



## Stress-induced vulnerability to develop cocaine addiction depends on cofilin modulation

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### ABSTRACT

Actin dynamics in dendritic spines can be associated with the neurobiological mechanisms supporting the comorbidity between stress exposure and cocaine increase rewards. The actin cytoskeleton remodeling in the nucleus accumbens (NA) has been implicated in the expression of stress-induced cross-sensitization with cocaine. The present study evaluates the involvement of cofilin, a direct regulator of actin dynamics, in the impact of stress on vulnerability to cocaine addiction. We assess whether the neurobiological mechanisms that modulate repeated-cocaine administration also occur in a chronic restraint stress-induced cocaine self-administration model. We also determine if chronic stress induces alterations in dendritic spines through dysregulation of cofilin activity in the NA core. Here, we show that the inhibition of cofilin expression in the NA core using viral short-hairpin RNA is sufficient to prevent the cocaine sensitization induced by chronic stress. The reduced cofilin levels also impede *a*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor surface expression enhancement and promote the reduction of head diameter in animals pre-exposed to stress after a cocaine challenge in the NA core. Moreover, downregulation of cofilin expression prevents facilitation of the acquisition of cocaine self-administration (SA) in male rats pre-exposed to chronic stress without modifying performance in sucrose SA. These findings reveal a novel, crucial role for cofilin in the neurobiological mechanisms underpinning the comorbidity between stress exposure and addiction-related disorders.

### 1. Introduction

There is converging epidemiological evidence of high comorbidity between stressful life events and posttraumatic stress disorder (PTSD) with substance use disorders (SUDs) (Boden et al., 2012; Clark et al.,

2001; Ouimette et al., 1998; Sinha, 2001). Stressful experiences play a critical role throughout the cycle of addiction, from the development of addictive disorders to maintenance, relapse, and recovery from SUDs, suggesting that the brain circuitry regulating reward and reinforcement is a critical hub for the effects of stress on behavior ((Boyson et al., 2014;

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Garcia-Keller et al., 2016; Miczek et al., 2008; Piazza and Moal, 1998). In rodent models, it has been shown that exposure to stress is able to promote the capacity of psychostimulants to release dopamine in the nucleus accumbens (NA) (Kalivas and Stewart, 1991; Pierce and Kalivas, 1997) and thus to enhance the locomotor response to a psychostimulant, a phenomenon known as cross-sensitization (Marinelli and Piazza, 2002; Pacchioni et al., 2007; Sorg and Kalivas, 1991). Our previous reports in animal models of stress and substance use showed enduring neurobiological adaptations at glutamatergic synapses in the NA core, which underlie the development of behavioral sensitization (Esparza et al., 2012; Garcia-Keller et al., 2013) and facilitate the acquisition of cocaine, alcohol and heroin self-administration (SA) (Carter et al., 2020; Garcia-Keller et al., 2016, 2019b).

Behavioral alterations associated with sensitization are mediated by enduring structural modifications in NA neurons. Indeed, long-term exposure to cocaine increases dendritic complexity and spine density and induces changes in the spine morphology of NA medium spiny neurons (MSNs) (Russo et al., 2010) that are responsible, in part, for the enhanced cocaine reward and also promote behavioral responses (Dietz et al., 2012; Shen et al., 2009; Toda et al., 2006; Verma et al., 2019). Modifications in dendritic spines depend on actin cytoskeleton remodeling (Dietz et al., 2012; Matus, 2005; Russo et al., 2010; Shen et al., 2009; Toda et al., 2006; Wang et al., 2005). Thus, actin cycling is crucial for the correct physiology of the spine (Rust, 2015) and is controlled by actin-binding proteins that maintain equilibrium between monomeric globular G-actin and filamentous F-actin (Dos Remedios et al., 2003).

Cofilin is a low molecular weight actin-modulating protein, a member of the actin depolymerizing protein family, an essential regulator of spine morphology and functional aspects of synaptic plasticity (Kruyer et al., 2019; Rust, 2015). The actin-binding of cofilin is controlled via phosphorylation (inactivation, p-cofilin) and dephosphorylation (activation) of a conserved serine residue at position 3 (Ser3). This phosphorylation cycle of cofilin is a critical event that leads to reorganization of the actin cytoskeleton, and is also involved in the post-synaptic transport and membrane transport of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) in synaptic plasticity (Bamburg, 1999; Kruyer et al., 2019; Lu et al., 2019; Mizuno, 2013; Rust, 2015).

Previous studies proposed that chronic cocaine administration regulates actin turnover in the NA associated with changes in F-actin levels and the phosphorylated state of cofilin (Dietz et al., 2012; Toda et al., 2006). Like cocaine administration, repeated exposure to stressors induces enduring adaptations in dendritic branching, shape and the number of spines in NA MSNs (Qiao et al., 2016; Ruisoto and Contador, 2019). However, the molecular mechanisms mediating the long-lasting changes in behavioral responses to cocaine induced by stress are not yet completely understood. Previously, we reported that chronic stress is associated with long-term changes in p-cofilin protein levels, regulating the actin cytoskeleton in the NA core (Esparza et al., 2012).

Here, we explore molecular and cellular events that are implicated in the reorganization of the actin cytoskeleton in dendritic spines and AMPA receptor expression during sensitization to cocaine induced by chronic stress. Moreover, we characterize the critical role of cofilin in the stress-induced facilitation of acquisition of cocaine self-administration behavior and we establish a crucial role of cofilin in the vulnerability to develop cocaine addiction. This work contributes to the hypothesis that modifications in the tight regulation of actin dynamics in dendritic spines may be associated with the neurobiological mechanisms underpinning the comorbidity between stress exposure and drug reward.

## 2. Materials and methods

### 2.1. DNA construct and viral vectors

The cofilin short-hairpin RNA (shRNA) plasmid was constructed in

psilencer 1.0 U6 vector. 5'gggcaaggagattctgtagg and 3'ctaccagaattctctgtccc were used as targeting sequences following experimental procedures described by (Chuang et al., 2013). The DNA fragments were generated following procedures described by (Bisbal et al., 2008) and were used for developing pseudotyped lentiviral (LV) vectors named shCtrl (empty) and shcofilin (shRNA cofilin).

### 2.2. Animal housing

Young adult male Sprague-Dawley rats (two months old, 250–300g; CIPReB Facultad de Ciencias Médicas, Universidad Nacional de Rosario) were housed in cages of 12x30x50 cm in the Facultad de Ciencias Químicas vivarium under a 12:12h dark/light cycle, with free access to food and water. All experiments were performed in the light cycle, except in the case of SA experiments. All animal experiments were approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas (CICUAL), Universidad Nacional de Córdoba (RES HCD 1220/18), which is consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.3. Stress protocol

Animals were randomly assigned to NS (non-stress) and S (chronic stress) groups. The animals assigned to the chronic stress group were restrained daily for 2 h in a Plexiglass restraining device for seven consecutive sessions between 10 a.m. and 2 p.m. The Plexiglass cylinders were designed to allow the rats' tails to emerge from the rear. The animals appeared healthy, as shown by their coat texture and minimal, reversible changes in body weight (Cancela et al., 1996; Esparza et al., 2012). Control animals remained undisturbed in their home cages for the full period, except for weekly weighing and regular husbandry. Animals were randomly assigned to the behavioral, biochemical or neurochemical studies.

### 2.4. Intracranial viral infusion

The day after the last stress session (day 8), all animals underwent stereotaxic surgery to perform bilateral microinjections of shCtrl or shcofilin in the NA core according to coordinates: +1.6 mm anterior;  $\pm$ 1.5 mm lateral (at an angle of 6° from vertical) and –6.6 mm ventral from bregma (Paxinos G & Watson C., 2007). The microinjections at the NA core site were verified for all experimental procedures.

### 2.5. Cocaine-induced locomotion

Three weeks after the intracranial injection of LV particles, locomotor activity was monitored in automated activity boxes equipped with photocells and quantified as total photocell counts (interruption of a beam that resulted in a photocell count). The animals were allowed to habituate in the activity chambers for 30 min, then 1 h was registered in response to saline (Sal) and 2 h in response to cocaine (Coc, 15 mg/kg i. p.). The behavior was recorded for 120 min over 10 min interval-bins (Esparza et al., 2012).

### 2.6. Operant conditioning chambers

Experiments were conducted in operant response test chambers (Med Associates, USA), each placed in a ventilated, sound-attenuating cubicle. Each operant chamber was equipped with two levers: one active and one inactive. Depression of the active lever activated the infusion pump or a food container trough onto which liquid sucrose was delivered. Responses on an inactive lever were counted but had no consequences. A cue light and a speaker were above the active lever. The house light was turned on at the start of each test session. Scheduling of experimental events and data collection was accomplished using Med Associates software.

