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# Corticostriatal Up-Regulation of Activity-Regulated Cytoskeletal-Associated Protein Expression after Repeated Exposure to Cocaine

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## ABSTRACT

We provide evidence that cocaine evokes short- and long-lasting increases in activity-regulated cytoskeletal-associated protein (Arc) expression after a finely tuned, time-dependent and regional-selective expression profile. Acute experiments revealed that cocaine up-regulates Arc expression primarily in striatum and prefrontal cortex through a dopamine D1-dependent mechanism and a combination of D1- and D2-dependent mechanisms, respectively. Aside from cocaine-dependent Arc elevation, we show for the first time that D1 and D2 receptors tonically regulate basal Arc expression following a regional-selective profile. As opposed to the effects of a single cocaine injection on Arc expression, which dissipate within 24 h, subchronic (five daily injections) or chronic (14 daily injections) cocaine administration, with animals sacrificed hours or days after the last treatment, demonstrated that Arc expression is still up-regulated long after treatment cessation, suggesting that adaptive changes have been set in motion by the prolonged administration of the psychostimulant. In summary, our findings are the first to demonstrate that repeated exposure to cocaine leads to long-lasting dysregulation of Arc expression in the corticostriatal network, thus establishing a molecular basis to explain, at least partially, the impaired synaptic transmission caused by cocaine abuse at this level. Furthermore, given the role exerted by Arc in cytoarchitectural rearrangements, it is conceivable to speculate that it mediates changes in synaptic connectivity brought about by cocaine. Our findings thus pinpoint this molecule as a neuropathological underpinning and molecular bridge that connects short- and long-term neuronal modifications associated with cocaine abuse.

Cocaine is a psychostimulant agent highly abused worldwide that places an enormous social and economic burden on modern society. Addiction to cocaine is both mental and physical and largely depends upon neurochemical, structural, and behavioral brain alterations (Nestler, 2005).

One of the main interests has indeed been the comprehension of the early changes set in motion soon after the exposure to cocaine, because it is believed that such alterations can set the stage for long-lasting modifications. To this end, attention has been focused on immediate early genes (IEGs) that are divided into 1) genes that encode transcription factors, which indirectly alter the expression of a given target gene, and 2) genes that encode effector proteins, which act directly on cellular homeostasis and function.

In recent years, much has been learned about the functions of IEGs. Cocaine acutely induces the expression of c-fos, zif/268, and Fra-2 (Bhat and Baraban, 1993; Liu et al., 2005), mainly at the striatal level. However, the expression of these IEGs is reduced as a consequence of prolonged treatment (Bhat et al., 1992; Freeman et al., 2002), clearly indicating that the role of these proteins is limited to the initial phase of the action of the psychostimulant. A novel effector IEG,

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**ABBREVIATIONS:** IEG, immediate early gene; Arc, activity-regulated cytoskeletal-associated protein; SCH 23390, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; SKF 81297, (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, analysis of variance.

called activity-regulated cytoskeletal-associated protein (Arc) has been cloned (Lyford et al., 1995); it has the peculiar characteristic of being localized at dendritic processes, a feature that allows the local synthesis of the protein in case of demand. In fact, Arc expression is increased after high-frequency activation of the perforant pathway projections (Link et al., 1995; Steward et al., 1998) or after acute electroconvulsive stimulation in the rat (Larsen et al., 2005), pointing to Arc as a reliable index of activity-dependent synaptic modifications.

Because of the high sensitivity to changes in neuronal activity and its peculiar localization, Arc could represent a preferential target for drugs of abuse. Although the effects of different drugs of abuse have been studied after both acute and chronic paradigms (Kodama et al., 1998; Schiltz et al., 2005; Schochet et al., 2005), Arc expression has thus far been investigated after a single cocaine treatment only (Fosnaugh et al., 1995), leaving unanswered the question as to whether Arc contributes to the long-term action of this psychostimulant. This information could be highly relevant because repeated exposure to cocaine alters the morphology of dendrites and spines in different brain regions (Robinson and Kolb, 1999; Robinson et al., 1999, 2001; Norrholm et al., 2003), an effect that could be mediated, at least in part, by Arc, as it is known to interact with components of the cytoskeleton such as F-actin and microtubule-associated protein-2 (Lyford et al., 1995; Fujimoto et al., 2004). In addition, recent data revealed that Arc also interacts with elements of the postsynaptic signaling machinery such as $\alpha$-calcium calmodulin kinase II, postsynaptic density-95, and $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (Okuno et al., 2004; Shepherd et al., 2004), which are known to be modulated by cocaine (Park et al., 2002; Licata et al., 2004; Yao et al., 2004), further pointing to Arc as a critical crossroad of multiple signals that converge into the stabilization of synaptic changes promoted by the psychostimulant.

Therefore, the purpose of the present study was to analyze the effects of prolonged treatments with cocaine on Arc expression to evaluate whether cocaine may affect synaptic plasticity through changes in Arc expression. In addition, we expanded our overall knowledge on the effects of a single cocaine injection by evaluating the time-dependent and regional-specific profile of Arc modulation, information that could be relevant in clarifying the mechanisms of the putative effects brought about by a prolonged treatment with this psychostimulant.

## Materials and Methods

### Materials

General reagents were purchased from Sigma-Aldrich (Milan, Italy), and molecular biology reagents were obtained from Cellbio (Pero, Milan, Italy) and Promega (Milan, Italy). Selective antagonists and agonists of dopaminergic D1 and D2 receptors were purchased from Sigma-Aldrich.

### Drug Treatments

**Single Injection.** We investigated the effect of a single injection of cocaine through two different approaches. First, we analyzed the dose-response profile, with rats receiving a single injection of cocaine at different doses (5, 10, and 20 mg/kg); animals were sacrificed by decapitation 2 h after the single administration. In the second experiment, rats received a single injection of cocaine (5 mg/kg) and were sacrificed 0.5, 2, 6, and 24 h after the treatment to evaluate the temporal profile of Arc expression produced by the psychostimulant.

