NAc Shell Arc/Arg3.1 Protein Mediates Reconsolidation of Morphine CPP by Increased GluR1 Cell Surface Expression: Activation of ERK-Coupled CREB is Required

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Abstract

Background: Relapse into drug abuse evoked by reexposure to the drug-associated context has been a primary problem in the treatment of drug addiction. Disrupting the reconsolidation of drug-related context memory would therefore limit the relapse susceptibility.

Methods: Morphine conditioned place preference (CPP) was used to assess activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) and correlative molecule expression in the Nucleus accumbens (NAc) shell during the reconsolidation of morphine CPP. U0126 and Arc/Arg3.1 antisense oligodeoxynucleotide were adapted to evaluate the role and the underlying mechanism of Arc/Arg3.1 during the reconsolidation.

Results: The retrieval of morphine CPP in rats specifically increased the Arc/Arg3.1 protein level in the NAc shell, accompanied simultaneously by increases in the phosphorylation of extracellular signal-regulated kinase1/2 (pERK1/2), the phosphorylation of Cyclic Adenosine monophosphate (cAMP) response element-binding (pCREB), and the up-regulation of the membrane α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors GluR1 subunit level. Intra-NAc shell infusion U0126, an inhibitor of the Mitogen-activated protein kinase (MEK), prevented the retrieval-induced up-regulation of pERK1/2, pCREB, Arc/Arg3.1, and membrane GluR1 immediately after retrieval of morphine CPP. The effect of disrupting the reconsolidation of morphine CPP by U0126 could last for at least 14 days, and could not be evoked by a priming injection of morphine. Furthermore, the specific knockdown of Arc/Arg3.1 in the NAc shell decreased the membrane GluR1 level, and impaired both the reconsolidation and the reinstatement of morphine CPP.

Conclusions: Arc/Arg3.1 in the NAc shell mediates the reconsolidation of morphine-associated context memory via up-regulating the level of membrane of GluR1, for which the local activation of the ERK-CREB signal pathway, as an upstream mechanism of Arc/Arg3.1, is required.

Keywords: Activity regulated cytoskeleton-associated protein/activity-regulated gene 3.1, extracellular signal-regulated kinase, cAMP response element-binding protein, GluR1, conditioned place preference, reconsolidation
Introduction

Repeated drug administration can develop an intense associative memory between drug and drug-related contexts. Once the association is learned, the drug users, whether humans or laboratory animals, can engage in drug-seeking behavior without drugs being present when they are reexposed to the context (O’Brien et al., 1992; Shaham et al., 2003). The contextual cues–induced relapse to drug abuse among addicts has become a major challenge to the treatment of drug addiction. Similar to other types of memories, drug-associated memory can be retrieved or reactivated and return to a labile, sensitive state. This reactivated memory often undergoes another process of consolidation, called reconsolidation, to be restabilized (Misanin et al., 1968; Nader, 2003). One of the animal models often used to study this context-induced drug seeking is morphine conditioned place preference (CPP), analogous to the drug environment associations that could trigger relapse in humans (O’Brien et al., 1992). During the conditioning phase, animals receive a drug in one context and vehicle in another to acquire a drug-associated memory. Following conditioning, memory can be retrieved when the animals are reexposed to the contexts in a drug-free state, and then entered into the process of reconsolidation (Bardo et al., 1995).

In previous studies, the molecular mechanisms underlying memory reconsolidation have been focused on de novo protein-synthesis, transcription factors implication, signal-pathway activation, and immediate-early gene (IEG) expression (Tronson and Taylor, 2007). In comparison, pharmacological options, such as protein synthesis inhibitor anisomycin, administered after reactivation of cocaine or morphine CPP, impaired drug seeking via disruption of the reconsolidation (Milekic et al., 2006; Lin et al., 2014). Apparently, drug-related memory can be inhibited or erased by interrupting its reconsolidation process. Although a growing body of studies have demonstrated that the reconsolidation of addiction memory after recall may rely on common neurobiological mechanisms, including N-methyl-D-aspartic acid-receptor driven protein kinase signal cascades, up-regulation of transcriptional factors, and temporal regulation of mRNA and protein synthesis (Tronson and Taylor, 2007; Nader and Einarsson, 2010), little is known about the specific downstream genes that are critical for the process.

Activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) is a particularly interesting IEG that may be induced by neuronal activity and then impact synaptic plasticity (Lyford et al., 1995; Steward et al., 1998). Previous studies have shown that Arc/Arg3.1 protein is involved in multiple forms of synaptic plasticity, including long-term potentiation, long-term depression, and the stable expansion of dendritic spines (Guzowski et al., 2000; Pandey et al., 2008; Waung et al., 2008). And Arc/Arg3.1 likely underlies the consolidation and reconsolidation of some forms of memory, including spatial memory, fear conditioning, and object recognition (Steward, 2002; Gusev and Gubin, 2010; Chia and Otto, 2013). Recently, the Arc/Arg3.1 protein has been reported to be involved in the molecular mechanisms of drug addiction (Hearing et al., 2011). Also, our previous study showed that Arc/Arg3.1 protein in the NAc shell is specifically designed to explore the effects of NAc-shell Arc/Arg3.1 in the memory reconsolidation of morphine-associated context using morphine-induced CPP.

Materials and Methods

Animals

Male Sprague Dawley rats, weighing 220–240g, 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 were maintained on a reverse light-dark cycle (12:12h, lights on at 19:00h). 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.

Place Preference Apparatus

The CPP apparatus is a black, rectangular polyvinyl chloride box (79.5 cm long × 23.0 cm wide × 25.0 cm high) containing three chambers (A, B, and C) separated by guillotine doors (Lv et al., 2011). 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 numbers of entrances into each of the three compartments were recorded by infrared beam crossings.

Cannula Implantation and Microinfusions

Rats weighing 260–280g 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). Stainless steel guide cannulas (0.67 mm in outer diameter) were bilaterally implanted 1.5 mm above the NAc shell. The coordinates ( Paxinos and Watson, 1997) were anterior/posterior, +1.6 mm; medial/lateral, ±0.9 mm; and dorsal/ventral, −6.5 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 cannula patent and prevent infection. Rats were given at least 5–7 days to recover before the conditioning procedures. The intra-nuclear infusions were performed as previously (Lv et al., 2011).

Oligodeoxynucleotide Design and Preparation

Arc/Arg3.1 antisense oligodeoxynucleotide (AS) and scrambled oligodeoxynucleotide (CS) (Shanghai Sangon Synthesis Technology Services Ltd.) were designed as described by
Conditioned Place Preference

Pretest
On day 0, 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 number of entrances into each of the three compartments were recorded. Fourteen of the initial 280 rats were excluded because of a strong unconditioned preference (>540s). 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.

Conditioning
On day 1, the rats were confined to the corresponding conditioning chambers (A or C) for 30 min immediately after the injection of morphine (5 mg/kg, i.p.) and then returned to their home cages. On day 2, the rats were injected with saline (1 ml/kg, i.p.) and confined to the other conditioning chamber. On the subsequent conditioning days, each rat was trained for 6 consecutive days with alternate injections of morphine and saline. The control group received saline every day. The neutral zone was never used during conditioning and was blocked by guillotine doors.

Test
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 numbers of entrances into each compartment were recorded. The procedure for the test was the same as the procedure for the initial baseline preference assessment, which also acted as a reactivation session (Robinson et al., 2011). The CPP score was defined as the time (in seconds) spent in the drug-paired chamber minus time spent in the saline-paired chamber during CPP testing.

Experimental Design
After the establishment of CPP, rats were randomly divided into three group sets.

Experiment 1
This experiment measured Arc/Arg3.1, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) GluR1 cell-surface expression, and extracellular signal-regulated kinase (ERK) cAMP response element-binding (CREB) activation in the NAc shell during the reconsolidation of morphine CPP. For Western blotting, half of the first group of rats was sacrificed by decapitation 2 h after the morphine CPP test. The other half, which did not conduct the CPP test, was sacrificed at the corresponding time.