## 2.7. Cocaine self-administration

Cocaine SA was performed as described in detail previously (Garcia-Keller et al., 2016). Briefly, eight days after implantation of permanent catheters in the right jugular vein, rats began daily 2 h SA training on a fixed ratio (FR1), in which one press on the active lever was reinforced with an infusion of cocaine (0.3 mg/kg/infusion, followed by 10 s timeout period), paired with a white cue light above the active lever and a discrete tone cue. Rats were trained for 10 days and they reached the SA criterion when they obtained >15 infusions of cocaine on at least two consecutive days. The exclusion criteria used during the SA experiment was if the animal pressed the inactive lever >30 times (<10% exclusion).

## 2.8. Oral sucrose self-administration

On day 16, eight days after intracranial microinjections of LV in the NA core, rats began daily 1 h sucrose oral SA on a FR1, in which pressing the active lever was reinforced with a sucrose delivery (0.1 ml of 10% sucrose solution available for 10 s in a well on the side of the chamber, followed by 10 s timeout period), paired with a white cue light above the active lever and a discrete tone cue. Rats were trained for 10 days and they reached the SA criterion when they obtained >15 intakes on at least two consecutive days. The exclusion criteria used was if the animals during the SA experiment pressed the inactive lever >30 times (<10% exclusion) (You et al., 2018; Zhang et al., 2015).

## 2.9. Experimental design

All experimental designs are detailed in the supplementary material section.

## 2.10. Surface biotinylation and western blotting

Surface biotinylation and western blotting experiments were performed as described in detail previously (Esparza et al., 2012). Briefly, animals were decapitated, and the NA core was dissected. The bilateral slices were pooled. Bilateral NA tissue was homogenized, and protein concentrations were determined using a protein assay (Bio-Rad, Hercules, CA, USA). Then the soluble fraction was mixed with SDS sample buffer protein and loaded onto precast Tris-HCl polyacrylamide gel. The samples were transferred to PVDF membrane and blocked in blocking buffer. Blocked membranes were incubated overnight at 4 °C with primary antibodies and then for 1 h with secondary antibodies. The bands were visualized using chemiluminescent peroxidase substrate. Bands were quantified with ImageJ Software and normalized to  $\alpha$ -tubulin to control for equal loading. Antibody data are detailed in the supplementary material.

## 2.11. Tissue preparation and DiI labeling

Morphological analysis and spine type classification were performed as previously published (Calfa et al., 2012; Giachero et al., 2015). Briefly, animals received a challenge injection of saline or cocaine and, 24 h later, animals were deeply anesthetized before being perfused transcardially. Sections of the brain were made with a vibratome. Dendritic portions of NA core cells were stained with saturated solution of lipophilic dye (DiI, Invitrogen Carlsbad, CA, USA) in fish oil (Pozzo-Miller et al., 1999) by microinjection with a patch pipette and positive pressure application. Z-sections from labeled dendritic segments were collected using a confocal microscope (Carl Zeiss LSM 800 inverted microscope).

## 2.12. Statistical analyses

Statistical analyses were performed using GraphPad Prism 6

(GraphPad software, La Jolla, CA). Behavioral, biochemical and neurochemical data were analyzed by one-way or two-way analysis of variance (ANOVA). Bonferroni's post-hoc test was applied for multiple comparisons and  $p < 0.05$  was considered statistically significant. Data were expressed as mean  $\pm$  SEM. Results of dendritic spines were analyzed by Rstudio software (version 3.5.3) and the statistical package nlme (Pinheiro et al., 2020) version 3.1–148. To analyze the differences between spine density means, a three-factor linear model was fitted. Differences in distribution were evaluated using the Kolmogorov-Smirnov (K-S) test. The p-values were adjusted using the Bonferroni correction method when applicable.

## 3. Results

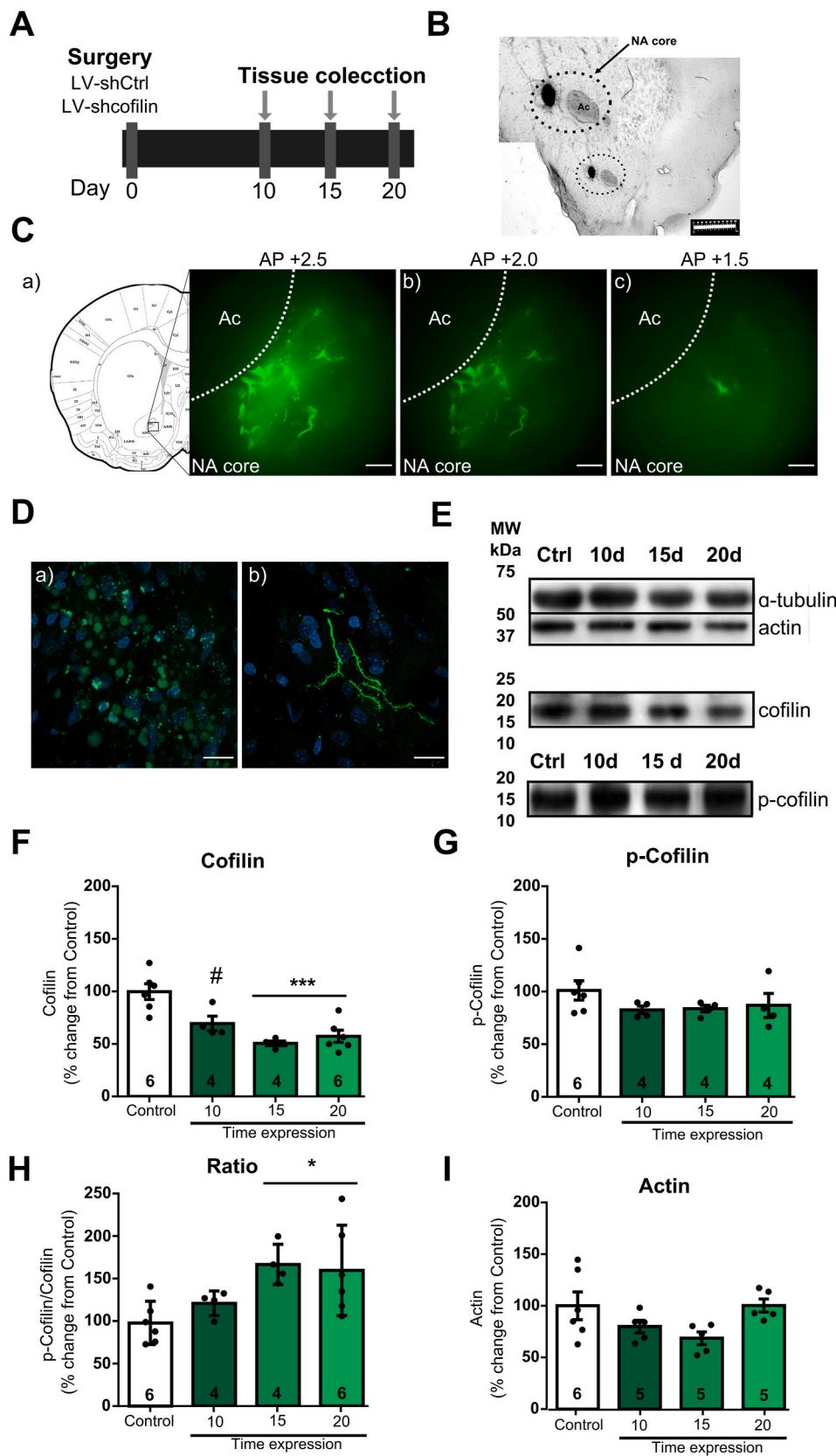
### 3.1. Time course analysis of protein expression after lentiviral injections in the NA core

To characterize the involvement of cofilin in the vulnerability to develop cocaine addiction induced by stress, we inhibited cofilin expression with a LV short-hairpin RNA, named shcofilin, in the NA core and verified the downregulation of cofilin expression at 10, 15 and 20 days after LV microinjection, since all our experiments were performed three weeks after the last stressful event. The experimental timeline showing animals treated with shCtrl (empty virus), denominated as control, represents the basal levels of protein expression (Fig. 1A). Fig. 1B shows a representative image of the LV particles infusion site stained with cresyl violet and a magnification of the dotted line representing the NA core area (arrow). Fig. 1C (a-c) shows coronal sections of the NA core infected with shcofilin-GFP across the different anterior-posterior axis. Fig. 1D (a-b) shows confocal images of infected cells expressing shcofilin-GFP (Green) and nuclear staining (DAPI in Blue) at NA core. Fig. 1E shows representative western blots of drug-naïve NA core tissue samples. Fig. 1F–H shows the levels of expression of total cofilin (cofilin), p-cofilin, p-cofilin/cofilin ratio and actin proteins after the microinjection of shcofilin in the NA core.