To evaluate the role of dopaminergic receptors in acute cocaine-induced Arc elevation, selective blockers of D1 (SCH 23390; 1 mg/kg) and D2 (raclopride; 2 mg/kg) dopaminergic receptors were administered 30 min before treatment with cocaine (5 mg/kg), alone, or concomitantly. To examine the tonic role of dopaminergic receptors on Arc expression, animals were treated with a single injection of the selective D1 agonist SKF 81297 (3 mg/kg), the selective D1 antagonist SCH 23390 (1 mg/kg), or the selective D2 agonist quinpirole (1 mg/kg) and sacrificed 2 h after the injection.

**Repeated Injections.** We performed two different treatments with cocaine (subchronic and chronic); in the subchronic administration, animals were subjected to five consecutive daily injections (5 mg/kg) and sacrificed 2 and 72 h after the fifth administration. In the chronic cocaine treatment, animals were subjected to 14 consecutive daily injections (5 mg/kg) and were sacrificed 2, 72, and 336 h (14 days) after the last drug administration. For each time point, in the acute and subchronic or chronic experiments, there was a saline-treated group of animals that matched each cocaine-treated group.

After animal sacrifice, brain regions were immediately dissected, frozen on dry ice, and stored at −70°C. Dissections were performed according to the atlas of Paxinos and Watson (1996). The prefrontal cortex, weighing approximately 7 mg, was dissected from 2-mm slices (prefrontal cortex defined as Cg1, Cg3, and infralimbic cortex subregions corresponding to plates 6–9), whereas hippocampus and striatum were grossly dissected. All animal handling and experimental procedures were performed in accordance with the EC guidelines (EEC Council Directive 86/609, 1987) and the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

### RNA Preparation

The tissue from different brain structures was homogenized in 4 M guanidinium isothiocyanate (containing 25 mM sodium citrate, pH 7.5, 0.5% sarcosyl, and 0.1% 2-mercaptoethanol), and total RNA was isolated by phenol-chloroform extraction. Quantitation was carried out by spectrophotometric analysis, and RNA aliquots were reprecipitated in ethanol for RNase protection assays.

### cRNA Probes and RNase Protection Assay

A transcription kit (MAXI script; Ambion, Austin, TX) was used to generate cRNA probes, and [$^{32}$P]CTP was used as a radiolabeled nucleotide. The following plasmids were employed in the RNase protection assay: Arc cDNA plasmid containing a portion of rat 5′ coding region (a generous gift of Dr. P. Worley, Johns Hopkins University, Baltimore, MD) and pTRI-GAPDH-Rat (Ambion) containing a portion of rat GAPDH. The cRNA probes and relative protected fragment were as follows: Arc, 630, protected fragment, 620; GAPDH, 359, protected fragment, 316.

The RNase protection assay was performed on a 10-$\mu$g sample of total RNA as described previously (Riva et al., 1996). In brief, after ethanol precipitation, total RNA, was dissolved in 20 $\mu$l of hybridization solution containing 50,000 cpm of $^{32}$P-labeled Arc and 50,000 cpm of $^{32}$P-labeled GAPDH cRNA probe. After being heated at 85°C for 10 min, the cRNA probes were allowed to hybridize the endogenous RNAs at 45°C overnight. At the end of hybridization, the solution was diluted with 200 $\mu$l of RNase digestion buffer containing a 1/400 dilution of an RNase cocktail (RNase A and RNase T1) and incubated for 30 min at 30°C. Proteinase K and SDS were then added to the sample, and the mixture was incubated at 37°C for an additional 15 min. At the end of incubation, the sample was extracted with phenol/chloroform and precipitated with ethanol. The pellet, containing the RNA/RNA hybrids, was dried and resuspended in loading buffer, boiled at 95°C for 5 min, and separated on 5% polyacrylamide gel under denaturing conditions.

### RNA Calculation

The levels of mRNA for Arc or GAPDH were calculated using the Quantity One software from Bio-Rad (Milan, Italy). To ensure that the autoradiographic bands were in the linear range of intensity, different exposure times were used. GAPDH was employed as an internal standard for RNase protection assay because its expression was not regulated by single or repeated drug treatments. Results were compiled as the unitless ratio of Arc/GAPDH mRNA.

### Preparation of Protein Extracts and Western Blot Analysis

Tissues were homogenized in six volumes (v/w) of hypotonic lysis buffer [150 mM Tris, 150 mM NaCl, 5 mM EDTA, 1% protease inhibitor cocktail (Sigma-Aldrich), pH 7.6], and the tissue was lysed by sonication. The suspension was centrifuged at 20,000*g* for 20 min (4°C). Total protein content was measured in the homogenate by the Bio-Rad Protein Assay.

Western blot analysis was performed on the homogenate. Total protein concentrations were adjusted to the same amount for all samples (10 $\mu$g per lane). All samples were run on an SDS-10% polyacrylamide gel under reducing conditions, and proteins were then electrophoretically transferred onto nitrocellulose membranes (Bio-Rad). Blots were blocked with 10% nonfat dry milk and then incubated with primary antibody. Arc-native form was detected by evaluating the band density at 55 kDa, probing with a rabbit polyclonal antibody (1:4000, 1 h, room temperature) (generous gift from Dr. P. Worley). Results were standardized to a $\beta$-actin control protein that was detected by evaluating the band density at 43 kDa after probing with a polyclonal antibody with a 1:10,000 dilution (Sigma-Aldrich). Membranes were incubated for 1 h at room temperature with a 1:10,000 dilution of peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology Inc., Danvers, MA) for Arc or with a 1:10,000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma-Aldrich) for $\beta$-actin. Arc immunocomplexes were visualized by chemiluminescence using the ECL Western Blotting kit (Amersham Life Sciences, Milan, Italy) according to the manufacturer's instructions. The analysis of Arc protein levels was performed only on striatum for both the acute and prolonged treatments, because the prefrontal cortex weight, nearly 7 mg, does not allow the concomitant and accurate determination of both mRNA and protein levels of Arc.

### Statistical Analysis

Data are presented as means and standard errors, with each individual group comprising 5 to 13 samples. Data were analyzed using one-way ANOVA followed by Dunnett's *t* test. Significance for all tests was assumed at $p < 0.05$.

