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The dendritic spine morphogenic effects of repeated cocaine use occur through the regulation of serum response factor signaling

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Abstract

The nucleus accumbens (NAc) is a primary brain reward region composed predominantly of medium spiny neurons (MSNs). In response to early withdrawal from repeated cocaine administration, *de novo* dendritic spine formation occurs in NAc MSNs. Much evidence indicates that this new spine formation facilitates the rewarding properties of cocaine. Early withdrawal from repeated cocaine also produces dramatic alterations in the transcriptome of NAc MSNs, but how such alterations influence cocaine's effects on dendritic spine formation remain unclear. Studies in non-neuronal cells indicate that actin cytoskeletal regulatory pathways in nuclei have a direct role in the regulation of gene transcription in part by controlling the access of co-activators to their transcription factor partners. In particular, actin state dictates the interaction between the serum response factor (SRF) transcription factor and one of its principal co-activators, MAL. Here we show that cocaine induces alterations in nuclear F-actin signaling pathways in the NAc with associated changes in the nuclear subcellular localization of SRF and MAL. Using *in vivo* optogenetics, the brain region-specific inputs to the NAc that mediate these nuclear changes are investigated. Finally, we demonstrate that regulated SRF expression, in turn, is critical for the effects of cocaine on dendritic spine formation and for cocaine-mediated behavioral sensitization. Collectively, these findings reveal a mechanism by which nuclear-based changes influence the structure of NAc MSNs in response to cocaine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

MEC and EJN conceived the study and wrote the manuscript. MEC, DMW, AMG, ZJW, CKL, RCB and DMD generated data. RLN designed essential constructs specifically for the experiments in this study.

INTRODUCTION

Dendritic spines, the smallest known information processing units in the brain, are small protrusions that radiate from dendrites and are the sites of most excitatory connections in the central nervous system. Alterations in spine density and morphology are a common feature of numerous neuropsychiatric disorders.^{1,2} Spines contain a distinct head region that is attached to the dendrite shaft via the spine neck. Spine head size is generally reflective of synaptic strength such that spines with large heads typically have a larger content of functional surface AMPA receptors than spines with small heads.³

The nucleus accumbens (NAc) is a primary brain reward region and the chief component of the ventral striatum. Alterations in dendritic spine morphogenesis in medium spiny neurons (MSNs), the principal neuron subtype of the NAc, accompany withdrawal from repeated cocaine administration.^{4–7} The duration of withdrawal from cocaine is a defining determinant of the nature of spine alterations in NAc MSNs such that early (hours, days) cocaine withdrawal is associated with the formation of *de novo* thin spines typified by a small head region, whereas more protracted (weeks) cocaine withdrawal is associated with enlargements of dendritic spine heads and associated increased strength of NAc MSN synapses.^{7–11} Much evidence supports a role for alterations in synaptic connectivity and strength in NAc MSNs, in contributing to distinct cocaine-mediated behavioral responses.^{6,12–19}

Early withdrawal from repeated cocaine also produces striking alterations in the transcriptome of NAc MSNs, which are thought to be mediated by alterations in transcription factor binding profiles to gene promoter regions.²⁰ Nevertheless, how such alterations influence spine formation in NAc MSNs remains largely uncharacterized. Here we show that cocaine alters both upstream and downstream regulatory pathways of the serum response factor (SRF) transcription factor in the NAc and we characterize the specific NAc circuits that mediate these changes. In addition, we show that SRF is essential for cocaine-mediated spine morphogenesis in NAc MSNs following early withdrawal. Finally, we reveal an essential role for NAc SRF in cocaine-mediated locomotor behavior. Collectively, these findings exemplify the means through which cocaine-driven alterations in the transcriptional landscape regulate the structure of NAc MSNs during early stages of withdrawal and the consequent effects on drug-mediated behavioral responses.

MATERIALS AND METHODS

The detailed description of the materials and methods is provided in the Supplementary Information.

Experimental animals

For all biochemistry experiments using intraperitoneally injected saline or cocaine, C57BL/6J male mice (Jackson Labs, Bell Harbor, ME, USA) were used (10–16 weeks of age; age-matched across experimental conditions). Floxed *SRF* mice (RRID:IMSR_JAX:006658) and floxed *miR-132/212* mice (Mirc19^{tm1Rhg} RRID:MGI_4868401) used in these

studies have been described previously.^{21,22} Male Sprague–Dawley rats (300 g; Harlan Laboratories, Indianapolis, IN, USA) were used for all self-administration experiments.

Western blottings

Primary antibodies were labelled using secondary antibodies conjugated to peroxidase (Vector Labs, Burlingame, CA, USA). Membrane proteins were developed with chemiluminescence (Thermo Scientific, Waltham, MA, USA) and protein levels quantified using Image J (US National Institutes of Health, Bethesda, MD, USA) densitometry. Protein levels were normalized to those of total actin, which itself was not altered under the experimental conditions studied.

Immunoprecipitation

Rock1 was immunoprecipitated from NAc P1 fractions using previously described methods²³ with a protein G agarose kit (Roche, Basel, Switzerland, 11719386001).

Cell fractionation

NAc tissue was homogenized in 60 µl HEPES-buffered sucrose (320 mM sucrose with 4 mM HEPES with protease and phosphatase inhibitors, pH 7.4) using a teflon homogenizer (40 strokes at 400 r.p.m.). Homogenates then underwent a centrifugation protocol to produce P1, S2 and P2 fractions as detailed previously.²³

G-actin/F-actin assay

A G-actin/F-actin *in vivo* assay kit from Cytoskeleton, Denver, CO, USA (BK037) was used to isolate G-actin and F-actin fractions following the manufacturer's protocol and as described previously.²³

Immunohistochemistry

Active Neural Wiskott-Aldrich syndrome protein (N-WASP) was labeled using a conformation-specific rabbit anti-active N-WASP antibody (1:2000), a gift from John Condeelis and described previously.²⁴ Chicken anti-NeuN (Millipore (Billerica, MA, USA) catalog number ABN91 RRID:AB_11205760) was used to label neuronal somas. Quantification of active N-WASP intensity was accomplished by analyzing a single image in the middle of each individual cell's Z-plane projection, where the middle was defined as the region in which the soma size is at its maximum. This approach prevents cells with greater spread into the Z-plane from artificially increasing fluorescent intensity measurements as detailed previously.²³ Within each experiment, all microscope parameters, including magnification, pinhole size, digital gain and offset, were kept constant. All imaging and quantification was performed blind to experimental conditions.

Spine imaging and analysis

Dendritic spines were imaged and analyzed from NAc MSNs using established procedures.^{6,23,25} Dendrites were imaged only if: (1) uniform green fluorescent protein (GFP) distribution was apparent, (2) they could be traced back to their parent soma and (3) they were a minimum of 50 µm away from the soma. One to two dendrites per cell were imaged,

and total spine density and the density of thin, stubby and mushroom spines were semi-automatically analyzed using NeuronStudio (<http://research.mssm.edu/cnic/tools-ns.html>). All imaging and quantification were done blind to experimental conditions.

HSV surgeries and infusions

For delivery of herpes simplex virus (HSVs) to the NAc, ketamine/xylazine anesthetized mice were infused with 0.5 μ l of virus per hemisphere delivered at a rate of 0.1 μ l infused per minute. This was followed by a 5 min wait period in which no infusions were given. Relative to the bregma, the stereotaxic coordinates used to target the NAc are: anterior/posterior, 1.6 mm; medial/lateral 1.5 mm; dorsal/ventral -4.4 mm, at a 10° fixed angle. The proper targeting of HSVs to the NAc was assessed using fluorescent microscopy.

Adeno-associated virus optogenetic stereotaxic surgery and viral vectors

Adeno-associated virus (AAV)-CamKIIa-ChR2-EYFP-CaMKIIa-ChR2-EYFP (University of North Carolina) was injected in the basolateral amygdala (BLA), ventral hippocampus or medial prefrontal cortex (mPFC) using stereotaxic surgery. Virus (0.5 μ l) was delivered per hemisphere at a flow rate of 0.1 μ l min^{-1} followed by a 5 min inactive period to allow for viral diffusion. After a minimum of 6 weeks post AAV infusion, medial NAc targeting optical fibers (200 μ m core 0.22 NA; Thor Labs, Newton, NJ, USA) enveloped by ceramic zirconia ferrules (Precision Fiber Products, Milpitas, CA, USA) were implanted onto the skull.

Optogenetic stimulation parameters

Fiber patch cords (Precision Fiber Products) were connected to a blue laser diode (473 nm; OEM Laser Systems, Bluffdale, UT, USA) and stimulator. For each stimulation session, 60 light bursts of 5 ms (intensity 15–20 mW) at a frequency of 30 Hz were delivered every 20 s for 20 min on 7 consecutive days as described previously.²³ For mock simulation conditions, the same viral conditions were used as in stimulated mice and the optical fibers connected to patch cords; however, the laser was not activated.

Quantitative chromatin immunoprecipitation

Quantitative chromatin immunoprecipitation methods are contained in the Supplementary Information. Primer sequences and locations within promoter regions are shown in Supplementary Table 1.

Cocaine self-administration

Cocaine self-administration procedures for the 24 h and 30-day withdrawal rats have been described in detail previously.²³ Briefly, rats were implanted with chronic indwelling jugular catheters and allowed for a 1-week recovery. Rats were trained to self-administer cocaine or saline for 5 days during 1 h sessions where responses to the active snout-poke hole caused infusions followed by a 30 s time out period using a FR1 schedule, which was increased per day to an FR5 schedule. Following acquisition, rats underwent 10 days of extended access saline or cocaine in which rats had 6 h access according to an FR5 reinforcement schedule.

