

# A Critical Role for the GluA1 Accessory Protein, SAP97, in Cocaine Seeking

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A growing body of evidence indicates that the transport of GluA1 subunit-containing calcium-permeable AMPA receptors (CP-AMPA) to synapses in subregions of the nucleus accumbens promotes cocaine seeking. Consistent with these findings, the present results show that administration of the CP-AMPA antagonist, Naspmm, into the caudal lateral core or caudal medial shell of the nucleus accumbens attenuated cocaine priming-induced reinstatement of drug seeking. Moreover, viral-mediated overexpression of 'pore dead' GluA1 subunits (via herpes simplex virus (HSV) GluA1-Q582E) in the lateral core or medial shell attenuated the reinstatement of cocaine seeking. The overexpression of wild-type GluA1 subunits (via HSV GluA1-WT) in the medial shell, but not the lateral core, enhanced the reinstatement of cocaine seeking. These results indicate that activation of GluA1-containing AMPARs in subregions of the nucleus accumbens reinstates cocaine seeking. SAP97 and 4.1N are proteins involved in GluA1 trafficking to and stabilization in synapses; SAP97-GluA1 interactions also influence dendritic growth. We next examined potential roles of SAP97 and 4.1N in cocaine seeking. Viral-mediated expression of a microRNA that reduces SAP97 protein expression (HSV miSAP97) in the medial accumbens shell attenuated cocaine seeking. In contrast, a virus that overexpressed a dominant-negative form of a 4.1N C-terminal domain (HSV 4.1N-CTD), which prevents endogenous 4.1N binding to GluA1 subunits, had no effect on cocaine seeking. These results indicate that the GluA1 subunit accessory protein SAP97 may represent a novel target for pharmacotherapeutic intervention in the treatment of cocaine craving.

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## INTRODUCTION

Nucleus accumbens AMPA receptors (AMPA) play a critical role in the reinstatement of cocaine seeking, an animal model of relapse. The nucleus accumbens is divided into two major subregions, the core and shell. Increased AMPA receptor transmission in both accumbens subregions contributes to cocaine reinstatement (Schmidt and Pierce, 2010). Thus, microinjection of the AMPA receptor antagonist, CNQX, into either the core or shell attenuated cocaine priming-induced reinstatement (Famous *et al*, 2008). Consistent with these findings, administration of AMPA itself into either accumbens subregion elicited cocaine seeking (Ping *et al*, 2008).

There are four AMPA receptor subunits, GluA1–4. All AMPARs become permeable to sodium and potassium on activation. However, the absence of GluA2 subunits from the AMPAR tetramer results in calcium permeability. GluA2-lacking calcium-permeable AMPARs (CP-AMPA) have larger single channel conductance and faster kinetics relative to calcium-impermeable (CI) AMPARs that include GluA2 subunits (Cull-Candy *et al*, 2006; Lee, 2012; Tanaka *et al*, 2000). The balance of AMPARs in the nucleus accumbens is shifted to CP-AMPA following extended-access cocaine self-administration followed by a prolonged period of forced cocaine abstinence. Thus, increased cell surface GluA1, but not GluA2, expression was observed in the nucleus accumbens (Conrad *et al*, 2008) and electrophysiological studies demonstrated inward rectification of evoked AMPA receptor EPSCs, a hallmark of CP-AMPA, in the accumbens of cocaine-experienced rats (Conrad *et al*, 2008; McCutcheon *et al*, 2011c). These increases in accumbens CP-AMPA expression and function were behaviorally relevant in that microinjection of the CP-AMPA antagonist Naspmm into the accumbens core blocked incubated cue-induced cocaine seeking (Conrad *et al*, 2008). Interestingly,

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short-access (2 h/day) cocaine self-administration had no influence on CP-AMPA-mediated transmission in the nucleus accumbens (Purgianto *et al*, 2013), which indicates that extended-access (6 h/day) cocaine self-administration in adult rats is required to produce prolonged increases in CP-AMPA function in the absence of extinction (Wolf and Tseng, 2012).

A separate line of investigation revealed that dynamic changes in accumbens AMPAR transmission contribute to the reinstatement of cocaine seeking following extinction of short-access cocaine self-administration. Under extinction conditions, drug seeking promoted by non-contingent cocaine administration was associated with increased surface expression of GluA1 subunits in the accumbens shell (Anderson *et al*, 2008). Moreover, suppression of accumbens GluA1 transcription (Ping *et al*, 2008) or impairment of the transport of GluA1-containing AMPA receptors to synapses in the accumbens shell during extinction (Anderson *et al*, 2008) attenuated the reinstatement of drug seeking induced by a cocaine-priming injection. To expand on these findings, the current experiments used pharmacological and viral vector approaches to assess the role of GluA1-mediated transmission in accumbens subregions in cocaine seeking using a short-access self-administration/extinction/reinstatement paradigm.