## Results

In this report, we investigated the effect of different paradigms of cocaine administration on Arc expression. We first examined the effect of a single injection of different doses of cocaine (5, 10, and 20 mg/kg) on Arc mRNA levels by sacrificing animals 2 h after treatment. An overall increase of Arc gene expression was observed in the brain regions examined with specific patterns of induction. In rat striatum, cocaine dose-dependently increased Arc mRNA levels (5 mg/kg = +231%, $p < 0.01$; 10 mg/kg = +311%, $p < 0.01$; 20 mg/kg = +435%, $p < 0.001$) (Fig. 1). Arc gene expression was increased similarly in the prefrontal cortex at 5 and 10 mg/kg (5 mg/kg = +304%, $p < 0.001$; 10 mg/kg = +271%, $p < 0.001$) (Fig. 1), whereas, at the highest dose employed (20 mg/kg), the increase was much more pronounced (20 mg/kg = +894%, $p < 0.001$) (Fig. 1). In the hippocampus, the increase in Arc gene expression was instead more attenuated (5 mg/kg = +21%, not significant; 10 mg/kg = +51%, $p < 0.01$; 20 mg/kg = +64%, $p < 0.01$) (Fig. 1).

To analyze the temporal profile of cocaine treatment, we decided to focus on the lowest dose capable of elevating Arc gene expression (i.e., 5 mg/kg). Examination of the temporal profile revealed regionally selective patterns of Arc induction (Fig. 2A). Striatal Arc gene expression began to increase 30 min after injection (+163%, $p < 0.05$), peaked 2 h later (+231%, $p < 0.01$), and waned at later time points, whereas in the prefrontal cortex, Arc mRNA levels were increased only 2 h after injection (+304%, $p < 0.01$). In the hippocampus, cocaine induced a biphasic pattern of Arc gene expression that was increased 30 min and 24 h after injection, whereas at the intermediate time points, it was not significantly different from controls (Fig. 2A). To verify whether changes in Arc mRNA levels were accompanied by correspondent modifications of the related protein, we measured the striatal levels of Arc protein that were up-regulated only 2 h after treatment (+143%, $p < 0.05$), with no significant changes versus controls at the other time points examined (Fig. 2B).

Cocaine is a potent and rather selective blocker of the dopamine transporter, although recent data have demonstrated that the psychostimulant also interacts with serotonin and norepinephrine transporters (Rocha et al., 1998). It is thus conceivable that the marked induction produced by cocaine on Arc mRNA levels could be driven mainly by the

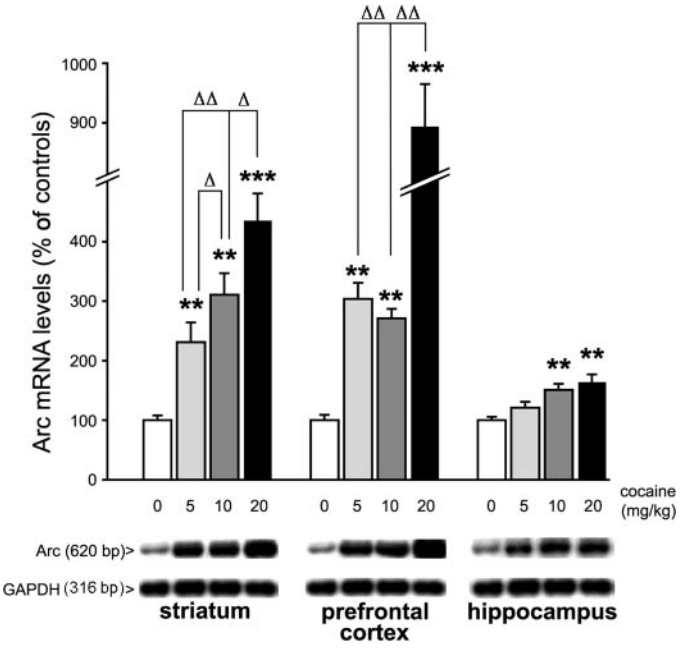


**Fig. 1.** Dose-response effect of a single cocaine injection on Arc mRNA levels in different rat brain regions. The figure shows the increase of Arc mRNA levels produced by a single injection of different doses of cocaine (5, 10, or 20 mg/kg) compared with control (vehicle-injected) animals (0) in striatum, prefrontal cortex, and hippocampus measured 2 h after the injection. The results, expressed as a percentage of control rats, represent the mean ± S.E.M. of 6 to 13 independent determinations. **, $p < 0.01$ and ***, $p < 0.001$ versus control rats; Δ, $p < 0.05$ and ΔΔ, $p < 0.01$ versus cocaine-treated animals (one-way ANOVA with Dunnett's *t* test). Underneath the bar graph are shown the correspondent representative bands of Arc and GAPDH mRNA levels in the different brain areas examined, as obtained by RNase Protection assay. The length of the protected fragments is 620 and 316 bp, respectively; 10 $\mu$g of total RNA was used for the determination. The autoradiographic film was exposed at −70°C with an intensifying screen for 8 h (Arc) or 6 h (GAPDH).

potentiation of dopaminergic neurotransmission. To test this hypothesis, we pretreated the animals with selective inhibitors of dopaminergic D1 (SCH 23390; 1 mg/kg) or D2 (raclopride; 2 mg/kg) receptors and sacrificed the animals 2 h after cocaine injection (5 mg/kg). In striatum, the increased expression of Arc elicited by cocaine is completely abrogated by D1, but not D2, receptor antagonism (Fig. 3A), whereas in the prefrontal cortex, the increased expression produced by cocaine can be ascribed to stimulation of both D1 and D2 receptors, because the specific receptor antagonists, when administered alone or concomitantly, only partially attenuated the increase caused by cocaine (Fig. 3).

