our evidence suggests that induction of c-*fos* in the striatum by psychomotor stimulants depends on dopamine D1 receptor stimulation. Therefore, both cAMP/A kinase and calcium or C kinase signaling may underlie the induction, for D1 receptors are positively coupled to adenylate cyclase (29) and to inositol phospholipid (30) signaling pathways. It has been shown in *in vitro* experiments that the transcription of c-*fos* can be induced through interaction of nuclear proteins with cAMP-responsive regulatory elements (CREs) present in the gene (31), and some C kinase effects have been reported as well (32). Our findings also implicate D2 dopamine receptors in the psychomotor stimulant induction of c-*fos*, at least in the caudoputamen. D2 receptors also act on cAMP/A kinase and calcium or C kinase signaling pathways through negative direct [inhibitory guanine nucleotide-binding (G_i) protein] or indirect coupling (33, 34). D1-selective but not D2-selective dopamine agonists have been shown to activate c-*fos* in the dopamine-depleted striatum of rats treated unilaterally with the neurotoxin 6-hydroxydopamine (6).

The molecular sequels to this c-*fos* induction by psychostimulants are unknown, but they may involve further protein synthesis. Fos is known to act in heterodimeric association with other members of the Jun/AP-1 family of nuclear proteins to control the transcriptional activity of a number of other genes (35). Interestingly, both Fos (32, 36) and dopamine receptors (25, 37) have been directly implicated in regulation of neuropeptide genes including those of opioid peptides that are abundant in many medium-sized neurons of the striatum. Moreover, the opiate antagonist naloxone (38, 39) and the mixed opiate agonist–antagonist buprenorphine (40) block some of the addictive/reinforcing effects of cocaine, and morphine itself induces c-*fos* in the striatum (41). There thus may be a link between psychostimulant induction of c-*fos* and subsequent changes in neuropeptide expression in the striatum.

Our findings suggest that the mechanisms of c-*fos* induction triggered by cocaine and by amphetamine, though sharing some characteristics, are nevertheless distinct both pharmacologically and anatomically. First, the effects of cocaine on c-*fos* in the striatum are mediated by reserpine-sensitive pools of monoamine, whereas striatal c-*fos* induction by amphetamine is not blocked by reserpine. These findings raise the interesting possibility that the long-standing pharmacological classification of reserpine-sensitive and reserpine-insensitive pools of releasable catecholamine (42, 43) and their mobilization by psychostimulants (44, 45) may be related to the patterns of proto-oncogene induction described here. Second, the induction of c-*fos* by amphetamine was especially prominent in the striosomal system, whereas the induction of c-*fos* by cocaine occurred widely in both striatal compartments. This result suggests that the striosome and matrix compartments of the caudoputamen, with their different limbic and sensory-motor affiliations with cortex and subcortex (24, 46), contribute differently to the functional effects of cocaine and amphetamine.

Striosomes and matrix are known to have contrasting dopaminergic properties as judged by their profiles of *in vitro* binding for dopamine D1- and D2-selective ligands (47–49) and monoamine uptake-site ligands (50, 51) and by the characteristics of their mesostriatal innervations (52–56). Together with the differential effects of amphetamine and cocaine on monoamine release (greater for amphetamine) and reuptake (greater for cocaine) (42, 57), these characteristics could be critical to the pharmacological as well as the spatial distinctiveness of the psychostimulant effects. Understanding these differences could have particular functional impor-

tance, given that amphetamine and cocaine, although showing cross-tolerance, have appreciably different effects on behavior (58).

The enduring behavioral changes that follow exposure to amphetamine and cocaine are at once the most puzzling and potentially the most detrimental effects of these psychomotor stimulants. Our findings raise the possibility that dopamine-regulated plasticity in gene transcription in the striatum may underlie some of these long-term influences on neural activity and behavior.

