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Research report

Expression of activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) in the nucleus accumbens is critical for the acquisition, expression and reinstatement of morphine-induced conditioned place preference

Xiu-Fang Lv, Ya Xu, Ji-Sheng Han, Cai-Lian Cui *

Neuroscience Research Institute and Department of Neurobiology, Peking University Health Science Center, Key Laboratory of Neuroscience, the Ministry of Education and Ministry of Public Health, 38 Xueyuan Road, Beijing 100191, PR China

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ABSTRACT

Activity-regulated cytoskeleton-associated protein (Arc), also known as activity-regulated gene 3.1 (Arg3.1), is an immediate early gene whose mRNA is selectively targeted to recently activated synaptic sites, where it is translated and enriched. This unique feature suggests a role for Arc/Arg3.1 in coupling synaptic activity to protein synthesis, leading to synaptic plasticity. Although the Arc/Arg3.1 gene has been shown to be induced by a variety of abused drugs and its protein has been implicated in diverse forms of long-term memory, relatively little is known about its role in drug-induced reward memory. In this study, we investigated the potential role of Arc/Arg3.1 protein expression in reward-related associative learning and memory using morphine-induced conditioned place preference (CPP) in rats. We found that (1) intraperitoneal (i.p.) injection of morphine (10 mg/kg) increased Arc/Arg3.1 protein levels after 2 h in the NAc core but not in the NAc shell. (2) In CPP experiments, Arc/Arg3.1 protein was increased in the NAc shell of rats following both morphine conditioning and the CPP expression test compared to rats that received the conditioning without the test or those that did not receive morphine conditioning. (3) Microinjection of Arc/Arg3.1 antisense oligodeoxynucleotide (AS) into the NAc core inhibited the acquisition, expression and reinstatement of morphine CPP; however, intra-NAc shell infusions of the AS only blocked the expression of CPP. These findings suggest that expression of the Arc/Arg3.1 protein in the NAc core is required for the acquisition, context-induced retrieval and reinstatement of morphine-associated reward memory, whereas Arc/Arg3.1 protein expression in the NAc shell is only critical for the context-induced retrieval of memory. As a result, Arc/Arg3.1 may be a potential therapeutic target for the prevention of drug abuse or the relapse of drug use.

1. Introduction

Opiate addiction is a chronic relapsing disorder characterized by compulsive drug seeking and consumption and a high risk of relapse even after long periods of abstinence, which is often precipitated by drug-associated cues [1]. It has been reported that repeated drug exposure elicits changes in molecular, neuronal and synaptic plasticity of the central nervous system during the development of addiction. These changes in synaptic plasticity following repeated drug use share similarities with the synaptic changes observed in physiological learning and memory and may therefore play an essential role in the development and persistence of drug addiction. The mesolimbic reward system, which extends from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) with projections to the limbic system and the orbitofrontal cortex, is the common neuronal pathway for physiological learning and memory and drug addiction [2]. The NAc is a major target and central component of the mesolimbic reward system. Multiple forms of synaptic plasticity have been studied, including changes in the density of dendritic spines [3], long-term potentiation (LTP) and long-term depression (LTD); commonly abused drugs have been also shown to affect these parameters [4,5]. Recently, studies have emphasized the role of the NAc as a whole, including both the shell and core components, in the processes leading to drug abuse and subsequent addiction. The core and the shell have heterogeneous structures with distinct immunohistochemical characteristics, neuronal morphologies and afferent and efferent connections [6–9]. Studies have demonstrated that the NAc core and shell differentially modulate the effects of opiates and psychostimulant drugs induced by conditioned behavioral responses [10,11].

Induction of immediate early genes (IEGs) is viewed as an important step in the formation of long-lasting neuroadaptations

* Corresponding author at: Neuroscience Research Institute, Peking University, 38 Xueyuan Road, Beijing 100191, PR China. Tel.: +86 10 8280 1120; fax: +86 10 8280 1120.
E-mail addresses: clcui@bjmu.edu.cn, clcuimclean@gmail.com (C.-L. Cui).

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underlying learning and memory as well as the persistence of some psychoactive drugs [12,13]. Much attention has been paid to the effects of psychostimulants on the transcription factor-coding IEGs, such as c-fos and cAMP response element binding protein (CREB), but less is known about their influence on the effector IEGs [14,15]. Among the effector IEGs, activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) is a unique IEG that may be induced by neuronal activity [16–18] and specifically trafficked and localized to recently potentiated synapses, where it may interact with structural proteins and proteins critical to synaptic plasticity [18–20]. Although the exact function of Arc/Arg3.1 remains unclear, studies have demonstrated that this protein is involved in multiple forms of synaptic plasticity, including LTP elicited by high-frequency stimulation (HFS) [21], LTD mediated by the activation of metabotropic glutamate receptors (mGluRs) [22] and the stable expansion of dendritic spines induced by the activation of BDNF signaling [23]. Arc/Arg3.1 knockout mice failed to develop long-lasting memories in implicit and explicit learning tasks, although their short-term memory was intact [24]. Studies using Arc/Arg3.1 antisense oligodeoxynucleotide (AS) infusion have shown that Arc/Arg3.1 expression in the dorsal hippocampus is necessary for inhibitory avoidance memory [25], and Arc/Arg3.1 expression in the lateral amygdala is necessary for the consolidation of Pavlovian fear conditioning [26]. Together, these results demonstrate a critical role for Arc/Arg3.1 in enduring synaptic plasticity and long-term memory.

Although Arc/Arg3.1 gene expression is induced in the NAc by a variety of drugs (such as cocaine, morphine and amphetamine) [27–29], relatively little is known about the role of the Arc/Arg3.1 protein in drug-related learning and memory. The present study was designed to determine the role of the Arc/Arg3.1 protein in the NAc subregions, namely the core and the shell, in the acquisition, expression and reinstatement of morphine-induced CPP, a widely accepted animal model of drug-associated reward memory.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats, weighing 220–240 g, were used in this study. These rats were obtained from the Laboratory Animal Center of the Peking University Health Science Center. The rats were housed five per cage in a temperature (23 ± 2 °C) and humidity (50 ± 5%) controlled animal facility with food and water provided ad libitum and maintained on a reverse light-dark cycle (12:12 h, lights on at 7 p.m.). Behavioral experiments were conducted during the dark cycle, and rats were handled for 5 days prior to the experiments. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local committee of animal use and protection. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Place preference apparatus

The CPP apparatus is a black, rectangular polyvinyl chloride (PVC) box (79.5 cm long × 23.0 cm wide × 25.0 cm high) containing three chambers (A, B and C) separated by guillotine doors [30]. Two large black conditioning chambers (A and C, 280 mm × 220 mm × 225 mm) were separated by a small gray center choice chamber (B, 135 mm × 220 mm × 225 mm). Chamber A had four light-emitting diodes (LEDs) forming a square on the wall and a stainless steel mesh floor (225 mm × 225 mm), and chamber C had four LEDs forming a triangle on the wall and a stainless-steel rod floor (15 mm apart). In contrast, chamber B had a plain floor. Fourteen photobeams were placed across the chambers 47.5 mm apart. Using a computer interface, the time spent in each chamber and the number of entrances into each of the three compartments were recorded by infrared beam crossings.

2.3. Cannula implantation and microinfusions

Rats weighing 260–280 g received cannula implants and were handled for 2 days prior to surgery. The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and secured in a Kopf stereotaxic apparatus (Kopf Instrument, Tujunga, CA). Stainless steel guide cannulas (0.67 mm in outer diameter) were bilaterally implanted 1.5 mm above the NAC shell or core. The NAc shell coordinates [31] were anterior/posterior, +1.6 mm; medial/lateral, ±0.9 mm; and dorsal/ventral, −6.5 mm. The coordinates for the NAc core were anterior/posterior, +1.6 mm; medial/lateral, ±2.0 mm; and dorsal/ventral, −6.0 mm. The cannulas were fixed with screws to the skull with dental cement. Internal cannulas were replaced with dummy cannulas, which were 0.5 mm longer than the guide cannulas, to keep the cannulas patent and prevent infection. Rats were given at least 5–7 days to recover before the conditioning procedures.

In studies involving intra-nuclear infusions, the dummy cannulas were removed and infusion cannulas (0.3 mm in outer diameter) were inserted. The cannulas were connected to 1.0-μl Hamilton syringes via PE 20 tubing. The tubing was back-filled with saline, with a small air bubble separating the saline from the drug solution. Drugs were infused with an infusion pump at a speed of 0.25 μl/min. A total volume of 1 μl of 1 nmol/μl Arc/Arg3.1 AS or CS was infused into each nucleus. After infusion, the cannula was left in place for an additional minute to allow the solution to diffuse away from the cannula tip. The dummy cannula was then replaced, and the rat was returned to its home cage.

