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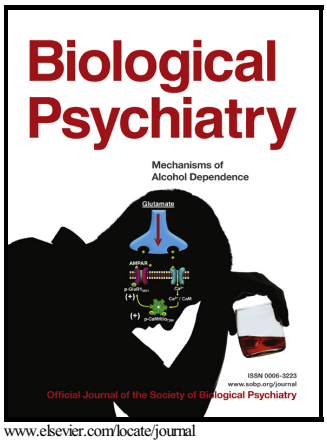

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Activity-regulated cytoskeleton-associated protein accumulates in the nucleus in response to cocaine and acts as a brake on chromatin remodeling and long-term behavioral alterations

Salery Marine[1,2,3], Dos Santos Marc[1,2,3], Saint-Jour Estefani[1,2,3], Moumné Lara[1,2,3], Pagès Christiane[1,2,3], Kappès Vincent[1,2,3], Parnaudeau Sébastien[1,2,3], Caboche Jocelyne[1,2,3]*, Vanhoutte Peter[1,2,3]*

[1]INSERM, UMR-S 1130, Neuroscience Paris Seine, Institute of Biology Paris Seine, F-75005, Paris, France

[2]CNRS, UMR 8246, Neuroscience Paris Seine, F-75005, Paris, France

[3]Sorbonne Universités, UPMC Université Paris 06, UM CR18, Neuroscience Paris Seine, F-75005, Paris, France

*co-last authors

Corresponding author : peter.vanhoutte@upmc.fr; phone : +33 4 27 53 52; fax : +33 44 27 25 08.

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ABSTRACT

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 Q-RT-PCR, 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* mRNA and proteins are rapidly induced in the striatum after acute cocaine administration, via an ERK-dependent *de novo* protein synthesis. Although detected in dendrites, Arc accumulates in the nucleus in active zones of transcription where it colocalizes with phosphorylated histone-H3 (pH3), an important component of nucleosomal response. *In vitro,* Arc overexpression down-regulates pH3 without modifying ERK phosphorylation in the nucleus. *In vivo*, *Arc*-deficient mice display decreased heterochromatin domains, a high RNA-Pol 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.

INTRODUCTION

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 AMPA 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 impact on transcription (20), while 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 MSN, where it is localized within active zones of transcription. Using either overexpression of Arc *in vitro*, or

its knock-down *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 from 8-weeks old C57BL/6 mice, GFP-Arc knock-in mice and their wild type 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 one-week interval. Spontaneous activity was recorded for 60 min before the first cocaine injection and locomotor activity was measured for 1 h. 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 pre-conditioning 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 post-conditioning phase was conducted exactly as the pre-conditioning phase with free

access to both chambers. The CPP score was calculated as the difference between time spent in cocaine paired chamber during post-conditioning minus pre-conditioning. See supplementary methods for details on behavioral tests.

For measurements of mRNA levels, mice were sacrificed at the indicated time after cocaine or saline injections, and dissected striata were snap-frozen before being processed for RT-qPCR. For immunohistochemistry, mice were anesthetized and perfused transcardially with 4% paraformaldehyde. See supplementary methods and materials for details.

Image acquisition and analysis

Immunoreactive cells were quantified in the dorso-medial striatum (DM) and Nucleus Accumbens Shell (ShNAcc). Confocal images (SP5, Leica) were acquired bilaterally with a 40X oil immersion objective. Quantifications were performed using ImageJ software taking into account the cells with immunofluorescence above a fixed threshold

Nucleus vs neuropil 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 in order to discriminate proteins levels in neuropile or in the nucleus. The percent of positive nuclei represents the percent of nuclei upon a threshold defined by user relative to the total number of nuclei.

Arc and phospho-H3 colocalization within the nucleus: Confocal images were acquired in the DM bilaterally and images were taken with a 63X oil immersion objective, Zoom 4, pixel size: x = 0.06µm, y = 0.06µm, z = 0.21 µm. A deconvolution step was performed using Maximum Likelihood Estimation algorithm with Huygens 3.6 Software (Scientific Volume Imaging) as described in (21). This treatment aims at limiting light diffraction, which increases the accuracy of colocalization analyzes. 3D segmentation of intra nuclear Arc and pH3 spots at high resolution has been computed thanks to the 3D ImageJ Suite plugins (22) as

already described in (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 3D automatically without a user-defined threshold (23). Colocalization of Arc and pH3 spots is automatically analyzed using the same plugins, two spots with overlapping voxels are considered as colocalizing.

RESULTS

Cocaine-induced Arc expression in the striatum relies on ERK-dependent transcription and *de novo* protein synthesis

In order 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 dorso-medial striatum (DM) and the nucleus accumbens shell (ShNAcc) (**Fig.1A**) and core (not shown) with a peak from 30 to 60 minutes and a return to basal levels at 2h (**Fig. S1A-D**). This induction occurred downstream from both dopamine 1 (D1R) and NMDA-glutamate receptors (NMDAR) in medium-sized spiny neurons (MSN) expressing c-Fos (**Fig. 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 minutes prior to cocaine and mice sacrificed 1 hour later. Cocaine-induced Arc-expression was abolished by anisomycin in both the DM and ShNAcc (**Fig. 1B**). Of note, Arc immunoreactivity was also strongly reduced by anisomycin in saline-treated mice, which is consistent with the short half-life of this IEG. A major pathway involved in cocaine-mediated long-term cellular adaptations is the ERK cascade, which behaves as an integrator of D1R and NMDAR signaling (24-27). The administration of SL 327, a selective inhibitor of the ERK pathway, prior to cocaine abolished the induction of *Arc* mRNA and proteins (**Fig. 1C-D**).

Altogether, these data show that *Arc* mRNA and proteins are transiently induced by acute cocaine in the striatum via an ERK-dependent *de novo* protein synthesis downstream from both D1R and NMDAR.

Cocaine preferentially increases Arc expression in the nucleus of striatal neurons

There are accumulating evidences that Arc induction in the dendritic compartment plays a key role in neuronal plasticity (13). However, whether it is induced locally in dendrites in response to cocaine remains unknown. Confocal imaging was performed in order to determine the impact of cocaine on the cellular localization of Arc. One hour after cocaine, DiI labeling of the dendritic shaft and spines (21) coupled to Arc staining showed that Arc-positive puncta were present in the dendrites and spines (**Fig. 2A**). Nevertheless, a strong immunolabeling was also visible in the nucleus (visualized by Hoechst staining) of striatal neurons (**Fig. 2B**). Arc cellular distribution within the nucleus and outside of it, in the soma and dendritic tree [referred to as neuropile (see **Fig.2C**)], was analyzed using a computer-based routine that was set up from confocal images of Arc and Hoechst co-staining. The Arc signal that colocalized with Hoechst was subtracted from total Arc staining to obtain the neuropile staining ("neuropile"; **Fig. 2C**). By contrast, the signal colocalized with Hoechst allowed the determination of Arc-positive nuclei ("nuclei"; **Fig. 2C**). In the DM, total Arc (**Fig. 2D**) and neuropile (**Fig. E**) fluorescence intensity significantly increased 60 minutes post-cocaine, and then returned to basal levels. Cocaine appeared to have a stronger effect on the nuclear expression of Arc (**Fig. 2F**), which was increased as soon as 30 minutes post-cocaine and further augmented at 60 minutes to return to basal levels within 2 hours. Of interest, in the ShNAcc, cocaine only increased the nuclear pool of Arc albeit fewer Arc

positive cells were detected than in the DM (**Fig. S2A-D**). This indicates that Arc proteins accumulate earlier and preferentially in the nucleus when compared to soma and neuropile in response to cocaine.