Our results revealed a decrease in cofilin expression levels in the NA core 10, 15 and 20 days after the microinjection of shcofilin LV particles (one-way ANOVA followed by Bonferroni's post-hoc. Treatment  $F_{(3-16)} = 12.39$   $p = 0.0002$ ) (Fig. 1F). Additionally, we examined p-cofilin expression levels and we observed no changes in its expression in the NA core after infection with shcofilin (one-way ANOVA followed by Bonferroni's post-hoc. Treatment  $F_{(3-14)} = 1.218$   $p = 0.3397$ ) (Fig. 1G), but we report an increase in p-cofilin/cofilin ratio at 15 and 20 days after shcofilin microinjection (one-way ANOVA followed by Bonferroni's post-hoc. Treatment  $F_{(3-16)} = 4.534$   $p = 0.0175$ ) (Fig. 1H). Total actin levels remained unchanged during the time measured (one-way ANOVA followed by Bonferroni's post-hoc. Treatment  $F_{(3-17)} = 2.813$   $p = 0.0705$ ) (Fig. 1I). These data are consistent with the ability of shcofilin *in vivo* to induce long-lasting changes in cofilin protein expression. Moreover, rats microinjected with shCtrl revealed no changes in protein levels compared with naïve rats at any timepoint (Fig. S1).

### 3.2. Cofilin downregulation inhibits stress-induced sensitization to cocaine

It is well known that acute stress, chronic stress or cocaine exposure increase the locomotor response to an acute cocaine injection (Esparza et al., 2012; Garcia-Keller et al., 2013, 2016; Pacchioni et al., 2007), all leading to structural and functional synaptic alterations in NA MSNs (Anderson and Self, 2017; Garcia-Keller et al., 2016, 2019a, 2020; Khibnik et al., 2016; Qiao et al., 2016; Verma et al., 2019). However, several questions remain unanswered regarding how stress induces changes in the neuronal architecture and plasticity during cross-sensitization between stress and cocaine. Cofilin has been proposed as a target involved in plastic changes related to cocaine and stress (Esparza et al., 2012; Kruyer et al., 2019; Toda et al., 2006). Thus, we proposed to determine whether cofilin is necessary for stress-induced



**Fig. 1.** Time course analysis of protein expression after lentiviral injections in NA core. Data are expressed as mean  $\pm$  SEM of the number of determinations depicted in each graphic. The results were analyzed by one-way ANOVA followed by Bonferroni's post-hoc. (A) Experimental design. (B) Representative image of the lentiviral particles infusion site stained with cresyl violet. The dotted line represents the NA core area (arrow). (C) (a,b,c) Representative images of a coronal sections of the NA core with shcofilin-infected cells expressing GFP across the different anterior-posterior axis. Scale bar = 50  $\mu$ m. (D) (a,b) Confocal images showing shcofilin infected cells expressing GFP (Green) and DAPI (Blue) at NA core. Scale bar = 5  $\mu$ m. (E) Representative western blots of each protein.  $\alpha$ -tubulin was used as loading control for Western blot experiments. The standard molecular weight band coincides with the expected molecular weight of each protein studied. A single band was observed for  $\alpha$ -tubulin (top), cofilin (middle) and phosphorylated cofilin, p-cofilin (bottom). (F) Cofilin expression levels after intra-NA core injection of shcofilin, n = 4–6 in each group. (G) p-Cofilin expression levels after intra-NA core injection of shcofilin, n = 4–6 in each group. (H) p-Cofilin/Cofilin ratio after intra-NA core injection of shcofilin, n = 4–6 in each group. (I) Actin expression levels after intra-NA core injection of shcofilin, n = 4–6 in each group. \* $p < 0.05$ , compared with control. # $p < 0.05$ , compared with control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vulnerability to developing cocaine addiction and its association with the structural changes occurring in MSNs of NA. To achieve this, we followed the timeline described in Fig. 2A. Microinjection sites in the NA core are depicted in Fig. 2B.

Our results revealed that the expression of cross-sensitization between stress and cocaine was prevented by an intra-accumbens microinjection of shcofilin three weeks before the cocaine challenge. Shcofilin did not alter the locomotor response elicited by acute saline in either prior chronic stress or non-stress animals, nor did it alter the motor response elicited by acute cocaine in non-stress animals (two-way ANOVA with repeated measures (RM) over time (non-stress/stress x shCtrl/shcofilin): time,  $F_{(17-493)} = 22.75, p < 0.0001$ ; non-stress/stress,  $F_{(3-29)} = 7.326, p = 0.0008$ ; interaction,  $F_{(51-493)} = 1.719, p = 0.0022$ ) (Fig. 2C). Bonferroni's post-hoc comparisons indicated that the stress shCtrl group shows significantly higher photocell counts at 70, 80 and 90 min than the stress shcofilin and all the remaining groups (Fig. 2C). These comparisons also showed that counts at between 70 and 150 min in the stress shCtrl group were significantly higher than those of the non-stress shCtrl group (Fig. 2C). The total photocell counts over 120 min after cocaine injection also showed a higher response of the stress shCtrl group compared with all the remaining groups (two-way ANOVA (non-stress/stress x shCtrl/shcofilin): treatment  $F_{(3-58)} = 74.21, p < 0.0001$ ; non-stress/stress  $F_{(1-58)} = 6.621, p = 0.0127$ ; interaction  $F_{(3-58)} = 4.352, p = 0.0078$ ). Bonferroni's post-hoc comparisons indicated a significant difference ( $p < 0.05$ ) in the total cumulative counts between the stress shCtrl group and all the remaining groups after cocaine injection (Fig. 2D).

### 3.3. Cofilin downregulation modulates structural plasticity in response to cocaine in stress animals

In order to assess the influence of cofilin in spine morphology

associated with stress-induced sensitization to cocaine we analyzed cumulative plots of head diameter ( $d_h$ ) of total spines (Fig. 3) and separately mushroom, thin and stubby spines (Fig. S2) following a single cocaine administration after 1 day of withdrawal. Thus, MSNs in the NA core brain slices were injected with DiI to fill the cells and quantify dendritic spine morphology. Fig. 3A–B shows dendritic segments from each saline (A) or cocaine (B) treatment groups of MSNs in the NA core.

We found that chronic stress in animals microinjected with shCtrl (S/shCtrl-Sal, dotted pink line vs NS/shCtrl-Sal, dotted blue line) induced a leftward shift in the cumulative curve in total (Fig. 3C,  $p < 0.0004$ ), mushroom (Fig. S2A,  $p < 0.0004$ ) and thin  $d_h$  (Fig. S2B,  $p = 0.02$ ), with no significant changes in stubby  $d_h$  (Fig. S2C,  $p = 0.1136$ ). While, after cocaine, stress exposure (S/shCtrl-Coc, pink line vs NS/shCtrl-Coc, blue line) produced a rightward shift in the cumulative curve in total (Fig. 3D,  $p < 0.0252$ ) and mushroom  $d_h$  (Fig. S2D,  $p < 0.0252$ ), with no changes in thin  $d_h$  (Fig. S2E,  $p = 1.5496$ ) or stubby  $d_h$  (Fig. S2F,  $p = 1.0972$ ). Collectively, these spine data indicated that changes in structural plasticity associated with stress-induced sensitization to cocaine were related to an enhancement of total  $d_h$  correlated with augmented mushroom  $d_h$  spines in NA MSNs after cocaine. Then, we examined the effect of cofilin downregulation on non-stress rats, after saline or cocaine injection. We found that shcofilin (NS/shcofilin-Sal, dotted gray line vs NS/shCtrl-Sal, dotted blue line) induced a leftward shift in the cumulative curve of total spine  $d_h$  (Fig. 3C,  $p < 0.0004$ ), as well as in all three type of spines (Fig. S2A–C,  $p < 0.0004$ ). Nevertheless, non-stress rats microinjected with shcofilin and exposed to cocaine showed a rightward shift in the cumulative curve of total compared with the control shCtrl group (Fig. 3D,  $p < 0.0004$ , NS/shCtrl-Coc, blue line vs NS/shcofilin-Coc, gray line) and all three types of spines  $d_h$  (Fig. S2D–F,  $p < 0.0004$ ). This rightward shift was better understood when we analyzed the effect of cocaine in non-stress groups microinjected with shCtrl or shcofilin (Fig. 3E and Fig. S2G–I). Cocaine exposure in non-stress groups