The mechanisms underlying the enhanced role of accumbens GluA1-containing AMPARs during cocaine priming-induced reinstatement of drug seeking remain unclear. SAP97 is a membrane-associated guanylyl kinase (MAGUK) protein that is sufficient (Cai *et al*, 2002; Howard *et al*, 2010), if not always necessary (Kim *et al*, 2005), for trafficking of GluA1-containing AMPARs to synapses. Similarly, 4.1N is an actin-binding protein that also contributes to the trafficking and stabilization of GluA1-containing AMPARs at the cell surface (Lin *et al*, 2009; Shen *et al*, 2000). Both SAP97-GluA1 and 4.1N-GluA1 interactions contribute to various forms of neuronal plasticity (Lin *et al*, 2009; Zheng *et al*, 2011; Zheng and Keifer, 2014). Here, viral vectors were used to reduce SAP97 expression or impair the interaction between 4.1N and GluA1 subunits prior to reinstatement test sessions to determine the functional significance of these accessory proteins in cocaine seeking.

## MATERIALS AND METHODS

### Animals and Housing

Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 225–250 g were obtained from Taconic Laboratories (Germantown, NY). Animals were individually housed with food and water available *ad libitum*. A 12 h light/dark cycle was used and all experiments were performed during the light cycle. All experiments used Med-Associates (East Fairfield, VT) operant chambers equipped with response levers, a house light and a pump for injecting drugs intravenously. Operant chambers were enclosed within ventilated, sound-attenuating chambers. All experimental procedures were consistent with the ethical guidelines of the US National Institutes of Health and were approved by the Perelman School of Medicine Institutional Animal Care and Use Committee at the University of Pennsylvania.

## Drugs and Viruses

Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD) and dissolved in bacteriostatic 0.9% saline. The first experiments examined the role of accumbens CP-AMPA receptors in cocaine reinstatement. 1-Naphthyl acetyl spermine trihydrochloride (Naspm) is a synthetic analog of Joro spider toxin that acts as a CP-AMPA antagonist (Tsubokawa *et al*, 1995). Naspm was obtained from Sigma-Aldrich (St Louis, MO) and was dissolved in phosphate-buffered saline (PBS). In the nucleus accumbens, CP-AMPA receptors are either GluA1 homomers or GluA1/A3 heteromers (Boudreau *et al*, 2007). Therefore, we next manipulated GluA1 expression in the accumbens prior to cocaine reinstatement using viral vectors. All constructs were packaged into a herpes simplex virus (HSV) p1005 plasmid, driven by an IE 4/5 promoter. The first virus produces 'pore dead' GluA1 subunits (Bachtell *et al*, 2008). These pore dead GluA1 subunits have a mutation of the glutamine (Q) at site 582 to a glutamic acid (E) (ie, GluA1-Q582E). This amino-acid residue is part of the pore-forming region. As glutamine is a polar, uncharged amino acid, whereas glutamic acid is negatively charged, this mutation results in GluA1-Q582E-containing AMPA receptors that cannot pass ions (Dingledine *et al*, 1999). An HSV that overexpresses wild-type GluA1 (GluA1-WT) was generated for the next experiment (Carlezon *et al*, 1997). The final two experiments used viral vectors to manipulate the GluA1 accessory proteins 4.1N or SAP97. The 4.1N virus (4.1N-CTD) overexpresses a dominant-negative form of the 4.1N C-terminal domain (residues 744–879), which binds GluA1 subunits and prevents interaction with endogenous 4.1N (Fukatsu *et al*, 2004; Shen *et al*, 2000). For SAP97, the HSV virus expresses a miRNA (5'-CAGTGACTGCCTTA AAGAATA-3') against SAP97, which suppresses translation of the SAP97 protein (both isoforms) (Zhang *et al*, 2015). All viruses were designed to co-express enhanced green fluorescent protein (eGFP) driven by a separate CMV promoter. Control viruses express a scrambled sequence plus eGFP.