Arc is localized at the vicinity of active chromatin markers in the nucleus

We studied the precise localization of Arc within the nucleus at high magnification and observed a perfect exclusion of Arc from heterochromatin, which is characterized by puncta with a bright Hoechst staining (**Fig. 3A**). Heterochromatin corresponds to inactive zone of transcription where DNA is highly packed into chromatin, a DNA/protein complex comprising histones and components of the transcriptional machinery. Activity-dependent remodeling of chromatin towards a permissive state for transcription involves post-translational modifications of histones, also called nucleosomal response. Ser10-Histone H3 phosphorylation (pH3) labeling, a marker of nucleosomal response is rapidly (30 minutes) activated downstream ERK and mitogen and stress-activated kinase 1 (MSK-1) in response to cocaine (28). The distribution of pH3 and Arc puncta was analyzed in 3D within the nuclei. At 30 minutes post-cocaine, Arc and pH3 strongly colocalized, with 70% of Arc-positive puncta colocalized with pH3 (**Fig. 3B-C).** One-hour post-cocaine, these Arc/pH3 positive puncta were significantly reduced, with only 50 % of colocalization (**Fig. 3D-E**). Consistently, the comparative kinetics of these markers showed that both signals were detectable at 30 minutes post-cocaine in the same cells (**Fig. 3F**; left panel), whereas pH3 was decreased at 1h meanwhile Arc was maintained (**Fig. 3F,** right panel), in accordance with the decrease of Arc/pH3- colocalization.

Unraveling the role of nuclear Arc in striatal neurons in vitro.

Because our data indicated that cocaine-induced Arc accumulation in the nucleus preferentially occurred within active zones of transcription in striatal neurons, we hypothesized that Arc could be a regulator of chromatin remodeling. To test this, we used striatal cultured neurons treated with glutamate (10 μM), which induces increases in pH3 levels downstream from ERK-MSK1 activation (29-30). The impact of Arc expression on these cellular events, was studied after transfection of a cDNA encoding Arc fused to GFP, followed by live imaging. Under basal conditions, the GFP-Arc signal was detectable in all cellular compartments, with higher levels in the soma (**Fig.4A**; **left panel**). Incubation with glutamate triggered a progressive accumulation of GFP-Arc in the nucleus (**Fig.4A-B**), thus rendering this *in vitro* model ideally suited to evaluate the potential impact of Arc on glutamate-mediated increase in pH3. In response to glutamate, striatal neurons transfected with GFP alone displayed increased pH3 labeling **(Fig. 4C, upper panel; 4D)**. This response was decreased in neurons expressing GFP-Arc **(Fig. 4C, lower panel; 4D**), thus indicating that Arc overexpression alters the nucleosomal response and inhibits pH3. This effect was not due to attenuation of glutamate-induced neuronal activity, since neither ERK1/2 phosphorylation nor its nuclear translocation was found in the presence of GFP-Arc (**Fig. 4E-F**).

Deletion of Arc alters heterochromatin domains in vivo.

Our results support that Arc is a potential modulator of chromatin remodeling. To test this *in vivo*, we took advantage of the GFP-Arc knock-in mice in which the coding sequence of Arc has been replaced by the GFP gene (31). In homozygous knock-in (KI) mice, Arc is absent and GFP serves as a reporter of Arc promoter's activity. One-hour post-injection, Wt mice presented an induction of Arc in the DM (**Fig. S3A-B**) with no detectable levels of GFP (**Fig.**

S3C-D). The mirror image was observed in homozygous GFP-Arc KI (referred to as KI) mice in which cocaine increased GFP but not Arc expression. To study the role of Arc on cocaine-induced chromatin remodeling, we focused on heterochromatin domains that can detected through Hoechst staining (32). In Wt mice, cocaine reduced the number of Hoechst-positive puncta in MSN **(Fig. 5A-C)** along with the area occupied by these puncta in Arc-positive nuclei **(Fig. 5D-E)**, thus illustrating that cocaine induces DNA decompaction. In Arc-GFP KI mice a significant reduction of the number (**Fig. 5A-C**) and size (**Fig. 5D-E**) of Hoechst positive puncta were found in saline- mice. The number of punctate was even further decreased after cocaine treatment (**Fig. 5B**). This indicates that the removal of Arc alters the basal organization of chromatin, reduces DNA compaction and hence potentially sensitizes neuronal cells to favor transcription.

RNA polymerase II (RNA-Pol II) is a central player of the transcriptional machinery, which activity is influenced by chromatin organization (33). To evaluate the role of cocaine on RNA-Pol II activity, we used an antibody that recognizes the phosphorylated form of RNA-Pol II on its c-terminal domain (34). In Wt mice, cocaine triggered a strong increase of RNA-Pol II phosphorylation **(Fig. 5F-G)**, which is consistent with the wide spectrum of activity-dependent gene induced by cocaine (2). By contrast, KI mice showed a constitutively high level of RNA-Pol II activity with no further increase detectable upon cocaine exposure **(Fig. 5F-G)**. This indicates that Arc has an unexpected broad inhibitory effect on chromatin remodeling and RNA Pol II-mediated transcription.

Arc deficiency favors the development of cocaine-induced long-term behavioral alterations.

Chromatin remodeling is critical for behavior alterations induced by drugs of abuse (2). Hence, we studied the role of Arc in the development of long-lasting adaptations at the behavioral level in GFP-Arc mice. We first measured the basal locomotor activity of these