2.4. Oligodeoxynucleotide design and preparation

2.4.1. Oligodeoxynucleotide design

Arc/Arg3.1 antisense oligodeoxynucleotide (AS) and scrambled oligodeoxynucleotide (CS) (Shanghai Sangon Synthesis Technology Services Ltd.) were designed as described by Guzowski et al. [21]. The Arc/Arg3.1 AS encoded an antisense sequence for the Arc/Arg.31 mRNA sequence near the translation start site [16]. The CS, which does not show significant homology to any sequences in the GenBank database, served as a control. Both AS and CS contained phosphorothioate linkages on the three terminal bases of both the 5′ and 3′ ends and phosphodiester internal bonds, as this nucleotide design has been reported to be more stable and less toxic than fully phosphorothioated designs. The following sequences were used ("~" denotes a phosphorothioate linkage): 5′-G~T~C~CAGCTCCATCTGCT~C~G~C-3′ (AS) and 5′-C~G~T~GCACCTCTCGCAGC~T~T~C-3′ (CS). This AS has been shown to effectively knock down Arc/Arg3.1 protein expression in the hippocampus and amygdala and exhibits a high degree of specificity for Arc/Arg3.1 relative to other IEGs [26]. The AS and CS were purified by high performance liquid chromatography (HPLC), their concentrations were determined spectrophotometrically, and their integrity was confirmed by denaturing gel electrophoresis.

2.4.2. Verification of AS/CS diffusion and knockdown

To verify the diffusion, biotinylated Arc/Arg3.1 AS was infused bilaterally (1 nmol; 1 μl) into the NAc core. 1, 3 or 6 h later, rats were rapidly and deeply anesthetized with chloral hydrate (250 mg/kg, i.p.) and perfused through the heart with phosphate buffered saline (PBS), followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and post-fixed in 4% paraformaldehyde–PB overnight at 4 °C. The next day, the brains were transferred to a 30% sucrose solution overnight until they sank. Cryostat sections containing the NAc core were cut at a thickness of 30 μm and were processed using a standard ABC-DAB reaction to visualize the extent of the distribution and cellular uptake of the AS.

To verify the effectiveness of knocking down Arc/Arg3.1 protein expression, naive rats were cannulated as described above and given an intra-NAc core infusion of Arc/Arg3.1 AS into one side of the brain and a Arc/Arg3.1 CS infusion into the contralateral side at the dose and volume used in the behavioral experiments (both 1 nmol; 1 μl). Rats were then sacrificed 1, 3 or 6 h later. Punches taken from around the cannula tips were assayed for Arc/Arg3.1 protein expression using western blotting.

2.5. Single dose morphine treatment

The effect of a single injection of morphine was investigated using two different approaches. First, rats were administered 10 mg/kg (i.p.) morphine (morphine hydrochloride purchased from Qinghai Pharmaceutical General Factory was dissolved in sterile saline) and sacrificed 1, 2, 4 or 8 h after the treatment to evaluate the temporal profile of Arc/Arg3.1 protein expression produced by a single dose of morphine. In the second experiment, we analyzed the dose–response profile, with rats receiving a single injection of morphine at different doses (0, 5, 10 or 20 mg/kg). Rats were sacrificed by decapitation 2 h after the single morphine administration.

2.6. Conditioned place preference

2.6.1. Preconditioning

In the preconditioning phase, rats were allowed to freely explore the entire apparatus for 15 min to assess the unconditioned chamber preference. The time (in seconds) spent in each compartment and the shuttle times were recorded. Rats that exhibited a strong unconditioned preference were excluded. Conditioning was performed using an unbiased, counterbalanced protocol. In the following experiments, rats in every group spent similar amounts of time in both chambers A and C in the pretest.

2.6.2. Conditioning

On the first day, the rats were confined to the corresponding conditioning chambers for 30 min immediately after the injections of morphine (5 mg/kg, i.p.) and then

returned to their home cages. On the second day, the rats were injected with saline (1 ml/kg, i.p.) and confined to another conditioning chamber. On the subsequent conditioning days, each rat was trained for 6 consecutive days with alternate injections of morphine and saline [32]. The control group received saline every day. The neutral zone was never used during conditioning and was blocked by guillotine doors.

To measure the effect of Arc/Arg3.1 protein expression on the acquisition of morphine-induced CPP, rats received a bilateral microinfusion (1 nmol/μl/side) of AS or CS 3 h before each morphine conditioning. The control group received the same microinfusions as the morphine group.

2.6.3. Post-conditioning

On day 9, one day after the last conditioning trial, rats were placed in the neutral area with the guillotine doors removed and allowed free access to all compartments in a drug-free state for 15 min. The time spent in each compartment and the shuttle times were recorded. The procedure for the expression test was the same as the procedure for the initial baseline preference assessment. The CPP score was defined as the time spent in the morphine-paired chamber divided by the total time spent in both the morphine and the saline-paired chambers during CPP testing.

For western blotting, the rats were sacrificed by decapitation 2 h after the expression test. To evaluate the effect of Arc/Arg3.1 protein expression on the expression of morphine CPP, both groups of rats received bilateral microinfusions (1 nmol/μl/side) of AS or CS 3 h before the expression test. The time spent in each compartment was recorded.

2.6.4. Extinction of conditioned place preference

On day 10, rats were given extinction training. Rats were injected with saline and alternately confined to the previously morphine- or saline-paired compartment for 30 min daily from days 10 to 17 (an 8-day period of extinction). The rats then received another CPP test (extinct test) on day 18. After an 8-day period of extinction training, most of the rats did not present conditioned preference for the drug-paired chamber, as described previously [33].

2.6.5. Reinstatement of conditioned place preference

On day 19, one day after the extinct test, rats were tested for the reinstatement of CPP immediately after receiving a priming injection of morphine (2.5 mg/kg, i.p.) or saline (1 ml/kg, i.p.). During this reinstatement test, rats were allowed free access to the entire apparatus for 15 min, and the time spent in each chamber was measured.

To measure the effect of Arc/Arg3.1 protein expression on reinstatement after the extinction of morphine-induced CPP, rats received bilateral microinfusions (1 nmol/μl/side) of AS or CS 3 h the morphine priming injection. The control group received the same microinfusions as the morphine group.

To rule out the possibility that Arc/Arg3.1 AS affects acquisition, expression and reinstatement of morphine CPP by influencing locomotion, we analyzed the shuttle times of every CPP test in all groups of rats using two-way RM ANOVA in each behavioral experiment. No significant differences were found in shuttle times within all groups or between each CPP test and its pretest (data not shown).

2.7. Tissue preparation and western blotting

For western blotting experiments, rats were given an overdose of chloral hydrate (250 mg/kg, i.p.), and their brains were quickly removed and frozen in N-hexane (−70 °C) for approximately 40 s; the brains were then stored at −80 °C until further use. Bilateral tissue punches (12/16 gauge) of the NAc core and shell were obtained from 60-μm thick sections taken on a sliding freezing microtome. Punches were sonicated in 120–150 μl ice-cold RIPA buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% NP-40 and 0.1% SDS (Beijing Applygen Technologies Inc, Beijing, China). The homogenate was then centrifuged at 12,000 × *g* for 5 min, and the supernatant saved for analysis. Protein concentrations were determined using the BCA assay (Pierce, Rockford, IL). Sample buffer was immediately added to the homogenates, and the samples were boiled for 5 min. Protein extracts (30 μg) were then electrophoresed in 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes. Blots were blocked in TBS buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.05% Tween 20) with 5% dry milk and incubated with an anti-Arc/Arg3.1 antibody (1:800; Santa Cruz Biotechnology). Blots were then incubated with an anti-rabbit secondary antibody conjugated to horseradish peroxidase (HRP; 1:2000; Zhongshan Biotechnology, Beijing, China) and developed using the West Dura chemiluminescent substrate (Pierce Laboratories). Densitometry was determined based on band intensity, and relative protein expression was quantified by densitometry using the Total Lab 2.01 analysis system (Phoretix, UK). To control for inconsistencies in loading, optical densities were normalized to β-actin protein expression. Data for treated animals were normalized to the average value of the naive controls.

2.8. Histology

Histological verification of cannula implantation was performed after conditioned place preference testing. Rats were anesthetized and perfused as described above. Coronal sections (30-μm thick) were cut on a cryostat (−20 °C) and mounted on slides coated with gelatin. Cannula placements were assessed by Nissl staining using light microscopy. The locations of the representative cannula tips are shown in Fig. 1. Only those rats whose cannulas were correctly placed were used for data analysis.

