

# The Activity-Regulated Cytoskeletal-Associated Protein Arc Is Expressed in Different Striosome–Matrix Patterns Following Exposure to Amphetamine and Cocaine

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**Abstract:** The activity-regulated, cytoskeletal-associated gene, *arc*, is a brain-enriched immediate-early gene whose expression is rapidly induced in the striatum by dopamine receptor agonists. This rapid induction of *arc* in the striatum is similar to that of other early response genes such as *c-fos*, *junB*,  $\Delta$ *fosB*, *fra*, and *NGFI-A*, which code for transcription factors. Unlike these proteins, however, Arc is a cytoskeletal protein expressed not only in the nucleus of neurons but also in their dendrites. We investigated the patterns of Arc expression evoked in the rat striatum by acute exposures to two psychomotor stimulants, cocaine and amphetamine. Cocaine induced *arc* in striatal neurons that were broadly distributed within both striosome and matrix compartments of the caudoputamen. Amphetamine also evoked Arc expression in striatal projection neurons, but these were heavily concentrated in the striosomal compartment and only sparsely in the matrix compartment in the rostral striatum. The contrasting patterns of Arc expression evoked by cocaine and amphetamine parallel those of c-Fos, JunB, FRA, and NGFI-A expression induced by these two psychomotor stimulants. This difference in the action of cocaine and amphetamine at the level of protein expression may be linked to the different effects of these psychomotor stimulants on behavior. **Key Words:** Cytoskeletal protein—Dopamine—Striatum—Gene expression—Psychomotor stimulants—Striosome.

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Amphetamine and cocaine are two widely abused psychomotor stimulant drugs that produce marked alterations in behavior and mood in humans (Johanson et al., 1976; Robinson and Becker, 1986; Fischmann, 1987; Kalivas and Stewart, 1991; Cole et al., 1992; Koob, 1992; Hyman, 1996; Koob and Le Moal, 1997; Nestler and Aghajanian, 1997; Pierce and Kalivas, 1997; Wise, 1998; Gainetdinov et al., 1999). A single moderate dose of cocaine or amphetamine typically leads to increased activity in adults and to reports of euphoria and general well-being. With higher doses, motor activity becomes repetitive, producing motor stereotypies. Extremely high

doses produce convulsions, hyperthermia, coma, and death. These behavioral effects in the human are paralleled in other animals. In addition, in both humans and other species, exposure to cocaine and amphetamine increases the probability of a drug-seeking response and can lead to drug addiction (Johanson et al., 1976; Fischmann, 1987; Koob and Le Moal, 1997).

At the cellular level, cocaine and amphetamine both increase synaptic dopamine levels in nigrostriatal and mesolimbic circuits, but via different mechanisms: Cocaine blocks the reuptake of synaptic dopamine by the dopamine transporter, whereas amphetamine releases dopamine and reverses its transport via the dopamine transporter (Hyman, 1996; Koob and Le Moal, 1997). Cocaine and amphetamine also increase synaptic levels of serotonin and norepinephrine. It is the increased amount of dopamine that is thought to be critical for the reinforcing effects of these drugs, and dopamine and serotonin for the motor effects (Robinson and Becker, 1986; Ritz et al., 1987; Kalivas and Stewart, 1991; Giros et al., 1996; Hyman, 1996; Gainetdinov et al., 1999).

Both cocaine and amphetamine also induce the expression in the brain of immediate-early genes such as *c-fos*, *junB*,  $\Delta$ *fosB*, *fra*, and *NGFI-A*, and this expression is especially strong in the dopamine-recipient striatum (Graybiel et al., 1990, 1995; Hope et al., 1994; Hughes and Dragunow, 1995; Hyman, 1996; Koob and Le Moal, 1997; Gerfen et al., 1998; Kelz et al., 1999). These genes

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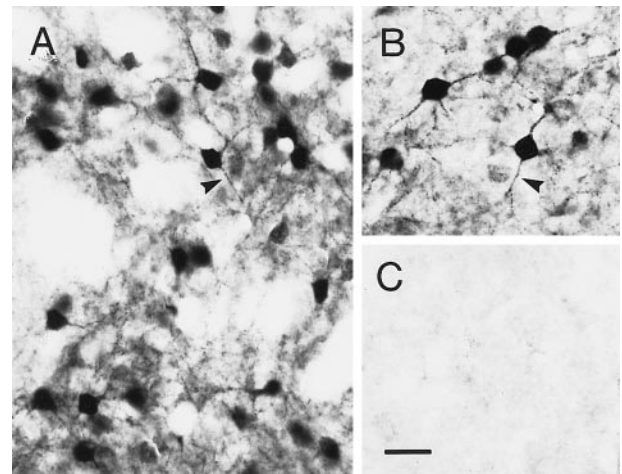
*Abbreviations used:* *arc*, activity-regulated, cytoskeletal-associated gene; MOR1,  $\mu$ -opiate receptor; PBS-TX, 0.01 M sodium phosphate, 0.15 M sodium chloride, 0.03 M potassium chloride, and 0.2% Triton X-100.