mice and found that KI mice displayed a significantly lower spontaneous locomotion than Wt littermates **(Fig. 6A-B)**. Mice were then subjected to a protocol of psychomotor sensitization induced by two injections of cocaine at 15mg/kg with a one-week interval, which triggers a robust psychomotor sensitization in Wt mice (25; **Fig. 6C**). After the first administration of cocaine (15 mg/kg), the acute locomotor response increased in both genotypes with a higher response in KI mice (**Fig. 6D**). After the second injection, both groups of mice showed a behavioral sensitization with a significantly higher locomotion measured in KI mice (**Fig. 6D**). We then used a lower dose of cocaine (7.5mg/kg), which triggers a moderate behavioral sensitization in Wt mice and may unmask a facilitating effect of Arc deficiency on the development of psychomotor sensitization. In these conditions, KI mice displayed an acute response that was undistinguishable from Wt mice, but their sensitized response to the second injection was significantly greater than in Wt mice (**Fig. 6E**). These data indicate that the absence of Arc favors the development of psychomotor sensitization. At this low dose of cocaine, pH3 immunoreactivity was not significantly increased, whatever the genotypes (**Fig. S3F**). However, the expression of c-Fos, which is known to facilitate psychomotor sensitization (34), was sensitized at this dose, with a stronger increase in KI when compared to Wt mice (**Fig. 6F**). This may be due to the observed decrease of DNA compaction in these KI mice, which is further decrease upon exposure to 7.5 mg/kg of cocaine (**Fig. 6G**). The impact of Arc deficiency on behavioral alteration induced by cocaine, was further assessed in the Conditioned Place Preference (CPP) paradigm, which measures the ability of mice to associate the rewarding properties of the drug with environmental cues. With a low dose of 2.5 mg/kg of cocaine, which does not trigger CPP in Wt mice, KI mice displayed a clear preference for cocaine-paired chamber in KI mice (**Fig. 6H-I**). Altogether, these data demonstrate that Arc deficiency exacerbates molecular and behavioral alterations induced by cocaine.

DISCUSSION

The present work demonstrates a nuclear accumulation of Arc in MSN in response to cocaine. Arc was restricted to active zones of transcription where it colocalized with pH3. *In vitro*, Arc overexpression in cultured striatal neurons altered glutamate-induced pH3, while sparing activation of ERK. *In vivo*, mice deficient for Arc showed a decrease of heterochromatin domains associated to a high RNA-Pol II activity. These mice showed an exacerbated psychomotor sensitization and CPP induced by low doses of cocaine.

With regard to signaling cascades driving Arc expression, a D1R antagonist was previously shown to abolish Arc induction by acute cocaine (3). Herein, we confirm this observation and further demonstrate that Arc induction also strictly depends on NMDAR stimulation. Cocaine-mediated expression of Arc is thus at the crossroad of dopamine and glutamate inputs converging onto MSN. Consistently, Arc transcription and translation occurred downstream from ERK activity, which behaves as a key integrator of D1R and NMDAR signaling in response to cocaine (24-27, 36). The promoter of Arc comprises two Serum Response Element (SRE) DNA regulatory elements that binds a ternary complex formed by the transcription factor Elk-1 and two molecules of Serum Response Factor. Mutation of one of these SRE sites blunted the late phase of cerebellar long-term depression (37), indicating that SRE-dependent transcription of Arc is critical for neuronal plasticity. We previously showed that the phosphorylation of Elk-1 by ERK, which is necessary and sufficient to trigger SRE-dependent transcription (38), was involved in cocaine-mediated Arc induction (39). Our data thus support that cocaine-mediated Arc transcription critically relies on the ERK/Elk-1 signaling module.

Herein, we demonstrated that Arc accumulates in the nucleus of MSN *in vivo* in response to cocaine. Arc has been detected in the nucleus of cultured hippocampal neurons (18) where it translocates in an activity-dependent manner (19). Accordingly, we also observed an activity-

dependent nuclear accumulation of Arc by live imaging of cultured MSN. The role of this nuclear pool of Arc remained controversial and emerged only recently, while its functions at synaptic sites have been extensively explored. Initial observations indicated that changes in neuronal activity increased Arc in both nuclei and soma, but Arc was enriched in dendrites where it co-precipitates with F-actin (40-41). Since then, most of its functions in neuronal plasticity have been described at the level of synapses and involve regulation of cytoskeleton dynamics, AMPAR trafficking and synaptic strength (14-17). Although cocaine increases dendritic Arc expression in the DM, we surprisingly found that the major impact of cocaine on Arc expression occurred in the nucleus. This was even clearer in the ShNAcc where nuclear Arc expression was increased in the absence of change in dendrites. With respect to its biochemical properties and a lack of DNA interaction consensus sequence, Arc is not likely to bind DNA or act as a transcription factor in the nucleus (40). What is known so far is that Arc binds to nuclear spectrin and PML bodies (19). PML bodies regulate transcription, notably by controlling the availability and histone acetyl transferase (HAT) activity of CBP and p300 (42). In cultured hippocampal neurons, increased neuronal activity triggers a nuclear localization of Arc within PML bodies, which decreases CBP-mediated transcription of GluA1 AMPAR subunit (19). By contrast, by interacting with the Histone Acetyl Transferase Tip60 at PML bodies Arc increases the acetylation of H4K12 (43), a histone mark associated with increased transcription and learning and memory. We report here that Arc colocalizes with pH3, within active zones of transcription in the nucleus of MSN after exposure to cocaine. In MSN, this phosphorylation event involves the nuclear kinase MSK-1 downstream from ERK and is critical for gene regulation at specific loci (28-29). Cocaine-induced PH3 *in vivo* also results from DARPP32-induced inhibition of phosphatases (44). Arc overexpression in cultured MSN significantly decreased glutamate-induced pH3, thus supporting that Arc plays a negative retro-control on transcription. This negative feedback is unlikely due to an

effect of Arc overexpression on AMPAR trafficking since ERK activation and nuclear translocation, which involve glutamate-induced endocytosis of AMPAR (45), were preserved. By contrast, Arc slightly inhibited glutamate-induced MSK-1 activation in the nucleus **(Fig. S2E)**. However, this inhibitory effect was weaker than the decrease of pH3 itself, and independent on DARPP32 phosphorylation and nuclear translocation (data not shown) supporting that additional mechanisms are engaged by Arc to alter pH3. Given the tight relation between Arc and actin dynamics in the cytoplasm (41), a tentative explanation could be that Arc also interacts with nuclear actin and as such controls the cyto-architecture of the nucleus and hence chromatin remodeling. Such mechanisms could also account for the alterered accessibility of MSK-1 to ERK-mediated phosphorylation in the nucleus.

In vivo, acute cocaine triggered a decrease in heterochromatin domains in the nucleus of Wt MSN. Persistent decreases in heterochromatin also occur after repeated cocaine, which suggests a potential role of heterochromatin organization in the long-term effects of cocaine (32). The decompaction of DNA induced by cocaine in Wt mice was associated with an increased RNA-Pol II activity, which is in agreement with the boost of activity-dependent transcription induced by cocaine (1,2). In both saline- and cocaine-treated KI mice, we found a significant decrease of heterochromatin domains, thereby indicating a relaxing of chromatin in the absence of Arc. Upon cocaine administration, the constitutive decrease of heterochromatin was combined with high levels of RNA-Pol II activity, an intracellular event that favors transcription. Altogether, these data support that Arc exerts a negative retro-control on transcription, by limiting chromatin decompaction and RNA-Pol II activity. Such impact of Arc on these nuclear events may have important implications for its well-known homeostatic functions.