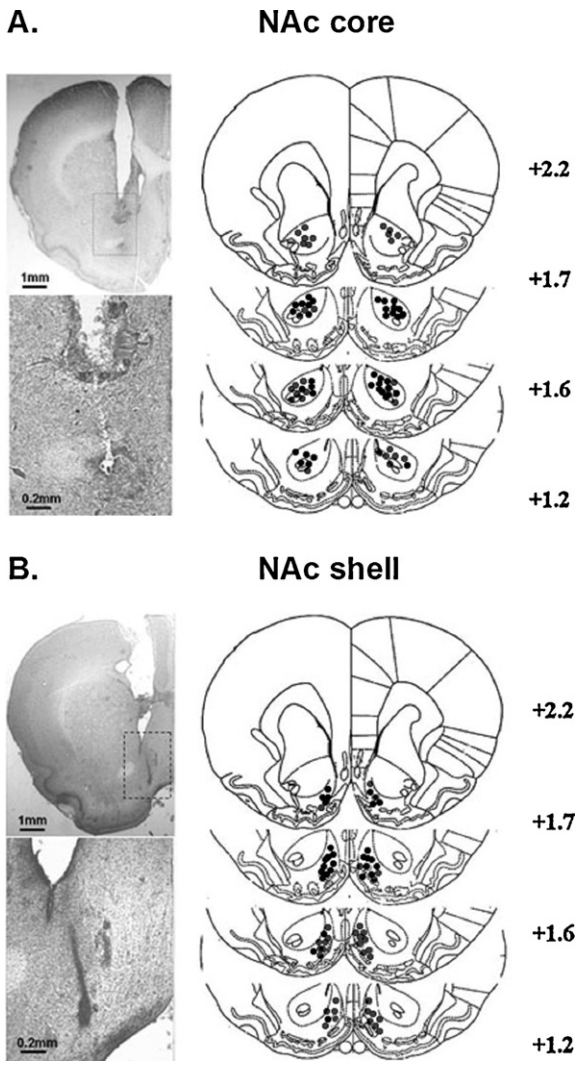

Fig. 1. Representative cannula placements and microinjection sites in the NAc. The NAc core (A) and shell (B) of rats with morphine-induced CPP, microinjected with Arc/Arg3.1 AS or CS (1 nmol/μl/side).

2.9. Statistical analysis

Data were expressed as the means ± SEM and analyzed using analysis of variance (ANOVA) with the appropriate between- and within-subjects factors for the different experiments. One-way ANOVA were used to analyze the differences in the dose-dependent effects of a single treatment with morphine. Post hoc analyses of significant effects from the ANOVA were performed using the Newman–Keuls test. The results from the CPP test and other immunoblotting experiments were analyzed with two-way ANOVA followed by the Bonferroni post-test. Data were processed using Graph Pad Prism 5.0 software. Statistical significance was set to $p < 0.05$.

3. Results

3.1. Effect of a single dose of morphine on Arc/Arg3.1 protein expression in the NAc

To evaluate the temporal profile of Arc/Arg3.1 protein expression in the NAc induced by a single dose of morphine, rats were divided into two groups, receiving either morphine (10 mg/ml/kg) or an equal volume of saline ($n = 5$). The rats' NAc tissues (core and shell) were collected from each group at four time points, 1, 2, 4 or 8 h after the injection of morphine or saline. Arc/Arg3.1 protein

expression in the NAc was quantified using SDS-PAGE and western blotting. Statistical analysis (two-way ANOVA) revealed a significant difference in Arc/Arg3.1 protein levels in the NAc core between morphine- and saline-treated rats ($F(1, 44) = 7.30$, $p < 0.01$). The Bonferroni post hoc test showed an increase in Arc/Arg3.1 protein expression after 2 h in morphine treated rats ($t = 2.720$, $p < 0.05$). There were no significant differences among the various time points in Arc/Arg3.1 expression in the NAc core ($F(3, 44) = 2.22$, $p > 0.05$) (Fig. 2A). In the NAc shell, no significant differences in Arc/Arg3.1 protein expression were found between morphine- and saline-treated rats ($F(1, 44) = 2.090$, $p > 0.05$) or among the various time points ($F(3, 44) = 1.182$, $p > 0.05$) (Fig. 2B).

To analyze the dose–response profile in the NAc core, rats receiving a single injection of morphine at different doses were sacrificed by decapitation 2 h after morphine administration. The statistical analysis (one-way ANOVA) revealed significant differences among groups ($F(3, 16) = 4.231$, $p < 0.05$). Compared to the controls, post hoc tests showed that Arc/Arg3.1 protein levels were increased in rats at all three doses of morphine (0 mg vs. 5 mg, $q = 3.966$, $p < 0.05$; 0 mg vs. 10 mg, $q = 4.187$, $p < 0.05$; 0 mg vs. 20 mg, $q = 4.175$, $p < 0.05$), but no differences were found among the three morphine doses. These results are presented in Fig. 2C. These data suggest that a single dose of morphine increased Arc/Arg3.1 protein levels in the NAc core, but not in the shell, 2 h after morphine administration. There were no time- or dose-dependent effects.

3.2. Effect of the morphine CPP test on Arc/Arg3.1 protein expression in the NAc

For the morphine-induced CPP test, rats were divided into morphine and saline groups. The baseline level of unconditioned preference was measured on the preconditioning day, and all rats spent a similar amount of time in each chamber ($t = 0.2025$, $p > 0.05$). After 8 days of alternative morphine (5 mg/kg, i.p.) and saline (1 ml/kg, i.p.) treatments or saline conditioning alone (controls), rats underwent the CPP expression test on day 9 (Fig. 3A). Statistical analysis (two-way RM ANOVA) revealed a significant effect from the interaction between the treatments (saline vs. morphine) and the tests (pretest vs. test) ($F(1, 17) = 36.72$, $p < 0.0001$). Significant differences were found within the pretest and the test ($F(1, 17) = 35.14$, $p < 0.0001$) and between saline- and morphine-treated rats ($F(1, 17) = 15.44$, $p < 0.01$). The Bonferroni post hoc test demonstrated that morphine-conditioned rats spent significantly more time in the drug-paired context during the expression test than saline-treated rats ($t = 6.509$, $p < 0.001$). The results presented in Fig. 3B show that 5 mg/kg morphine successfully induced CPP, demonstrating that this is a valid model for the following experiments.

Because Arc/Arg3.1 protein was increased by 5 mg/kg morphine in the NAc core in this experiment, rats were divided into six subgroups to evaluate the Arc/Arg3.1 protein levels in the NAc after the morphine CPP expression test. We designated conditioning and testing as the within-subjects factors, and saline and morphine treatments were designated as between-subjects factors. Statistical analysis (two-way ANOVA) revealed no difference in Arc/Arg3.1 protein expression in the NAc core between saline and morphine treatments ($F(1, 20) = 0.3670$, $p > 0.05$) or between the conditioning and the test ($F(2, 20) = 0.2666$, $p > 0.05$). In the NAc shell, significant differences in Arc/Arg3.1 protein expression were found between saline and morphine treatments ($F(1, 24) = 5.861$, $p < 0.05$) and between the conditioning and the test ($F(2, 24) = 7.184$, $p < 0.01$). The Bonferroni post hoc test demonstrated that Arc/Arg3.1 protein expression was increased in rats that received both the morphine conditioning and the CPP expression tests compared to the morphine-treated rats that did not receive conditioning ($t = 3.643$, $p < 0.01$) or the morphine-conditioned rats that did not experience the expression test ($t = 3.764$, $p < 0.01$). These results are presented in Fig. 3C and D. These data suggest that Arc/Arg3.1 protein expression was increased in the NAc shell (but not in the NAc core) after context-induced drug seeking.