Experiment 2
This experiment measured the effect of the MEK inhibitor, U0126, on the reconsolidation of morphine CPP. Drugs were infused using an infusion pump at 0.25 µl/min. A total volume of 0.5 µl of 2 µg/µl U0126 or vehicle was infused into the NAc shell of both the morphine and saline groups of rats immediately after the CPP test on day 9. Then, half of the rats in each group were sacrificed by decapitation 2 h after the microinfusions for Western blotting. The other half had post-CPP tests on day 10 (post-test 1), day 24 (post-test 2), and day 25 (priming 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.].
anti-β-Actin antibody (1:5000; Sigma). After washing three times for 5 min in TBS/0.1% Tween-20, blots were then incubated with anti-rabbit or -mouse secondary antibody conjugated to horseradish peroxidase (1:2000; Zhongshan Biotechnology) 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). 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.

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 Figure 1B and C. Only those rats whose cannulas were correctly placed were used for data analysis.

Drugs

Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang. U0126 was purchased from Upstate. Morphine was dissolved in 0.9% saline and U0126 was dissolved in dimethyl sulfoxide (DMSO; Sigma) and diluted to 2 µg/µl in 5% DMSO and 6% Tween 80 in 1× phosphate buffer saline (PBS) before use. BS3® was purchased from Thermo Fisher Scientific Inc.

Statistical Analysis

Data were expressed as the means ± standard error of the mean and analyzed using analyses of variance (ANOVA) with the appropriate between- and within-subjects factors for the different experiments. One-way ANOVAs were used to analyze the Arc/Arg3.1 and GluRs protein levels. Post hoc analyses of significant effects from the ANOVA were performed using the Bonferroni test. The results from the CPP test and other immunoblotting experiments were analyzed with two-way ANOVAs followed by the Bonferroni post-test. Data were processed using Graph Pad Prism 5.0 software. Statistical significance was set to p < 0.05.

Results

Changes of Arc/Arg3.1 Protein, AMPA GluR1 Cell-Surface Expression Level and ERK, CREB Phosphorylation in NAc Shell During the Reconsolidation of Morphine CPP

Following 8 days of alternative morphine and saline or saline conditioning alone, rats underwent the CPP expression test on day 9 (Figure 2A). Memory was retrieved when reexposed to the contexts in a drug-free state, and then entered into the process of reconsolidation. Statistical analysis (two-way ANOVA) revealed a significant effect from the interaction [F(1, 36) = 33.39; p < 0.001] between the treatments (saline vs. morphine) and the tests (pretest vs. test). Significant differences were found between saline- and morphine-treated rats [F(1, 36) = 36.24, p < 0.001] and within the pretest and the test [F(1, 36) = 42.49, p < 0.0001]. 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 = 8.343, p < 0.001; Figure 2B).

To evaluate the Arc/Arg3.1 protein level, AMPA GluR1 cell-surface expression level, and ERK-CREB phosphorylation in the NAc shell during the reconsolidation of morphine CPP, we designated test and no-test as the between-subjects factors, and saline and morphine treatments as within-subjects factors. Rats were sacrificed by decapitation 2 h after the test for Western blotting. Statistical analysis (two-way ANOVA) revealed a significant effect from the interaction [F(1, 16) = 6.712, p < 0.05] between the tests (test vs. no-test) and treatments (saline vs. morphine) in Arc/Arg3.1 protein expression. Significant differences were found between tests [F(1, 16) = 15.04, p < 0.01] and within treatments [F(1, 16) = 8.92, 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 = 8.343, p < 0.001; Figure 2B).