For cue-induced reinstatement, rats underwent 14 days of saline or cocaine self-administration in which infusions were accompanied by a 5 s illumination of a stimulus light above the active snout-poke hole and the house light was turned off for the duration of the time-out period (30 s). Rats were then exposed to multiple within-session extinction sessions, during which the chambers were dark and responses were recorded but resulted in no programmed consequences. On the subsequent day, rats were given a 1 h test of cue-induced reinstatement during which active responses produced cues previously paired with drug delivery (that is, illumination of the stimulus light above the active snout-poke hole for 5 s and the house light extinguished for 30 s). NAc was then isolated 24 h after the cue session.

RESULTS

miR-132/212 control NAc actin regulatory pathways

The microRNAs, miR-132 and miR-212, attenuate cocaine's reinforcing and rewarding properties when induced in the dorsal striatum²⁶ and we showed recently that loss of these microRNAs in the NAc increases cocaine place conditioning.²³ As such, the identification of striatal signaling pathways whose expression or function are altered by the loss of miR-132/212 has been used as an initial means of identifying candidate cocaine regulatory pathways.²³ Actin, a core cytoskeleton component, is present in all neuronal compartments and exists in either a monomeric form (G-actin) or in higher-order filaments composed of multiple monomeric actin subunits (F-actin),² and studies have revealed an important role for the regulation of synaptic actin in the effects of miR-132/212 on neuronal function.²⁷

Actin is also enriched in neuronal nuclei where its role extends beyond structural support, as actin state is a critical determinant of gene transcription.^{28–30} We were thus interested in determining whether miR-132/212 shape nuclear actin pathways in NAc neurons.

Phalloidin, a toxin that directly and specifically binds F-actin, is typically incapable of binding nuclear F-actin owing to the altered size or conformation of nuclear F-actin as compared with F-actin in non-nuclear cellular compartments.³¹ Indeed, phalloidin failed to label nuclear F-actin in NAc neurons (Supplementary Figures 1a and b). Nevertheless, the facilitation of F-actin formation in neuronal nuclei can be assessed by the activity of actin-binding proteins that directly polymerize actin monomers into F-actin filaments. The N-WASP actin-binding protein initiates *de novo* F-actin formation by linking G-actin monomers to the Arp2/3 complex, thereby forming an actin nucleation core that favors elongation.² To determine whether active N-WASP levels are altered upon the loss of miR-132/212 in NAc neurons, we infused HSV vectors expressing Cre recombinase and GFP (HSV-Cre-GFP) into the NAc of *loxP*-flanked (floxed) *miR-132/212* mice enabling NAc-specific loss of these microRNAs; HSV-GFP was used as a control (Supplementary Figures 2a and b). HSVs specifically infect neurons and we found that HSV-Cre-GFP is effective in causing the loss of miR-132/212 in floxed mice (Supplementary Figure 2c). Using immunohistochemistry we found that Cre-driven loss of *miR-132/212* increased nuclear N-WASP activity in NAc neurons (Supplementary Figures 2d–f).

Whereas N-WASP facilitates F-actin formation, other actin regulatory proteins limit G-actin pools by enhancing the stability of existing F-actin structures. Notably, PDZ-RhoGEF was

recently identified as an F-actin-binding protein that induces F-actin bundling,³² which enhances filament stability.³³ *Arhgef11*, which encodes PDZ-RhoGEF, is a predicted target of both miR-132 and miR-212, and we recently showed that knockout of these microRNAs after several weeks increases PDZ-RhoGEF levels in NAc whole-cell lysates.²³ Whether these microRNAs also affect PDZ-RhoGEF levels more rapidly and whether any altered expression occurs in the nucleus remain unknown. To this end, we infused HSV-Cre-GFP or HSV-GFP into the NAc of floxed *miR-132/212* mice and 5 days later harvested NAc tissue (Supplementary Figure 3a). P1 (crude nuclear) fractions were then isolated from NAc lysates using established methods.²³ The rapid loss of *miR-132/212* increased PDZ-RhoGEF in NAc P1 fractions (Supplementary Figures 3b and c). As a control, we examined levels of a related actin regulatory protein, RasGRF1,² which is not a predicted target of these microRNAs. Consistent with this, we found no alterations of RasGRF1 levels upon the loss of *miR-132/212* (Supplementary Figures 3b and c).

Cocaine regulates nuclear actin pathways in the NAc

The combined actions of active N-WASP and PDZ-RhoGEF could enable synergistic effects such that nuclear F-actin formation and subsequent F-actin stability, respectively, are augmented by the loss of *miR-132/212* in NAc neurons. To determine whether N-WASP and PDZ-RhoGEF are enriched in distinct actin subtype pools in NAc nuclei, NAc P1 fractions were further processed allowing for the isolation of G-actin-enriched and F-actin-enriched subfractions (Figure 1a). Consistent with its role in facilitating G-actin polymerization,² we found that N-WASP was more highly enriched in the G-actin subfraction as compared with the F-actin subfraction. Conversely, PDZ-RhoGEF was significantly enriched in the F-actin subfraction consistent with its known F-actin-binding capabilities³² (Figures 1b and c). Although we previously showed that early withdrawal from repeated cocaine administration increases nuclear PDZ-RhoGEF levels in NAc,²³ whether N-WASP activity is similarly regulated by cocaine is not known. To this end, mice were withdrawn from repeated non-contingent (experimenter-administered) cocaine for 24 h and NAc tissue immunostained for active N-WASP. We found that cocaine increased active N-WASP levels in NAc neuronal nuclei (Figures 1d–f).

Within the nucleus, actin structure is a critical regulator of gene transcription, particularly that mediated by the SRF transcription factor.^{34,35} Notably, MAL (also known as MRTF-A or MKL1), a principal co-activator of SRF, directly binds G-actin.³⁶ Increases in nuclear G-actin content reduce SRF-mediated gene transcription by the sequestering of MAL from SRF, as well as by fostering MAL nuclear export.^{35,37,38} MAL also interacts with F-actin³⁹ and nuclear F-actin polymerization enhances SRF-mediated gene transcription through MAL.⁴⁰ Thus, nuclear signaling pathways that limit the G-actin pool, by either facilitating F-actin formation or by limiting the breakdown of F-actin, can facilitate MAL-SRF interactions and concomitant SRF-mediated gene transcription.⁴¹ In G-actin and F-actin subfractions derived from NAc P1 fractions, MAL is particularly enriched in the F-actin subfraction (Figures 1g and h). The F-actin subfraction is also enriched in histones, which are minimally expressed or entirely absent in the G-actin subfraction (Figures 1g and h). Surprisingly, opposite to its MAL co-activator, SRF is enriched in NAc P1 G-actin subfractions (Figures 1g and h), suggesting that SRF's presence in the F-actin subfraction

where MAL and histones are enriched could be a limiting factor for SRF-mediated gene transcription. Indeed, the overexpression of the enzymatic GEF domain (DHPH) of PDZ-RhoGEF, which has been previously shown to facilitate SRF-mediated gene transcription,⁴² increases the abundance of SRF in P1 F-actin subfractions (Supplementary Figures 4a–c). Consistent with this, we found that, although early withdrawal from repeated non-contingent cocaine administration does not affect total MAL-SRF expression in NAc P1 fractions (Supplementary Figures 5a–g), cocaine increased the abundance of SRF in the NAc P1 F-actin subfraction relative to saline (Figures 1i–k and Supplementary Figures 6a and b). These results parallel our previous findings of an increase in P1 F-actin MAL enrichment following early cocaine withdrawal despite no change in total expression in the P1 fraction.²³ To evaluate the specificity of the cocaine effects, we also examined SRF's localization in actin subfractions following withdrawal from chronic morphine and found no regulatory effects of morphine (Supplementary Figures 7a–c).

Cocaine induces the expression of Rock1 in the NAc

The serine/threonine kinase Rock1 promotes F-actin stability by phosphorylating and inhibiting the actin-binding protein cofilin, a negative regulator of F-actin growth.² Rock1 in the nucleus induces SRF-mediated gene transcription via its ability to limit the available G-actin pool and hence the constraints on MAL-SRF interactions.⁴³ Within gene promoters, SRF directly binds to canonical sites termed serum response elements (SREs) and *in silico* analysis indicates that the *Rock1* promoter contains multiple putative SREs (Figure 2a). As F-actin is extremely labile, sustainment of F-actin regulatory pathways would be important in the maintenance of SRF-mediated signaling by preventing the replenishment of G-actin pools. To determine whether SRF binding to putative SREs in the *Rock1* promoter is altered by cocaine, mice were withdrawn from repeated non-contingent cocaine administration for 24 h, and binding of SRF to putative SREs assessed in NAc extracts using chromatin immunoprecipitation. We found no evidence of SRF binding to two of the three putative SREs; however, at a putative SRE located 599 base pairs (bp) downstream from the *Rock1* transcription start site, SRF binding exceeded that of an IgG control specifically in cocaine-treated mice. Further, the binding of SRF at this site was greater in cocaine-treated mice as compared with saline-treated mice (Figure 2b).