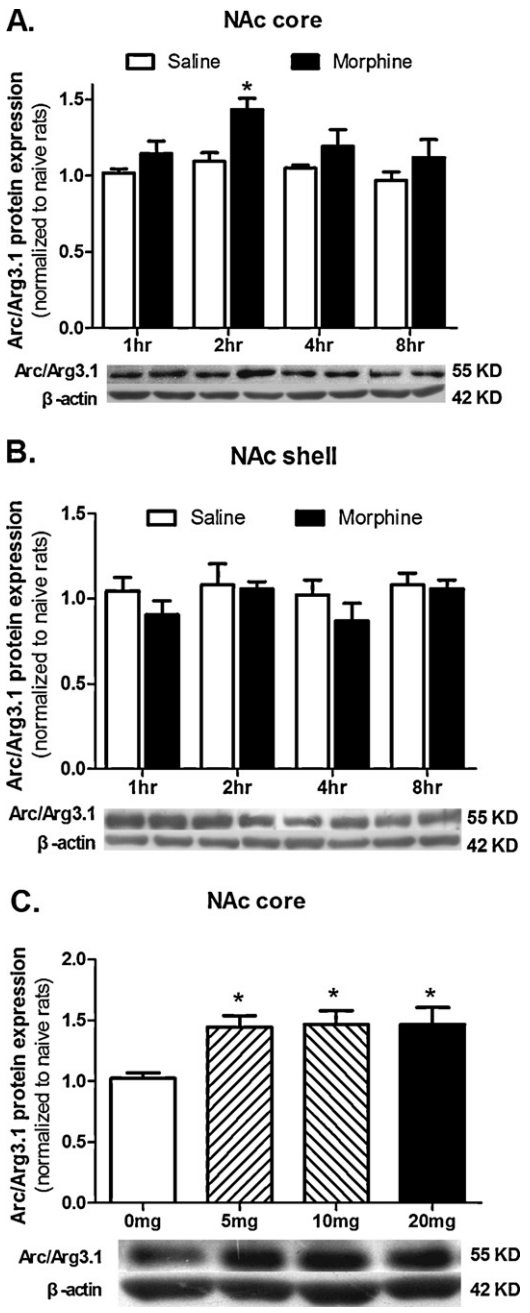

Fig. 2. Effect of a single dose of morphine on Arc/Arg3.1 protein expression in the NAc.
(A) Arc/Arg3.1 protein expression in the NAc core increased significantly 2 h after a 10 mg/kg morphine injection. No time-dependent effects were observed. (B) Morphine (10 mg/kg) had no effect on Arc/Arg3.1 protein expression in the NAc shell. (C) All three doses of morphine (5, 10 and 20 mg/kg) increased Arc/Arg3.1 protein expression in the NAc core, and there were no dose-dependent effects. Data are expressed as the means ± SEM, $n = 5$–8. $^{*}p < 0.05$, compared to the saline-treated group.

3.3. Effect of Arc/Arg3.1 AS on Arc/Arg3.1 protein levels in the NAc

To verify the diffusion of AS/CS in subregions of the NAc, we visualized the extent of the distribution and the cellular uptake

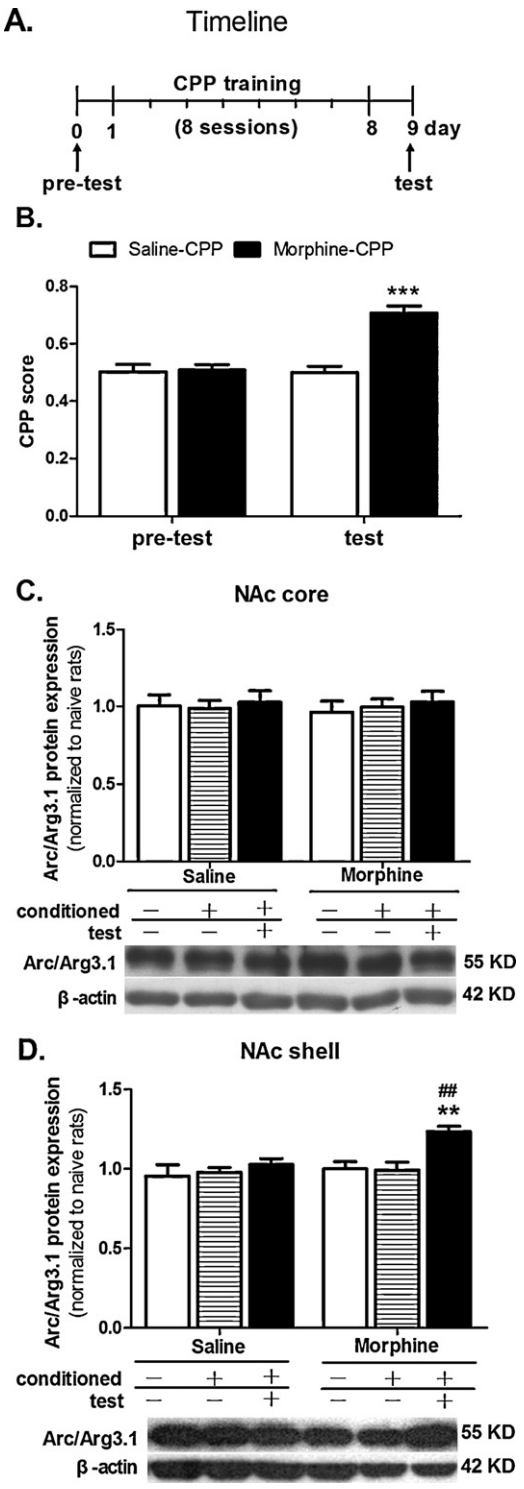

Fig. 3. Re-exposure to the morphine conditioning context induces drug seeking and Arc/Arg3.1 protein expression in the NAc.
(A) Diagram outlining the behavioral procedures. (B) After 8 days of drug treatment, rats that received alternating injections of morphine (5 mg/kg) and saline (1 ml/kg) showed a significant preference for the morphine-paired chamber. Data are expressed as the means ± SEM, $n = 5$. $^{***}p < 0.001$, compared to the saline-treated group. (C) Re-exposure to the morphine conditioning context had no effect on Arc/Arg3.1 protein expression in the NAc core of rats in all groups. (D) Re-exposure to the morphine conditioning context significantly increased Arc/Arg3.1 protein expression in the NAc shell of morphine-conditioned rats. Data are expressed as the means ± SEM, $n = 5$. $^{**}p < 0.01$, compared to the morphine-treated group without conditioning and re-exposure; $^{\#\#}p < 0.01$, compared to the morphine-conditioned group without re-exposure.

of biotinylated AS (bio-AS) by means of immunohistochemistry. Result showed that the bio-AS remained strictly confined to the borders of the NAc core. At higher magnification, Arc/Arg3.1 bio-AS was localized to cell bodies and dendrites, indicating that the bio-AS was actively taken up by cells (Fig. 4A, B and C). Labeling of the Arc/Arg3.1 bio-AS was less pronounced at 1 and 6 h after infusion.

To verify the effectiveness of Arc/Arg3.1 AS (1 nmol/μl/side) in knocking down Arc/Arg3.1 protein levels, the AS was microinfused into one side of the NAc core in naive rats, and the CS was infused into the contralateral side. Rats were then sacrificed 1, 3 or 6 h later, and western blotting was performed on the isolated tissue. Statistical analysis (two-way ANOVA) revealed a significant difference in Arc/Arg3.1 protein levels across the three time points ($F(2, 20) = 4.515$, $p < 0.05$) and between the microinjections of AS and CS ($F(1, 20) = 9.591$, $p < 0.01$). Post hoc tests showed that Arc/Arg3.1 protein levels were decreased at 3 h ($t = 3.281$, $p < 0.05$) following the microinjection of Arc/Arg3.1 AS as compared to the CS microinjection. There were no significant effects at either 1 h ($t = 1.687$, $p > 0.05$) or 6 h ($t = 0.007425$, $p > 0.05$) after infusion (Fig. 4D).

Collectively, these findings indicate that Arc/Arg3.1 AS/CS is anatomically restricted to the NAc core after the infusion of this dose and volume (1 nmol; 1 μl) and taken up by NAc core cells, resulting in the significant and transient knockdown of Arc/Arg3.1 protein at 3 h after AS microinfusion.

3.4. Effect of Arc/Arg3.1 AS microinjection into the NAc core or shell on the acquisition of morphine CPP

In this experiment, the CPP conditioning was executed as described above. During the acquisition period, rats undergoing morphine CPP received bilateral microinfusions (1 nmol/μl/side) of AS or CS 3 h before every i.p. injection of morphine, and the saline-treated control rats received the same microinfusions as the morphine group. The CPP expression test was executed on day 9, and the CPP score was calculated (Fig. 5A).

When AS/CS was microinfused into the NAc core, statistical analysis (two-way RM ANOVA) revealed a significant interaction effect between the treatments and the times ($F(3, 32) = 4.104$, $p < 0.05$). Significant differences in the CPP score were found within groups ($F(3, 32) = 3.593$, $p < 0.05$) and between the pretest and the test ($F(1, 32) = 11.03$, $p < 0.01$). Compared to the pretest, the post hoc test showed there were no significant increases in the CPP score in the expression test of morphine-conditioned rats microinfused with Arc/Arg3.1 AS ($t = 1.600$, $p > 0.05$), whereas a significant increase in the CPP score was found in the expression test of rats microinfused with CS ($t = 4.752$, $p < 0.001$).

