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Activity-Regulated Cytoskeleton-Associated Protein Accumulates in the Nucleus in Response to Cocaine and Acts as a Brake on Chromatin Remodeling and Long-Term Behavioral Alterations

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BACKGROUND: Addiction relies on persistent alterations of neuronal properties, which depends on gene regulation. Activity-regulated cytoskeleton-associated protein (Arc) is an immediate early gene that modulates neuronal plasticity underlying learning and memory. Its role in cocaine-induced neuronal and behavioral adaptations remains elusive.

METHODS: Acute cocaine-treated mice were used for quantitative reverse-transcriptase polymerase chain reaction, immunocytochemistry, and confocal imaging from striatum. Live imaging and transfection assays for Arc overexpression were performed from primary cultures. Molecular and behavioral adaptations to cocaine were studied from *Arc*-deficient mice and their wild-type littermates.

RESULTS: *Arc* messenger RNA and proteins are rapidly induced in the striatum after acute cocaine administration, via an extracellular-signal regulated kinase–dependent de novo protein synthesis. Although detected in dendrites, Arc accumulates in the nucleus in active zones of transcription, where it colocalizes with phospho-Ser10-histone H3, an important component of nucleosomal response. In vitro, *Arc* overexpression downregulates phospho-Ser10-histone H3 without modifying extracellular-signal regulated kinase phosphorylation in the nucleus. In vivo, *Arc*-deficient mice display decreased heterochromatin domains, a high RNA-polymerase II activity and enhanced c-Fos expression. These mice presented an exacerbated psychomotor sensitization and conditioned place preference induced by low doses of cocaine.

CONCLUSIONS: Cocaine induces the rapid induction of Arc and its nuclear accumulation in striatal neurons. Locally, it alters the nucleosomal response, and acts as a brake on chromatin remodeling and gene regulation. These original observations posit Arc as a major homeostatic modulator of molecular and behavioral responses to cocaine. Thus, modulating Arc levels may provide promising therapeutic approaches in drug addiction.

Keywords: Addiction, Arc, Cocaine, Extracellular-signal regulated kinase, ERK, Signaling, Striatum

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Long-term behavioral alterations induced by drugs of abuse rely on molecular adaptations within specific brain areas that belong to the reward circuitry (1). Within these structures, early changes in gene expression occur soon after cocaine exposure and set the stage for long-lasting modifications of neuronal activity and behavior (2). This early transcription is characterized by the induction of immediate early genes (IEGs), which encode either transcription factors, including c-Fos, and Zif268, regulating a second wave of genes, or effector proteins acting directly on cellular functions and homeostasis. Activity-regulated cytoskeleton-associated protein (Arc), which belongs to this second category of IEGs, is rapidly induced by cocaine in cortical and striatal regions and cocaine-associated stimuli (3–9). However, the role of striatal Arc in the development of neuronal and behavioral adaptations to cocaine is not known.

In the hippocampus, Arc is highly regulated by changes in neuronal activity, including high-frequency stimulation of the perforant path or electroconvulsive shock (10–12), thus positioning Arc as a reliable index of activity-dependent synaptic modifications. Because of its characteristic dendritic localization, where it controls glutamate alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) trafficking, synaptic strength, and long-term neuronal plasticity (13–17), much attention has been paid on Arc's functions at synapses. However recent studies indicate that it accumulates within the nucleus (18–20), where its functions remain controversial with some evidence in favor of its positive

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impact on transcription (20), although others support a negative role of Arc on transcription (19).

Herein, we identified unexpected functions of Arc in response to cocaine. By studying the dendritic versus nuclear localization of Arc, we made the original observation that cocaine dramatically increases the expression of the protein in the nucleus of medium-sized spiny neurons (MSN), where it is localized within active zones of transcription. Using either overexpression of Arc in vitro, or its knockout in vivo, we show that Arc acts as a brake on gene regulation and long-term behavioral adaptations induced by cocaine. We conclude that the rapid and transient induction of Arc within the nucleus contributes to the dynamic of chromatin remodeling, and modulates behavioral responses to cocaine. This new facet of Arc's functions in the nucleus is likely to contribute to its homeostatic role within neurons.