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1. Koob, G. F. & Bloom, F. E. (1988) *Science* **242,** 715–723.
2. Ritz, M. C., Lamb, R. J., Goldberg, S. R. & Kuhar, M. J. (1987) *Science* **237,** 1219–1223.
3. Groves, P. M., Ryan, L. J. & Linder, J. C. (1987) *NIDA Res. Monogr.* **78,** 132–142.
4. Wise, R. W. (1984) *NIDA Res. Monogr.* **50,** 15–33.
5. Young, S. T., Porrino, L. J. & Iadarola, M. J. (1989) *Soc. Neurosci. Abstr.* **15,** 1091.
6. Robertson, H. A., Peterson, M. R., Murphy, K. & Robertson, G. S. (1989) *Brain Res.* **503,** 346–349.
7. Mueller, R. A., Grimes, L. M., Criswell, H., Carter, L. S., McGimsey, W. C., Stumpf, W. & Breese, G. R. (1989) *Soc. Neurosci. Abstr.* **15,** 430.
8. Johnson, K. & Robertson, H. A. (1989) *Soc. Neurosci. Abstr.* **15,** 782.
9. Peterson, M., Moratalla, R., Graybiel, A. M. & Robertson, H. A. (1990) *Can. J. Neurol. Sci.*, in press.
10. Mitchell, P. J. & Tjian, R. (1989) *Science* **245,** 371–378.
11. Goelet, P., Castelluci, V. F., Schacher, S. & Kandel, E. R. (1986) *Nature (London)* **322,** 419–422.
12. Berridge, M. (1986) *Nature (London)* **323,** 294–295.
13. Cole, A. J., Saffen, D. W., Baraban, J. M. & Worley, P. F. (1989) *Nature (London)* **340,** 474–476.
14. Sonnenberg, J. L., Macgregor-Leon, P. F., Curran, T. & Morgan, J. I. (1989) *Neuron* **3,** 359–365.
15. Sagar, S. M., Sharp, F. R. & Curran, T. (1988) *Science* **240,** 1328–1331.
16. Iorio, L. C., Barnett, A., Leitz, F. H., Houser, V. P. & Korduba, C. A. (1983) *J. Pharmacol. Exp. Ther.* **226,** 462–468.
17. Iwanami, S., Takashima, M., Hirata, Y., Hasegawa, O. & Usuda, S. (1981) *J. Med. Chem.* **24,** 1224–1230.
18. Fuller, R. W. (1980) *Annu. Rev. Pharmacol. Toxicol.* **20,** 111–127.
19. Gerfen, C. R., Baimbridge, K. G. & Miller, J. J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8780–8784.
20. Graybiel, A. M. & Ragsdale, C. W., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5723–5726.
21. Baldino, F., Jr., Chesselet, M.-F. & Lewis, M. E. (1989) *Methods Enzymol.* **168,** 761–777.
22. Graybiel, A. M. (1989) in *Neural Mechanisms in Disorders of Movement*, eds. Crossman, A. & Sambrook, M. A. (Libbey, London), pp. 3–15.
23. Gerfen, C. R. (1989) *Science* **246,** 385–388.
24. Donoghue, J. P. & Herkenham, M. (1986) *Brain Res.* **365,** 397–403.
25. Graybiel, A. M. (1990) *Trends Neurosci.* **13,** 244–254.
26. Bischoff, S., Heinrich, M., Sonntag, J. M. & Krauss, J. (1986) *Eur. J. Pharmacol.* **129,** 367–370.
27. Miller, J. C. (1990) *J. Neurochem.* **54,** 1453–1455.
28. Dragunow, M., Robertson, G. S., Faull, R. L. M., Robertson, H. A., Jansen, K., Emson, P. C. & Augood, S. (1990) *Neuroscience*, in press.
29. Stoof, J. C. & Kebabian, J. W. (1981) *Nature (London)* **294,** 366–368.
30. Mahan, L. C., Burch, R. M., Monsma, F. J., Jr., & Sibley, D. R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2196–2200.