When the AS/CS was microinfused into the NAc shell, statistical analysis (two-way RM ANOVA) revealed a significant interaction effect between treatments and times ($F(3, 36) = 6.169$, $p < 0.01$). Significant differences in the CPP scores were found within groups ($F(3, 36) = 12.55$, $p < 0.001$) and between the pretest and the test ($F(1, 36) = 14.11$, $p < 0.001$). Compared to the pretest, the post hoc test showed a significant increase in the CPP score in morphine-conditioned rats microinfused with both Arc/Arg3.1 AS ($t = 3.371$, $p < 0.01$) and CS ($t = 4.595$, $p < 0.001$). These data suggest that the microinfusion of Arc/Arg3.1 AS into the NAc core, but not the shell, blocked the acquisition of morphine CPP (Fig. 5B and C).

3.5. Effect of Arc/Arg3.1 AS microinjection into the NAc core or shell on the expression of morphine CPP

In this experiment, rats were divided into morphine- and saline-treated CPP groups. After 8 days of CPP conditioning, rats

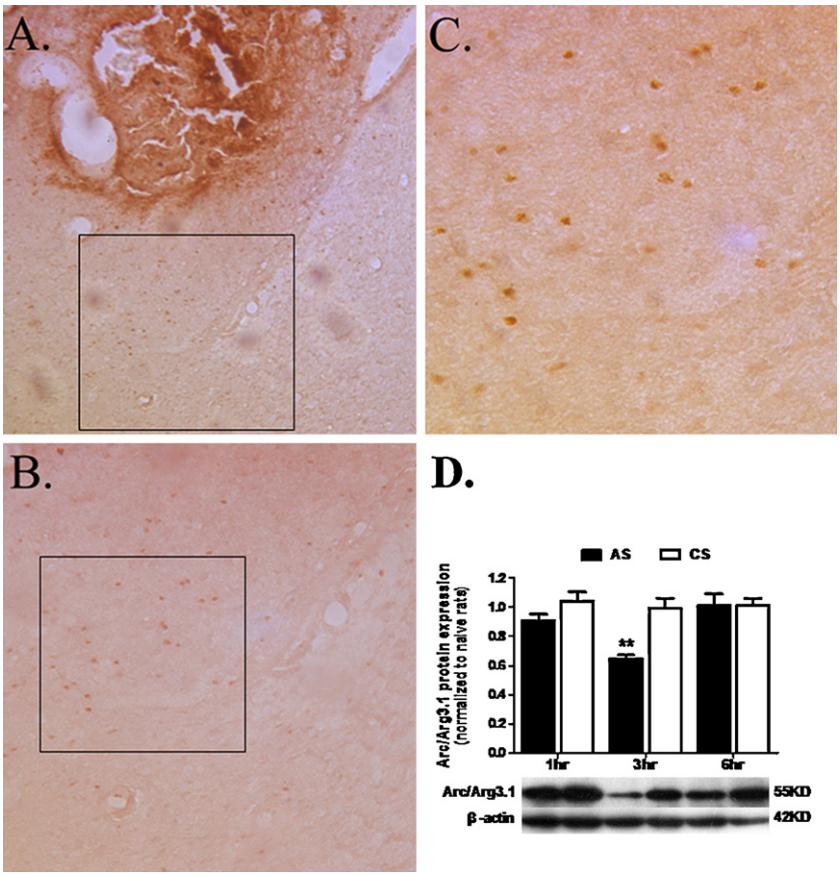

Fig. 4. Arc/Arg3.1 AS/CS (1 nmol/μl/side) knocks down Arc/Arg3.1 protein levels in the NAc core.
(A) Representative photomicrographs of a rat infused with biotinylated Arc/Arg3.1 AS (1 nmol; 1 μl) and sacrificed 3 h later. Note that the biotinylated AS diffusion is restricted to the NAc core. (B and C) Higher magnifications (20× and 40×, respectively) of the boxed regions of the NAc core neurons containing biotinylated AS. (D) Arc/Arg3.1 protein expression from NAc core homogenates of rats sacrificed either 1 ($n=5$), 3 ($n=3$) or 6 ($n=5$) h after infusion of either CS or AS. Data are expressed as the means ± SEM, $^{**}p<0.01$, compared to the CS infused side. AS, microinfused antisense ODN; CS, microinfused scrambled ODN.

received bilateral microinfusions (1 nmol/μl/side) of AS or CS 3 h before the expression test on day 9 (Fig. 6A). When microinfused into the NAc core, statistical analysis (two-way RM ANOVA) revealed a significant interaction effect between treatments and times ($F(3, 36)=5.365$, $p<0.001$). Significant differences in the CPP score were found within groups ($F(3, 36)=8.124$, $p<0.05$) and between the pretest and the test ($F(1, 36)=11.34$, $p<0.01$). Compared to the pretest, the post hoc test showed no significant increases in the CPP score in the expression test in morphine-conditioned rats microinfused with Arc/Arg3.1 AS ($t=1.639$, $p>0.05$), whereas a significant increase in the CPP score was found in the expression test in rats microinfused with CS ($t=4.958$, $p<0.001$) (Fig. 6B).

Similar findings were obtained when Arc/Arg3.1 AS was microinfused into the NAc shell. Statistical analysis (two-way RM ANOVA) revealed a significant interaction effect between treatments and times ($F(3, 36)=2.912$, $p<0.05$). Significant differences in the CPP score were found within groups ($F(3, 36)=8.407$, $p<0.001$) and between the pretest and the test ($F(1, 36)=7.040$, $p<0.05$). Compared to the pretest, the post hoc test showed no significant increases in the CPP score in the expression test in morphine-conditioned rats microinfused with Arc/Arg3.1 AS ($t=1.502$, $p>0.05$), whereas there was a significant increase in the CPP score in the expression test in morphine-conditioned rats microinfused with CS ($t=3.757$, $p<0.01$) (Fig. 6C). These data suggest that Arc/Arg3.1 AS, microinfused into both the NAc core and shell, impaired the expression of morphine CPP.

3.6. Effect of Arc/Arg3.1 AS microinjection into the NAc core or shell on the reinstatement of morphine CPP

In this experiment, rats were divided to morphine- and saline-treated CPP groups, and the CPP conditioning was executed as described above. After the expression test, rats were given 8 days of extinction training and received another CPP test (extinct test) on day 18 (Fig. 7A). Statistical analysis (two-way RM ANOVA) revealed a significant effect from the interaction between treatments and test ($F(2, 172)=24.09$, $p<0.0001$). Significant differences in the CPP score were found both within the pretest, expression test and ($F(2, 172)=26.95$, $p<0.0001$) and between morphine- and saline-treated rats ($F(1, 172)=25.47$, $p<0.0001$). Compared to the saline group, the post hoc test showed a significant increase in the CPP score in the expression test in morphine group ($t=8.571$, $p<0.001$). There was no significant difference in the CPP score in the extinct test in the morphine group compared to the saline group ($t=0.5795$, $p>0.05$). Five out of the 97 rats in this study were excluded because they still presented a conditioned preference for the drug-paired chamber in the extinct test. These data suggested that morphine CPP was successfully extinguished after 8 days of extinction training, and these rats were used in the following experiments (Fig. 7B).

On day 19, rats in both the morphine and the saline CPP groups received bilateral microinfusions (1 nmol/μl/side) of Arc/Arg3.1 AS or CS into the NAc core or shell 3 h before a priming dose of morphine (2.5 mg/kg) injection and following the priming test. Following microinfusions into the NAc core, statistical analysis (two-way RM ANOVA) revealed a significant effect from the inter-

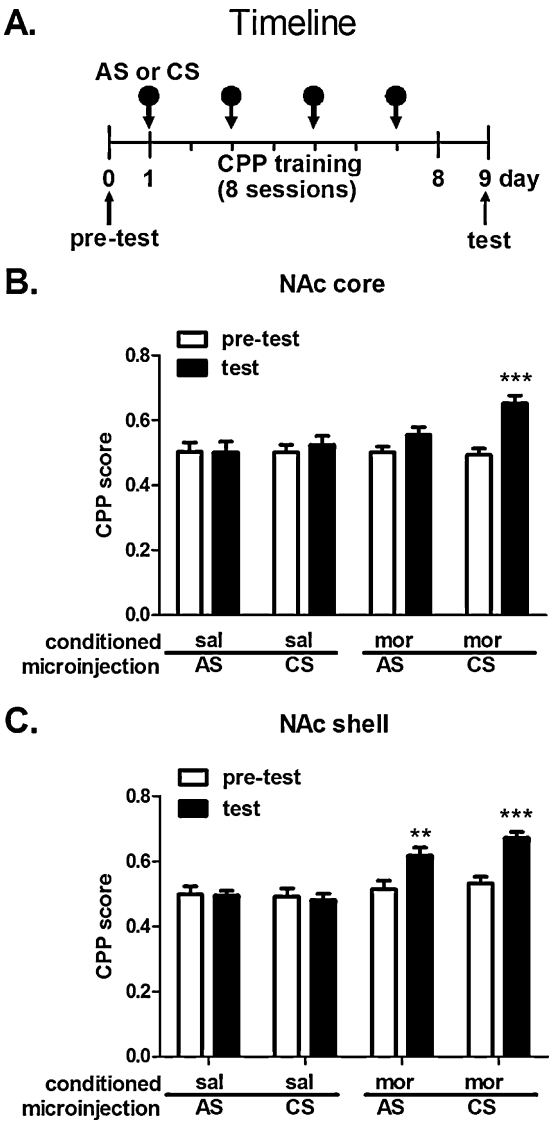

Fig. 5. Effect of Arc/Arg3.1 AS/CS (1 nmol/μl/side) microinfused into the NAc on the acquisition of morphine-induced CPP.
(A) Diagram outlining the behavioral procedures. (B) Arc/Arg3.1 AS (but not CS) microinjection into the NAc core blocked the acquisition of morphine CPP and had no effect on saline CPP. (C) Arc/Arg3.1 AS and CS microinjections into the NAc shell had no effect on the acquisition of morphine or saline CPP. Data are expressed as the means ± SEM, $n = 8$–10. $^{**}p < 0.01$, $^{***}p < 0.001$, compared to the pretest. AS, microinfused antisense ODN; CS, microinfused scrambled ODN; sal, saline conditioning; mor, morphine conditioning.