Arc expression is correlated to neuronal processing underlying learning and memory storage (46,47) and Arc deficient mice have severe deficit in the consolidation of long-term synaptic

plasticity and memory (48). In the striatum, Arc induction is associated with early phase of learning as well as inversion phase during reversal learning, suggesting a role in striatal plasticity underlying learning acquisition (49,50). In the context of addiction, Arc is increased by cocaine (3-5) or upon re-exposure to a context previously associated with the drug (6,7). In rats trained to self-administer cocaine, the local inhibition of Arc in the dorso-lateral part of the striatum did not affect cocaine-seeking but blocked extinction of this behavior (9), a result in agreement with the increased Arc expression during reversal learning and changes of rules (50).

To the best of our knowledge, no causal link has been established between Arc expression and the development of long-term behavioral alterations to cocaine. Arc induction has been correlated to the level of psychomotor sensitization and the rate of cocaine delivery (51). With regard to self-administration, individuals considered as vulnerable showed lower *Arc* mRNA expression in the DM, whereas resilient rats displayed higher levels (52). Accordingly, we showed here that psychomotor sensitization by low doses of cocaine was higher in KI mice when compared to their Wt littermate, revealing a pre-sensitization to the drug in the absence of Arc. This effect was unlikely due to global changes in excitability since KI mice did not show any increase in striatal GluA1 mRNA (**Fig. S3E**). By contrast, the expression of c-Fos that facilitates for psychomotor sensitization (35), was significantly higher in KI mice when compared to Wt littermate at these low doses of cocaine. Furthermore, at doses of cocaine that did not induce CPP in Wt animals, KI mice displayed a clear preference for the cocaine-paired chamber. Altogether these data are in agreement with an inhibitory role of Arc on chromatin decompaction and RNA-Pol II activity at specific loci. These observations strongly support that, upon cocaine administration, the rapid induction of Arc acts as a brake on chromatin remodeling and could contribute to the behavioral homeostatic response to cocaine by limiting activity-dependent transcription of genes. These new facets of Arc's functions

suggest that modulating levels of Arc may provide interesting therapeutic approaches in drug addiction.

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FIGURE LEGENDS:

Figure 1: Cocaine-induced Arc expression in the striatum relies on ERK-dependent transcription and *de novo* protein synthesis. (A) Illustrative confocal images of the Arc staining in the DM (left) and ShNAcc (right) from the areas delineated in Fig. S1B (scale bar: 30 µm). (**B**) Quantifications of Arc-positive cells in the DM and ShNAcc from mice treated with saline or cocaine for 1h with or without a 30 min pre-treatment with 100 mg/kg of anisomycine (aniso); n = 4-5 mice per group; two-way ANOVA: interaction between treatment and pretreatment, $F_{(1,12)} = 45.47$, $p < 0.001$; effect of treatment, $F_{(1,12)} = 74.46$, $p < 0.001$; effect of pretreatment, $F_{(1,12)} = 142.64$, $p < 0.001$, followed by post-hoc comparisons (Bonferroni's test). ***$p < 0.001$, cocaine group vs saline group; °°$p < 0.01$, °°°$p < 0.001$, vehicle vs anisomycine pre-treated animals. Impact of a pre-treatment with SL327 (50 mg/kg) on cocaine-induced Arc protein (**C**); n = 8 mice per group; one-way ANOVA in the DM: $F_{(2,21)} = 23.54$, $p < 0.001$ and in the ShNAcc $F_{(2,16)} = 13.67$, $p < 0.001$, followed by post-hoc comparisons (Bonferroni). ***$p < 0.001$, saline or SL327 pre-treated cocaine groups vs saline pre-treated saline group.) and *Arc* mRNA (**D**); n = 5 mice per group; one-way ANOVA: $F_{(2,12)} = 32.47$, $p < 0.001$, followed by post-hoc comparisons (Bonferroni). ***$p < 0.001$, saline or SL327 pre-treated cocaine groups vs saline pre-treated saline group and at 45 or 60 min cocaine post-injection, respectively.

Figure 2: Cocaine induces a preferential increase of Arc in the nucleus of striatal neurons.

(A) Maximum z projection of a stack of confocal images of a dendritic fragment visualized from a MSN stained with DiI (red) and the corresponding Arc labeling (green) detected 1h after cocaine administration. The merge of the two signals illustrates that Arc is expressed in the dendritic shaft and in dendritic spines (*) as expected (Scale bar: 10 µm). **(B)** Single confocal plan from a striatal slice prepared 1h after a cocaine administration. Nuclei are

counterstained with Hoechst (blue) and Arc is detected in red. The merge shows that Arc is expressed in somatic, dendritic and nuclear, compartments (scale bar: 10 µm). **(C)** Diagram summarizing the principle of the custom routine used to separate the Arc signal outside and within nuclei. A single confocal plan of Arc (red) and Hoechst (blue) signals is acquired after double staining from the same striatal slice (left panel; scale bar 30 µm). After automatic detection of the nuclei, the corresponding signal is subtracted from the image acquired in the red channel. The resulting image corresponds to the Arc signal in all sub-cellular compartments except the nucleus (neuropile) as illustrated in the insert (scale bar: 10 µm). To isolate the Arc signal in the nucleus, the signal corresponding to the nucleus (detected in the blue channel) is kept in the red image. Based on this automated approach the mean intensity of the total **(D)**, neuropile (**E**) and nuclear **(F)** Arc signals were measured in the DM and expressed as percentage of increase relative to the corresponding saline condition at each time point. n = 6-8 mice per group; two-way ANOVA: for total Arc signal: interaction between treatment and time, $F_{(5,54)} = 3.56$, $p < 0.01$; effect of treatment, $F_{(1,54)} = 6.88$, $p < 0.05$; effect of time, $F_{(5,54)} = 4.65$, $p < 0.01$, for non-nuclear Arc signal: interaction between treatment and time, $F_{(5,55)} = 3.18$, $p < 0.05$; effect of treatment, $F_{(1,55)} = 10.25$, $p < 0.01$; effect of time, $F_{(5,55)} = 3.18$, $p < 0.05$; for nuclear Arc signal: effect of interaction $F_{(5,55)} = 6.072$, $p < 0.001$; effect of treatment, $F_{(1,55)} = 20.25$, $p < 0.001$; effect of time, $F_{(5,55)} = 6.072$, $p < 0.001$ followed by post-hoc Bonferroni comparisons. ***$p < 0.001$, *$p < 0.05$ cocaine group vs saline group; °°°$p < 0.001$, each time point vs 15' group. **(G)** The percentage of Arc-positive nuclei (i.e. above a fixed intensity threshold) has been calculated at each time point after saline or cocaine administration. n = 6-8 mice per group; two-way ANOVA: interaction between treatment and time $F_{(4,48)} = 5.95$, $p < 0.001$; effect of treatment, $F_{(1,48)} = 34.01$, $p < 0.001$; effect of time, $F_{(4,48)} = 12.66$, $p < 0.001$, followed by post-hoc comparisons (Bonferroni). ***$p < 0.001$, cocaine group vs saline group; °°°$p < 0.001$, each time point vs 15' group.