code for transcription factors that act in the nucleus on downstream target genes, including those encoding neuropeptides and other neurotransmitter-related molecules expressed in the striatum (Graybiel, 1990; Gerfen et al., 1991). Despite the fact that amphetamine and cocaine can affect similar genes in the striatum, the anatomical patterns of striatal gene expression are different for the two psychomotor stimulants. Cocaine treatment across a range of doses induces these genes in neurons that are distributed broadly through the striatum, but amphetamine treatment induces the gene expression in a compartmental pattern in which there is differentially high expression in striosomes rostrally (Graybiel et al., 1990; Moratalla et al., 1992, 1996). This difference is not yet understood at the molecular level, but it is likely to be important at the systems level: Corticobasal ganglia circuits involving striosomes differ sharply from those of the surrounding matrix compartment (Graybiel et al., 1990; Gerfen, 1992), and in chronically treated animals, evidence suggests that such compartmental differences in striatal cell activation are correlated with different behavioral effects (Canales and Graybiel, 2000).

In the study reported here, we asked whether these differential distribution patterns would also hold for the induction of *arc* (activity-regulated, cytoskeletal-associated gene), a recently discovered immediate-early gene that is regulated in neurons by synaptic activity (Fosnaugh et al., 1995; Lyford et al., 1995). The special interest of *arc* is that *arc* mRNA and Arc protein are found in neuronal dendrites. High-frequency stimulation has been found to localize Arc selectively to activated dendrites (Steward et al., 1998). *Arc* encodes a protein with modest homology to  $\alpha$ -spectrin that co-precipitates with F-actin and localizes to the extranuclear region of neurons, suggesting that Arc is a cytoskeletal protein (Lyford et al., 1995). In their initial work on *arc*, Fosnaugh et al. (1995) demonstrated that *arc* is rapidly induced in the striatum by a single dose of cocaine. We show here that the expression of this activity-dependent cytoskeletal protein is broadly induced in the rostral striatum by cocaine, but that amphetamine differentially activates its expression preferentially in rostral striosomes. These contrasting activation patterns, similar to those found for nuclear transcription factors, suggest that amphetamine and cocaine induce a coordinate immediate-early gene response that may exert rapid compartment-specific effects on dendritic function in striatal neurons.

## MATERIALS AND METHODS

Drug-naive adult male Sprague-Dawley rats (180–200 g) and c57b6 mice (20–25 g) were housed in the MIT animal facility in pairs for a minimum of 2–4 days before the start of each experiment and were maintained under a 12-h light/dark cycle with free access to food and water. Each animal was then given a single intraperitoneal injection of 5 or 10 mg/kg D-amphetamine sulfate (Sigma), 25 mg/kg cocaine hydrochloride (Sigma), or 0.9% saline solution. Drugs were dissolved in 0.9% saline. These doses were chosen as representative of a



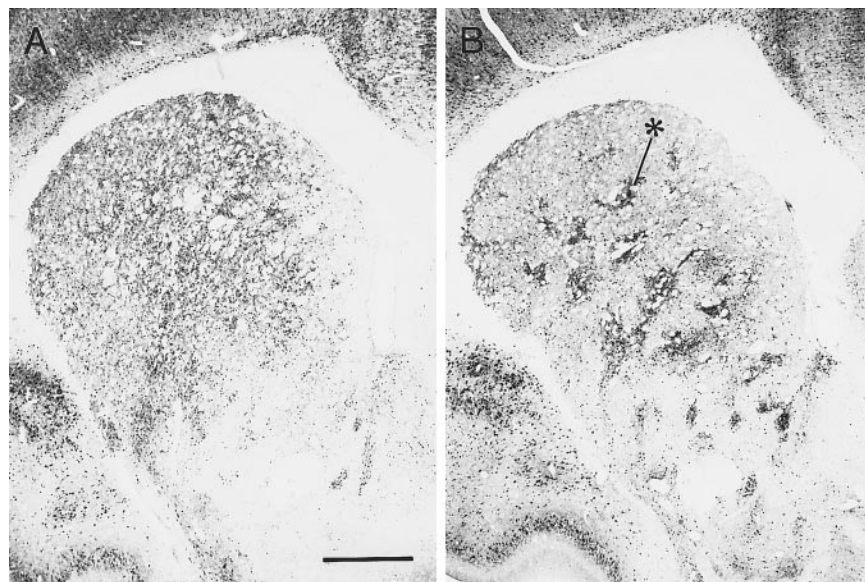
**FIG. 1.** Expression of Arc in medium-sized neurons of the rat striatum following a single exposure to amphetamine (5 mg/kg) or cocaine (25 mg/kg). **A** and **B**: Photomicrographs illustrate Arc immunostaining in neurons of the caudoputamen following amphetamine (**A**) and cocaine (**B**) treatments. The cell body and proximal dendrites of the neurons are stained as is the surrounding neuropil; arrowheads indicate dendrite in **A** and **B**. **C**: Photomicrograph illustrates near absence of staining in the caudoputamen in saline-treated control rat. Bar = 20  $\mu$ m.