It is noteworthy that the selective D1 antagonist SCH 23390 reduced Arc gene expression below control levels, an effect that can be observed in striatum but not in prefrontal cortex (Fig. 3). To further investigate the dopaminergic receptor D1-dependence of basal Arc gene expression, we measured Arc mRNA levels by selectively stimulating or blocking D1 receptors in the absence of cocaine. Figure 4 shows that a regionally selective, tonic dopaminergic control over Arc gene expression exists. In fact, stimulation of D1 receptors with the highly selective agonist SKF 81297 (3 mg/kg) increased Arc mRNA levels in striatum and prefrontal cortex (striatum = +235% versus controls, $p < 0.01$; prefrontal cortex = +215% versus controls, $p < 0.01$) (Fig. 4A), whereas the selective D1 antagonist SCH 23390 (1 mg/kg) reduced Arc gene expression in both brain regions (striatum = −67% versus controls, $p < 0.01$; prefrontal cortex = −41% versus

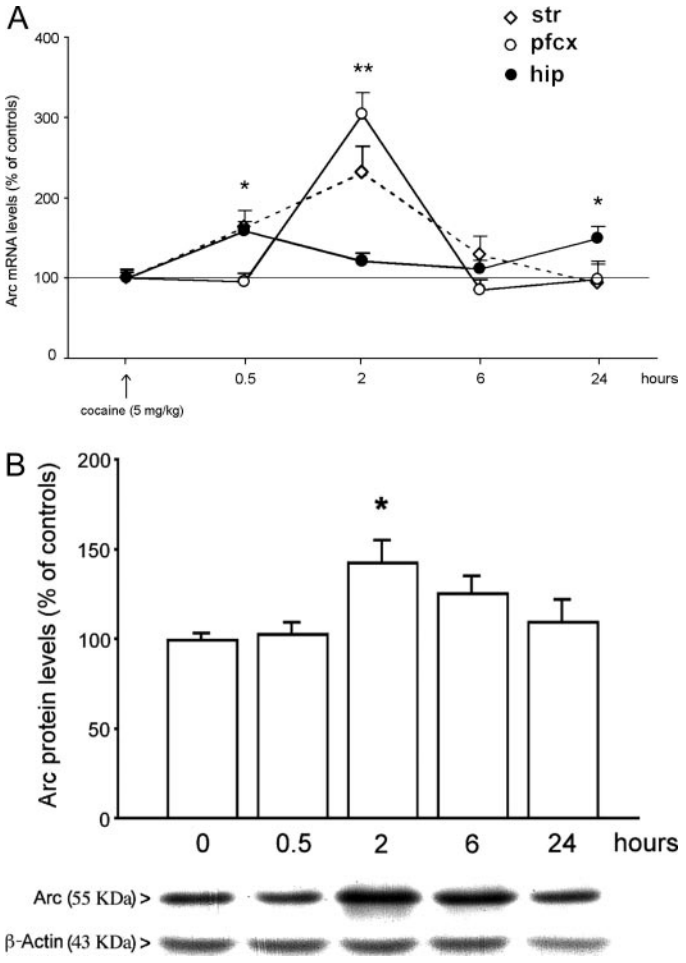


**Fig. 2.** Temporal modulation of Arc expression by single cocaine injection in different rat brain regions. A, Arc mRNA levels measured in striatum (str), prefrontal cortex (pfcx), and hippocampus (hip) at different time points (0.5, 2, 6, and 24 h) after a single injection of cocaine (5 mg/kg) or vehicle. The results, expressed as a percentage of control (vehicle-injected) rats (0), represent the mean ± S.E.M. of 7 to 12 independent determinations. *, $p < 0.05$ and **, $p < 0.01$ versus control rats (one-way ANOVA with Dunnett's $t$ test). B, Arc protein levels measured in striatum at different time points (0.5, 2, 6, and 24 h) after a single injection of cocaine (5 mg/kg) or vehicle. The results, expressed as a percentage of control rats (0), represent the mean ± S.E.M. of 7 to 12 independent determinations. *, $p < 0.05$ versus control rats (one-way ANOVA with Dunnett's $t$ test).