To assess the specificity of our chromatin immunoprecipitation findings, as a positive control we examined SRF binding to an SRE on the *Fosb* promoter, which has been shown to bind SRF in a manner unaffected by cocaine (–440 bp from the *Fosb* transcription start site)⁴⁴ (Supplementary Figure 8a). Consistent with this, we found that the magnitude of SRF binding to this SRE is similar in NAc between saline- and cocaine-treated mice (Supplementary Figure 8b). As a negative control we examined SRF binding to multiple sites on the promoter of *Arhgef11* (Supplementary Figure 8c), as *in silico* analysis indicates that this gene's promoter contains no putative SREs. We found no evidence of SRF binding to the *Arhgef11* promoter under saline or cocaine conditions (Supplementary Figures 8d and e).

To determine whether Rock1 protein levels are altered by cocaine, mice were withdrawn from repeated non-contingent cocaine for 24 h and NAc tissue fractionated into the P1, S2

(cytoplasmic) and P2 (synaptoneurosomal) compartments (Figure 2c). We found that cocaine selectively increased Rock1 levels in the P1 fraction (Figures 2d–f). Within NAc P1 fractions, we found that actin is recovered in immunoprecipitates of Rock1 (Figure 2g), indicating that Rock1 associates with actin in this fraction. To determine whether Rock1 preferentially interacts with a particular actin subtype, NAc P1 fractions were subfractionated into G-actin and F-actin (Figure 2h), and we found that Rock1 is most highly enriched in the F-actin subfraction (Figures 2i and j), consistent with its role in altering the function of F-actin binding proteins.² Following 24 h withdrawal from non-contingent cocaine, the abundance of Rock1 in the P1 F-actin subfraction is increased as compared with saline (Figures 2k and l and Supplementary Figures 9a and b), indicating that cocaine-mediated increases in P1 fraction Rock1 levels are primarily due to increased levels in the F-actin subfraction.

Self-administered cocaine regulates MAL-SRF expression in the NAc

Paradigms in which rodents self-administer drugs of abuse are valuable in modeling specific aspects of human drug addiction in that rodents initiate a voluntary act to receive the drug and the escalation of cocaine intake over time models aspects of human compulsive drug consumption.⁴⁵ To determine whether long-access cocaine self-administration affects MAL or SRF levels in NAc, rats self-administered saline or cocaine for 10 days, followed by a 24 h withdrawal. NAc tissue was then dissected and fractionated into the P1 and S2 fractions (Figure 3a). We found that early withdrawal from self-administered cocaine increased MAL levels in the P1 fraction with a strong trend for an increase in SRF levels as well (Figures 3b and c). On the other hand, neither levels of MAL nor SRF in the S2 fraction were affected by cocaine self-administration (Figures 3d and e). Next, we assessed the relationship between levels of these proteins in the P1 and S2 fractions, and found a significant increase in the P1 to S2 ratio for SRF and a strong trend for MAL after cocaine treatment (Figure 3f).

Cues associated with drug use in humans function as triggers for relapse after periods of drug abstinence.⁴⁶ To determine whether cues associated with cocaine intake in rodents are in and of themselves capable of triggering altered expression of MAL or SRF, we used a cue-induced reinstatement paradigm in which the ability of a light cue previously associated with drug infusion to trigger biochemical alterations was examined. Animals were studied 24 h after re-exposure to the cue (Figure 3g). We found that re-exposure to the associative light cue was sufficient to trigger a selective increase of MAL in the P1, but not S2, fraction of NAc in rodents that had previously self-administered cocaine as compared with saline. However, no changes in SRF levels were detected in either fraction (Figures 3h–k).

To determine whether the biochemical changes we observed are specific for early withdrawal periods, rats were subjected to 30-day withdrawal from repeated self-administered cocaine (Figure 3l). Surprisingly, protracted cocaine withdrawal produced a significant reduction in SRF levels and a trend toward reduced MAL levels in NAc P1 fractions. No changes in the S2 fraction were observed (Figures 3m–p). Further, we found that long-term cocaine withdrawal decreased the ratio of SRF in the P1 fraction relative to S2, with no significant change in MAL (Figure 3q). Different from self-administered

cocaine, protracted withdrawal from non-contingent cocaine did not affect NAc SRF or MAL expression (Supplementary Figures 10a–d).

Glutamatergic NAc circuits regulates MAL-SRF expression

Recruitment and engagement of glutamatergic signaling in NAc neurons mediates many of the rewarding properties of cocaine.^{47–53} The NAc receives direct glutamatergic input from numerous structures, including the mPFC, the BLA and the ventral hippocampus.^{54,55} Although stimulation of glutamatergic inputs to the NAc produces reward behavior in rodents irrespective of their brain region of origin,⁵⁶ pathway-specific effects have been reported and remain incompletely understood.^{57–60} To determine whether stimulating glutamatergic inputs to the NAc alters the expression of MAL or SRF in a pathway-specific manner, we used *in vivo* optogenetics to achieve circuit-specific activation of individual inputs to the NAc using stimulation parameters similar to those previously shown to be behaviorally reinforcing.^{23,56} Mice underwent a single 20 min stimulation session per day for 7 days; NAc tissue was dissected 24 h after the last stimulation session and P1 NAc fractions subsequently isolated. Stimulation of channel rhodopsin (ChR2)-expressing terminals in the NAc that project from the mPFC (Figures 4a and b) increased SRF levels in NAc P1 fractions as compared with mock-stimulated mice, whereas MAL levels were unaltered (Figures 4c and d). Conversely, stimulation of ChR2-expressing terminals in the NAc that project from the BLA (Figures 4e and f) increased MAL levels in NAc P1 fractions, with no effect on SRF (Figures 4g and h). The stimulation of ventral hippocampus inputs to the NAc did not alter the expression of either MAL or SRF (Supplementary Figures 11a–d).

As the stimulation of mPFC projection terminals in the NAc increases SRF expression in NAc P1 fractions, we determined whether SRF preferentially associates with a particular actin pool in NAc P1 fractions after stimulation. To this end, we stimulated ChR2-expressing terminals in the NAc that project from the mPFC and isolated G-actin and F-actin subfractions from NAc P1 fractions (Figure 4i). Similar to early withdrawal from repeated cocaine administration (Figures 1j and k), stimulation of mPFC to NAc inputs increased SRF F-actin to G-actin ratios (Figures 4j and k), indicating a preferential enrichment of SRF in the vicinity of NAc nuclear F-actin pools. As cocaine also increases MAL enrichment in NAc nuclear F-actin pools,²³ we examined MAL's association with G-actin and F-actin following mPFC to NAc stimulation and found a trend toward an increase in the MAL F-actin to G-actin ratio (Figures 4l and m). Stimulation of BLA to NAc inputs did not alter the balance between SRF or MAL levels in NAc P1 F-actin vs G-actin subfractions (Supplementary Figures 12a–e).

SRF mediates cocaine's effects on dendritic spine morphogenesis

Recent work suggests that SRF regulates the expression of the activity-regulated cytoskeleton-associated protein Arc (Arg3.1).⁶¹ *In silico* analysis indicates that *Arc* contains two putative SREs in its promoter region (Figure 5a). Although assessment of binding to each individual SRE was not possible given their close proximity to each other, using chromatin immunoprecipitation we found that collective SRF binding to the putative *Arc* SREs was increased in NAc of repeated cocaine-treated mice relative to saline-treated

controls at the 24 h withdrawal timepoint (Figure 5b). To determine whether cocaine alters Arc protein expression, mice were withdrawn for 24 h from repeated cocaine and NAc tissue fractionated into P1, S2 and P2 fractions. We found that cocaine selectively increased Arc levels in the P2 fraction (Figures 5c and d), suggestive of a role for synaptosomal Arc in cocaine action. As we also identified a cocaine-induced SRF binding site in the *Rock1* promoter (Figure 2b), we tested further the involvement of SRF in the regulation of Rock1 and Arc protein levels. To this end, we infused HSV-Cre-GFP into the NAc of *loxP*-flanked (floxed) *SRF* mice, which we previously showed is effective in reducing NAc P1 SRF levels,²³ and acutely withdrew mice from repeated cocaine administration. HSV-GFP was used as a control. We found that SRF loss reduced Rock1 and Arc levels in the P1 and P2 fractions, respectively (Supplementary Figures 13a–f).

Dendritic spines can be divided into three distinct functional subclasses that include stubby spines, which have a clear spine head but lack a neck, and neck-bearing thin and mushroom spines where the size of the head region (typically larger in mushroom spines) is among the primary distinguishing features of these spine types.⁶² Arc has a characterized role in augmenting the selective formation of *de novo* thin dendritic spines with no effect on mature mushroom spines.⁶³ Early (hours, days) withdrawal from repeated cocaine administration is similarly typified by selective increases in thin dendritic spine density on NAc MSNs with minimal effects on mature spine numbers.^{6,8,23} However, how manipulations of SRF in adult neurons affect spine morphogenesis remains unknown. To assess this, we infused HSV-Cre-GFP or HSV-GFP into the NAc of floxed *SRF* mice and acutely withdrew them from repeated cocaine administration (Figure 5e). Total spine density and the density of individual spine subtypes was then assessed using established procedures^{6,23,25} (Figures 5f–h). We found that cocaine significantly increased total dendritic spine density on NAc MSNs expressing HSV-GFP relative to saline-treated mice; however, MSNs expressing HSV-Cre-GFP were unresponsive to the spine morphogenic effects of cocaine (Figures 5i and j). Further, we found that cocaine increased the density of thin spines (which includes filopodia) on NAc MSNs expressing HSV-GFP, with no effect on the densities of either stubby or mushroom spines (Figures 5k–m). Consistent with a selective induction of thin spines, we found that in HSV-GFP-expressing NAc MSNs mean spine head diameter was lower in cocaine-treated relative to saline-treated mice (mean $\pm$ s.e.m.; GFP saline, 0.477 ± 0.016 μ m; GFP cocaine, 0.436 ± 0.011 μ m; $*P < 0.05$). In neurons expressing HSV-Cre-GFP, cocaine failed to alter the density of thin spines (Figure 5k), with no effect on mean spine head diameter (mean $\pm$ s.e.m.; Cre saline, 0.488 ± 0.015 μ m; Cre cocaine, 0.534 ± 0.016 μ m; $P > 0.05$), indicating that SRF is essential for cocaine-mediated *de novo* thin spine formation in NAc MSNs.