## Surgery

Rats were handled daily and allowed 1 week to acclimate to their home cages on arrival. Prior to all surgeries, rats were anesthetized with 80 mg/kg ketamine (i.p.; Sigma-Aldrich) and 12 mg/kg xylazine (i.p.; Sigma-Aldrich). An indwelling silastic catheter (CamCaths, UK) was inserted into the right jugular vein and sutured in place. The catheter was routed to a mesh backmount platform that was implanted subcutaneously dorsal to the shoulder blades. Catheters were flushed daily with 0.3 ml of an antibiotic (Timentin, 0.93 mg/ml, Henry Schein, Melville, NY) dissolved in heparinized saline. The catheters were sealed with plastic obturators when not in use.

For animals destined for the Naspm experiments, immediately following catheter insertion the rats were mounted in a stereotaxic apparatus and two stainless-steel guide cannulae (24 gauge) were implanted bilaterally 2 mm dorsal to the medial nucleus accumbens shell, accumbens core or dorsal striatum. Guide cannulae were cemented in place by affixing dental acrylic to stainless-steel screws

secured in the skull. The coordinates for the ventral ends of the guide cannulae, relative to bregma (Paxinos and Watson, 1997), were as follows: accumbens shell = +1.0 mm A/P,  $\pm$  1.0 mm M/L, -5.0 mm D/V; accumbens core = +1.0 mm A/P,  $\pm$  2.5 mm M/L, -5.0 mm D/V; dorsal striatum = +1.0 mm A/P,  $\pm$  3.0 mm M/L, -2.0 mm D/V. An obturator (33-gauge wire) was inserted into each guide cannula to prevent occlusion.

### Cocaine Self-Administration and Extinction

Following a 7-day recovery period from surgery, rats were placed in operant chambers and allowed to lever press for intravenous cocaine infusions (0.25 mg/59  $\mu$ l saline over 5 s) over a 2-h time period daily for 21 days. Each self-administration session began with the i.v. administration of 59  $\mu$ l cocaine (0.25 mg) to fill the catheter. Rats initially self-administered cocaine under a fixed ratio (FR) 1 schedule of reinforcement (limited to 30 injections per 120 min). Once animals achieved stable responding, as defined by at least 20 infusions over the 2 h session for 2 consecutive days, they were transitioned to a FR5 schedule of reinforcement (and again limited to 30 injections per 120 min). In our experience, use of an FR5 self-administration schedule results in more robust reinstatement responses subsequently. For both schedules of reinforcement, a 20 s time-out period followed each cocaine infusion, during which time active, drug-paired lever responses were tabulated but had no scheduled consequence. Each operant chamber was also equipped with an inactive lever. Responses made on the inactive lever, which had no scheduled consequence, were also recorded during all self-administration sessions. Following 21 days of cocaine self-administration, drug-taking behavior was extinguished by replacing cocaine with 0.9% bacteriostatic saline. Extinction continued until responding on the active lever was <15% of the responses on the last day of cocaine self-administration.

### Sucrose Self-Administration and Extinction

After a 7-day post-surgery recovery period, rats were food deprived (15–20 g of lab chow daily, Harlan Teklad, Wilmington, DE) and allowed to administer sucrose pellets on an FR1 schedule, where they received one 45-mg sucrose pellet (Research Diets, New Brunswick, NJ) each time they pressed the active lever. After receiving over 20 pellets per session over two consecutive self-administration sessions, the rats then advanced to an FR5 schedule. Rats self-administered sucrose pellets for a total of 14 days with a maximum of 30 pellets per session. Following 14 days of sucrose self-administration, the subjects were shifted into extinction where active lever pressing no longer resulted in sucrose pellet administration. Extinction of sucrose-taking behavior continued until responding on the active lever was 15% or less of that on the last self-administration day.

### Cocaine and Sucrose Reinstatement—Naspm Microinjections

During the reinstatement phase, the obturators were removed from the guide cannulae and 33-gauge stainless-steel microinjectors (Small Parts, Logansport, IN) were

inserted into the guide cannula. The microinjectors extended 2 mm below the ventral end of the guide cannulae into the caudal medial nucleus accumbens shell, caudal lateral accumbens core, or dorsal striatum. Bilateral infusions of 0.5  $\mu$ l of Naspm (40  $\mu$ g) or PBS occurred over a 120-s time period. This dose of Naspm was previously shown to modulate cocaine-mediated behavior (Conrad *et al*, 2008). Following microinfusion of either Naspm or PBS, the microinjectors remained in place for 60 s to allow the solution to diffuse away from the tips of the microinjectors before removal. For cocaine reinstatement, 10 min following microinjection a systemic priming injection of cocaine (10 mg/kg, i.p.) was administered immediately prior to a reinstatement test session. For sucrose reinstatement, the experimenter remotely administered one sucrose pellet every 2 min for the first 10 min of the reinstatement test session.