range of dose levels for amphetamine (2.5–15 mg/kg) and cocaine (5–50 mg/kg) at which the two psychomotor stimulants induce contrasting patterns of induction of nuclear immediate-early genes in the striatum (Moratalla et al., 1992; R. Moratalla and A. M. Graybiel, unpublished observations). Two hours after injection, animals were deeply anesthetized and fixed by transcardial perfusion (4% paraformaldehyde, 0.1 M sodium cacodylate, pH 7.4), and the brains were cut into 20- $\mu$ m-thick sections on a sliding microtome. Experimental protocols were in accord with the guidelines outlined in the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Massachusetts Institute of Technology Committee on Animal Care.

Free-floating sections were washed in a solution of 0.01 M sodium phosphate, 0.15 M sodium chloride, 0.03 M potassium chloride, and 0.2% Triton X-100 (Sigma) (PBS-TX), incubated successively in 3% hydrogen peroxide (10 min) and 5% normal goat serum (30 min) in PBS-TX, and incubated for 5 days with polyclonal Arc antiserum (1:100 in PBS-TX) characterized by Lyford et al. (1995). Sections were then incubated successively in biotinylated goat anti-rabbit secondary antibody (1 h), streptavidin (1 h), and a solution of 0.002 M ammonium nickel sulfate, 0.0006 M diaminobenzidine, 0.08 M potassium phosphate, and 0.02 M sodium phosphate. A similar protocol was followed for staining with polyclonal  $\mu$ -opiate receptor antiserum (MOR1; at 1:100,000; kindly donated by Dr. R. P. Elde, University of Minnesota, Minneapolis, MN, U.S.A.).

## RESULTS

Both cocaine and amphetamine induced the expression of Arc in medium-sized neurons in the caudoputamen (Fig. 1A and B). The immunoreactive neurons had the morphology of medium-sized projection neurons, which make up most of the neuronal population in the



**FIG. 2.** Induction of Arc immunoreactivity in compartment-selective patterns in the rat striatum. **A:** Acute treatment with cocaine (25 mg/kg) induces a diffuse pattern of Arc expression in the caudoputamen. **B:** Acute treatment with amphetamine (5 mg/kg) induces Arc expression in clusters of neurons in the caudoputamen. Asterisk shows example of a cluster of Arc-positive neurons. Bar = 0.5 mm.

striatum, and they were stained strongly throughout the cell body. The immunostaining clearly extended into the dendrites of these neurons, although it faded distally (Fig. 1A and B). There was at most faint immunostaining in the striatum of the saline-treated controls (Fig. 1C). Arc immunostaining was visible in the neocortex and elsewhere in the brains of the drug-treated animals but was not analyzed for this report.

The striatal distributions of Arc-immunoreactive neurons differed sharply in the cocaine-treated and amphetamine-treated rats (Fig. 2). In cocaine-treated animals, Arc-immunoreactive neurons were present throughout most of the caudoputamen at rostral levels (Fig. 2A) and in a large centromedial region of the caudoputamen farther caudally. Arc-positive neurons appeared in the ventral caudoputamen and in the ventral striatum as well (Fig. 2A), but the levels of increased staining there were much lower than in the dorsal two-thirds of the caudoputamen.