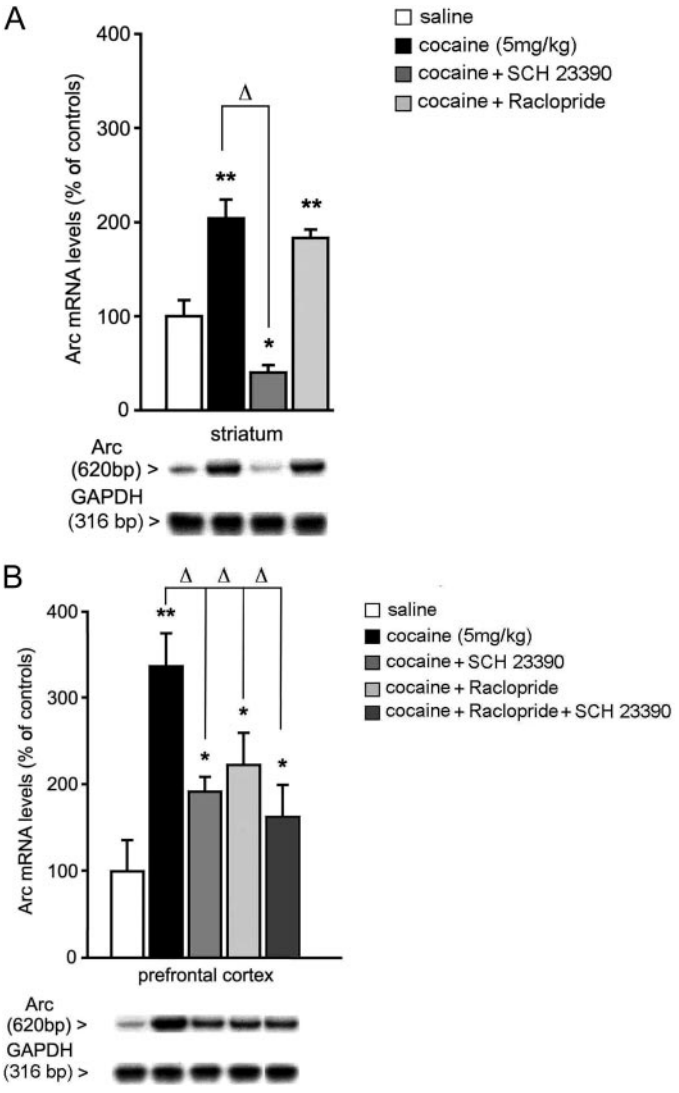


**Fig. 3.** Modulation of Arc gene expression by dopaminergic receptor blockade after single injection with cocaine. A, selective D1 receptor antagonist SCH 23390 (1 mg/kg) or D2 receptor antagonist raclopride (2 mg/kg) administered 30 min before cocaine injection (5 mg/kg). The animals were sacrificed 2 h after cocaine treatment, and Arc gene expression was measured in striatum. B, selective D1 receptor antagonist SCH 23390 (1 mg/kg) or D2 receptor antagonist raclopride (2 mg/kg) administered 30 min before cocaine injection (5 mg/kg). The animals were sacrificed 2 h after cocaine treatment, and Arc gene expression was measured in prefrontal cortex. Because the elevation of Arc gene expression could not be ascribed to a single dopaminergic receptor, SCH 23390 and raclopride were concomitantly administered 30 min before cocaine injection (5 mg/kg), and the animals were sacrificed 2 h after cocaine treatment. The results, expressed as a percentage of control (vehicle-injected) rats, represent the mean ± S.E.M. of 6 to 10 independent determinations. *, $p < 0.05$ and **, $p < 0.01$ versus control rats; Δ, $p < 0.01$ versus cocaine-treated animals (one-way ANOVA with Dunnett's $t$ test).

controls, $p < 0.01$) (Fig. 4). No effect of D1 receptor activation or blockade was observed in the hippocampus (data not shown). To draw a complete picture of the dopaminergic regulation of Arc in the corticostriatal circuit, we analyzed the expression of Arc after stimulation of D2 receptors with the selective agonist quinpirole (1 mg/kg). Figure 4B shows that quinpirole dramatically reduced (−78% versus controls, $p < 0.01$) striatal Arc mRNA levels, whereas in the prefrontal cortex, the D2 receptor activation produced only a slight, but not significant, increase in Arc gene expression (Fig. 4B).

To verify whether the immediate early gene Arc could be up-regulated after prolonged cocaine exposure, we incorporated cocaine treatments of different length (5 or 14 days), sacrificing the animals at different time points after the last injection (2 and 72 h and 14 days). Such an approach was used to distinguish the cumulative effect of prolonged treatment (72 h and 14 days) from the effect of the last treatment (2 h); in these experiments, we focused on the brain regions that showed a clear effect of cocaine on Arc expression (i.e., striatum and prefrontal cortex).

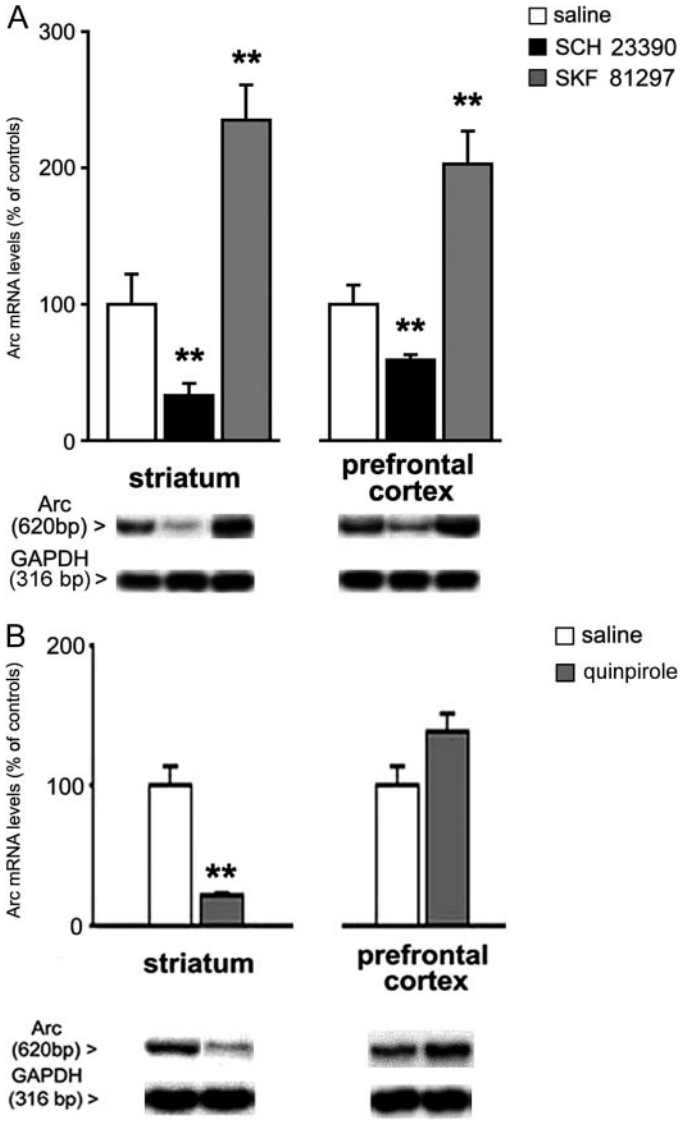


**Fig. 4.** Modulation of Arc gene expression by dopaminergic receptors. A, selective D1 agonist SKF 81297 (3 mg/kg) and selective D1 antagonist SCH 23390 (1 mg/kg) were injected, and animals were sacrificed 2 h later for the analysis of Arc mRNA levels in striatum and prefrontal cortex. B, selective D2 agonist quinpirole (1 mg/kg) was injected, and animals were sacrificed 2 h later for the analysis of Arc mRNA levels in striatum and prefrontal cortex. The results, expressed as a percentage of control (vehicle-injected) rats, represent the mean ± S.E.M. of six to nine independent determinations. **, $p < 0.01$ versus control rats (one-way ANOVA with Dunnett's $t$ test).

Five consecutive daily injections of cocaine elicited a different pattern of Arc gene expression, if compared with the single injection of the psychostimulant. In fact, striatal Arc mRNA levels were increased at 2 h (+222%, $p < 0.05$) and 72 h (+230%, $p < 0.05$) (Fig. 5A) after the last treatment, but no changes in the IEG expression were observed in the prefrontal cortex, which differs from the acute experiments (Fig. 2A). The analysis of Arc protein showed a marked increase of the expression 2 h after treatment in striatum that subsided after 72 h (Fig. 5B).