During the cocaine reinstatement test sessions, satisfaction of the response requirement (ie, five presses on the active lever) resulted in saline rather than cocaine infusion. During sucrose reinstatement active lever responses had no scheduled consequences. For both cocaine and sucrose reinstatement, each reinstatement test session was followed by extinction sessions until responding was again <15% of the response rate maintained by cocaine or sucrose self-administration. Generally, 1–2 days of extinction were necessary to reach extinction criterion between reinstatement test sessions. The FR5 schedule was used throughout the extinction and reinstatement phases of these experiments. Naspm or PBS were administered during the reinstatement phase in a counterbalanced fashion.

### Cocaine and Sucrose Reinstatement—Viral Vectors

Only one viral vector or its control was microinjected bilaterally per animal in a between-subjects design. Following recovery from surgery the obturators were removed from the guide cannulae and 33-gauge stainless-steel microinjectors were inserted into the caudal lateral nucleus accumbens core or caudal medial shell as described above. Bilateral infusions of HSV GluA1-Q582E, HSV GluA1-WT, HSV 4.1N-CTD, HSV eGFP, HSV miSAP97, or HSV scrambled miSAP97 occurred over a 10-min time period for a total volume of 2.0  $\mu$ l per side. Following the injections, microinjectors were left in place for 120 s to allow the solution to diffuse away from the tips of the cannula before they were removed. Animals underwent daily extinction sessions until reaching the first reinstatement test session 3 days (GluA1-Q582E, GluA1-WT, 4.1N-CTD) or 4 days (miSAP97, scramble) post infusion (see Results for additional details). Animals then underwent daily extinction sessions until the second reinstatement test 7 days post injection. Cocaine and sucrose reinstatement were precipitated as described above.

### Verification of Cannulae Placements

After the completion of all behavioral experiments, the animals were given an overdose of pentobarbital (100 mg/kg, i.p.) and brains were removed and stored in formalin for at least 3 days. Subsequently, coronal sections (100  $\mu$ m) were taken at the level of the striatum with a Vibratome (Technical Products International; St Louis, MO). The sections were mounted on gelatin-coated slides. Cannula

placements as well as potential drug- or cannula-induced neuronal damage were subsequently assessed. Cell death and associated gliosis also were determined. Animals with cannulae placements outside of the areas of interest, or with excessive mechanical damage/gliosis, were excluded from subsequent data analysis.

### Viral Expression

To ascertain viral expression via green fluorescent protein, we injected (2  $\mu$ l/side) HSV GluA1-Q582E, HSV GluA1-WT, HSV 4.1N-CTD, or HSV miSAP97 into the nucleus accumbens of separate, drug-naive rats. At 3 days (HSV GluA1-Q582E, HSV GluA1-WT, HSV 4.1N-CTD), 4 days (HSV miSAP97), or 7 days (all viruses) after injection, animals received 100 mg/kg pentobarbital (i.p.) before perfusion with 120 ml ice-cold PBS followed by 60 ml 4% PFA dissolved in ice-cold PBS. Brains were removed and placed in 4% PFA for 24 h before storage in 30% sucrose dissolved in PBS with 1% sodium azide. Coronal sections (10–30  $\mu$ m) were taken using a cryostat (Cryotome FE, Thermo Fisher Scientific, Waltham, MA) and mounted directly onto polarized glass slides. Dry slides were washed in 1  $\times$  PBS, and then blocked for 1 h in 0.1% triton and 3% normal donkey serum in 1  $\times$  PBS. We then added primary antibody (Anti-GFP, Ms/Rb—Millipore, Billerica, MA—diluted 1:1000 in 0.1% triton+3% donkey serum in PBS) to the slides and incubated overnight at 4  $^{\circ}$ C. The next day, the slides were washed in 1  $\times$  PBS before incubation in secondary fluorescent antibody at room temperature for 2 h (Alexa Fluor 488, 1:500, Jackson ImmunoResearch, West Grove, PA). After 2 h, slides were washed in 1  $\times$  PBS before being cover-slipped using Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and imaged for GFP expression using fluorescent microscopy.