In the amphetamine-treated rats, Arc-immunoreactive neurons were grouped in distinct 20- to 80- $\mu$ m-wide clusters in the rostral caudoputamen (Fig. 2B). Each cluster of Arc-immunostained neurons was also distinguished by having a more darkly stained neuropil than that of surrounding regions. Few Arc-positive neurons

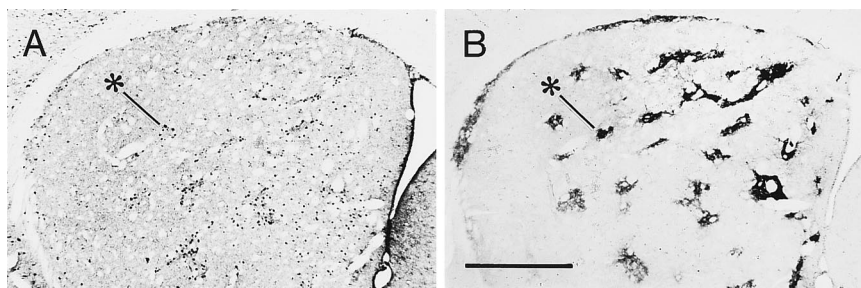
were present outside these clusters, so that the entire rostral striatum presented a patchy staining pattern (Fig. 2B). In progressively more caudal sections, the patchiness of the Arc immunostaining diminished, and Arc-positive neurons became more common in the centromedial region of the caudoputamen until, at caudal levels, the pattern of neuronal staining in the caudoputamen became indistinguishable from that found at corresponding levels in the cocaine-treated rats. Similar patterns were visible in mice (Fig. 3A).

To determine whether the Arc-positive cell clusters seen after administration of amphetamine corresponded to striosomes, pairs of adjacent sections from the striatum of amphetamine-treated animals were stained, respectively, for MOR1 as a marker of striosomes, or for Arc. Figure 3 shows the results in sections from the mouse: The clusters of Arc-positive neurons corresponded precisely to MOR1-positive striosomes.

## DISCUSSION

The striking differences in the patterns of Arc expression triggered by exposure to cocaine and amphetamine parallel those found for c-Fos, JunB, FRA, and NGFI-A. A patchy, striosome-predominant pattern of expression

**FIG. 3.** Comparison of amphetamine-induced Arc immunostaining and immunostaining for MOR1, a marker of striosomes. **A:** Patchy distribution of Arc immunostaining in the caudoputamen of a mouse given a single dose of amphetamine (10 mg/kg) is shown. **B:** Section serially adjoining that shown in A illustrates MOR1-immunoreactive striosomes. Asterisks indicate examples of corresponding striosome in A and B. Bar = 0.5 mm.



in the rostral caudoputamen is evoked by amphetamine, but a noncompartmental pattern of expression occurs at these same rostral levels following acute treatment with cocaine. For Arc, the immunopositive striosomes differentially labeled as a result of amphetamine administration were defined not only by stained nuclei, but also by cytoplasmic staining of medium-sized neurons and by a more darkly stained neuropil. We were not able to achieve complete staining in the dendrites of these neurons either in the rat or in the mouse, but the extensive cytoplasmic immunoreactivity that we did detect, particularly in the rat, is consistent with evidence that Arc is a cytoskeletal protein.

Our findings suggest that acute exposures to cocaine and to amphetamine may have different effects at the level of the cytoskeleton as well as at the level of transcriptional control. Indirect evidence suggests that changes to the cytoskeleton may be important in the molecular description of how psychomotor stimulants act (Ohmi and Nakamura, 1991; Johnson and Byerly, 1993; Rosenmund and Westbrook, 1993; Torres and Rivier, 1993; Johnston and Morris, 1994a,b; Nestler and Aghajanian, 1997). Our results suggest that *arc* induction may be involved in these molecular cascades, and that its effects would be exerted differentially on compartmentally distinct subpopulations of striatal projection neurons depending on whether the psychomotor stimulant were cocaine or amphetamine.

In prior studies, it has been unclear how the ability of psychomotor stimulants to regulate immediate-early genes influences the rapid behavioral responses to these drugs. The activation of immediate-early genes has been interpreted primarily as a potential first stage in a chain of transcriptional events that could influence long-term plasticity in neurons (Morgan and Curran, 1989; Hughes and Dragunow, 1995; Kelz et al., 1999). Arc mRNA has been shown to accumulate selectively in regions of the dendritic tree that have received recent synaptic activity, providing a potential mechanism to confer synapse-specific effects of the gene (Steward et al., 1998). Our findings suggest that both cocaine and amphetamine can rapidly influence the composition of this activity-related protein in dendrites of striatal neurons, and that this influence can be exerted differentially on striosome- and matrix-based circuits of the basal ganglia. This differential influence may help account for the differences in behavior rapidly induced by the two psychomotor stimulants (Randrup and Munkvad, 1972).

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