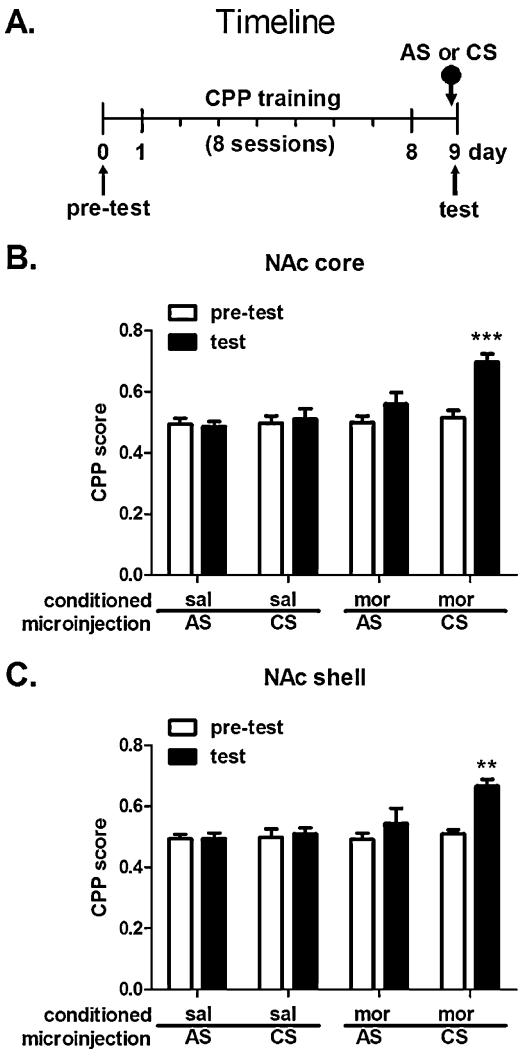

Fig. 6. Effect of Arc/Arg3.1 AS/CS (1 nmol/μl/side) microinfused into the NAc on the expression of morphine-induced CPP.
(A) Diagram outlining the behavioral procedures. (B) Arc/Arg3.1 AS (but not CS) microinjection into the NAc core inhibited the expression of morphine CPP and had no effect on saline CPP. (C) Arc/Arg3.1 AS (but not CS) microinjection into the NAc shell inhibited the expression of morphine CPP and had no effect on saline CPP. Data are expressed as the means ± SEM, $n = 10$. $^{**}p < 0.01$, $^{***}p < 0.001$, compared to the pretest. AS, microinfused antisense ODN; CS, microinfused scrambled ODN; sal, saline conditioning; mor, morphine conditioning.

action between treatments and times ($F(3, 31) = 3.250$, $p < 0.05$). Significant differences in the CPP score were found both within groups ($F(3, 31) = 6.845$, $p < 0.01$) and between the extinct test and the priming test ($F(1, 31) = 5.225$, $p < 0.05$). Compared to the previous extinct test, the Bonferroni post hoc test showed no significant increase in the CPP score in the priming test in morphine-conditioned rats microinfused with Arc/Arg3.1 AS ($t = 1.308$, $p > 0.05$), while a significant increase in the CPP score in the priming test was found in morphine-conditioned rats with CS ($t = 3.665$, $p < 0.01$) (Fig. 7C).

Following microinjection of Arc/Arg3.1 AS/CS (1 nmol/μl/side) into the NAc shell, statistical analysis revealed a significant effect from the interaction between treatments and times ($F(3, 29) = 6.419$, $p < 0.01$). Significant differences in the CPP score were found within groups ($F(3, 29) = 5.324$, $p < 0.01$) and between the extinct test and the priming test ($F(1, 29) = 12.35$, $p < 0.01$). Compared to the extinct test, the post hoc test showed that the CPP score was significantly increased in the priming test in the morphine CPP group following both Arc/Arg3.1 AS ($t = 3.998$, $p < 0.01$) and CS ($t = 4.280$, $p < 0.001$) microinfusions (Fig. 7D). These data suggest that Arc/Arg3.1 AS microinjection into the NAc core, but not the shell, blocked the reinstatement of morphine CPP by a low priming dose of morphine after extinction.

4. Discussion

4.1. A single morphine injection increased Arc/Arg3.1 protein content in the NAc core

Although Arc/Arg3.1 is an effector IEG induced by many psychostimulants and cocaine-associated reward memory [34–36], to our knowledge, its induction following morphine-associated reward memory has not been previously investigated. Additionally, little is known about the function of this protein in drug-induced long-term memory. In this study, we demonstrated for the first time that a single dose of morphine increases Arc/Arg3.1 protein

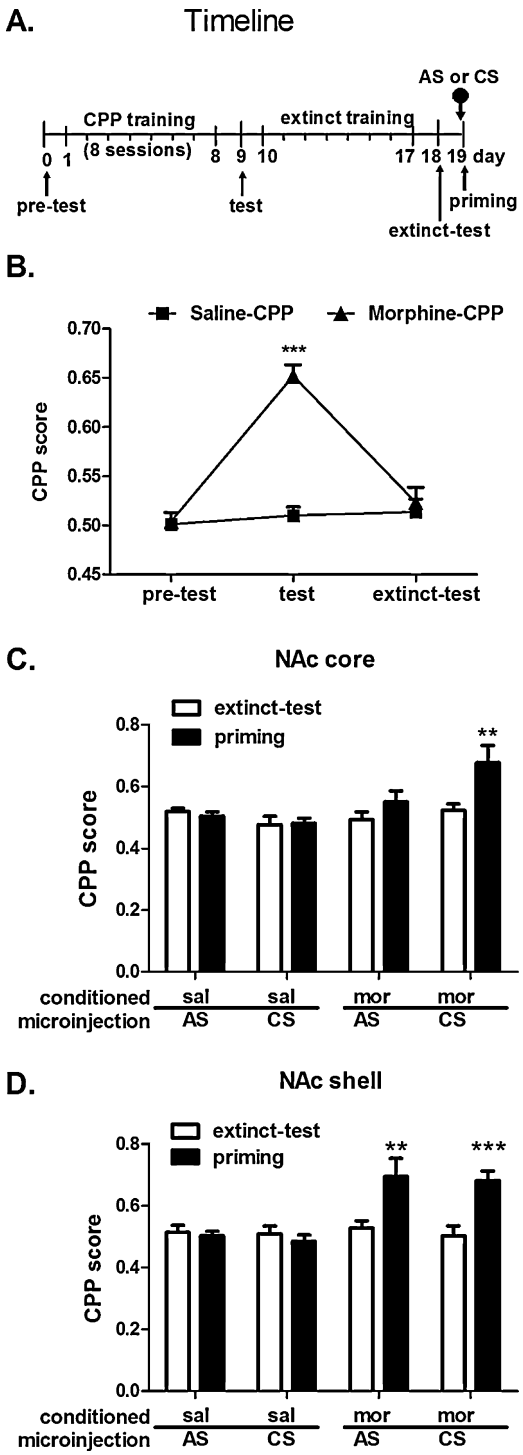

Fig. 7. Effect of Arc/Arg3.1 AS/CS (1 nmol/μl/side) microinfused into the NAc on the reinstatement of morphine-induced CPP.(A) Diagram outlining the behavioral procedures. (B) All groups of rats established morphine CPP after 8 days of morphine (5 mg/kg) conditioning. Rats did not show place preference following 8 days of extinction training. Data are expressed as the means ± SEM, $n = 42–46$. $^{***}p < 0.001$, compared to the saline CPP group. (C) Arc/Arg3.1 AS (but not CS) microinjection into the NAc core inhibited the reinstatement (2.5 mg/kg morphine priming) of morphine CPP and had no effect on saline CPP. (D) Arc/Arg3.1 AS and CS microinjections into the NAc shell had no effect on the reinstatement (2.5 mg/kg morphine priming) of morphine CPP or saline CPP. Data are expressed as the means ± SEM, $n = 7–10$ per group. $^{**}p < 0.01$, $^{***}p < 0.001$, compared to the extinct test. AS, microinfused antisense ODN; CS, microinfused scrambled ODN; sal, saline conditioning; mor, morphine conditioning.