We then investigated the effects of a more prolonged treatment with cocaine (14 daily injections) on Arc expression, sacrificing the animals at different times of withdrawal after the last treatment (2, 72, and 336 h). In striatum, Arc mRNA levels were significantly increased over control levels at the different experimental conditions (2 h = +136%, $p < 0.05$; 72 h = +140%, $p < 0.05$; 336 h = +143%, $p < 0.05$) (Fig. 6A), whereas in the prefrontal cortex, Arc gene expression was up-regulated 2 and 72 h, but not 336 h, after the last drug was administered (2 h = +146%, $p < 0.05$; 72 h = +153%, $p < 0.01$) (Fig. 6A). It is noteworthy that Arc protein levels in striatum were significantly enhanced 2 and 72 h (2 h = +122%, $p < 0.05$; 72 h = +120%, $p < 0.05$) after the last treatment but not 2 weeks later (Fig. 6B). No changes were observed in hippocampal Arc expression after both 5- and 14-day treatments at any of the time points investigated (data not shown).

## Discussion

We provide detailed evidence that Arc undergoes dynamic and regional-selective changes in the corticostriatal network as a consequence of both short- and long-term treatment with cocaine. A single injection of cocaine revealed that Arc responds to alterations in neurotransmitter release, with a primary role for dopamine, whereas prolonged treatment indicated for the first time that cocaine specifically targets Arc, perhaps leading to modified cellular responses and corticostriatal synaptic efficacy. These findings identify Arc as a molecular switch from cocaine-induced neuronal activity to cocaine-driven long-term adaptations. Given the role of Arc in brain plasticity, we propose that increased Arc gene expression long after the cessation of cocaine treatment does not represent a homeostatic cellular response such as, for instance, the changes in Cdk5 (Bibb et al., 2001) or fibroblast growth factor-2 (Fumagalli et al., 2006), but rather a relevant cocaine-induced cellular imprinting that contributes to enduring synaptic plasticity.

Indeed, there seems to be fundamental, regional differences in Arc response to cocaine, indicating a potential relationship to highly directed targeting of cerebral regions innervated by dopaminergic fibers. Regardless of the dose used, single cocaine injection markedly up-regulated Arc mRNA levels in striatum and prefrontal cortex, whereas in hippocampus, where dopaminergic innervation is scarce, the increase in Arc expression is more attenuated. The possibil-

ity that enhanced Arc gene expression is triggered by similar mechanisms (i.e., dopaminergic mechanisms) is strengthened by the almost identical temporal profile, with a peak of expression 2 h after treatment that dissipates at the other time points investigated. The dramatic increase in Arc expression produced by a single cocaine injection seems to arise from regionally distinct mechanisms, with the striatum relying on D1-dependent activity, whereas D1 and D2 dopamine receptors may cooperate in the modulation of Arc in prefrontal cortex, as shown by the selective stimulation of both receptor subtypes in cocaine-free animals. This interpretation is in agreement with evidence that, in prefrontal cortex, low dopamine concentrations preferentially stimulate D1 receptors, whereas both dopamine receptor subtypes are activated with increased dopaminergic tone, as achieved after cocaine injection (Trantham-Davidson et al., 2004).

In contrast to striatum and prefrontal cortex, hippocampal Arc expression undergoes a biphasic activation, with a first peak 30 min after injection that subsides within 2 h and a second peak 24 h later. This biphasic response is reminiscent of that observed by Ramirez-Amaya et al. (2005), who elegantly showed two temporally coincident waves of hippocampal Arc expression after a single spatial exploration. These results reflect the activation of common signaling pathways and point to Arc as a common substrate of experience- and cocaine-mediated genomic alterations.

Furthermore, our data reveal the previously unappreciated role for ongoing synaptic activity at dopaminergic receptors in maintaining basal expression of Arc. This novel finding implies that dysregulation of D1 or D2 receptor signaling may have functional implications for cellular homeostasis. For example, Zahrt et al. (1997) have shown that supranormal activation of D1 receptors in the prefrontal cortex causes impairments in spatial working memory performance, a situation that, based on our data and on the role of Arc in cognition, could be mediated, at least partially, through changes in Arc expression.

Repeated administration of cocaine causes structural and functional modifications through altered gene or protein expression. After subchronic or chronic treatments, we measured Arc expression at early and late withdrawal times (2 and 72 h and 14 days) from the last drug administration.

Subchronic cocaine administration (5 injections, 5 mg/kg, once a day) markedly increased Arc mRNA levels in striatum but not in prefrontal cortex, revealing that whereas the extent of striatal Arc activation was similar to the effect of the single injection, the overall regional pattern of induction was different. In addition, the duration of Arc mRNA elevation