Figure 3: Within the nucleus Arc is excluded from heterochromatin and localized at the vicinity of an active chromatin marker. (A) Representative confocal images obtained from a striatal slice prepared from a mouse that has been sacrificed 1h post-cocaine injection. Nuclei were counterstained with Hoechst (blue) and Arc is labeled in red (scale bar: 10 µm). Right panel is a close-up of the nucleus with double labeling (scale bar: 2 µm). The fusion of the two channels illustrates the exclusion of Arc puncta from nuclear domains with dense Hoechst labeling that corresponds to inactive zones of transcription (referred to as heterochromatin). Mice were sacrificed 30 min **(B)** or 1h (**C**) after saline or cocaine administration and confocal sections were acquired from slices counterstained with Hoechst (blue), Arc (green) and phospho-Ser^{10} histone H3 (pH3; red; scale bar: 2 µm). Right panels are 3D views of a pile of confocal Arc and pH3 images at these two time points post-cocaine. The proximity of Arc and pH3 was analyzed in 3D at 30 minutes post-cocaine **(D; left graph)**; n = 17 nuclei per group; unpaired t-test. ***$p < 0.001$, Arc colocalized with pH3 vs pH3 colocalized with Arc or 1h **(D; right graph);** n = 21 nuclei per group; unpaired t-test. **$p < 0.01$, Arc colocalized with pH3 vs pH3 colocalized with Arc. **(E)** The number of cells that are immunoreactive for pH3 or Arc only and the ones that are positive for both Arc and pH3 was assessed by immunohistochemistry from mice that have been sacrificed 30 min (left) or 1h (right) after cocaine administration. n = 4-5 mice per group; unpaired t test for each marker. **$p < 0.01$, *$p < 0.05$, cocaine group vs saline group.

Figure 4: Overexpression of Arc alters histone H3, but not ERK1/2, phosphorylation *in vitro.* (A) Cultured striatal neurons were transfected with cDNA encoding Arc fused to GFP. Live imaging was performed and images were acquired before (basal) and at the indicated time point after application of glutamate 10 µM. (**B**) Quantifications of the GFP-Arc

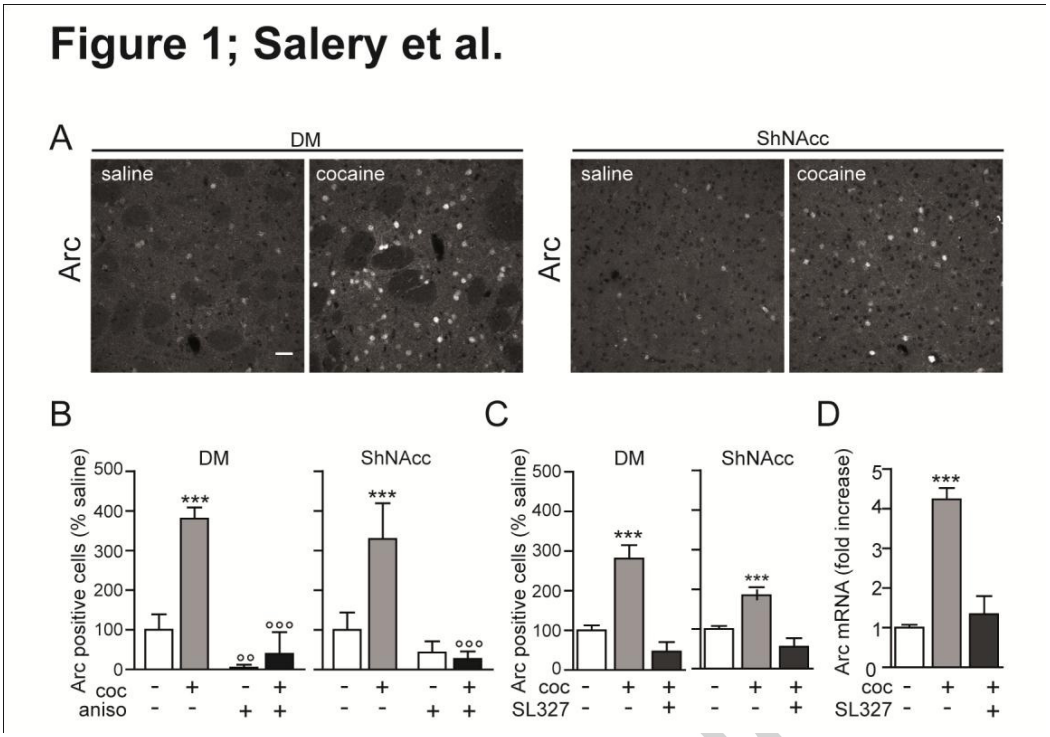
fluorescence profile overtime (F/F0; error bars are s.e.m) within the nucleus (white dotted line). The black curve is the average intensity of the nuclear GFP-Arc from 4 transfected neurons including the one shown in (**A**), represented by the red curve which shown in red. Note the progressive nuclear accumulation of the GFP-Arc signal in response to glutamate. **(C)** Shown are representative pictures of neurons transfected with GFP (top panels) or GFP-Arc (bottom panels), treated with glutamate (glu 10 μM) for 30 minutes to induce histone Ser^{10}-H3 phosphorylation (pH3). Nuclei are counterstained with Hoechst (blue), pH3 is red, GFP in green and the merge of pH3 and GFP is show on the right panels (Scale bar: 10 μm). **(D)** Quantifications of the intensity of the pH3 labeling in transfected cells stimulated of not with glutamate. Results are expressed as % increase when compared to control neurons transfected with GFP. n = 3-4 independent experiments per group; two-way ANOVA: interaction between treatment and transfection, $F_{(1,16)} = 3.48$, NS; effect of treatment, $F_{(1,16)} = 18.54$, $p < 0.001$; effect of transfection, $F_{(1,16)} = 4.01$, ns, followed by post-hoc comparisons (Bonferroni). ***$p < 0.001$, control group (cont) vs glutamate group (glu); °$p < 0.05$, GFP vs GFP-Arc plasmid. (**D**) Illustrations of neurons transfected as described in **(B)** and treated with glutamate for 10 minutes to induce the phophorylation of ERK1/2 (pERK1/2; red; scale bar: 10 μm). **(E)** Analyses of the intensity of the pERK1/2 labeling in transfected cells stimulated of not with glutamate. n = 3-4 independent experiments per group; two-way ANOVA: interaction between treatment and transfection, $F_{(1,12)} = 0.15$, ns, effect of treatment, $F_{(1,12)} = 54.94$, $p < 0.001$; effect of transfection, $F_{(1,12)} = 0.52$, ns, followed by post-hoc comparisons (Bonferroni). ***$p < 0.001$, control group (cont) vs glutamate group (glu).