NAc SRF is essential for cocaine-induced locomotor sensitization

The formation of new dendritic spines on NAc MSNs is correlated with locomotor sensitization in response to repeated cocaine.^{49,64} As we show that the loss of SRF prevents the normal upregulation of new thin dendritic spines by cocaine, we were interested in determining whether SRF loss in the NAc impacts cocaine-mediated locomotor responses. To test this, HSV-Cre-GFP or HSV-GFP was infused into the NAc of floxed *SRF* mice and cocaine locomotor responses were measured using an escalating dose paradigm in which

mice were injected with a moderate cocaine dose (8.5 mg kg^{-1}) for 4 days followed by injection with a higher cocaine dose (15 mg kg^{-1}) for 4 days (Supplementary Figure 14a). We found that SRF loss does not affect locomotor responses to the 8.5 mg kg^{-1} cocaine dose, similar to previous results using moderate cocaine doses.⁴⁴ However, SRF loss significantly reduced locomotor responses to the 15 mg kg^{-1} dose (Supplementary Figure 14b). Further analysis indicates that the loss of SRF in the NAc blocks locomotor sensitization in response to an escalation in cocaine dose (Supplementary Figure 14c).

Although the above findings indicate that SRF is not important for locomotor responses to moderate cocaine doses during a mouse's first exposure to cocaine, it is nevertheless possible that SRF could have a role in controlling responses to lower cocaine doses in mice with a prior history of cocaine intake. To test this, HSV-Cre-GFP or HSV-GFP was infused into the NAc of floxed *SRF* mice and mice were injected in their home cage with a high cocaine dose for 7 days. Following 8 days of withdrawal, locomotor responses to a low cocaine dose challenge were assessed in a novel cage (Supplementary Figure 15a). The loss of SRF in the NAc reduced locomotor responses to the cocaine challenge (Supplementary Figure 15b and c).

DISCUSSION

Although the mechanisms by which cocaine regulates SRF signaling were previously largely uncharacterized, knowledge of this is important given that NAc SRF is a critical mediator of cocaine's rewarding properties.⁶⁵ We previously showed that cocaine increases the physical interaction between MAL and SRF in NAc nuclei.²³ Here we extend these findings by detailing the effects of cocaine on MAL-SRF signaling in the NAc, we assess cocaine's ability to regulate SRF binding to gene promoter regions, we reveal the role for glutamatergic neurotransmission in regulating NAc MAL-SRF expression, we demonstrate the requirement of SRF in cocaine-mediated structural alterations in NAc MSNs and we reveal a previously unknown role for NAc SRF in behavioral sensitization (see Supplementary Figure 16 for summary).

We found that repeated non-contingent cocaine administration increases the expression and/or activity of F-actin regulatory molecules, including N-WASP and Rock1, in the NAc. The synergistic actions of these molecules would be expected to promote F-actin formation and stability, and as such likely limit the normal G-actin constraints on MAL. In addition, we found that cocaine increases the abundance of SRF in nuclear F-actin subfractions, which likely places SRF in close proximity to F-actin-enriched MAL. In contrast, morphine did not alter the abundance of SRF in G-actin or F-actin nuclear pools indicating that regulation of SRF's subcellular localization is at least partially specific to cocaine and not a common feature of all drugs of abuse. We showed recently that cocaine increases the physical interaction between MAL and SRF in the NAc,²³ and the enrichment of SRF in F-actin nuclear pools by cocaine is one mechanism by which this could occur. The enrichment in F-actin pools could also place SRF in closer proximity to potential DNA-binding sites given that we show histones are nearly exclusively present in nuclear F-actin regions. Given F-actin's lability, the sustainment of F-actin promoting regulatory pathways is important for the maintenance of SRF-mediated signaling. In this regard, it is of particular interest that

cocaine induces the binding of SRF to the *Rock1* promoter, as such action could trigger a positive feedback loop, whereby increased SRF expression or activity directly contributes to the sustenance of nuclear F-actin, which in turn further maintains heightened SRF activity.

Actin state also influences the shuttling of MAL and SRF to and from the nucleus, as increased nuclear G-actin levels are associated with increased SRF and MAL nuclear export, and hence reduced nuclear levels. Opposite to this, nuclear F-actin formation facilitates nuclear MAL accumulation.^{40,43} During early withdrawal from non-contingent cocaine we found that SRF levels are increased in nuclear F-actin subfractions with no alteration in that associated with G-actin. The fact that SRF levels in the G-actin fraction are not decreased by cocaine suggests that the rise in F-actin SRF is not likely due to the shuttling of SRF from G-actin to F-actin pools. As SRF undergoes constitutive cycling between the cytoplasm and nucleus,⁴³ upon entering the nucleus it is possible that there is greater opportunity for SRF to interact with F-actin and reduced opportunity to interact with G-actin following cocaine treatment. The interaction with F-actin would not only place SRF in close proximity to DNA binding regions but also potentially confer greater stability to SRF in that it is more immune to nuclear export. In this regard, it is interesting that during early withdrawal from non-contingent cocaine SRF nuclear to cytoplasmic ratios increase. Similarly, during early withdrawal from self-administered cocaine SRF nuclear to cytoplasmic ratios increase, with a decrease during long-term withdrawal. Parallel alterations in MAL were also seen, but these changes failed to achieve statistical significance. We previously found that, in response to self-administered cocaine, NAc nuclear expression of PDZ-RhoGEF is increased during early withdrawal and decreased during long-term withdrawal.²³ Such bidirectional changes could influence the relative ratios of SRF and MAL in the nucleus vs the cytoplasm via PDZ-RhoGEF's effects on nuclear F-actin stability.

In response to early withdrawal from self-administered cocaine, MAL expression is increased in NAc P1 fractions with a strong trend for an increase in SRF as well. Interestingly, protracted (30 days) withdrawal decreases NAc SRF levels with a trend toward a loss of MAL. It is possible that more robust increases in SRF during early withdrawal and decreases in MAL during long-term withdrawal would result from slightly different withdrawal times or by using different cocaine doses. The consequences of SRF loss during long-term withdrawal are not entirely clear at present. Drug seeking behavior intensifies with longer withdrawal periods, increasing the propensity toward relapse.^{66,67} It is possible the loss of SRF dampens the heightened drug-seeking behavior typical of protracted cocaine withdrawal. Indeed, adaptations in NAc MSNs that oppose relapse have been observed after long-term cocaine withdrawal.¹⁵ As long-term withdrawal from non-contingent cocaine does not affect MAL-SRF expression, this adaptation might be unique to self-administration paradigms. The voluntary nature of the drug intake could account for differences relative to experimenter-administered paradigms; however, differences in the amount of cocaine intake and the duration of cocaine availability could be important factors as well. Species differences might also be of importance as the non-contingent studies were performed in mice and the contingent studies in rats.

BLA innervation of a subtype of NAc MSNs is increased during early cocaine withdrawal⁶⁸ and BLA to NAc signaling is critical for responsivity to reward-predictive cues,⁶⁹ including

cue-induced cocaine reinstatement.⁵⁹ We found that exposure to a cocaine-predictive cue induces MAL expression in the NAc. As we also show that the stimulation of BLA to NAc terminals, but not those from other regions, increases NAc MAL expression, it is conceivable that cue-induced MAL expression is driven by BLA to NAc signaling. Following 24 h withdrawal from chronic self-administered and experimenter-administered cocaine mPFC to NAc glutamatergic transmission is enhanced.⁷⁰ As we found stimulation of mPFC to NAc inputs increased SRF expression, whereas the stimulation of other NAc inputs exerted no effects on SRF, cocaine-mediated increases in mPFC to NAc glutamatergic transmission potentially influence NAc SRF levels.

Finally, we show that SRF is a critical mediator of cocaine's effects on new thin spine formation in the NAc following early withdrawal and is critical for cocaine locomotor sensitization. Interestingly, the induction of weak synapses in NAc neurons during early cocaine withdrawal preferentially occurs in neurons that are the most strongly activated by cocaine.⁷¹ Recent studies suggest that the progression from thin spines during early cocaine withdrawal to mature spines during long-term withdrawal is best conceptualized as a form of 'metaplasticity' such that the sprouting of new weak connections serves as a locus at which subsequent synaptic strengthening can occur.^{9,72} Indeed, although the formation of new thin spines in NAc MSNs during early withdrawal is associated with increased cocaine-associated behavioral responses, such as drug seeking and locomotor responses,^{6,16,23} the transition to synaptic maturation in most NAc neural circuits during longer withdrawal periods (for example, 1–2 months) further heightens such behavioral responses.^{14,15} The fact that SRF is essential for cocaine-mediated formation of new thin spines during early withdrawal highlights an important role for this molecule in generating the framework through which behavioral responses to cocaine are established. Indeed, we found that SRF loss in the NAc attenuates cocaine-mediated locomotor responses to a high dose of cocaine and blocks sensitization responses resulting from an escalation in cocaine dose. Further, SRF loss inhibited locomotor responses to a low dose challenge in mice with a history of prior cocaine intake. Previous work has shown that the combined loss of SRF plus CREB severely reduces place preference for cocaine at multiple doses, indicative of a synergistic role for these molecules in regulating cocaine's rewarding properties.⁶⁵ While numerous studies support the idea that alterations in structural plasticity, including *de novo* spine formation, are key determinants of cocaine-mediated behavioral responses,^{4,6,11,23,49,64} understanding how SRF loss impacts cocaine-mediated alterations in NAc MSN synaptic function merits further attention and remains an interesting question for future studies.