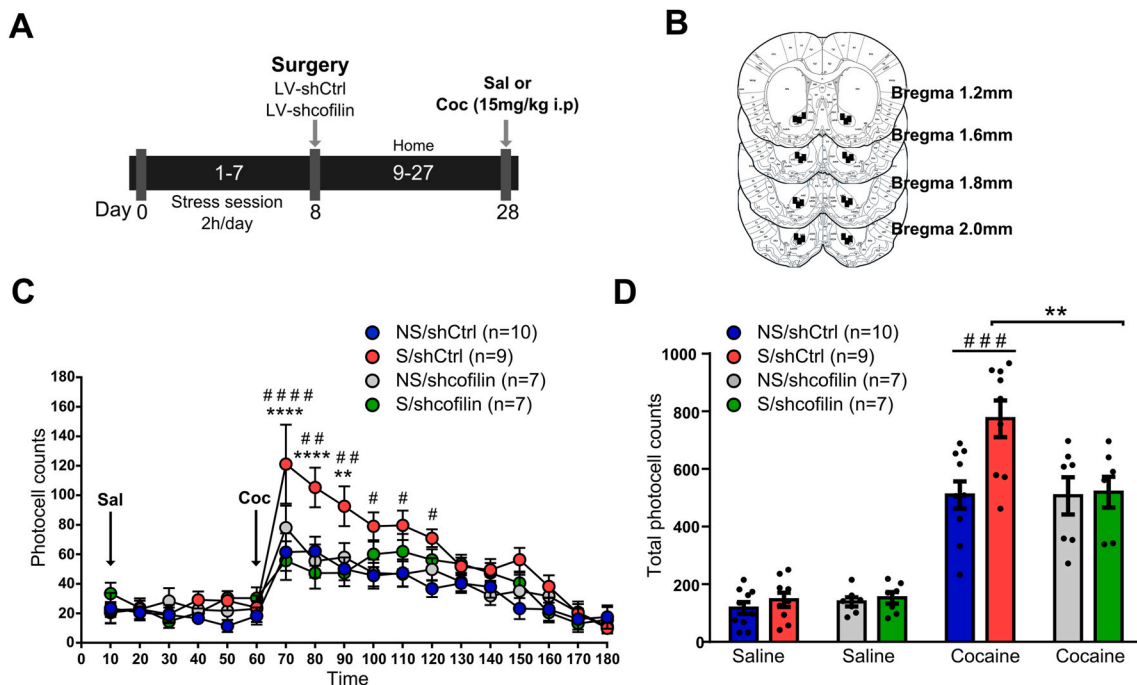
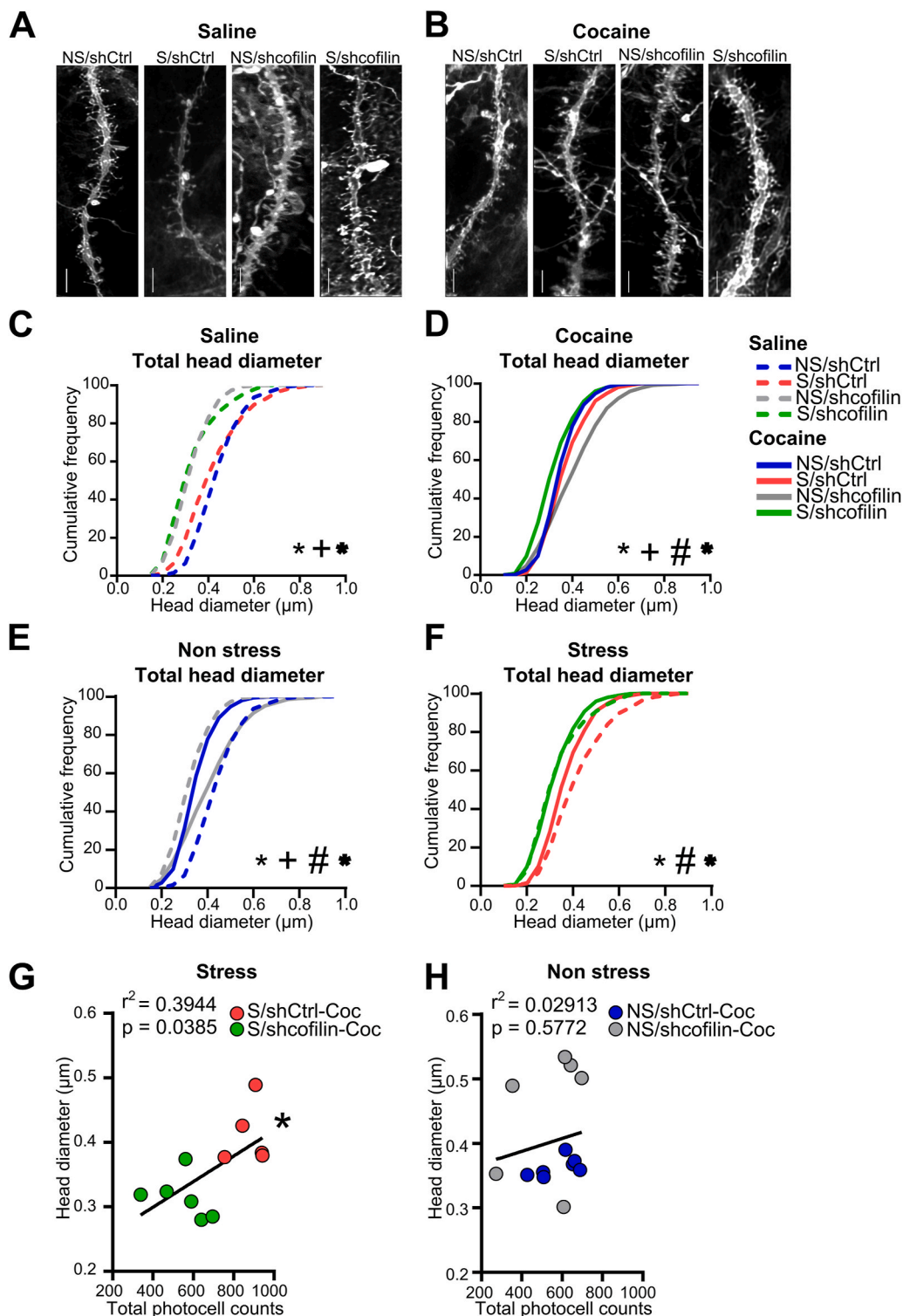


Fig. 2. Cofilin downregulation inhibits stress-induced sensitization to cocaine.

(A) Experimental design. (B) Location of lentiviral microinjection in the NA core of rats included in the data analyses. Schematic line drawings, adapted from Paxinos and Watson (2007). Numbers to the right indicate millimeters from bregma. (C) Time course of horizontal photocell counts after saline and cocaine (15 mg/kg i.p.) injections. Values represent the mean  $\pm$  SEM of horizontal photocell counts over 10 min periods. Timeframe from 10 to 60 min corresponds to saline administration at 0 min, and timeframe from 70 to 180 min to cocaine administration. The results were analyzed by two-way ANOVA with repeated measures (RM) over time and Bonferroni's post-hoc. \* $p < 0.05$ , compared with NS/shcofilin and S/shcofilin, # $p < 0.05$ , compared with NS/shCtrl (n = 7–10 in each group). (D) Total horizontal photocell counts 120 min as mean  $\pm$  SEM, after the saline or cocaine injection analyzed by two-way ANOVA and Bonferroni's post-hoc. \* $p < 0.05$ , compared with NS/shcofilin and S/shcofilin. # $p < 0.05$ , compared with NS/shCtrl and remaining groups after saline injection, n = 7–10 in each group.



**Fig. 3.** Cofilin downregulation modulates structural plasticity in response to cocaine in stress animals.

(A) Representative dendritic segments from each saline treatment group of MSNs in the NA core. Scale bar = 5  $\mu\text{m}$ . (B) Representative dendritic segments from each cocaine treatment group of MSNs in the NA core. Scale bar = 5  $\mu\text{m}$ . (C) Cumulative frequency plot of total spine head diameter ( $d_h$ ) 24 h after saline exposure analyzed by Kolmogorov-Smirnov (K-S) test. Total data analyzed 800–1000  $\mu\text{m}$  dendritic length per group,  $n = 5-7$  rats in each group. \*NS/shCtrl vs S/shCtrl; <sup>+</sup>NS/shCtrl vs NS/shcofilin; \*S/shCtrl vs S/shcofilin. (D) Cumulative frequency plot of total spine  $d_h$  24 h after cocaine exposure analyzed by Kolmogorov-Smirnov (K-S) test. Total data analyzed 800–1000  $\mu\text{m}$  dendritic length per group,  $n = 5-7$  rats in each group. \*NS/shCtrl vs S/shCtrl; <sup>+</sup>NS/shCtrl vs NS/shcofilin; <sup>#</sup>NS/shcofilin vs S/shcofilin; \*S/shCtrl vs S/shcofilin. (E) Cumulative frequency plot of total spine  $d_h$  of non-stressed animals 24 h after saline or cocaine exposure analyzed by Kolmogorov-Smirnov (K-S) test. Total data analyzed 800–1000  $\mu\text{m}$  dendritic length per group,  $n = 5-7$  rats in each group. \*NS/shCtrl-Sal vs NS/shCtrl-Coc; <sup>+</sup>NS/shcofilin-Sal vs NS/shcofilin-Coc; <sup>#</sup>NS/shCtrl-Sal vs NS/shcofilin-Sal and \*NS/shCtrl-Coc vs NS/shcofilin-Coc (F) Cumulative frequency plot of total spine  $d_h$  of stressed animals 24 h after saline or cocaine exposure analyzed by Kolmogorov-Smirnov (K-S) test. Total data analyzed 800–1000  $\mu\text{m}$  dendritic length per group,  $n = 5-7$  rats in each group. \*S/shCtrl-Sal vs S/shCtrl-Coc; <sup>+</sup>S/shcofilin-Sal vs S/shcofilin-Coc; <sup>#</sup>S/shCtrl-Sal vs S/shcofilin-Sal and \*S/shCtrl-Coc vs S/shcofilin-Coc. (G) Correlation between total spine  $d_h$  and total photocell counts in S/Coc groups,  $n = 5-6$  in each group. (H) Correlation between total spine  $d_h$  and total photocell counts in NS/Coc groups,  $n = 6-7$  in each group.

microinjected with shCtrl led to a leftward shift in the cumulative distribution in total spine  $d_h$  (Fig. 3E,  $p < 0.0004$ , NS/shCtrl-Sal (dotted blue line) vs NS/shCtrl-Coc (blue line), and in all three types of spines  $d_h$  (Fig. S2G-I,  $p < 0.0004$ ). However, in non-stress animals microinjected intra-accumbens with shcofilin (NS/shcofilin-Coc, gray line) cocaine injection induced a rightward shift in the cumulative distribution compared with the NS/shcofilin-Sal group (dotted gray line) in total spine  $d_h$  and in all three types of spines  $d_h$  (Fig. 3E and Fig. S2G-I,  $p < 0.0004$ ), almost reaching the basal levels observed in the control group

(NS/shCtrl-Sal), thus explaining why the behavioral response remained unaffected (Fig. 2C–D).