METHODS AND MATERIALS

Animals and Behavior

Experiments were carried out on 8-week-old C57BL/6 mice, green fluorescent protein (GFP)-Arc knockin (KI) mice, and their wild-type (WT) littermates in accordance with the European Community guidelines on the Care and Use of Laboratory Animals (86/609/EEC). Experiments were approved by the local ethic committee C2EA-05. Locomotor activity was measured as the number of interruptions by the mice of two adjacent beams in a circular corridor (Imetronic, Pessac, France) containing four infrared beams placed at every 90°. After 3 days of habituation, mice were subjected to the psychomotor sensitization protocol consisting of two cocaine injections separated by a 1-week interval. Spontaneous activity was recorded for 60 minutes before the first cocaine injection and locomotor activity was measured for 1 hour. One week later, a second session was performed as described for the first cocaine injection. The conditioned place preference (CPP) was evaluated in a Plexiglas Y-Shaped apparatus (Imetronic) consisting of two compartments distinguished by different patterns on floors and walls, separated by a small neutral area. After a preconditioning during which mice were placed in the neutral area and allowed to explore both chambers, mice were treated for 6 days consecutively with alternate injections of cocaine (2.5 mg/kg) or saline during the so-called conditioning phase. The postconditioning phase was conducted exactly as the preconditioning phase with free access to both chambers. The CPP score was calculated as the difference between the time spent in cocaine-paired chamber during postconditioning minus preconditioning. See the Supplement for details on behavioral tests.

For measurements of messenger RNA (mRNA) levels, mice were sacrificed at the indicated time after cocaine or saline injections, and dissected striata were snap-frozen before being processed for quantitative reverse-transcriptase polymerase chain reaction. For immunohistochemistry, mice were anesthetized and perfused transcardially with 4% paraformaldehyde. See the Supplement for details.

Image Acquisition and Analysis

Immunoreactive cells were quantified in the dorsomedial striatum (DM) and nucleus accumbens shell (ShNAcc). Confocal images (SP5; Leica, Wetzlar, Germany) were acquired bilaterally with a 40× oil immersion objective. Quantifications were performed using ImageJ software (National Institutes of Health, Bethesda, MD), taking into account the cells with immunofluorescence above a fixed threshold.

Nucleus Versus Neuropile Fluorescence Intensity Assessment. Images were acquired as described above. Image analyses were performed in ImageJ using custom-built procedures. Nuclei are segmented by a binarization of the Hoechst signal. The fluorescence was measured inside and outside the nuclei masks to discriminate protein levels in neuropile or in the nucleus. The percent of positive nuclei represents the percent of nuclei above a threshold defined by user relative to the total number of nuclei.

Arc and Phospho-Ser10-Histone H3 Colocalization Within the Nucleus. Confocal images were acquired in the DM bilaterally and images were taken with a 63× oil immersion objective, Zoom 4, pixel size: x = 0.06 μm, y = 0.06 μm, z = 0.21 μm. A deconvolution step was performed using the maximum likelihood estimation algorithm with Huygens 3.6 Software (Scientific Volume Imaging, Wetzlar, Germany) as described in Heck *et al.* (21). This treatment aims at limiting light diffraction, which increases the accuracy of colocalization analyzes. Three-dimensional segmentation of intranuclear Arc and phospho-Ser10-histone H3 (pH3) spots at high resolution has been computed thanks to the 3D ImageJ Suite plugins (22) as already described in Heck *et al.* (23). The local maxima inside the nucleus are detected and defined as seeds of the spots. Then an adaptive threshold is automatically calculated for each object based on their intensity by measuring the signal in concentric spheres created around the seeds. This allows detecting the contour in three dimensions automatically without a user-defined threshold (23). Colocalization of Arc and pH3 spots is automatically analyzed using the same plugins, and two spots with overlapping voxels are considered as colocalizing.

RESULTS

Cocaine-Induced Arc Expression in the Striatum Relies on Extracellular-Signal Regulated Kinase–Dependent Transcription and De Novo Protein Synthesis

To study the role of Arc in cocaine-induced cellular and molecular adaptations, we first established a precise time-window of its expression by acute cocaine in mice. Both Arc mRNA and protein were transiently induced by cocaine in the DM and the ShNAcc (Figure 1A) and core (not shown) with a peak from 30 to 60 minutes and a return to basal levels at 2 hours (Supplemental Figure S1A–D). This induction occurred downstream from both D_1 (D1R) and *N*-methyl-D-aspartate glutamate receptors (NMDAR) in MSN expressing c-Fos (Supplemental Figure S1E–G).

The signaling pathways that regulate Arc expression in the striatum in response to cocaine remain to be established. A protein synthesis inhibitor (anisomycin) was administered 30