Both acute and repeated cocaine administration increase F-actin levels with concomitant reductions in G-actin in NAc synaptosomes.⁷³ In the case of repeated cocaine, this F-actin increase is accompanied by alterations in the expression and activity of multiple actin binding proteins that would predictably facilitate the genesis of filopodia.⁷³ Here we found increased N-WASP activity and increased levels of Rock1 in NAc nuclei following early cocaine withdrawal, which would likely favor F-actin formation and stability, respectively. Taken together, these findings indicate that repeated cocaine administration potentially augments F-actin content in multiple compartments of NAc neurons. Interestingly, manipulation of F-actin in the NAc affects cocaine behavioral responses in a manner sensitive to cocaine history. Namely, inhibition of NAc F-actin increases reinstatement

behavior following extinction,⁷³ whereas NAc F-actin inhibition reduces locomotor sensitization in rodents that have received chronic cocaine.⁷⁴ In these experiments, the rapid effects (within 30 min) of F-actin inhibition on cocaine-elicited behaviors were assessed, on the time scale of alterations in spine morphogenesis as opposed to gene transcription. As we found that SRF loss in the NAc reduces locomotor sensitization and because SRF activity is highly regulated by nuclear actin state, it would be interesting to determine the precise contributions of nuclear F-actin in the NAc to cocaine behavioral responses and the comparative functions of nuclear versus synaptosomal F-actin in this regard. Addressing these questions will be aided by the development of investigative tools in which actin state can be manipulated in specific neuronal compartments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. *Nat Neurosci.* 2011; 14:285–293. [PubMed: 21346746]
2. Penzes P, Cahill ME. Deconstructing signal transduction pathways that regulate the actin cytoskeleton in dendritic spines. *Cytoskeleton (Hoboken).* 2012; 69:426–441. [PubMed: 22307832]
3. Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci.* 2001; 4:1086–1092. [PubMed: 11687814]
4. Robinson TE, Gorny G, Mitton E, Kolb B. Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse.* 2001; 39:257–266. [PubMed: 11169774]
5. Pulipparacharuvil S, Renthal W, Hale CF, Taniguchi M, Xiao G, Kumar A, et al. Cocaine regulates MEF2 to control synaptic and behavioral plasticity. *Neuron.* 2008; 59:621–633. [PubMed: 18760698]
6. Dietz DM, Sun H, Lobo MK, Cahill ME, Chadwick B, Gao V, et al. Rac1 is essential in cocaine-induced structural plasticity of nucleus accumbens neurons. *Nat Neurosci.* 2012; 15:891–896. [PubMed: 22522400]
7. Shen HW, Toda S, Moussawi K, Bouknight A, Zahm DS, Kalivas PW. Altered dendritic spine plasticity in cocaine-withdrawn rats. *J Neurosci.* 2009; 29:2876–2884. [PubMed: 19261883]
8. Graziane NM, Sun S, Wright WJ, Jang D, Liu Z, Huang YH, et al. Opposing mechanisms mediate morphine- and cocaine-induced generation of silent synapses. *Nat Neurosci.* 2016; 19:915–925. [PubMed: 27239940]
9. Dong Y, Nestler EJ. The neural rejuvenation hypothesis of cocaine addiction. *Trends Pharmacol Sci.* 2014; 35:374–383. [PubMed: 24958329]
10. Golden SA, Russo SJ. Mechanisms of psychostimulant-induced structural plasticity. *Cold Spring Harb Perspect Med.* 2012; 2
11. Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* 2010; 33:267–276. [PubMed: 20207024]

12. Stankeviciute NM, Scofield MD, Kalivas PW, Gipson CD. Rapid, transient potentiation of dendritic spines in context-induced relapse to cocaine seeking. *Addict Biol.* 2014; 19:972–974. [PubMed: 23648005]
13. Smith AC, Kupchik YM, Scofield MD, Gipson CD, Wiggins A, Thomas CA, et al. Synaptic plasticity mediating cocaine relapse requires matrix metalloproteinases. *Nat Neurosci.* 2014; 17:1655–1657. [PubMed: 25326689]
14. Lee BR, Ma YY, Huang YH, Wang X, Otaka M, Ishikawa M, et al. Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nat Neurosci.* 2013; 16:1644–1651. [PubMed: 24077564]
15. Ma YY, Lee BR, Wang X, Guo C, Liu L, Cui R, et al. Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. *Neuron.* 2014; 83:1453–1467. [PubMed: 25199705]
16. Brown TE, Lee BR, Mu P, Ferguson D, Dietz D, Ohnishi YN, et al. A silent synapse-based mechanism for cocaine-induced locomotor sensitization. *J Neurosci.* 2011; 31:8163–8174. [PubMed: 21632938]
17. Wang X, Cahill ME, Werner CT, Christoffel DJ, Golden SA, Xie Z, et al. Kalirin-7 mediates cocaine-induced AMPA receptor and spine plasticity, enabling incentive sensitization. *J Neurosci.* 2013; 33:11012–11022. [PubMed: 23825406]
18. Pascoli V, Turiault M, Luscher C. Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature.* 2012; 481:71–75.
19. Gipson CD, Kupchik YM, Shen H, Reissner KJ, Thomas CA, Kalivas PW. Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. *Neuron.* 2013; 77:867–872. [PubMed: 23473317]
20. Robison AJ, Nestler EJ. Transcriptional and epigenetic mechanisms of addiction. *Nat Rev Neurosci.* 2011; 12:623–637. [PubMed: 21989194]
21. Magill ST, Cambronne XA, Luikart BW, Lioy DT, Leighton BH, Westbrook GL, et al. microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. *Proc Natl Acad Sci USA.* 2010; 107:20382–20387. [PubMed: 21059906]
22. Ramanan N, Shen Y, Sarsfield S, Lemberger T, Schutz G, Linden DJ, et al. SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. *Nat Neurosci.* 2005; 8:759–767. [PubMed: 15880109]
23. Cahill ME, Bagot RC, Gancarz AM, Walker DM, Sun H, Wang ZJ, et al. Bidirectional synaptic structural plasticity after chronic cocaine administration occurs through Rap1 small GTPase signaling. *Neuron.* 2016; 89:566–582. [PubMed: 26844834]
24. Sukumvanich P, DesMarais V, Sarmiento CV, Wang Y, Ichetovkin I, Mouneimne G, et al. Cellular localization of activated N-WASP using a conformation-sensitive antibody. *Cell Motil Cytoskeleton.* 2004; 59:141–152. [PubMed: 15362118]
25. Golden SA, Christoffel DJ, Heshmati M, Hodes GE, Magida J, Davis K, et al. Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression. *Nat Med.* 2013; 19:337–344. [PubMed: 23416703]
26. Hollander JA, Im HI, Amelio AL, Kocerha J, Bali P, Lu Q, et al. Striatal microRNA controls cocaine intake through CREB signalling. *Nature.* 2010; 466:197–202. [PubMed: 20613834]
27. Impey S, Davare M, Lesiak A, Fortin D, Ando H, Varlamova O, et al. An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. *Mol Cell Neurosci.* 2010; 43:146–156. [PubMed: 19850129]
28. Ye J, Zhao J, Hoffmann-Rohrer U, Grummt I. Nuclear myosin I acts in concert with polymeric actin to drive RNA polymerase I transcription. *Genes Dev.* 2008; 22:322–330. [PubMed: 18230700]
29. Wu X, Yoo Y, Okuhama NN, Tucker PW, Liu G, Guan JL. Regulation of RNA-polymerase-II-dependent transcription by N-WASP and its nuclear-binding partners. *Nat Cell Biol.* 2006; 8:756–763. [PubMed: 16767080]
30. Grosse R, Vartiainen MK. To be or not to be assembled: progressing into nuclear actin filaments. *Nat Rev Mol Cell Biol.* 2013; 14:693–697. [PubMed: 24088744]