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Data are expressed as the means ± SEM, n = 5. *p < 0.05; **p < 0.01; ***p < 0.001, compared to the saline-treated group. (C) Reexposure to the morphine conditioning context induces drug seeking, increases activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) protein levels, and activates extracellular signal-regulated kinase (ERK) cAMP response element-binding (CREB) in the NAc shell. (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 ± standard error of the mean (SEM), n = 10. **p < 0.001, compared to the saline-treated group. 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as well as in post-test 2 \((t = 9.28, p < 0.001)\) and the priming test \((t = 1.27, p < 0.001; \text{Figure } 4B)\). These results suggested that the NAc shell, microinjected with U0126, can block the reconsolidation of morphine CPP for at least 14 days and cannot be reversed by a 2.5 mg/kg morphine priming injection. Two hours after the bilateral infusions of the MEK1/2 inhibitor U0126 or its vehicle to the NAc shell, rats were killed for analysis of ERK1/2 and CREB phosphorylation, Arc/Arg3.1 protein level, and AMPA GluR1 cell-surface expression level in the NAc shell. For ERK1/2 phosphorylation, statistical analysis (two-way ANOVA) revealed a significant effect from the interaction between conditionings and treatments \[F(1, 20) = 6.06, p < 0.05\]. Significant differences were found between the U0126 and its vehicle microinfusions \[F(1, 20) = 7.91, p < 0.05\] and within saline and morphine conditions \[F(1, 20) = 8.778, p < 0.01\]. The Bonferroni post hoc test demonstrated that microinjected U0126 significantly decreased the pERK1/2 level in the NAc shell in morphine-conditioned rats compared to those rats microinjected with the vehicle \((t = 3.73, p < 0.01; \text{Figure } 4C)\). For CREB phosphorylation, there were significant main effects between treatments \[F(1, 20) = 8.63, p < 0.01\] and within conditions \[F(1, 20) = 4.48, p < 0.05\], as well as a significant condition and treatment interaction \[F(1, 20) = 6.936, p < 0.05\]. The Bonferroni post hoc test demonstrated that U0126 can significantly reduce the amount of pCREB \((t = 3.94, p < 0.01; \text{Figure } 4D)\). Importantly, as \text{Figure } 4E shows, statistical analysis revealed a significant effect from the interaction between the treatments and the conditionings in Arc/Arg3.1 expression \[F(1, 20) = 6.13, p < 0.05\]. Significant differences were found between U0126 and its vehicle microinfusions \[F(1, 20) = 8.03, p < 0.01\] and within the saline and morphine conditions \[F(1, 20) = 10.72, p < 0.01\]. The Bonferroni post hoc test demonstrated that intra-NAc shell infusions of U0126 significantly decreased the Arc/Arg3.1 protein level in morphine-conditioned rats compared to those rats microinjected with the vehicle \((t = 3.767, p < 0.01)\). Finally, we detected the AMPA GluR1 cell-surface expression level in the NAc shell after the U0126 microinfusion. Statistical analysis (two-way ANOVA) revealed significant differences between the U0126 and its vehicle microinfusions \[F(1, 16) = 5.50, p < 0.05\] and within the saline and morphine conditions \[F(1, 16) = 6.69, p < 0.05\]. The Bonferroni post hoc test demonstrated that microinjected U0126 into the NAc shell significantly decreased the AMPA GluR1 cell-surface expression level in morphine-conditioned rats compared to those microinjected with the vehicle \((t = 3.11, p < 0.05; \text{Figure } 4F)\). These data suggested that the ERK-CREB signaling pathway was the upstream mechanism of Arc/Arg3.1 protein expression and AMPA GluR1
cell-surface expression in the NAc shell during the reconsolidation of morphine CPP.

**Effect of Arc/Arg3.1 AS on AMPA GluR1 Cell-Surface Expression and the Reconsolidation of Morphine CPP**

In this experiment, rats were divided into morphine- and saline-treated CPP groups. After 8 days of CPP conditioning, rats received bilateral microinfusions (1 nmol/µl/side) of AS or CS to the NAc shell immediately after the test on day 9. Then, half of rats were sacrificed by decapitation 2 h after the microinfusions for Western blotting and the other half received post-CPP tests on day 10, day 24, and day 25 (Figure 5A). Statistical analysis (two-way ANOVA) revealed a significant interaction effect between treatments and times \[F(12, 128) = 15.47, p < 0.0001\]. Significant differences in the CPP scores were found between AS and CS treatments \[F(3, 160) = 50.31, p < 0.0001\] and within the different tests \[F(4, 128) = 14.54, p < 0.0001\]. In the expression test, the post hoc test showed a significant increase in the CPP score in morphine-conditioned rats microinfused with either Arc/Arg3.1 AS (t = 7.60, p < 0.001) or CS (t = 7.94, p < 0.001) when compared to the saline groups. In post-test 1, the CPP score decreased in morphine-conditioned rats microinfused with Arc/Arg3.1 AS compared to CS (t = 8.55, p < 0.001), as well as in post-test 2 (t = 8.66, p < 0.001) and the priming test (t = 9.32, p < 0.001; Figure 5B). These data suggested that Arc/Arg3.1 AS, microinfused into the NAc shell, impaired reconsolidation of morphine CPP at least for 14 days and cannot be reversed by 2.5 mg/kg morphine injection.