Interestingly, when we examined the consequences of cofilin downregulation on stress-mediated spine morphology, we found that the shcofilin in stress rats (S/shCtrl-Sal, dotted pink line vs S/shcofilin-Sal, dotted green line) produced a leftward shift in total spine  $d_h$  (Fig. 3F,  $p = 0.0048$ ), mushroom, thin (Fig. S2J-K,  $p < 0.0004$ ) and stubby  $d_h$  (Fig. S2L,  $p = 0.0048$ ). Similarly, after cocaine, the cumulative plots in chronic stress rats microinjected with shcofilin (S/shcofilin-Coc,

green line vs NS/shcofilin-Coc, gray line) showed a leftward shift in total spine  $d_h$  (Fig. 3D,  $p < 0.0004$ ) across all three types of spines  $d_h$  (mushroom, thin and stubby, Fig. S2D-F,  $p < 0.0004$ ). However, the adaptive structural plasticity occurring after chronic stress and a single cocaine administration (S/shcofilin-Coc, green line vs S/shCtrl-Coc, pink line) revealed that cofilin downregulation induced a decrease in total spine  $d_h$  (Fig. 3D,  $p < 0.0004$ ), as well as in mushroom  $d_h$  (Fig. S2D,  $p < 0.0004$ ), thin  $d_h$  (Fig. S2E,  $p < 0.0004$ ) and stubby  $d_h$  (Fig. S2F,  $p = 0.0088$ ), suggesting a persistent reduction on spine  $d_h$  in stressed animals given by the reduction of cofilin expression in NA core after cocaine injection.

Furthermore, supporting a relationship between cofilin downregulation and  $d_h$  in regulating cocaine sensitization induced by stress, we were able to positively correlate the diminution of behavioral response to cocaine with smaller  $d_h$ , when we compared both stress groups exposed to cocaine (Fig. 3G,  $r^2 = 0.3944$ ,  $p = 0.0385$ ), while these changes were not observed in non-stress groups (NS-shCtrl vs NS-shcofilin, Fig. 3H,  $r^2 = 0.02913$ ,  $p = 0.5772$ ). These results highlight the role of cofilin in the maintenance of structural plasticity during stress-induced sensitization, as we are able to report modifications in spine head diameter after a cocaine challenge only in non-stress animals, while this response is blunted in previously stressed animals.

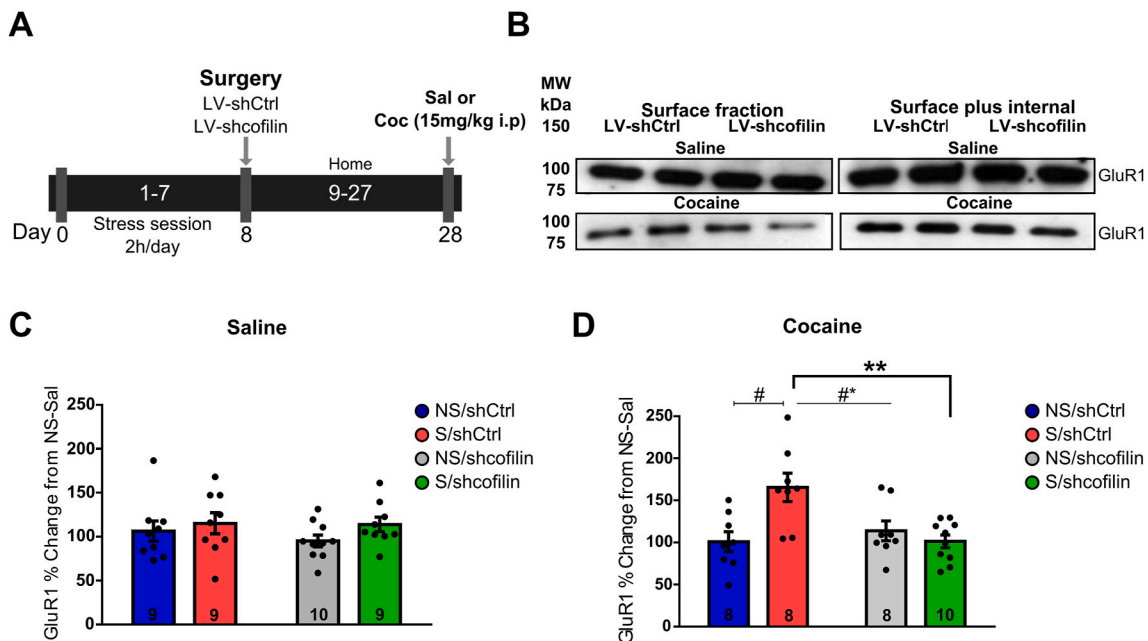
Moreover, we analyzed differences in spine density for each type of treatment, using a three-factor linear mixed model by Rstudio software (Fig. S3). These results revealed a three-factor interaction only in mushroom spine density (Table 1. SM, Three-factor linear model: cocaine  $F_{(1,145)} = 3.36$   $p = 0.069$ , lentivirus (LV)  $F_{(1,40)} = 11.77$   $p = 0.001$ , stress  $F_{(1,40)} = 1.92$   $p = 0.173$ . stress\*LV  $F_{(1,40)} = 3.97$   $p = 0.053$ , stress\*cocaine  $F_{(1,145)} = 0.25$   $p = 0.615$ , LV\*cocaine  $F_{(1,145)} = 1.21$   $p = 0.275$  and stress\*LV\*cocaine  $F_{(1,145)} = 5.97$   $p = 0.016$ ). Furthermore, modifications in total spine density are dependent on cocaine and lentivirus effect and not due to stress exposure (Table 1. SM, cocaine  $F_{(1,145)} = 9.45$   $p = 0.003$ , LV  $F_{(1,40)} = 10.80$   $p = 0.002$  and stress  $F_{(1,40)} = 4.02$   $p = 0.052$ . stress\*LV  $F_{(1,40)} = 1.55$   $p = 0.221$ , stress\*cocaine  $F_{(1,145)} = 1.84$   $p = 0.178$ , LV\*cocaine  $F_{(1,145)} = 0.83$   $p = 0.363$  and

stress\*LV\*cocaine  $F_{(1,145)} = 0.54$   $p = 0.464$ ). Indeed, we noticed an independent effect on stress and cocaine in thin spines, with a trend toward increased density in thin spines in stress animals after cocaine (Table 1 Table 1. SM, cocaine  $F_{(1,145)} = 5.24$   $p = 0.023$ , LV  $F_{(1,40)} = 1.53$   $p = 0.223$ , stress  $F_{(1,40)} = 9.67$   $p = 0.003$ . stress\*LV  $F_{(1,40)} = 1.43$   $p = 0.239$ , stress\*cocaine  $F_{(1,145)} = 2.51$   $p = 0.115$ , LV\*cocaine  $F_{(1,145)} = 0.10$   $p = 0.755$  and stress\*LV\*cocaine  $F_{(1,145)} = 0.00$   $p = 0.988$ ), and no changes in stubby spine density.

Thus, our results suggest that cofilin downregulation modulates actin remodeling in MSN, influencing the chronic stress-induced sensitization to psychostimulant response to cocaine.