31. Vartiainen MK. Nuclear actin dynamics—from form to function. *FEBS Lett.* 2008; 582:2033–2040. [PubMed: 18423404]
32. Banerjee J, Fischer CC, Wedegaertner PB. The amino acid motif L/IIxxFE defines a novel actin-binding sequence in PDZ-RhoGEF. *Biochemistry.* 2009; 48:8032–8043. [PubMed: 19618964]
33. Koskinen M, Hotulainen P. Measuring F-actin properties in dendritic spines. *Front Neuroanat.* 2014; 8:74. [PubMed: 25140131]
34. Posern G, Treisman R. Actin' together: serum response factor, its cofactors and the link to signal transduction. *Trends Cell Biol.* 2006; 16:588–596. [PubMed: 17035020]
35. Vartiainen MK, Guettler S, Larijani B, Treisman R. Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL. *Science.* 2007; 316:1749–1752. [PubMed: 17588931]
36. Guettler S, Vartiainen MK, Miralles F, Larijani B, Treisman R. RPEL motifs link the serum response factor cofactor MAL but not myocardin to Rho signaling via actin binding. *Mol Cell Biol.* 2008; 28:732–742. [PubMed: 18025109]
37. Kokai E, Beck H, Weissbach J, Arnold F, Sinske D, Sebert U, et al. Analysis of nuclear actin by overexpression of wild-type and actin mutant proteins. *Histochem Cell Biol.* 2014; 141:123–135. [PubMed: 24091797]
38. Stern S, Debre E, Stritt C, Berger J, Posern G, Knoll B. A nuclear actin function regulates neuronal motility by serum response factor-dependent gene transcription. *J Neurosci.* 2009; 29:4512–4518. [PubMed: 19357276]
39. Posern G, Miralles F, Guettler S, Treisman R. Mutant actins that stabilise F-actin use distinct mechanisms to activate the SRF coactivator MAL. *EMBO J.* 2004; 23:3973–3983. [PubMed: 15385960]
40. Baarlink C, Wang H, Grosse R. Nuclear actin network assembly by formins regulates the SRF coactivator MAL. *Science.* 2013; 340:864–867. [PubMed: 23558171]
41. Muehlich S, Hermanns C, Meier MA, Kircher P, Gudermann T. Unravelling a new mechanism linking actin polymerization and gene transcription. *Nucleus.* 2016; 7:121–125. [PubMed: 27104924]
42. Medina F, Carter AM, Dada O, Gutowski S, Hadas J, Chen Z, et al. Activated RhoA is a positive feedback regulator of the Lbc family of Rho guanine nucleotide exchange factor proteins. *J Biol Chem.* 2013; 288:11325–11333. [PubMed: 23493395]
43. Liu HW, Halayko AJ, Fernandes DJ, Harmon GS, McCauley JA, Kocieniewski P, et al. The RhoA/Rho kinase pathway regulates nuclear localization of serum response factor. *Am J Respir Cell Mol Biol.* 2003; 29:39–47. [PubMed: 12600823]
44. Vialou V, Maze I, Renthal W, LaPlant QC, Watts EL, Mouzon E, et al. Serum response factor promotes resilience to chronic social stress through the induction of DeltaFosB. *J Neurosci.* 2010; 30:14585–14592. [PubMed: 20980616]
45. Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. *Science.* 2004; 305:1014–1017. [PubMed: 15310906]
46. Bossert JM, Ghitza UE, Lu L, Epstein DH, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: an update and clinical implications. *Eur J Pharmacol.* 2005; 526:36–50. [PubMed: 16289451]
47. Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature.* 2008; 454:118–121. [PubMed: 18500330]
48. White SL, Ortinski PI, Friedman SH, Zhang L, Neve RL, Kalb RG, et al. A critical role for the GluA1 accessory protein, SAP97, in cocaine seeking. *Neuropsychopharmacology.* 2016; 41:736–750. [PubMed: 26149358]
49. Kalivas PW. The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci.* 2009; 10:561–572. [PubMed: 19571793]
50. Reissner KJ, Gipson CD, Tran PK, Knackstedt LA, Scofield MD, Kalivas PW. Glutamate transporter GLT-1 mediates N-acetylcysteine inhibition of cocaine reinstatement. *Addict Biol.* 2015; 20:316–323. [PubMed: 24612076]

51. Doyle SE, Ramoa C, Garber G, Newman J, Toor Z, Lynch WJ. A shift in the role of glutamatergic signaling in the nucleus accumbens core with the development of an addicted phenotype. *Biol Psychiatry*. 2014; 76:810–815. [PubMed: 24629536]
52. Loweth JA, Tseng KY, Wolf ME. Adaptations in AMPA receptor transmission in the nucleus accumbens contributing to incubation of cocaine craving. *Neuropharmacology*. 2014; 76(Pt B): 287–300. [PubMed: 23727437]
53. Schmidt HD, Pierce RC. Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. *Ann N Y Acad Sci*. 2010; 1187:35–75. [PubMed: 20201846]
54. Phillipson OT, Griffiths AC. The topographic order of inputs to nucleus accumbens in the rat. *Neuroscience*. 1985; 16:275–296. [PubMed: 4080159]
55. Friedman DP, Aggleton JP, Saunders RC. Comparison of hippocampal, amygdala, and perirhinal projections to the nucleus accumbens: combined anterograde and retrograde tracing study in the Macaque brain. *J Comp Neurol*. 2002; 450:345–365. [PubMed: 12209848]
56. Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A. Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. *Neuron*. 2012; 76:790–803. [PubMed: 23177963]
57. Pascoli V, Terrier J, Espallergues J, Valjent E, O'Connor EC, Luscher C. Contrasting forms of cocaine-evoked plasticity control components of relapse. *Nature*. 2014; 509:459–464. [PubMed: 24848058]
58. Stefanik MT, Moussawi K, Kupchik YM, Smith KC, Miller RL, Huff ML, et al. Optogenetic inhibition of cocaine seeking in rats. *Addict Biol*. 2013; 18:50–53. [PubMed: 22823160]
59. Stefanik MT, Kalivas PW. Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. *Front Behav Neurosci*. 2013; 7:213. [PubMed: 24399945]
60. Stefanik MT, Kupchik YM, Kalivas PW. Optogenetic inhibition of cortical afferents in the nucleus accumbens simultaneously prevents cue-induced transient synaptic potentiation and cocaine-seeking behavior. *Brain Struct Funct*. 2016; 221:1681–1689. [PubMed: 25663648]
61. Kawashima T, Okuno H, Nonaka M, Adachi-Morishima A, Kyo N, Okamura M, et al. Synaptic activity-responsive element in the Arc/Arg3.1 promoter essential for synapse-to-nucleus signaling in activated neurons. *Proc Natl Acad Sci USA*. 2009; 106:316–321. [PubMed: 19116276]
62. Nimchinsky EA, Sabatini BL, Svoboda K. Structure and function of dendritic spines. *Annu Rev Physiol*. 2002; 64:313–353. [PubMed: 11826272]
63. Peebles CL, Yoo J, Thwin MT, Palop JJ, Noebels JL, Finkbeiner S. Arc regulates spine morphology and maintains network stability in vivo. *Proc Natl Acad Sci USA*. 2010; 107:18173–18178. [PubMed: 20921410]
64. Li Y, Acerbo MJ, Robinson TE. The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. *Eur J Neurosci*. 2004; 20:1647–1654. [PubMed: 15355332]
65. Vialou V, Feng J, Robison AJ, Ku SM, Ferguson D, Scobie KN, et al. Serum response factor and cAMP response element binding protein are both required for cocaine induction of DeltaFosB. *J Neurosci*. 2012; 32:7577–7584. [PubMed: 22649236]
66. Lu L, Grimm JW, Dempsey J, Shaham Y. Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology (Berl)*. 2004; 176:101–108. [PubMed: 15071719]
67. Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*. 2001; 412:141–142. [PubMed: 11449260]
68. MacAskill AF, Cassel JM, Carter AG. Cocaine exposure reorganizes cell type- and input-specific connectivity in the nucleus accumbens. *Nat Neurosci*. 2014; 17:1198–1207. [PubMed: 25108911]
69. Ambroggi F, Ishikawa A, Fields HL, Nicola SM. Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron*. 2008; 59:648–661. [PubMed: 18760700]

70. Suska A, Lee BR, Huang YH, Dong Y, Schluter OM. Selective presynaptic enhancement of the prefrontal cortex to nucleus accumbens pathway by cocaine. *Proc Natl Acad Sci USA*. 2013; 110:713–718. [PubMed: 23267100]
71. Koya E, Cruz FC, Ator R, Golden SA, Hoffman AF, Lupica CR, et al. Silent synapses in selectively activated nucleus accumbens neurons following cocaine sensitization. *Nat Neurosci*. 2012; 15:1556–1562. [PubMed: 23023294]
72. Lee BR, Dong Y. Cocaine-induced metaplasticity in the nucleus accumbens: silent synapse and beyond. *Neuropharmacology*. 2011; 61:1060–1069. [PubMed: 21232547]
73. Toda S, Shen HW, Peters J, Cagle S, Kalivas PW. Cocaine increases actin cycling: effects in the reinstatement model of drug seeking. *J Neurosci*. 2006; 26:1579–1587. [PubMed: 16452681]
74. Toda S, Shen H, Kalivas PW. Inhibition of actin polymerization prevents cocaine-induced changes in spine morphology in the nucleus accumbens. *Neurotox Res*. 2010; 18:410–415. [PubMed: 20401643]

Figure 1.