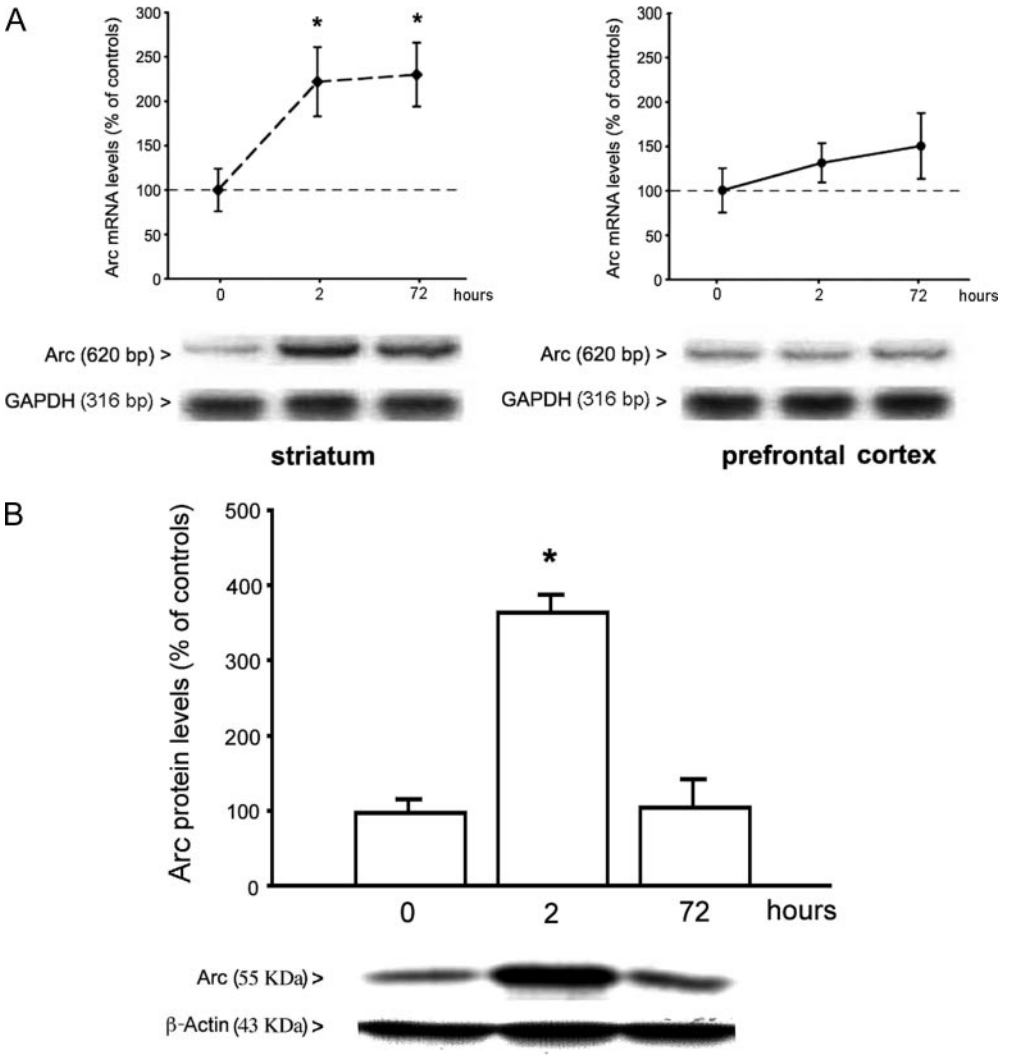


**Fig. 5.** Modulation of Arc expression by subchronic cocaine administration. Cocaine (5 mg/kg) was administered once daily for 5 days, and the animals were sacrificed 2 or 72 h after the last injection. A, effect of subchronic cocaine treatment (5 mg/kg) on Arc mRNA levels measured in striatum and prefrontal cortex. The results, expressed as a percentage of control (vehicle-injected) rats, represent the mean ± S.E.M. of 7 to 12 independent determinations. *, $p < 0.05$ versus control rats (one-way ANOVA with Dunnett's $t$ test). B, effect of subchronic cocaine treatment (5 mg/kg) on Arc protein levels in striatum. The results, expressed as a percentage of control (vehicle-injected) rats (0), represent the mean ± S.E.M. of five to eight independent determinations. *, $p < 0.05$ versus control rats (one-way ANOVA with Dunnett's $t$ test).

persists in striatum up to 72 h after the fifth drug treatment, as opposed to the acute effect that vanished within 24 h.

After 2-week treatment with cocaine, the overall extent of Arc mRNA up-regulation was less in striatum and prefrontal cortex compared with the single injection, presumably reflecting adaptive mechanisms set in motion by the chronic cocaine exposure. Whereas acutely striatal Arc protein shows an expression profile similar to the mRNA induction, differences from the correspondent mRNA were observed in the subchronic and chronic paradigms. In fact, Arc protein is increased 2 h, but not 72 h, after five injections and 2 and 72 h, but not 14 days, after the 2-week treatment. The discrepancy between Arc mRNA and protein at certain time points could reflect different kinetics of activation. However, we have to take into account that a low dose of cocaine was used in the repeated treatments (5 mg/kg); thus, we cannot rule out the possibility that a more robust drug regimen could elicit a longer-lasting enhancement of Arc protein levels. On the other hand, because Arc mRNA half-life is short (Steward and Worley, 2001), repeated administration of cocaine might prolong Arc mRNA half-life, for example by reducing Arc mRNA turnover or inhibiting protein synthesis, a mechanism that is not activated after a single injection. This possibility is in agreement with the study from Ichikawa et al. (2003), who showed that, after "in vitro" stimulation of neuronal activity, Arc mRNA accumulates because of protein synthesis inhibition.

It is noteworthy that whereas the rapid increase of Arc gene expression after acute cocaine injection is on the time scale of other immediate early genes, the persistence of cocaine-induced Arc elevation long after drug removal is at variance with other genes of this family whose increase dissipates as a result of repeated cocaine stimulation (Bhat et al., 1992; Freeman et al., 2002), confirming the peculiar and multifaceted role of Arc in drug abuse.

Preclinical and clinical data revealed that frontostriatal dysfunctions may occur as a consequence of drug abuse (Bolla et al., 1998; Jentsch and Taylor, 1999), showing that cocaine-driven alterations in corticostriatal pyramidal neurons play a crucial role in drug seeking (Nestler, 2001). Therefore, craving-related activation of cortical and striatal structures in cocaine addicts has been demonstrated (Grant et al., 1996; Kilts et al., 2001). Because protracted alterations in gene expression may represent a critical factor in drug craving