### 3.4. Inhibition of Cofilin in the NA core prevents the enhancement of AMPAR surface expression in stressed rats after cocaine

Glutamate signaling in the NA plays an important role in the behavioral and molecular plasticity observed in behavioral sensitization to cocaine (Loweth et al., 2014; Wolf and Ferrario, 2010; Wolf and Tseng, 2012). Our previous report showed that actin reorganization promotes an increase in AMPAR surface expression in the NA core in stress animals after cocaine (Esparza et al., 2012). To examine the role of cofilin in the NA core in the expression of the AMPAR in stressed animals, we performed an intra-accumbens microinjection with shcofilin and, three weeks after chronic stress, surface expression of the AMPAR subunit GluR1 was evaluated 45 min after a challenge with cocaine or saline (Fig. 4A). Representative Western blots of the surface fraction and surface plus internal fractions are shown in Fig. 4B. Non-stress/stress shCtrl and non-stress/stress shcofilin that received an injection of saline did not show modifications in the surface expression of the AMPAR subunit GluR1 (two-way ANOVA (non-stress/stress x shCtrl-Sal/shcofilin-Sal) revealed no effect of non-stress vs stress group  $F_{(1,33)} = 2.020$ ,  $p = 0.1646$ , interaction  $F_{(1,33)} = 0.2593$ ,  $p = 0.6140$  and shcofilin vs shCtrl  $F_{(1,33)} = 0.4025$   $p = 0.5302$ ) (Fig. 4C). However, shCtrl animals pre-exposed to stress showed an enhancement in surface expression of the GluR1 containing AMPAR after a cocaine challenge,



**Fig. 4.** Cofilin downregulation in NA core prevents the enhancement of AMPAR surface expression in stressed rats exposed to cocaine. (A) Experimental design. (B) Representative western blots of GluR1 surface fraction and GluR1 surface plus internal.  $\alpha$ -tubulin was used as loading control for Western blot experiments. (C) AMPAR subunit GluR1 in rats pretreated with shCtrl or shcofilin into the NA core, 45 min after a saline injection, analyzed by two-way ANOVA and Bonferroni's post-hoc comparison,  $n = 9-10$  in each group. (D) AMPAR subunit GluR1 in rats pretreated with shCtrl or shcofilin into the NA core, 45 min after a cocaine injection, analyzed by two-way ANOVA and Bonferroni's post-hoc,  $n = 8-10$  in each group. Data are expressed as mean  $\pm$  SEM of the number of determinations depicted in each graphic. \* $p < 0.05$ , compared with S/shcofilin. \*\* $p < 0.05$ , compared with NS/shcofilin. # $p < 0.05$ , compared with NS/shCtrl.

while the remaining groups were unchanged (two-way ANOVA (non-stress/stress  $\times$  shCtrl-Coc/shcofilin-Coc) revealed effect of non-stress vs stress  $F_{(1-30)} = 4.643$ ,  $p = 0.0393$ , interaction  $F_{(1-30)} = 10.17$ ,  $p = 0.0033$  and shcofilin vs shCtrl  $F_{(1-30)} = 4.528$   $p = 0.0417$ ) (Fig. 4D). These findings suggest that cofilin downregulation impedes the GluR1 surface enhancement in the NA core described in stressed animals after a cocaine challenge.

### 3.5. Inhibition of cofilin expression in the NA core prevents the facilitatory influence of stress during the acquisition of cocaine self-administration

Stress plays a powerful role in the initiation, escalation, and relapse to drug abuse (Garcia-Keller et al., 2016, 2019a; Kim et al., 2009; Shaham et al., 2000; Sinha, 2007, 2009). Here, we show that cofilin, has a crucial role in the vulnerability to develop SUDs. One week after microinjection of LV particles, non-stress and stress rats underwent surgery for implantation of jugular catheters and, eight days later, began daily 2 h cocaine SA on a FR1 for 10 days (Fig. 5A). Three weeks after chronic stress, the acquisition of cocaine SA was facilitated in the group stress shCtrl (Fig. 5B). Stress-induced facilitation was significant when quantified by the number of active lever presses (two-way ANOVA with RM over time (non-stress/stress  $\times$  LV-shCtrl/shcofilin) revealed no effect of interaction  $F_{(27-207)} = 0.8880$   $p = 0.6288$ , but significant effects in: non-stress/stress  $F_{(3-23)} = 7.592$   $p = 0.0011$  and time  $F_{(9-207)} = 3.097$   $p = 0.0016$ ) (Fig. 5B) or cocaine infusions (two-way ANOVA with RM over time (non-stress/stress  $\times$  shCtrl/shcofilin) revealed no effect of interaction  $F_{(27-207)} = 0.6837$   $p = 0.8796$  but significant effects in non-stress/stress  $F_{(3-23)} = 6.118$   $p = 0.0032$  and time  $F_{(9-207)} = 3.235$   $p = 0.0011$ ) (Fig. 5C). Bonferroni's post-hoc comparisons indicated that the stress shCtrl group shows significantly higher performance on active lever presses between day 4 and day 9 of SA compared with the non-stress shCtrl group and all the remaining groups (Fig. 5B). Also, these differences were observed in cocaine infusions between stress/non-stress shCtrl groups during the same period of time (Fig. 5C). These comparisons showed no significant differences between the non-stress shCtrl and non-stress/stress shcofilin groups in the number of active lever presses or cocaine infusions during the 10 days of SA (Fig. 5B and C). Differences between the stress shCtrl and stress shcofilin groups in the number of active lever presses at day 6 and day 9 were significant (Fig. 5B). In addition, significant differences in the cocaine infusions were evidenced at 9 and 10 days of SA (Fig. 5C). Further, these differences were also evidenced as the total cumulative number of infusions taken by each treatment group (two-way ANOVA (non-stress/stress  $\times$  shCtrl/shcofilin)) revealed effect of interaction ( $F_{(1-23)} = 11.08$   $p = 0.0029$ ) and non-stress vs stress ( $F_{(1-23)} = 9.438$   $p = 0.0054$ , with no effect of treatment ( $F_{(1-23)} = 0.8539$   $p = 0.3651$ ) (Fig. 5D). The inactive lever pressing rate (two-way ANOVA with RM over time (non-stress/stress  $\times$  shCtrl/shcofilin)) showed no effect of interaction ( $F_{(27-207)} = 0.8078$   $p = 0.7392$ ) or treatment ( $F_{(3-23)} = 0.3498$   $p = 0.7896$ ), but significant effect of time ( $F_{(9-207)} = 2.430$   $p < 0.0121$ ) (Fig. 5B).

To further investigate the effect of the inhibition of cofilin on the acquisition of cocaine SA induced by stress, we analyzed the lever presses of individual rats of each group (Fig. 5E,G and Fig. S4 A and C) and the brain cocaine concentration across self-administration sessions was averaged within treatment groups (Fig. 5F,H and Fig. S4B,D). These findings revealed a faster lever-pressing response rate and stabilization of response for cocaine in stress shCtrl animals (Fig. 5E,G). The heat maps of the average modeled brain cocaine concentration showed stable brain cocaine levels during the first days with the highest levels on day 9, continuing with high stable brain cocaine concentration in the following days (Fig. 5F). Non-stress shCtrl animals, however, stabilized their lever-pressing response on day 7, reaching lower brain cocaine levels than the stress shCtrl group (Fig. S4A-B). The analysis of lever presses of individual rats and average brain concentrations of cocaine

over time during SA showed no apparent differences between the non-stress shCtrl group (Fig. S4A-B) and non-stress/stress shcofilin groups (Fig. S4C-D). Nevertheless, differences were evidenced between stressed shCtrl and shcofilin groups (Fig. 5E,G). Interestingly, both groups of animals stabilized their lever-pressing response rate around day 6 but, in the following days, the stress shCtrl animals reached much higher brain levels of cocaine than those treated with shcofilin (Fig. 5F,H).

Additionally, the same experimental groups were evaluated under a model of acquisition of oral sucrose SA (Fig. 6A). The sucrose SA acquisition after 10 sessions of training did not differ among the different groups (Fig. 6B–D). In these experiments, the active lever pressing rate during 1 h/day, under the FR1 schedule (two-way ANOVA with RM over time (non-stress/stress  $\times$  shCtrl/shcofilin)) revealed no effect of interaction ( $F_{(27-198)} = 0.9093$   $p = 0.5982$ ) or treatment ( $F_{(3-22)} = 0.05047$   $p = 0.9846$ ), but a significant effect of time ( $F_{(9-198)} = 19.38$   $p < 0.0001$ ) (Fig. 6B). Furthermore, the analyses of sucrose intake revealed no effect of interaction ( $F_{(27-198)} = 0.6134$   $p = 0.9338$ ) or treatment ( $F_{(3-22)} = 0.04324$   $p = 0.9877$ ), but a significant effect of time analyzed by a two-way ANOVA with RM over time (non-stress/stress  $\times$  shCtrl/shcofilin) time ( $F_{(9-198)} = 21.70$   $p < 0.0001$ ) (Fig. 6C). The analysis of total sucrose intake for each treatment group revealed no significant differences among groups (Fig. 6D). Further, the inactive lever pressing rate (two-way ANOVA with RM over time (non-stress/stress  $\times$  shCtrl/shcofilin)) showed no effect of interaction ( $F_{(27-198)} = 0.3239$   $p = 0.9995$ ) or treatment ( $F_{(3-21)} = 0.9087$   $p = 0.4536$ ), but significant effect of time ( $F_{(9-198)} = 5.827$   $p < 0.0001$ ) (Fig. 6B). Together, these data support the idea of a differential, specific role of cofilin in the acquisition of cocaine SA, given that all groups of animals are capable of self-administering sucrose independently of the treatment.