Figure 5: Deletion of Arc alters heterochromatin domains and promotes the phosphorylation of Polymerase II *in vivo*. (A) Homozygous Knock-in GFP-Arc (KI) and corresponding wild type littermate (Wt) were treated with saline or cocaine (20 mg/kg) and

sacrificed 1h post-injection. Shown are representative confocal images acquired in the DM of nulcei counterstained with Hoechst and immunolabeling of Arc or GFP performed from Wt and KI mice, respectively. Arrowheads indicate the nuclei that were used for subsequent analyzes in which low (saline-treated mice) or high (cocaine-treated mice) levels of Arc or GFP were detectable (scale bar: 2 μm). The number of Hoechst-positive puncta per nuclei was analyzed **(B)** as well as the distribution **(C)** of the different categories of nuclei according to the number of Hoechst puncta they display. n = 800-1000 neurons per group; Mann-Whitney U test between each group. *$p < 0.05$, saline group vs cocaine group; °°°$p < 0.001$, Wt vs KI. The total area corresponding to Hoechst-positive puncta within nuclei (in μm^2) was measured **(D)** as well as the distribution of nuclei **(E)** depending on the area occupied by heterochromatin domains. 800-1000 neurons per group; Mann-Whitney U test between each group. **$p < 0.01$, saline group vs cocaine group; °°°$p <0.001$, °°$p<0.01$, Wt vs KI. **(F)** Wt and KI mice were treated with saline or cocaine and sacrificed 1h later to study the phosphorylation of the polymerase II on the Ser^2 of its C-terminal domain (p-Pol II) by immunohistochemistry (Scale bar: 15 μm). (**G**) Quantifications p-Pol II positive cells; n = 3-4 mice per group; two-way ANOVA: interaction between treatment and genotype, $F_{(1,11)} = 2.27$, ns, effect of treatment, $F_{(1,11)} = 6.88$, $p < 0.05$; effect of genotype, $F_{(1,11)} = 9.12$, $p < 0.05$, followed by post-hoc comparisons (Bonferroni). *$p < 0.05$, saline group vs cocaine group; °$p < 0.05$, Wt vs KI.

Figure 6: Arc deficiency augments behavioral sensitization to low doses of cocaine. (A) Time course of basal locomotor activity of GFP-Arc Wt (black), and homozygous knock-in (KI; grey) mice and **(B)** corresponding area under the curve in arbitrary unit. n = 11 mice per group; unpaired t-test. **p < 0.01, Wt vs KI mice. **(C)** Diagram depicting the psychomotor sensitization protocol induced by two injections in which mice received a first injection of cocaine after a period of habituation and a second injection a week later of the same dose of cocaine. Mice of the two genotypes have been subjected to a protocol of psychomotor sensitization induced by two injections of cocaine at 15 mg/kg **(D)** or 7.5 mg/kg **(E)**. n = 11 mice per group; two-way ANOVA (repeated measure over time for matching data): for 15 mg/kg: interaction between genotype and time, $F_{(3,54)} = 6.76$, $p < 0.001$; effect of genotype, $F_{(1,54)} = 5.62$, $p < 0.05$; effect of time, $F_{(3,54)} = 56.25$ $p < 0.001$; for 7.5 mg/kg: interaction between genotype and time, $F_{(3,54)} = 5.53$, $p < 0.01$; effect of genotype, $F_{(1,54)} = 2.29$, NS; effect of time, $F_{(3,54)} = 54.94$ $p < 0.01$ followed by post-hoc Bonferroni comparisons . *p < 0.05; **p < 0.01, Day 1 vs Day 8 cocaine injection; °° p < 0.01, Wt vs KI. **(F)** Homozygous KI mice and corresponding Wt littermates treated with saline or cocaine (7.5 mg/kg) were sacrificed 30 minutes post-injection and the number of c-Fos positive cells were analyzed in the striatum. Data expressed as percent of the mean of the Wt saline group. n=4-5 mice per group; two-way ANOVA: interaction between treatment and genotype, $F_{(1,14)} = 8.97$, $p < 0.01$; effect of treatment, $F_{(1,14)} = 47.59$, $p < 0.001$; effect of genotype, $F_{(1,14)} = 4.03$, NS followed by post-hoc comparisons (Bonferroni). *p < 0.05; ***p < 0.001, saline vs cocaine-treated group; °°° p < 0.001, Wt vs KI. **(G)** DNA compaction state was assessed in these animals by analyzing the number of Hoechst-positive puncta per nuclei according to the number of Hoechst puncta they display. n = 800-1000 neurons per group; Mann-Whitney U test between each group. *p < 0.05, ***p < 0.001, saline group vs cocaine-treated group; °p < 0.05; °°°p < 0.001, Wt vs KI. **(H-I)** CPP induced by cocaine (n=11 mice per group) was

performed from Wt or homozygous KI mice in a three-pairing CPP paradigm. **(H)** Initial preference during preconditioning session (pre) and place preference on the test session (post) in Wt and homozygous KI mice were analyzed using two-way ANOVA (repeated measure over time for matching data): for Wt mice: interaction between genotype and time, $F_{(1,44)} = 0.11$, NS; effect of time, $F_{(1,44)} = 0.21$, NS; effect of treatment, $F_{(1,44)} = 0.32$, NS; for KI mice: interaction between genotype and time, $F_{(1,42)} = 5.72$, $p < 0.05$; effect of time, $F_{(1,42)} = 3.49$, NS; effect of treatment, $F_{(1,42)} = 3.18$, NS, followed by post-hoc comparison (Bonferroni). ** $p < 0.01$, cocaine vs saline group. **(I)** The CPP score (time spent in cocaine-paired chamber during post conditioning minus preconditioning) was analyzed using two-way ANOVA (repeated measure over time for matching data): interaction between genotype and time, $F_{(1,43)} = 2.59$, NS; effect of time, $F_{(1,43)} = 2.09$, NS; effect of treatment, $F_{(1,43)} = 8.33$, $p < 0.01$; followed by post-hoc comparison (Bonferroni). ** $p < 0.01$, cocaine vs saline group.