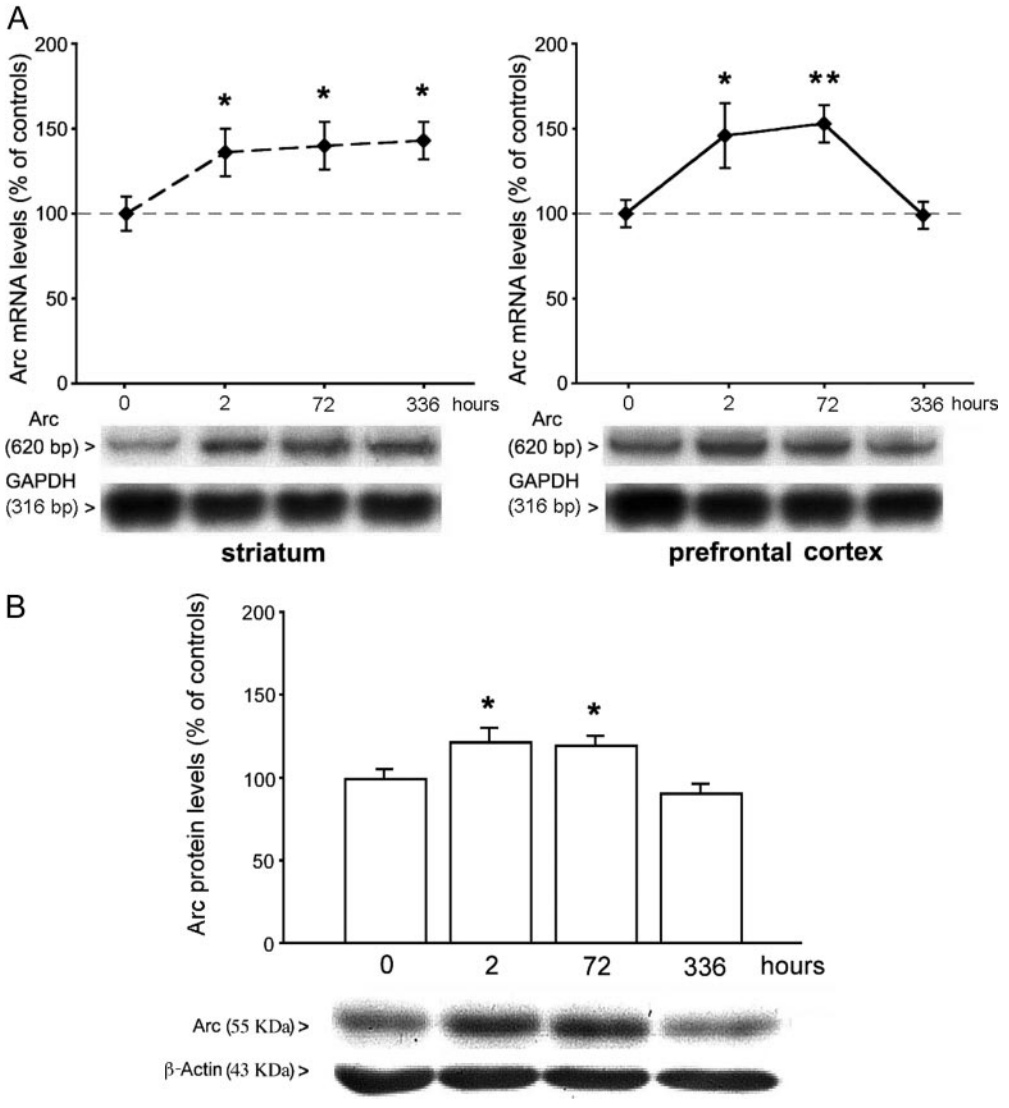


**Fig. 6.** Modulation of Arc expression by repeated exposure to cocaine. A, effect of cocaine (5 mg/kg), administered once daily for 14 days, on Arc expression in striatum and prefrontal cortex. The animals were sacrificed 2, 72, and 336 h (14 days) after the last drug administration. The results, expressed as a percentage of control (vehicle-injected) rats (0), represent the mean ± S.E.M. of 6 to 12 independent determinations. *, $p < 0.05$ and **, $p < 0.01$ versus control rats (one-way ANOVA with Dunnett's $t$ test). B, effect of chronic cocaine treatment (5 mg/kg) on Arc protein levels in striatum. The results, expressed as a percentage of control rats (0), represent the mean ± S.E.M. of six to nine independent determinations. *, $p < 0.05$ versus control rats (one-way ANOVA with Dunnett's $t$ test).

and dependence (Koob et al., 1998), the heightened and long-lasting neuronal activation of the frontostriatal network, as measured in our experiments by increased Arc expression, may result in exaggerated strengthening of synapses, thus setting the stage for drug seeking. Accordingly, Centonze et al. (2006) have elegantly shown that chronic treatment with cocaine prevents synaptic depotentiation at corticostriatal synapses, an effect that could dictate the persistence of addictive behaviors. Because the same loss of corticostriatal depotentiation was shown after chronic L-DOPA treatment (Picconi et al., 2003), a therapy that strongly promotes Arc up-regulation (Sgambato-Faure et al., 2005), we speculate that increased Arc expression herein reported may contribute, at least in part, to the impaired corticostriatal depotentiation after repeated cocaine administration.

It is noteworthy that Arc interacts with a component of the cytoskeleton, F-actin, which is increased as a consequence of long-term withdrawal from prolonged cocaine administration (Toda et al., 2006). The coincident increase of F-actin (Toda et al., 2006) and Arc (present report) long after drug discontinuation may suggest aberrant cytoskeletal reorganization, as demonstrated by previous studies (Robinson et al., 2001; Norrholm et al., 2003).

It has been proposed that drugs of abuse may engage the same pathways that subserve "physiological" learning and experience-dependent plasticity (Kelley, 2004; Robinson and Kolb, 2004), thus overriding "normal" functioning of synaptic networks, a possibility that is strengthened by the similar profile of Arc induction produced by spatial exploration (Ramirez-Amaya et al., 2005) and acute cocaine treatment (present study) in the hippocampus, as discussed above. To this end, Kolb et al. (2003) have elegantly shown that previous cocaine treatment offsets the ability of later experiences to drive physiological plasticity in the cerebral cortex. On this basis, we propose that the cocaine-induced elevation of Arc expression could increase the threshold, or represent an interfering stimulus, for physiological neuroplasticity that could presumably be impaired because of synaptic saturation.

Whatever the functional implications, our results provide critical information for long-term basic cell processes activated by the psychostimulant, yielding unique insights into the mechanism that might contribute to the long-term effects of cocaine and pinpointing Arc as a molecular bridge that—by connecting neuronal activity with synaptic plasticity under prolonged use of cocaine—may contribute to the enduring drug-seeking behavior.

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