expression at 2 h post-injection in the NAc core, but not in the NAc shell, of the rat. There were no time- or dose-dependent effects. The current results are similar to previous studies showing that a single injection of cocaine or amphetamine increases Arc/Arg3.1 protein expression in the striatum at 2 h post-injection [37,38]. In contrast, acute injection of morphine produced a marked stimulation of Arc/Arg3.1 protein expression in the striatum of mice at 4 h post-injection [39]. Administration of morphine (10 mg/kg s.c.) alone failed to induce an increase in Arc/Arg3.1 protein expression in the PFC, striatum, amygdala and hippocampus [40]. The use of different species, different opiate administration paradigms and different harvest time points in these studies might result in different changes in Arc/Arg3.1 protein expression in response to a single dose of morphine. Our present results suggest that increases in Arc/Arg3.1 protein expression in the NAc core following a single dose of morphine are probably involved in the changes in synaptic plasticity required for drug-associative learning. We therefore explored the role of Arc/Arg3.1 protein expression in the NAc in morphine-associated learning and memory using the CPP model.

4.2. Retrieval of morphine CPP selectively increased Arc/Arg3.1 protein levels in the NAc shell

Previous studies found that Fos protein levels were significantly elevated in the NAc shell after the expression of morphine-conditioned behavior [41]. Further, injections of the D1 receptor antagonist SCH23390 into the lateral or medial shell, but not the core, decreased the context-induced reinstatement of heroin seeking [42]. Therefore, the NAc shell is not only activated first after the expression test of morphine CPP but also plays an essential role in the context-induced relapse to drug seeking. The results from our experiments revealed that, when re-exposed to the context after 8 days of conditioning, rats exhibited significant place preference for the morphine-paired chamber, which was accompanied by an increase in Arc/Arg3.1 protein expression in the NAc shell but not in the NAc core. The elevation of Arc/Arg3.1 protein in the morphine-conditioned and test groups was the result of morphine conditioning and context-induced retrieval and not due to morphine treatments alone or context re-exposure. The unconditioned animals and the conditioned animals without re-exposure did not show increased Arc/Arg3.1 protein expression on the test day. This finding is similar to previous studies showing the cue-induced reinstatement of heroin self-administration and a significant increase in Arc/Arg3.1 mRNA in the NAc [43]. Due to these findings, we hypothesized that Arc/Arg3.1 protein expression in the NAc shell might play a role in the context-induced relapse of drug seeking.

4.3. Arc/Arg3.1 protein expression in the NAc subregions has different effects on the different stages of morphine CPP

Some studies have demonstrated that Arc/Arg3.1 is involved in spatial memory (Morris water maze), Pavlovian fear memory, object-recognition memory and alcohol addiction memory [24,26]. To demonstrate that Arc/Arg3.1 plays a critical role in morphine-associated addiction memory, we used Arc/Arg3.1 AS to knock down Arc/Arg3.1 protein levels in the NAc core or shell during the acquisition, expression and reinstatement of morphine-induced CPP, which are three important phases of classical learning and memory. The AS was designed following guidelines described by Guzowski et al. Our results showed that the most effective time point of AS administration was 3 h after the AS microinjection, rather than 6 h as previously reported [21]. This discrepancy is most likely due to the differences of the purifying method used, i.e., the high performance liquid chromatography used in this study (HPLC) versus the gel filtration method used previously.

Our present results showed that Arc/Arg3.1 protein expression in the NAc core is essential for the acquisition, expression and reinstatement of morphine CPP. Additionally, Arc/Arg3.1 protein expression in the NAc shell was only critical for the expression of morphine-induced CPP. Similar to our study, previous studies that lesioned NAc subregions revealed that the NAc core plays a critical role in the acquisition, retrieval and reinstatement of morphine-induced CPP after extinction, whereas the shell was shown to be more important than the core for the expression of morphine-induced CPP [44,45]. Our results suggest that the NAc shell plays a greater role in the context-induced expression of morphine-induced CPP than in acquisition or reinstatement. Conversely, intra-NAc shell infusions of the D1 receptor antagonist SCH 39166 and the D2 receptor antagoniss L-sulpiride impaired the acquisition, but not expression, of morphine-conditioned single-trial place preference [46]. Although experimental differences may account for these apparently conflicting findings, it is more likely that the core and the shell of the NAc each contribute to the acquisition, expression and reinstatement of drug seeking.

As summarized above, a single dose of morphine elevated Arc/Arg3.1 expression in the NAc core, whereas Arc/Arg3.1 expression was only increased in the NAc shell (not core) after the expression test of morphine CPP. Furthermore, the antisense infusions of Arc/Arg3.1 into either the shell or the core blocked the expression of morphine CPP. Previous studies on the neuroanatomical and neurochemical features of the NAc core and shell have revealed that the basal level of extracellular DA is about three times greater in core than in the shell, whereas the neurochemical milieu is more diverse and more sensitive to a variety of pharmacological and physiological stimuli in the shell than in the core [47]. In addition, the activation of NMDA receptors is necessary for the transcription of the Arc/Arg3.1 gene [16]. Additionally, Arc/Arg3.1 mRNA has been shown to be induced by cocaine in the striatum through a dopamine D1 receptor-dependent mechanism [37]. Therefore, it is possible that the NAc core first responds to DA that is released into the NAc following a single dose of morphine, and Arc/Arg3.1 protein levels are increased following dopamine D1 receptor activation. The DA released into the NAc core, which was induced by a 5-mg/kg injection of morphine, was sufficient to increase Arc/Arg3.1 protein expression; no greater effects were observed when the dosage was increased to 10 or 20 mg/kg.

It has been shown that the CPP expression test affects various neurotransmitters and the normal function of the NAc shell is "highjacked" by them. This may explain why Arc/Arg3.1 expression is increased only in the NAc shell (not core) through D1, NMDA and/or the activation of other receptors. However, the existence of an interaction between neurons projecting between the shell and the core suggests that significant coordination of the levels of activity in these two subregions may exist [48]. This may explain why antisense infusions of Arc/Arg3.1 into either the shell or the core block expression of the morphine CPP.

Further, the present study found that Arc/Arg3.1 protein expression in the NAc played a critical role in the acquisition, expression and reinstatement of morphine-associated reward memory. Other studies on the roles of "effector" immediate early genes (IEGs) have implicated these IEGs, such as Narp and Homer1/2, in drug dependence. Reti et al. have reported that Narp mediates the long-term, aversive behavioral effects induced by opiate withdrawal, whereas Narp knockout mice are markedly resistant to the extinction of morphine CPP [49,50]. In addition, the deletion of Homer1 or Homer2 in mice significantly increased the sensitivity to cocaine-induced locomotor activity, conditioned reward and augmented extracellular glutamate in the nucleus accumbens [51]. These findings, in addition to our findings, indicate that the "effector" IEGs play a critical role in drug addiction. As the effector IEGs plays a central role in synaptic activity and have no effect on the expression of other genes, local disruption of the "effector" IEGs during drug usage and drug addiction may prevent the development or relapse of drug addiction.

In conclusion, the present study demonstrated that Arc/Arg3.1 protein expression in the NAc core is critical for the acquisition, expression and reinstatement of morphine CPP, whereas the Arc/Arg3.1 protein expression in the NAc shell is only required for the expression of morphine-induced CPP. These findings suggest that the protein expression of Arc/Arg3.1, an "effector" IEG, plays a central role in the morphine-conditioned memory processes in the NAc.