Cocaine's effects on nuclear actin regulatory pathways in the NAc. **(a)** Experimental design. P1 fractions were isolated from NAc lysates and subsequently fractionated into G-actin and F-actin subfractions. **(b and c)** Western blottings show protein levels of N-WASP and PDZ-RhoGEF in the P1 G-actin and F-actin subfractions. Quantification indicates that N-WASP levels are more highly enriched in the P1 G-actin versus the F-actin subfraction (Student's $t_6 = 7.191$, $***P = 0.0004$, $n = 4$ brains), whereas PDZ-RhoGEF is more highly enriched in the P1 F-actin subfraction (Student's $t_6 = 4.619$, $**P = 0.0036$, $n = 4$ brains). **(d)** Mice were intraperitoneally (IP) injected with saline or cocaine (20 mg kg⁻¹) for 7 days followed by a 24 h withdrawal. Coronal NAc-bearing sections were immunostained for NeuN to label neuronal soma (and exclude glia) along with active N-WASP using a conformation-specific antibody. Sections were counterstained with DAPI to label nuclear regions. Images show 0.1 μ m steps in the Z-plane of individual NAc shell neuronal soma. Active N-WASP fluorescent intensity in the region defined by DAPI was used to assess levels in the nucleus, whereas levels in the NeuN region minus levels in the DAPI region was used to assess cytoplasmic levels. **(e)** Quantification reveals that cocaine increases active N-WASP levels in NAc neuronal nuclei (Student's $t_{99} = 4.177$, $***P < 0.0001$, $n = 51$ cells from 5 brains for saline and 50 cells from 5 brains for cocaine). **(f)** Quantification reveals that cocaine does not alter

active N-WASP levels in NAc neuronal cytoplasm (Student's $t_{99} = 0.02852$, $P = 0.98$, $n = 51$ cells from 5 brains for saline and 50 cells from 5 brains for cocaine). (g and h), Westerns blottings of G-actin and F-actin subfractions derived from P1 NAc fractions. Levels of MAL and histone H1 are enriched in the F-actin subfraction relative to that of G-actin (MAL, Student's $t_6 = 5.545$, $**P = 0.0015$, $n = 4$ brains; Histone H1, $t_6 = 2.473$, $*P = 0.0482$, $n = 4$ brains), whereas levels of SRF are enriched in the G-actin subfraction (Student's $t_6 = 6.809$, $***P = 0.0005$, $n = 4$ brains). Histone H3 was exclusively found in the F-actin subfraction. (i) Experimental design. Mice were IP injected with saline or cocaine (20 mg kg^{-1}) for 7 days followed by a 24 h withdrawal. P1 fractions were obtained from NAc lysate and subsequently subfractionated into G-actin and F-actin subfractions. (j and k) Proteins levels of SRF in the P1 F-actin and G-actin subfractions are shown following 24 h withdrawal from saline or cocaine treatment. Cocaine treatment increased the ratio of SRF present in the P1 F-actin subfraction relative to the G-actin subfraction (Student's $t_{59} = 2.602$, $**P = 0.0022$, $n = 29$ saline brains and 32 cocaine brains). All summary data are depicted as the mean+s.e.m.

Figure 2.

Cocaine alters Rock1 levels in the NAc. **(a)** Schematic showing the location of putative SREs on the *Rock1* promoter relative to the transcription start site (arrow). Chromatin immunoprecipitation (ChIP) of NAc SRF (or IgG as a control) following 24 h withdrawal from 7 days of IP saline or cocaine (20 mg kg^{-1}) treatment was used to assess differential binding of SRF to putative SREs on the *Rock1* promoter. **(b)** SRF binding to SRE sites 1 and sites 3 on *Rock1* promoter (as depicted in 2A) in NAc did not exceed that of an IgG control following either saline or cocaine treatment (2×2 analysis of variance (ANOVA) with Bonferroni *post hoc* saline IgG versus saline SRF, $P > 0.05$; cocaine IgG versus cocaine SRF, $P > 0.05$). SRF binding to SRE site 2 exceeded that of IgG specifically in cocaine-treated mice and SRF binding to this site was greater in cocaine-treated relative to saline-treated mice (2×2 ANOVA main effects of SRF pulldown $F_{1,24} = 6.993$, $*P = 0.0142$ and drug treatment $F_{1,24} = 4.959$, $*P = 0.0356$; Bonferroni *post hoc* saline IgG versus saline SRF, $P > 0.05$, cocaine IgG versus cocaine SRF, $**P < 0.01$; Bonferroni *post hoc* saline SRF versus cocaine SRF, $*P < 0.05$; $n = 6$ saline brains and 8 cocaine brains). **(c)** Experimental design. Mice were IP injected with saline or cocaine (20 mg kg^{-1}) for 7 days followed by a 24 h withdrawal. NAc lysate was fractionated into P1, S2 and P2 fractions. **(d)** Western blots and quantification showing that cocaine treatment increases Rock1 protein levels in the NAc P1 fraction relative to saline (Student's $t_{30} = 2.307$, $*P = 0.0282$, $n = 16$ saline brains and 16 cocaine brains). **(e)** Western blots and quantification showing that cocaine treatment does not

affect Rock1 protein levels in the NAc S2 fraction (Student's $t_{30} = 0.2209$, $P = 0.8266$, $n = 16$ saline brains and 16 cocaine brains). **(f)** Western blottings and quantification showing the cocaine treatment does not affect Rock1 protein levels in the NAc P2 fraction (Student's $t_{14} = 0.5617$, $P = 0.5832$, $n = 8$ saline brains and 8 cocaine brains). **(g)** Rock1 was immunoprecipitated from NAc P1 fractions and immunoprecipitates resolved via SDS-PAGE. Actin was recovered in Rock1 immunoprecipitates but not those from IgG control immunoprecipitates. **(h)** Experimental design. P1 fractions were isolated from NAc lysates and subsequently fractionated into G-actin and F-actin subfractions. **(i and j)** Western blottings and quantification showing Rock1 protein levels in NAc P1 fraction G-actin and F-actin subfractions. Rock1 was most highly enriched in the F-actin subfraction (Student's $t_6 = 2.619$, $*P = 0.0396$, $n = 4$ brains). **(k and l)** Proteins levels of Rock1 in the P1 F-actin and G-actin subfractions are shown following 24 h withdrawal from 7 days of IP saline or cocaine (20 mg kg^{-1}) treatment. Cocaine increased the ratio of Rock1 present in the P1 F-actin subfraction relative to the G-actin subfraction (Student's $t_{59} = 2.488$, $*P = 0.0157$, $n = 29$ saline brains and 32 cocaine brains). All summary data are depicted as the mean+s.e.m.

Figure 3.

Self-administered cocaine regulates MAL-SRF expression in the NAc. **(a)** Experimental design. Rats were implanted with intravenous catheters and some rats exclusively self-administered saline and others cocaine during daily 6 h sessions for 10 consecutive days. Twenty-four hours following the last session, NAc was dissected and fractionated into P1 and S2 fractions. **(b and c)**. Western blottings and quantification showing that cocaine increased levels of NAc P1 fraction MAL relative to saline rats (Student's $t_{17} = 2.127$, $*P = 0.0484$, $n = 8$ saline brains and 11 cocaine brains). P1 SRF showed a non-significant trend

toward increased levels in response to self-administered cocaine (Student's $t_{21} = 1.863$, $P = 0.0798$, $n = 8$ saline brains and 11 cocaine brains). (d and e) Western blottings and quantification of NAc S2 fraction MAL and SRF levels following 24 h withdrawal from chronic self-administered cocaine. Cocaine did not alter S2 fraction MAL or SRF levels relative to saline rats (MAL, Student's $t_{17} = 1.452$, $P = 0.1648$; SRF, $t_{17} = 1.566$, $P = 0.1357$, $n = 8$ saline brains and 11 cocaine brains). (f) Graphs show the ratio of MAL and SRF in the P1 fraction relative to the S2 fraction following 24 h withdrawal from self-administered cocaine. The ratio of SRF in the P1 fraction relative to the S2 fraction was increased in cocaine-treated rats as compared with those treated with saline (Mann-Whitney test, $U = 10.00$, $**P = 0.0057$, $n = 8$ saline brains and 11 cocaine brains). The ratio of MAL in the P1 fraction relative to the S2 fraction showed a trend toward an increase in cocaine-treated rats (Mann-Whitney test, $U = 21.00$, $P = 0.0632$, $n = 8$ saline brains and 11 cocaine brains). (g) Experimental design. Rats self-administered saline or cocaine for 14 days and a light cue was presented during each intravenous drug infusion. Rats were then subjected to 1 day of extinction in which lever presses were no longer rewarded with drug infusions and no light cues were administered. One day after extinction, rats were re-exposed to the same light cue that had previously indicated drug delivery; however, no saline or cocaine was administered. The NAc was isolated 24 h after cue re-exposure and P1 and S2 fractions derived. (h and i). Western blottings and quantification showing that exposure to a cocaine-associated cue is sufficient to induce expression of MAL, but not SRF, in NAc P1 fractions (MAL, Student's $t_{19} = 3.507$, $**P = 0.0024$, $n = 7$ saline brains and 14 cocaine brains; SRF, $t_{21} = 1.379$, $P = 0.1823$, $n = 8$ saline brains and 15 cocaine brains). (j and k). Western blottings and quantification showing that exposure to a cocaine-associated cue does not alter levels of MAL or SRF in NAc S2 fractions (MAL, Student's $t_{21} = 0.7803$, $P = 0.4439$; SRF, $t_{21} = 0.1518$, $P = 0.8808$, $n = 8$ saline brains and 15 cocaine brains). (l) Experimental design. Rats were implanted with intravenous catheters and rats allowed to exclusively self-administer saline or cocaine during daily 6 h sessions for 10 consecutive days. Thirty days after the last session, NAc tissue was dissected and fractionated into P1 and S2 fractions. (m and n). Western blottings and quantification showing protein levels of MAL and SRF in NAc P1 fractions following 30-day withdrawal from chronic self-administered cocaine. Cocaine decreased levels of NAc P1 fraction SRF relative to saline rats (Student's $t_{28} = 2.057$, $*P = 0.0491$, $n = 14$ saline brains and 16 cocaine brains). MAL showed a non-significant trend toward decreased levels in response to self-administered cocaine (Student's $t_{28} = 1.859$, $P = 0.0735$, $n = 14$ saline brains and 16 cocaine brains). (o and p) Western blottings and quantification of NAc S2 fraction MAL and SRF levels following 30-day withdrawal from chronic self-administered cocaine. Cocaine did not alter S2 fraction MAL or SRF levels relative to saline rats (MAL, Student's $t_{28} = 1.423$, $P = 0.1657$; SRF, $t_{28} = 1.491$, $P = 0.1472$, $n = 14$ saline brains and 16 cocaine brains). (q) Graphs show the ratio of MAL and SRF in the P1 fraction relative to the S2 fraction following 30-day withdrawal from self-administered cocaine. The ratio of SRF in the P1 fraction relative to the S2 fraction was decreased in cocaine-treated rats as compared with those treated with saline (Mann-Whitney test, $U = 60.00$, $*P = 0.0323$, $n = 14$ saline brains and 16 cocaine brains). The ratio of MAL in the P1 fraction relative to the S2 fraction did not differ between saline- and cocaine-treated rats (Mann-Whitney test, $U = 76.00$, $P = 0.1400$, $n = 14$ saline brains and 16 cocaine brains). All summary data are depicted as the mean+s.e.m.