On day 9, half the rats were sacrificed 2 hours after the bilateral infusions of the Arc/Arg3.1 AS or CS to the NAc shell. The other half of the rats were sacrificed by decapitation for analysis of Arc/Arg3.1 protein level, AMPA, and GluR1 cell-surface expression level in the NAc shells. For the Arc/Arg3.1 protein, statistical analysis (one-way ANOVA) revealed significant differences among groups \[F_{(3, 31)} = 10.42, p < 0.0001\]. Compared to the saline group, the Bonferroni post hoc test showed that the Arc/Arg3.1 protein level was increased in morphine CPP rats (t = 3.70, p < 0.01). In morphine CPP groups, the Arc/Arg3.1 protein level was decreased in rats microinfused with Arc/Arg3.1 AS (t = 4.03, p < 0.01) and CS (t = 4.18, p < 0.01; Figure 5C) compared to those without microinfusion. For AMPA GluR1 cell-surface expression, statistical analysis (one-way ANOVA) revealed significant differences among groups \[F_{(3, 31)} = 5.26, p < 0.01\]. The Bonferroni post hoc test showed a significant increase in the AMPA GluR1 cell-surface expression level in morphine CPP rats compared to rats in the saline CPP group (t = 2.91, p < 0.05). In morphine CPP groups, the AMPA GluR1 cell-surface expression level was decreased in rats microinfused with Arc/Arg3.1 AS (t = 3.36, p < 0.05) and or CS (t = 3.18, p < 0.05; Figure 5D) compared to those without microinfusion. These data suggested that Arc/Arg3.1 AS can inhibit Arc/Arg3.1 protein expression and AMPA GluR1 cell-surface expression induced by morphine CPP retrieval in the NAc shell.

**Discussion**

Previous study in our lab has demonstrated that the Arc/Arg3.1 protein level was increased in the NAc shell, but not the NAc core,
after the expression test of morphine CPP (Lv et al., 2011). The present study extended those observations and revealed that morphine context memory reactivation dramatically enhances the Arc/Arg3.1 protein level, accompanied by increases in mem- branous AMPA GluR1 expression, pERK1/2, and pCREB levels, specifically in the NAc shell. Interestingly, the changes of Arc/ Arg3.1-related molecules, including AMPA GluR1, pERK1/2, and pCREB, are selective in the test group of morphine CPP, but not the non-tests of the morphine- or saline-CPP groups, suggesting that the elevated Arc/Arg3.1-related molecules in animals treated with the retrieval cannot be attributed to the earlier training experience but to the exposure to a drug-paired context.

It has been reported that the activation of the ERK signaling pathway plays a critical role in memory reconsolidation. The reconsolidation of recognition memory is associated with an increase in the phosphorylation of ERK in the entorhinal cortex and hippocampal CA1, and inhibition of the ERK pathway with U0126 can block the reconsolidation. This process is shown to be dependent on the reactivation of memory trace by brief reexposure to the objects (Kelly et al., 2003). Additionally, transcription factors CREB is phosphorylated at Ser133 site by upstream kinases result in cellular gene expression, and ERK pathway activation has been coupled to CREB phosphorylation, which is widely implicated in many forms of neuronal plasticity and learning and memory (Hall et al., 2001). Targeted disruption of CREB in the hippocampus, amygdala, and prefrontal cortex could impair the reconsolidation of both auditory fear and contextual fear memories (Kida et al., 2002; Mamiya et al., 2009). A recent report showed that CREB is activated in the striatum of mice following a methamphetamine (METH) CPP expression test (Liu et al., 2014). In brief, the above data suggest that changes in ERK and CREB activity may be the underlying mechanism for the reconsolidation of drug addiction memory, regulating a variety of gene expressions related to addiction behavior; the target genes regulated by CREB need further study.