31. Greenberg, M. E., Green, L. A. & Ziff, L. B. (1985) *J. Biol. Chem.* **260,** 14101–14110.
32. Goodman, R. H. (1990) *Annu. Rev. Neurosci.* **13,** 111–127.
33. Vallar, L. & Meldolesi, J. (1989) *Trends Pharmacol. Sci.* **10,** 74–77.
34. Pizzi, M., Da Prada, M., Valerio, A., Memo, M., Spano, P. F. & Haefely, W. E. (1988) *Brain Res.* **456,** 235–240.
35. Curran, T. & Franza, B. R., Jr. (1988) *Cell* **55,** 395–397.
36. Sonnenberg, J. L., Rauscher, F. J., III, Morgan, J. I. & Curran, T. (1989) *Science* **246,** 1622–1625.
37. Jiang, H. K., McGinty, J. F. & Hong, J. S. (1990) *Brain Res.* **507,** 57–64.
38. Bain, G. T. & Kornetsky, C. (1987) *Life Sci.* **40,** 1119–1125.
39. Koob, G. F., Le, H. T. & Creese, I. (1987) *Neurosci Lett.* **79,** 315–320.
40. Mello, N. K., Mendelson, J. H., Bree, M. P. & Lukas, S. E. (1989) *Science* **245,** 859–862.
41. Chang, S. L., Squinto, S. P. & Harlan, R. E. (1988) *Biochem. Biophys. Res. Commun.* **157,** 698–704.
42. Glowinski, J., Iversen, L. L. & Axelrod, J. (1966) *J. Pharmacol. Exp. Ther.* **151,** 385–399.
43. Glowinski, J. (1970) in *Handbook of Neurochemistry*, ed. Lajtha, A. (Plenum, New York), Vol. 4, pp. 91–114.
44. Shore, P. A., McMillen, B. A., Miller, H. H. & Sanghera, M. K. (1979) in *Catecholamines, Basic and Clinical Frontiers*, eds. Usdin, E., Kopin, I. J. & Barchas, J. (Pergamon, New York), pp. 722–727.
45. McMillen, B. A. (1983) *Trends Pharmacol. Sci.* **4,** 429–432.
46. Ragsdale, C. W., Jr., & Graybiel, A. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6196–6199.
47. Loopuijt, L. D. (1989) *Brain Res. Bull.* **22,** 805–817.
48. Besson, M.-J., Graybiel, A. M. & Nastuk, M. (1988) *Neuroscience* **26,** 101–119.
49. Joyce, J. N., Sapp, D. W. & Marshall, J. F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8002–8006.
50. Graybiel, A. M. & Moratalla, R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9020–9024.
51. Lowenstein, P. R., Joyce, J. N., Coyle, J. T. & Marshall, J. F. (1990) *Brain Res.* **510,** 122–126.
52. Olson, L., Seiger, A. & Fuxe, K. (1972) *Brain Res.* **44,** 283–288.
53. Gerfen, C. R. (1984) *Nature (London)* **311,** 461–464.
54. Jimenez-Castellanos, J. & Graybiel, A. M. (1987) *Neuroscience* **23,** 223–242.
55. Langer, L. A. & Graybiel, A. M. (1989) *Brain Res.* **498,** 344–350.
56. Kemel, M. L., Desban, M., Glowinski, J. & Gauchy, C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9006–9010.
57. Fischman, M. W. (1987) in *Psychopharmacology: The Third Generation of Progress*, ed. Meltzer, H. Y. (Raven, New York), pp. 1543–1553.
58. Scheel-Krüger, J., Braestrup, C., Nielson, M., Golembiowska, K. & Mogilnicka, E. (1977) in *Cocaine and Other Stimulants, Advances in Behavior and Biology*, eds. Ellinwood, E. H., Jr., & Kilbey, M. M. (Plenum, New York), Vol. 21, pp. 373–407.