## 4. Discussion

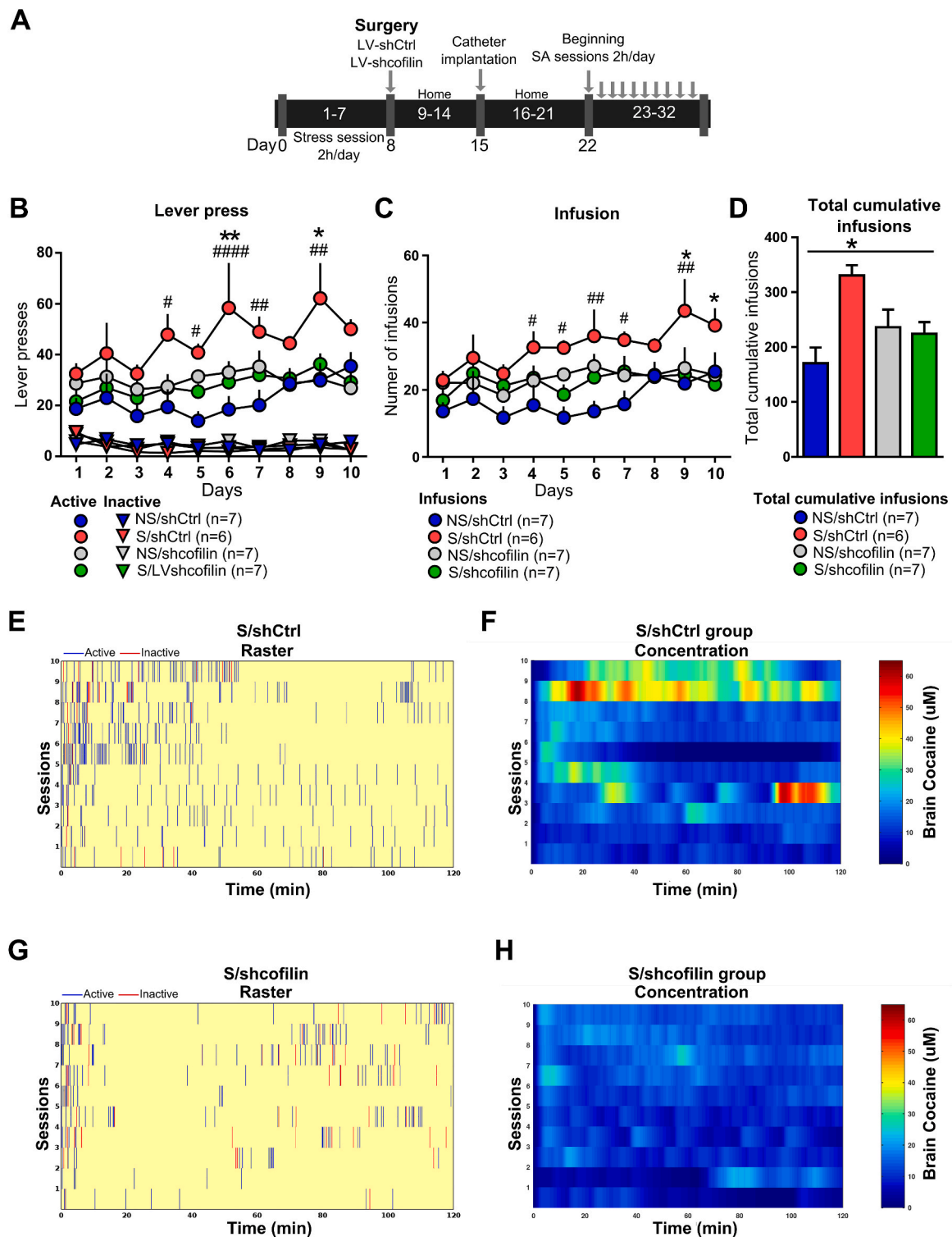
These results provide a novel characterization of the role of cofilin in stress-induced cocaine sensitization and the potentiation of cocaine SA using molecular, biochemical assays and behavioral approaches. Considering that cofilin is involved in spine structural plasticity regulating actin remodeling and AMPAR trafficking and accumulation within synapses in a dynamic sequence of activation and inactivation during long-term potentiation (LTP) (Bosch et al., 2014; Gu et al., 2010; Noguchi et al., 2016; Rust et al., 2010), we hypothesized that cofilin activity is critical for the development of SUDs induced by chronic stress.

We report that cofilin downregulation prevents stress-induced cocaine sensitization (Fig. 2C–D), and facilitation of cocaine self-administration (Fig. 5B–D). The specificity of this process was clearly demonstrated by the fact that sucrose administration remained unmodified in shcofilin-exposed animals (Fig. 6B–D). Furthermore, after cofilin downregulation in chronically stressed rats, the prevention of cross-sensitization to cocaine was accompanied by a smaller spine head diameter, allowing us to establish a positive correlation between the reduced behavioural response to cocaine and smaller spine head diameter. Moreover, cofilin downregulation prevents the AMPAR enhancement in the NA core described in stressed animals after a cocaine challenge. Our previous study showed that chronic stress downregulates p-cofilin expression and decreases F-actin levels in the NA core, and that, after a single cocaine injection in stressed animals the levels of F-actin returns to normal, accompanied with decreased levels of p-cofilin and p-cortactin protein expression (Esparza et al., 2012). Here, we report that pretreatment with shcofilin induces persistent cofilin downregulation in the NA core (Fig. 1F), suggesting enhanced stabilization of actin dynamics, due to increased p-cofilin/cofilin ratio (Fig. 1H), after stress exposure preventing cocaine sensitization (Fig. 2C–D).

### 4.1. Cofilin and dendritic spine plasticity

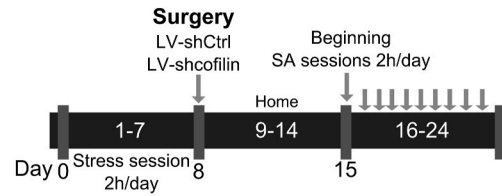
Chronic cocaine administration and stress exposure are able to



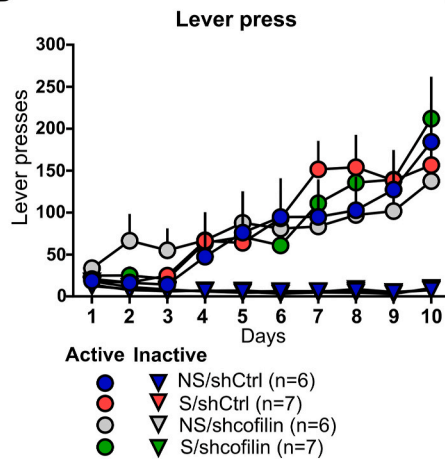


**Fig. 5.** Cofilin downregulation in NA core prevents the facilitatory influence of stress during the acquisition of cocaine self-administration. (A) Experimental design. (B) Number of active lever and inactive presses during acquisition of cocaine self-administration 2 h daily on a fixed ratio (FR1) over 10 days. The results were analyzed by two-way ANOVA with repeated measures (RM) over time followed by Bonferroni's post-hoc,  $n = 7-8$  in each group.  $^{\#}p < 0.05$ , compared with NS/shCtrl.  $^*p < 0.05$ , compared with S/shcofilin. Number of inactive lever presses revealed no significant differences among groups. (C) Number of cocaine infusions during acquisition of cocaine self-administration under FR1 schedule (two-way ANOVA with repeated measures (RM) over time followed by Bonferroni's post-hoc,  $n = 7-8$  in each group.  $^{\#}p < 0.05$ , compared with NS/shCtrl.  $^*p < 0.05$ , compared with S/shcofilin). (D) Total cumulative number of infusions taken by each treatment group after 10 days of cocaine self-administration (two-way ANOVA followed by Bonferroni's post-hoc.  $^*p < 0.05$ , compared with all the remaining groups). (E, G) Example raster plots of the best performing stress animal microinjected with shCtrl (E) or shcofilin (G). Presses on the active (blue) and inactive (red) lever. (F, H) Heat maps of average modeled brain cocaine concentration (uM) in stress animals microinjected with shCtrl (F) or shcofilin (H). Data are shown as mean ( $\pm$ S.E.M.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

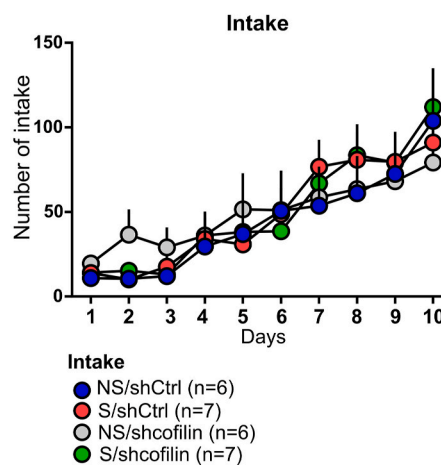
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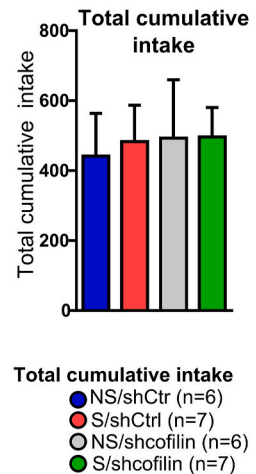
B