The present work showed that Arc/Arg3.1 protein induction during morphine CPP reconsolidation was accompanied by ERK-CREB signal pathway activation. Meanwhile, it inhibited ERK1/2 activation with MEK1/2 inhibitor U0126, attenuated CREB activation and Arc/Arg3.1 protein level, and disrupted the reconsolidation of morphine CPP. This behavioral result is similar to those pharmacological studies implying that the inhibition of ERK was almost simultaneous with the reduction of CREB activation and the impairment of contextual memory (Miller and Marshall, 2005; Fricks-Gleason and Marshall, 2011). However, there is some dispute about whether the Arc/Arg3.1 gene expression was regulated by CREB transcription factors. In Walterteit et al.’s (2001) study, they did not detect the CAMP response element (CRE) site within 1737 bp of the Arc/Arg3.1 promoter or the entire transcribed region of 3.5 kb, which does not exclude the possibility that unidentified CRE sites lie outside of this region. Kawashima et al. (2009) identified a major synaptic activity responsive element of the Arc/Arg3.1 gene, a cis-regulatory element consisting of closely localized binding sites for CREB, myocyte enhancer factor 2, and serum response factor that is located >5 kb upstream of the Arc/Arg3.1 transcription initiation site in the mouse genome. This data suggested that the single element is necessary and sufficient for replicating crucial properties of endogenous Arc/Arg3.1’s transcriptional regulation. The present
study further recommends that morphine CPP reconsolidation depends on ERK activation, which is required for CREB phosphorylation and induction of Arc/Arg3.1 protein expression.

As we know, Arc/Arg3.1 up-regulation is followed by contextual spatial memory retrieval (Zhang et al., 2005; Kee et al., 2007). These findings are in accord with our present results that a reexposure to the morphine-paired chamber obviously triggered the reconsolidation of morphine CPP and was accompanied by increasing Arc/Arg3.1 expression within the NAc shell in rats. If Arc/Arg3.1 itself plays a key role in the reconsolidation of morphine CPP, disruption of Arc/Arg3.1 expression should prevent these processes. Exactly this result has been obtained in that blockade of Arc/Arg3.1 induced by intra-NAc shell infusion of AS, which impaired the reconsolidation of morphine CPP in a long-term and irreversible manner. These results demonstrate that Arc/Arg3.1 is the key downstream molecule of the ERK-CREB pathway, which plays an essential role in the context-induced morphine CPP reconsolidation.

Studies on the molecular function of Arc/Arg3.1 showed that it is involved in multiple forms of synaptic plasticity (Guzowski et al., 2000; Pandey et al., 2008; Waung et al., 2008). One major mechanism that regulates synaptic strength involves the tightly regulated trafficking of u-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPARs) into and out of synapses (Anggono and Huganir, 2012). In the present study, to explore Arc/Arg3.1’s function, we detected AMPA GluR1, GluR2, and GluR3 on cell surface expression when the Arc/Arg3.1 protein was induced in the NAc shell during morphine CPP reconsolidation. We found that AMPA GluR1 protein expression, but not AMPA GluR2 and GluR3 expression, was significantly increased at the cell-surface following Arc/Arg3.1 protein induction. Disrupting the induction of the Arc/Arg3.1 protein can normalize the GluR1 cell-surface expression level and impair both reconsolidation and reinstatement of morphine CPP. This result suggests that Arc/Arg3.1 in the NAc shell mediated morphine-associated context memory reconsolidation via up-regulating the cell-surface expression of GluR1 in the NAc shell. Significantly, the data is different from the previous finding of Arc/Arg3.1 regulated AMPAR endocytosis (Hearing et al., 2011). Further studies are needed to elucidate the mechanisms of synaptic capture of AMPA GluR1.

Conclusions
In conclusion, our current study suggests that the Arc/Arg3.1 increase in the NAc shell following the reexposure of morphine-associated context relied on the ERK-CREB activation and coupled the elevation of GluR1 membrane surface expression to promote synaptic plasticity. The findings provide new evidence that Arc/Arg3.1 is the key molecule during the reconsolidation of morphine CPP, and local disruption of Arc/Arg3.1 hopefully prevents the reconsolidation of opioid-conditioned memory and further suppresses relapse of drug abuse.

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Statement of Interest
The funders had no participation in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors have no conflicts of interest (financial or otherwise) to declare related to the data presented in this manuscript.

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