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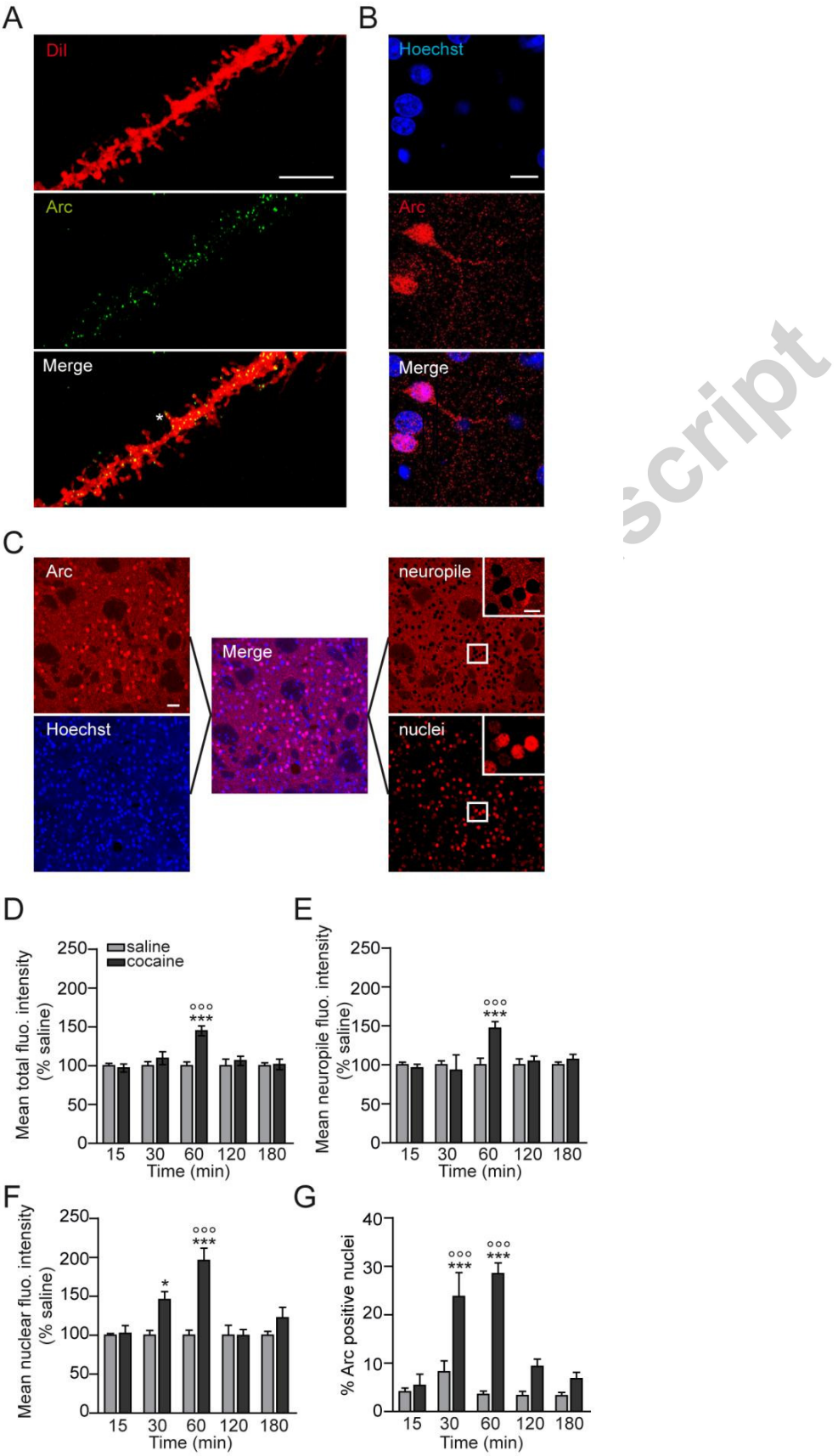

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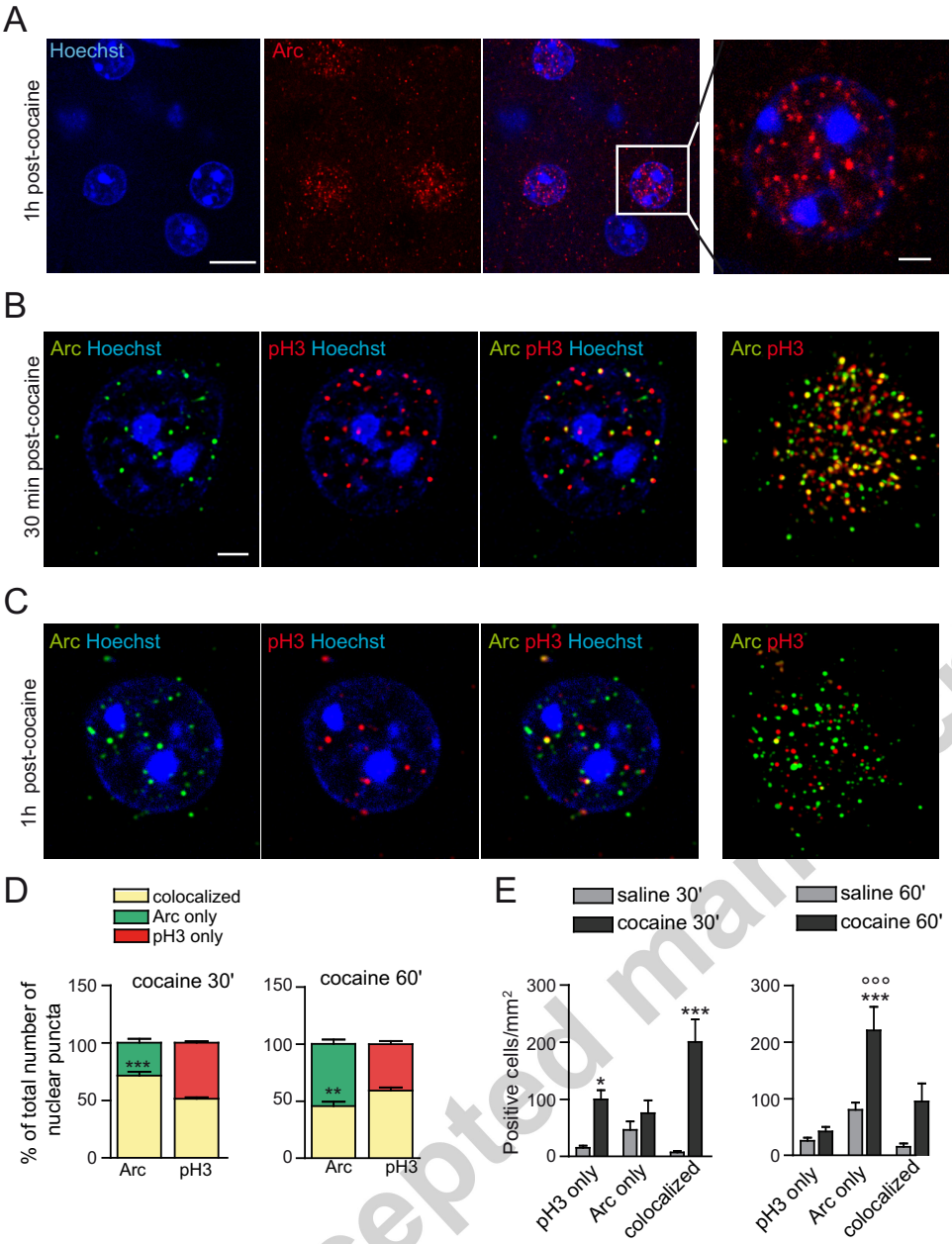