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References

[1] De Vries TJ, Shippenberg TS. Neural systems underlying opiate addiction. Journal of Neuroscience 2002;22:3321–5.
[2] Lisman JE, Grace AA. The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron 2005;46:703–13.
[3] 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 in Neurosciences 2010;33:267–76.
[4] Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, et al. Transition to addiction is associated with a persistent impairment in synaptic plasticity. Science (New York, NY) 2010;328:1709–12.
[5] Mameli M, Halbout B, Creton C, Engblom D, Parkitna JR, Spanagel R, et al. Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc. Nature Neuroscience 2009;12:1036–108.
[6] Jongenrelo AL, Voorn P, Groenewegen HJ. Immunohistochemical characterization of the shell and core territories of the nucleus-accumbens in the rat. European Journal of Neuroscience 1994;6:1255–64.
[7] Meredith GE, Agolia R, Arts MP, Groenewegen HJ, Zahm DS. Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. Neuroscience 1992;50:149–62.
[8] Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the accumbens part of the rat ventral striatum – immunohistochemical detection of retrogradely transported fluorogold. Journal of Comparative Neurology 1993;338:255–78.
[9] Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C. Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 1991;41:89–125.
[10] Di Ciano P, Everitt BJ. Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. Neuropsychopharmacology 2001;25:341–60.
[11] Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ. Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. Behavioral Neuroscience 2000;114:42–63.
[12] Morgan JI, Cohen DR, Hempstead JL, Curran T. Mapping patterns of C-Fos expression in the central-nervous-system after seizure. Science 1987;237:192–7.
[13] Worley PF, Bhat RV, Baraban JM, Erickson CA, Mcnaughton BL, Barnes CA. Thresholds for synaptic activation of transcription factors in hippocampus – correlation with long-term enhancement. Journal of Neuroscience 1993;13:4776–86.
[14] Moron JA, Gullapalli S, Taylor C, Gupta A, Gomes I, Devi LA. Modulation of opiate-related signaling molecules in morphine-dependent conditioned behavior: conditioned place preference to morphine induces CREB phosphorylation. Neuropsychopharmacology 2010;35:955–66.
[15] Burke WJ, Li SW, Zahm DS, Macarthur H, Kolo LL, Westfall TC, et al. Catecholamine monoamine oxidase a metabolite in adrenergic neurons is cytotoxic in vivo. Brain Research 2001;891:218–27.
[16] Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, et al. Arc, a growth-factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 1995;14:433–45.
[17] Waddell S, Jenkins JR. arg3+, a new selection marker system for Schizosaccharomyces pombe: application of ura4+ as a removable integration marker. Nucleic Acids Research 1995;23:1836–7.
[18] Steward O. Local synthesis of proteins at synaptic sites on dendrites: role in synaptic plasticity and memory consolidation? Neurobiology of Learning and Memory 2002;78:508–27.

[19] Steward O, Wallace CS, Lyford GL, Worley PF. Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron 1998;21:741–51.
[20] Steward O, Worley PF. A cellular mechanism for targeting newly synthesized mRNAs to synaptic sites on dendrites. Proceedings of the National Academy of Sciences of the United States of America 2001;98:7062–8.
[21] Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McGaugh JL, Worley PF, et al. Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. Journal of Neuroscience 2000;20:3993–4001.
[22] Waung MW, Pfeiffer BE, Nosyreva ED, Ronesi JA, Huber KM. Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron 2008;59:84–97.
[23] Pandey SC, Zhang HB, Ugale R, Prakash A, Xu TJ, Misra K. Effector immediate-early gene Arc in the amygdala plays a critical role in alcoholism. Journal of Neuroscience 2008;28:2589–600.
[24] Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C, et al. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 2006;52:437–44.
[25] McIntyre CK, Miyashita T, Setlow B, Marjon KD, Steward O, Guzowski JF, et al. Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 2005;102:10718–23.
[26] Ploski JE, Pierre VJ, Smucny J, Park K, Monsey MS, Overeem KA, et al. The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for memory consolidation of pavlovian fear conditioning in the lateral amygdala. Journal of Neuroscience 2008;28:12383–95.
[27] Freeman WM, Patel KM, Brucklacher RM, Lull ME, Erwin M, Morgan D, et al. Persistent alterations in mesolimbic gene expression with abstinence from cocaine self-administration. Neuropsychopharmacology 2008;33:1807–17.
[28] Marie-Claire C, Courtin C, Roques BP, Noble F. Cytoskeletal genes regulation by chronic morphine treatment in rat striatum. Neuropsychopharmacology 2004;29:2208–15.
[29] Chase T, Carrey N, Soo E, Wilkinson M. Methylphenidate regulates activity regulated cytoskeletal associated but not brain-derived neurotrophic factor gene expression in the developing rat striatum. Neuroscience 2007;144:969–84.
[30] Ma YY, Guo CY, Yu P, Lee DY, Han JS, Cui CL. The role of NR2B containing NMDA receptor in place preference conditioned with morphine and natural reinforcers in rats. Experimental Neurology 2006;200:343–55.
[31] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 3rd ed. Amsterdam: Elsevier Academic; 1997.
[32] German PW, Fields HL. Rat nucleus accumbens neurons persistently encode locations associated with morphine reward. Journal of Neurophysiology 2007;97:2094–106.
[33] Mueller D, Perdikaris D, Stewart J. Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. Behavioural Brain Research 2002;136:389–97.
[34] Hearing MC, Schochet TL, See RE, Mcginty JF. Context-driven cocaine-seeking in abstinent rats increases activity-regulated gene expression in the basolateral amygdala and dorsal hippocampus differentially following short and long periods of abstinence. Neuroscience 2010;170:570–9.
[35] Fumagalli F, Franchi C, Caffino L, Racagni G, Riva MA, Cervo L. Single session of cocaine intravenous self-administration shapes goal-oriented behaviours and up-regulates Arc mRNA levels in rat medial prefrontal cortex. International Journal of Neuropsychopharmacology 2009;12:423–9.
[36] Hearing MC, See RE, McGinty JF. Relapse to cocaine-seeking increases activity-regulated gene expression differentially in the striatum and cerebral cortex of rats following short or long periods of abstinence. Brain Structure & Function 2008;213:215–27.
[37] Fumagalli F, Bedogni F, Frasca A, Di Pasquale L, Racagni G, Riva MA. Corticostriatal up-regulation of activity-regulated cytoskeletal-associated protein expression after repeated exposure to cocaine. Molecular Pharmacology 2006;70:1726–34.
[38] Fosnaugh JS, Bhat RV, Yamagata K, Worley PF, Baraban JM. Activation of Arc, a putative effector immediate-early gene, by cocaine in rat-brain. Journal of Neurochemistry 1995;64:2377–80.
[39] Ziolkowska B, Urbanski MJ, Wawrzczak-Bargiela A, Bilecki W, Przewlocki R. Morphine activates arc expression in the mouse striatum and in mouse neuroblastoma Neuro2A MOR1A cells expressing mu-opioid receptors. Journal of Neuroscience Research 2005;82:563–70.
[40] Li M, Hou Y, Lu B, Chen J, Chi Z, Liu J. Expression pattern of neural synaptic plasticity marker-Arc in different brain regions induced by conditioned drug withdrawal from acute morphine-dependent rats. Acta Pharmacologica Sinica 2009;30:282–90.
[41] Harris GC, Aston-Jones G. Enhanced morphine preference following prolonged abstinence: association with increased Fos expression in the extended amygdala. Neuropsychopharmacology 2003;28:292–9.
[42] Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. Journal of Neuroscience 2007;27:12655–63.
[43] Kuntz KL, Patel KM, Grigson PS, Freeman WM, Vrana KE. Heroin self-administration: II. CNS gene expression following withdrawal and cue-induced drug-seeking behavior. Pharmacology Biochemistry and Behavior 2008;90:349–56.
[44] Yu Peng XW, Cui Cailian. Effects of electrolytic lesion of the NAc core or shell on morphine induced reward and sexual ability in rats. Chinese Journal of Drug Dependence 2007;16:341–6.
[45] Wang J, Zhao Z, Liang Q, Wang X, Chang C, Wang J, et al. The nucleus accumbens core has a more important role in resisting reactivation of extinguished conditioned place preference in morphine-addicted rats. Journal of International Medical Research 2008;36:673–81.
[46] Fenu S, Spina L, Rivas E, Longoni R, Di Chiara G. Morphine-conditioned single-trial place preference: role of nucleus accumbens shell dopamine receptors in acquisition, but not expression. Psychopharmacology (Berl) 2006;187:143–53.
[47] Zahm DS. Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Annals of the New York Academy of Sciences 1999;877:113–28.
[48] Dalley JW, Everitt BJ, Robbins TW. Impulsivity, compulsivity, and top-down cognitive control. Neuron 2011;69:680–94.
[49] Reti IM, Baraban JM. Opiate withdrawal induces Narp in the extended amygdala. Neuropsychopharmacology 2003;28:1606–13.
[50] Crombag HS, Dickson M, Dinenna M, Johnson AW, Perin MS, Holland PC, et al. Narp deletion blocks extinction of morphine place preference conditioning. Neuropsychopharmacology 2009;34:857–66.
[51] Szumlinski KK, Dehoff MH, Kang SH, Frys KA, Lominac KD, Klugmann M, et al. Homer proteins regulate sensitivity to cocaine. Neuron 2004;43:401–13.