C



D



**Fig. 6.** Inhibition of cofilin expression in NA core did not affect sucrose self-administration.

(A) Experimental design. (B) Number of active and inactive lever presses during acquisition of sucrose self-administration over 10 days on a fixed ratio (FR1) over 10 days. The results were analyzed by two-way ANOVA with repeated measures (RM) over time followed by Bonferroni's post-hoc,  $n = 6-7$  in each group. (C) Number of intakes during acquisition of sucrose self-administration under the FR1 schedule (two-way ANOVA with RM over time followed by Bonferroni's post-hoc,  $n = 6-7$  in each group). (D) Total cumulative sucrose intake for each treatment group after 10 days of sucrose self-administration. The results were analyzed by two-way ANOVA followed by Bonferroni's post-hoc. Data are shown as mean  $\pm$  S.E.M.

regulate actin turnover in the NA (Dietz et al., 2012; Golden and Russo, 2012; Shen et al., 2009; Toda et al., 2006, 2010). Exposure to either or both has been associated with an increase in spine density and reorganization of the dendritic structure of NA MSNs (Brown et al., 2011; Christoffel et al., 2011; Dietz et al., 2012; Dumitriu et al., 2012; Khibnik et al., 2016; Robinson and Kolb, 2004). Toda et al. (2006) reported that non-contingent acute cocaine administration enhanced p-cofilin, but withdrawal from repeated cocaine was associated with a decrease in p-cofilin, downregulated LIMK1 and therefore increased actin dynamics influencing cocaine-induced reinstatement of drug seeking. Similarly, downregulation of Rac1 promotes alterations in cofilin activity and changes in dendritic spine morphology (Dietz et al., 2012). Also, it was shown that p-cofilin upregulation through focal adhesion kinase (FAK) is required for cue-induced cocaine reinstatement in D1-MSN in the NA core (Garcia-Keller et al., 2020). Several studies reported no changes (Francis et al., 2017) or enhanced immature spine density in NA MSNs after social defeat stress (CSDS) through a Rac1-dependent mechanism involving the redistribution of synaptic cofilin in the NA (Golden et al., 2013). Further, Fox et al. (2018) report that dendritic atrophy of D1, but not D2 receptor containing MSNs is strongly associated with social avoidance after CSDS caused by upregulation of the GTPase RhoA and its downstream effector Rho-kinase (Fox et al., 2018). Likewise, after CSDS, D2-MSNs exhibit increased spine density, mostly thin and stubby, and this increased spine density negatively correlates with social interaction behavior (Fox et al., 2020). Additionally, in a previous publication (Garcia-Keller et al., 2016) we showed that an acute stress exposure was associated with increases in spine density, increased AMPA/NMDA currents and potentiation in acquisition of cocaine self-administration in the stress group. The fact that a single stressor produced such long-lasting alterations at glutamatergic synapses in the NA core and facilitated the acquisition of cocaine self-administration suggests

possible common substrates that may predispose individuals suffering from stress disorders to develop cocaine addiction. Here, after chronic restraint exposure, we report unchanged spine density in stress animals after cocaine, although there was a trend toward increased density in thin spines of stress animals after cocaine (Fig. S3A and C). Interestingly, we did not find modifications in mushroom spine density at this time point, but we found a three-factor interaction: chronic stress, the shcofilin and cocaine, only in this spine subtype, pointing up the importance of cofilin in the chronic stress-induced sensitized cocaine response (Fig. S3B). Moreover, we reported a reduction in  $d_h$  in the stress shCtrl group after saline (Fig. 3C and Fig. S2A-B), which may be associated with the decreased levels of p-cofilin and F-actin in the NA core described in our previous report (Esparza et al., 2012). Further, we found that cofilin downregulation promoted reduced behavioral response to cocaine in stress rats and this reduced response was positively correlated with a smaller spine  $d_h$ , supporting a possible causal association between spine head enlargement and stress-induced sensitization to cocaine. Our results highlight the importance of structural and functional changes induced by stress and, more importantly, provide evidence suggesting the involvement of cofilin in this process, as we show that cofilin downregulation impedes the actin remodeling necessary for rapid changes in spine morphology in response to cocaine and thereby prevents the sensitized response. In line with our observations, several evidences demonstrate that cofilin and its upstream effectors Rac1 and LIMK1 seemed to be implicated in the synaptic plasticity induced by different paradigms of chronic stress in NA (Golden et al., 2013), ventro-medial prefrontal cortex (vmPFC) (Fan et al., 2018), hippocampus (Chen et al., 2008; Lu et al., 2019) and mPFC (Gao et al., 2020). In addition, the RhoA-ROCK signalling pathway mediates depression-like behaviours, via dendritic remodeling of NA D1-MSNs (Fox et al., 2018). However, other articles have reported that chronic

stress-induced modifications were not directed by cofilin, but rather by myosin phosphatase targeting protein 1 (MYPT1), downstream of RhoA–ROCK kinases, underlying the spine loss in hippocampal neurons (Castañeda et al., 2015; Garcia Rojo et al., 2017). Further studies are needed to explain the divergent results regarding MSNs morphological changes induced by stress, specifically evaluating the role of DA receptor expression in relation to stress-induced drug-seeking behavior.

#### 4.2. Cofilin and AMPAR plasticity

In MSNs of the NA, the cell surface expression enhancement of AMPARs is required for cocaine-seeking behavior, and synaptic levels of AMPARs are upregulated after withdrawal from cocaine (Kalivas and Volkow, 2005; Wolf, 2010; Wolf and Ferrario, 2010; Wolf and Tseng, 2012). Here, we prevented the enhancement of AMPAR surface expression in the NA core (Fig. 4) previously observed in stressed rats exposed to cocaine by inhibiting cofilin expression (Esparza et al., 2012; Garcia-Keller et al., 2013). It has been reported that cofilin controls AMPAR surface expression via an actin-dependent mechanism, and that cofilin controls synaptic strength via AMPAR mobility (Bosch et al., 2014; Rust et al., 2010), evidencing an important function of cofilin in both structural and functional plasticity (Borovac et al., 2018). Moreover, changes in structural plasticity suggest a temporal sequence of cofilin activation and inactivation during LTP, relevant for initial spine enlargement and synaptic AMPAR accumulation (both dependent on cofilin activation), followed by consolidation of structural changes (dependent on cofilin inactivation) (Gu et al., 2010; Noguchi et al., 2016; Stefen et al., 2016). Previous literature described that repeated cocaine changes in AMPAR surface expression are paralleled by increases in AMPA currents, AMPA/NMDA ratio and the density of dendritic spines in accumbens MSNs directed by cofilin-induced changes in actin dynamics (Dietz et al., 2012; Kourrich et al., 2007; Robinson and Kolb, 2004; Shen et al., 2009; Verma et al., 2019). Here, we found that the reduced behavioral response to cocaine in pre-stress rats exposed to shcofilin with a smaller spine  $d_h$  also showed reduced levels of AMPAR surface expression (Fig. 4D) probably explained by the stabilization of actin remodeling and the reduction of AMPAR mobility (Rust, 2015; Rust et al., 2010). This result is in agreement with our previous observation describing an enhancement in AMPAR surface expression during the sensitization between stress and cocaine and increased PSD (Esparza et al., 2012).

#### 4.3. Conclusion

In conclusion, we have shown that cofilin is involved in the enhanced vulnerability to cocaine abuse induced by stress, as we were able to prevent it by diminishing cofilin levels of expression, thereby altering its precise regulation during behavioral sensitization to cocaine and the acquisition of cocaine SA.

Our work highlights the role of cofilin as molecular mediator of spine morphological changes and AMPAR surface expression, reinforcing the cocaine sensitization and cocaine SA induced by previous chronic stress exposure. Thus, our research provides new directions for the comprehension of the neurobiological basis of comorbidity between stress exposure and cocaine addiction and provides a platform for further studies on approaches to therapy for this dual pathology.

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#### Data accessibility

Data collected for this manuscript can be provided upon request from the corresponding author.

#### CRedit authorship contribution statement

**Daiana Rigoni:** Conceptualization, Methodology, Resources, Writing – original draft, Visualization. **Maria P. Avalos:** Methodology, Resources. **Maria J. Boezio:** Methodology, Resources. **Andrea S. Guzmán:** Methodology, Resources. **Gaston D. Calfa:** Methodology, Resources. **Eduardo M. Perassi:** Resources, Formal analysis. **Silvia M. Pierotti:** Resources, Formal analysis. **Mariano Bisbal:** Methodology, Resources, Writing – review & editing. **Constanza Garcia-Keller:** Resources, Writing – review & editing, Visualization. **Liliana M. Cancela:** Methodology, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Flavia Bollati:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Visualization, Project administration, Funding acquisition.

#### Declaration of competing interest

The authors report no biomedical financial interests or potential conflicts of interest.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

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