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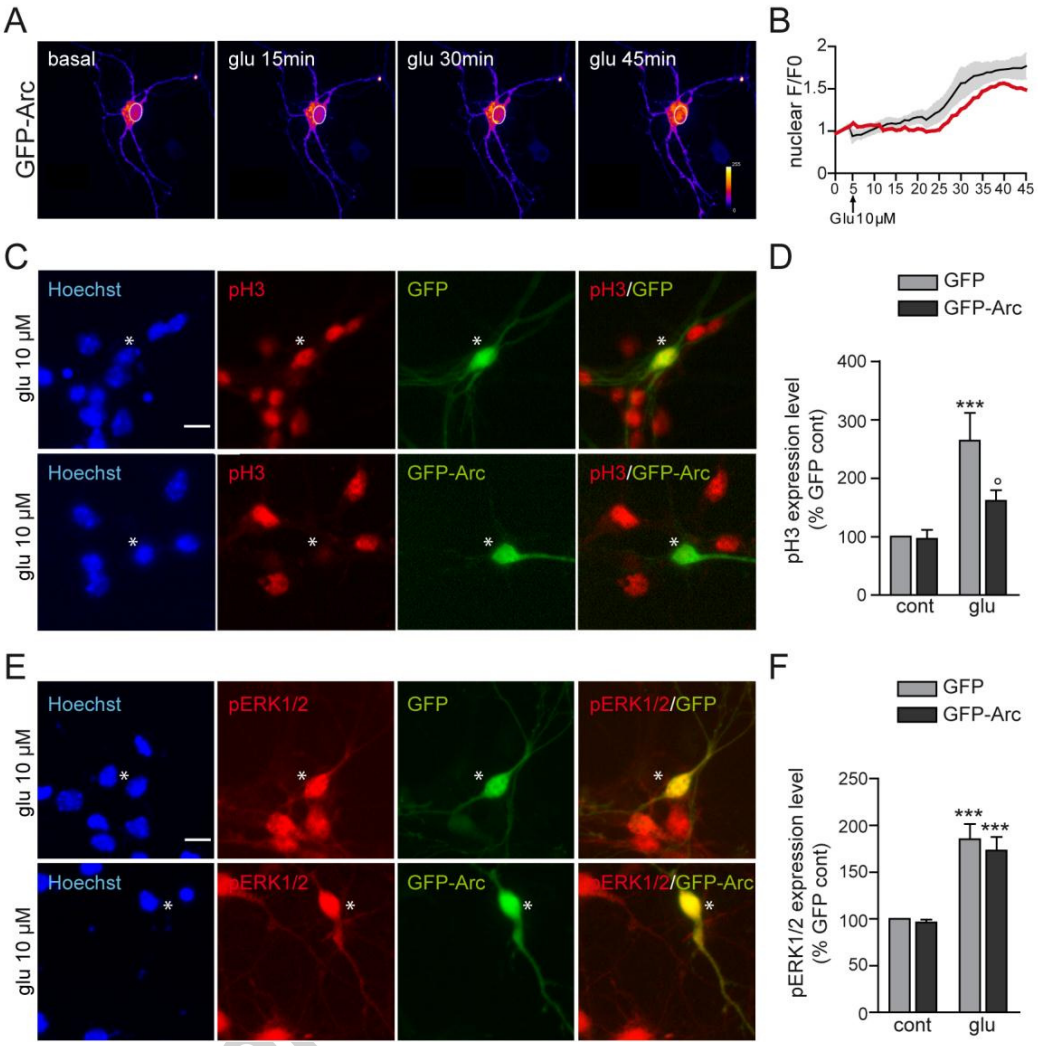

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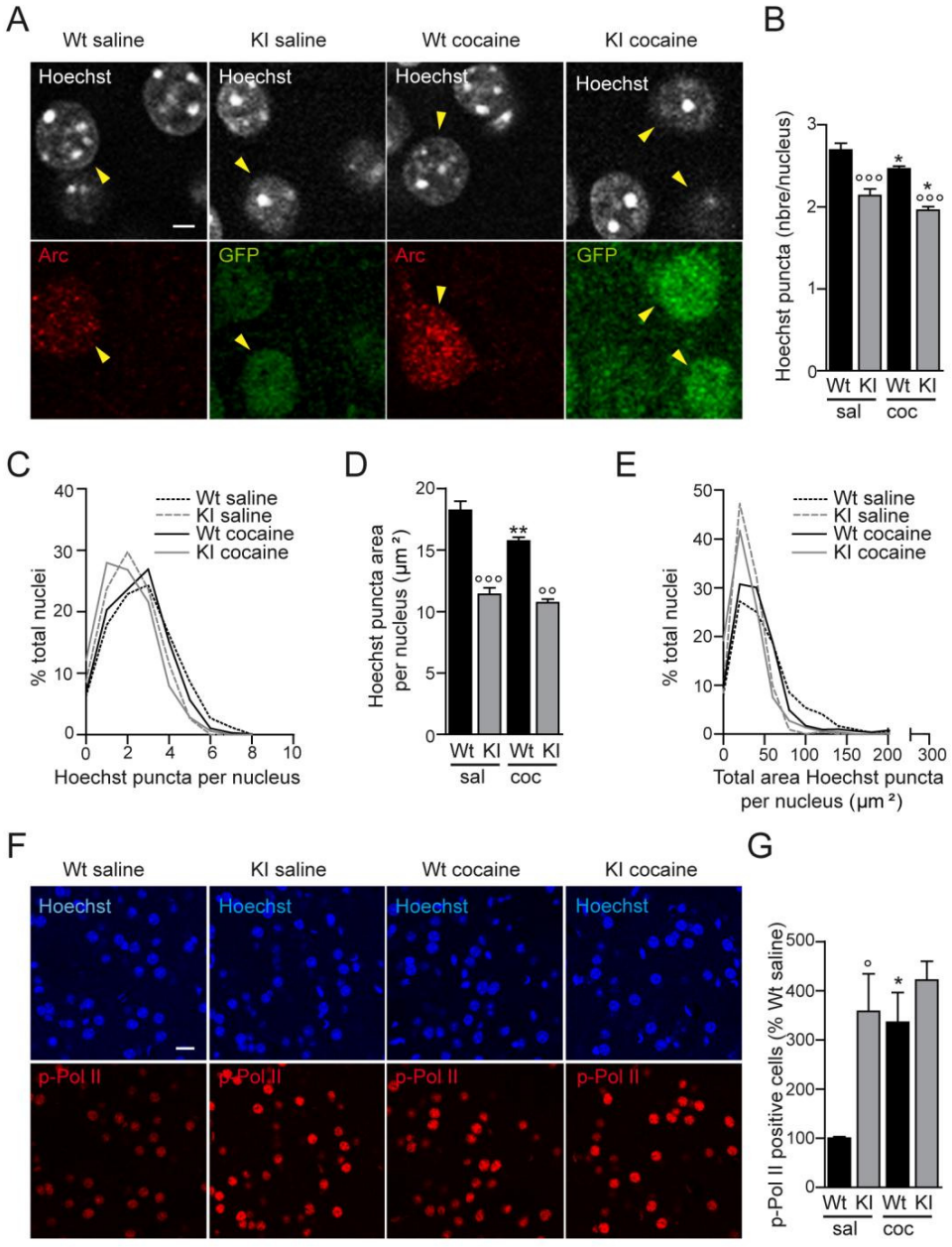

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