Figure 4.

Input-specific regulation of NAc MAL-SRF levels. **(a)** Experimental design. Adeno-associated virus 5 (AAV5)-CaMKIIa-ChR2-EYFP was infused into the mPFC and optical ferrules targeting the medial NAc were implanted. Six weeks post viral infusion, mice underwent 7 days of 20 min burst stimulation sessions using 473 nm (blue) laser light (60 bursts delivered in 300 ms; burst sessions delivered every 20 s) in which AAV5-expressing axon terminals in the medial NAc were stimulated. Mock stimulation in which the same surgeries and viral manipulations were administered to mice in absence of blue laser light stimulation was used as a control. Twenty-four hours after the final stimulation session, NAc tissue was dissected and P1 fractions obtained. **(b)** Images showing the targeting of mPFC neurons with AAV5-CaMKIIa-ChR2-EYFP (left image) and the corresponding axon terminals from this region that project to the NAc (right). Midline and anterior commissure (aca) landmarks are shown. **(c and d)** Western blottings and quantification showing that stimulation of mPFC axon terminals that project to the NAc increases SRF levels in NAc P1 fractions (Student's $t_{29} = 2.200$, $*P = 0.0359$, $n = 17$ stim brains and 14 mock-stim brains), with no significant effect on MAL levels (Student's $t_{29} = 1.077$, $P = 0.2906$, $n = 17$ stim brains and 14 mock-stim brains). **(e)** Experimental design. AAV5-CaMKIIa-ChR2-EYFP was infused into the BLA and medial NAc-targeting optical ferrules implanted. Medial

NAc-projecting axon terminals were stimulated during 20 min sessions for 7 consecutive days using the identical parameters and controls as detailed in 4a. Twenty-four hours after the final stimulation session, NAc tissue was dissected and P1 fractions obtained. **(f)** Images showing the targeting of BLA neurons with AAV5-CaMKIIa-ChR2-EYFP (left image) and the corresponding axon terminals from this region that project to the NAc (right). Piriform cortex (Pir), internal capsule (ic) and anterior commissure (aca) landmarks are shown. **(g and h)** Western blottings and quantification showing that stimulation of BLA axon terminals that project to the NAc increases MAL levels in NAc P1 fractions (Student's $t_{29} = 2.179$, $*P = 0.0376$, $n = 14$ stim brains and 17 mock-stim brains), with no significant effect on SRF levels (Student's $t_{30} = 0.5171$, $P = 0.3089$, $n = 14$ stim brains and 18 mock-stim brains). **(i)** Experimental design. AAV5-CaMKIIa-ChR2-EYFP was infused into the mPFC and axons that project from this region to the medial NAc were stimulated (or mock-stimulated) during 20 min sessions per day for 7-days and NAc tissue dissected 24 h following the final stimulation session. The stimulation parameters were identical to those described in 4a. G-actin and F-actin subfractions were derived from NAc P1 fractions. **(j and k)** Western blottings and quantification showing levels of SRF in NAc P1 fraction G-actin and F-actin subfractions following stimulation or mock-stimulation of axon terminals in the NAc that originate from mPFC neurons. Stimulation of mPFC inputs to the NAc increased the ratio of SRF in the P1 F-actin subfraction relative to the P1 G-actin subfraction (Student's $t_{21} = 2.423$, $*P = 0.0245$, $n = 12$ stim brains and 11 mock-stim brains). **(l and m)** Western blottings and quantification showing levels of MAL in NAc P1 fraction G-actin and f-actin subfractions following stimulation or mock-stimulation of axon terminals in the NAc that originate from mPFC neurons. Stimulation of mPFC inputs to the NAc showed a non-significant trend toward altering MAL in the P1 F-actin subfraction relative to the P1 G-actin subfraction (Student's $t_{21} = 1.678$, $P = 0.1074$, $n = 12$ stim brains and 12 mock-stim brains). All summary data are depicted as the mean+s.e.m.

Figure 5.

SRF regulates cocaine's spine morphogenic effects in the NAc. **(a)** Schematic shows the location of 2 putative SREs in the *Arc* promoter relative to the transcription start site (arrow). Chromatin immunoprecipitation (ChIP) for SRF (or IgG as a control) of NAc following 24-h withdrawal from 7 days of IP saline or cocaine (20 mg kg⁻¹) treatment was used to assess differential binding of SRF to putative SREs on the *Arc* promoter. Due to their close proximity to each other, binding at each individual SRE was not possible; collective binding across both SREs was thus assessed. **(b)** SRF binding to the putative SREs exceeded that of IgG in both saline- and cocaine-treated mice. SRF binding at these sites was greater in cocaine-treated relative to saline-treated mice (2 × 2 analysis of variance (ANOVA) main effects of SRF pulldown $F_{1,24} = 71.39$, *** $P < 0.0001$ and drug treatment $F_{1,24} = 4.39$, * $P = 0.0335$; Bonferroni post hoc saline IgG versus saline SRF, *** $P < 0.001$, cocaine IgG versus cocaine SRF, *** $P < 0.001$; Bonferroni post hoc saline SRF versus cocaine SRF, * $P < 0.05$; $n = 7$ saline brains and 7 cocaine brains). **(c and d)** Mice were IP injected with saline or cocaine (20 mg kg⁻¹) for 7 days followed by a 24 h withdrawal. NAc lysates were fractionated into P1, S2 and P2 fractions. Cocaine significantly increased Arc levels in the P2 fraction with no effect in the P1 or S2 fractions (2 × 2 ANOVA main effect of drug treatment $F_{1,54} = 5.336$, * $P = 0.0247$; Bonferroni post hoc P2 fraction saline versus cocaine,

****** $P < 0.01$; P1 and S2 fraction saline versus cocaine, $P > 0.05$; $n = 10$ saline brains and 10 cocaine brains). **(e)** Experimental design. The NAc of floxed *SRF* mice was infused with HSV-Cre-GFP to knock down SRF or HSV-GFP as a control. To allow for multiple cocaine doses over the transient lifespan of HSVs, starting 2 days post viral infusion mice received 5 saline or cocaine (20 mg kg^{-1}) IP injections over a 3-day period and brains perfused 4 h after the final injection. **(f)** Image shows the targeting of HSV-Cre-GFP to the NAc of a floxed *SRF* mouse. Anterior commissure (aca) landmark is shown. **(g)** Image shows a NAc MSN infected with HSV-GFP. **(h)** Image shows a dendritic segment from the neuron in 5 g. Different spine subclasses are noted. **(i)** Representative dendrite segments from floxed *SRF* mouse NAc MSNs expressing GFP or Cre-GFP from saline- or cocaine-treated mice. **(j)** Total spine density in HSV-GFP-expressing and HSV-Cre-GFP-expressing NAc MSNs from floxed *SRF* mice following saline or cocaine treatment. Cocaine increased total spine density in GFP-expressing, but not Cre-expressing neurons (2×2 ANOVA main effect of drug treatment $F_{1,45} = 5.179$, $*P = 0.0277$ and SRF expression $F_{1,45} = 6.353$, $*P = 0.0153$; Bonferroni *post hoc* GFP saline versus GFP cocaine, $**P < 0.01$; Cre saline versus Cre cocaine, $P > 0.05$; $n = 8$ cells from 3 mice (GFP saline), 12 cells from 3 mice (GFP cocaine), 17 cells from 4 mice (Cre saline) and 12 cells from 4 mice (Cre cocaine)). **(k)** Thin spine density in HSV-GFP-expressing and HSV-Cre-GFP-expressing NAc MSNs from floxed *SRF* mice following saline or cocaine treatment. Cocaine increased thin density in GFP-expressing, but not Cre-expressing neurons (2×2 ANOVA main effect of SRF expression $F_{1,45} = 8.901$, $**P = 0.0046$; Bonferroni *post hoc* GFP saline versus GFP cocaine, $**P < 0.01$; Cre saline versus Cre cocaine, $P > 0.05$; n same as in **j**). **(l)** Cocaine did not affect stubby spine density in GFP- or Cre-expressing NAc MSNs from floxed *SRF* mice (2×2 ANOVA main effect of SRF expression $F_{1,45} = 10.33$, $**P = 0.0024$; Bonferroni *post hoc* GFP saline versus GFP cocaine, $P > 0.05$; Cre saline versus Cre cocaine, $P > 0.05$; n same as in **j**). **(m)** Cocaine did not affect mushroom spine density in GFP- or Cre-expressing NAc MSNs from floxed *SRF* mice (2×2 ANOVA no main effect of SRF expression $F_{1,45} = 0.07613$, $P = 0.7839$; Bonferroni *post hoc* GFP saline versus GFP cocaine, $P > 0.05$; Cre saline versus Cre cocaine, $P > 0.05$; n same as in **j**). All summary data are depicted as the mean+s.e.m.