### Electrophysiology

Separate, drug-naive rats received bilateral injections of HSV GluA1-Q582E or GluA1-WT (2  $\mu$ l) into the medial accumbens shell. After 3 days, animals were decapitated under isofluorane anesthesia and brain slices prepared as described previously (Ortinski *et al*, 2013). In brief, the brain was removed and coronal slices (300  $\mu$ m) containing the nucleus accumbens were cut with a Vibratome (VT1000S, Leica Microsystems) in ice-cold artificial cerebrospinal fluid solution (ACSF), in which NaCl was replaced by an equiosmolar concentration of sucrose. ACSF consisted of 130 mM NaCl, 3 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 26 mM NaHCO<sub>3</sub>, 10 mM glucose, 1 mM MgCl<sub>2</sub>, and 2 mM CaCl<sub>2</sub> (pH 7.2–7.4 when saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub>). Whole-cell recordings were obtained using borosilicate glass pipettes filled with intracellular solution containing: 100 mM CsCH<sub>3</sub>O<sub>3</sub>S, 50 mM CsCl, 3 mM KCl, 0.2 mM BAPTA, 10 mM HEPES, 1 mM MgCl<sub>2</sub>, 2.5 mM phosphocreatine-2Na, 2 mM Mg-ATP, 0.25 mM GTP-Tris, 1 mM QX-314, 0.1 mM spermine, adjusted to pH 7.2–7.3 with CsOH (osmolarity 280–290 mOsm). Slices were viewed using infrared differential interference contrast optics under an upright microscope (Eclipse FN1, Nikon Instruments) with a 40 $\times$  water immersion objective. To avoid recording from damaged cells, neurons within 50  $\mu$ m of the injection

cannula track were excluded from the analysis. There were no differences on any of the measures between eGFP-negative cells in slices exposed to GluA1-Q582E and GluA1-WT. Therefore, these cells were pooled for analysis.

For evoked EPSCs (eEPSCs), a minimal stimulation intensity required to evoke a consistent post-synaptic response was determined. Peak eEPSC amplitudes were then measured at double and triple the minimal stimulation intensity (at 0.05–0.1 Hz) to construct the input/output curves. Average responses were then calculated based on 5–10 eEPSCs at each stimulation intensity. The rectification index of AMPA receptor-mediated eEPSCs was measured as the ratio of the peak current amplitude at –70 mV to the peak current amplitude at +40 mV. For rectification index, the measurements were based on 5–10 eEPSCs evoked at 0.1 Hz.

### Western Blot

Protein expression in the nucleus accumbens was assessed following HSV 4.1N-CTD HSV eGFP administration. Drug-naive animals received unilateral 2  $\mu$ l injections of HSV 4.1N-CTD or HSV miSAP97 into the medial accumbens shell in one hemisphere and HSV GFP into the medial shell of the other hemisphere. Following virus administration, the subjects were returned to their home cages for 3 days (HSV 4.1N-CTD) or 4 days (HSV miSAP97). At this point, the rats were killed via rapid, live decapitation; brains were immediately extracted and flash-frozen in isopentane. 300  $\mu$ m sections containing the nucleus accumbens were subsequently taken using a cryostat and the medial accumbens shell was dissected before processing whole-cell tissue for western blot as described previously (Anderson *et al*, 2008).

For all samples, protein concentration was quantified using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein (10–20  $\mu$ g) were loaded and separated in 10% Tris-Glycine gels (Life Technologies, Grand Island, NY) under reducing conditions, then transferred to nitrocellulose membranes using the i-Blot dry transfer system (Life Technologies). Membranes were blocked with Odyssey Blocking Buffer (Life Technologies), according to manufacturer instructions. Membranes were incubated overnight at 4  $^{\circ}$ C with selective antibodies to 4.1N (Rb 1:1000, Millipore) or SAP97 (Rb 1:1000, Abcam, Cambridge, England) as well as GAPDH (1:2000, Cell Signaling, Danvers, MA) diluted in Odyssey Blocking Buffer. After washing with TBST, membranes were then incubated with infrared secondary antibodies at room temperature for 1 h (1:5000, IR-dye 680 or IR-dye 800) before being imaged on an Odyssey fluorescent scanner (Li-Cor Biotechnology, Lincoln, NE). GAPDH expression was used as a loading control; proteins were normalized to GAPDH-integrated intensity values.

We used hippocampal cultures to test the efficacy of the HSV miRNA toSAP97. Pure hippocampal cultures were obtained from the University of Pennsylvania Neuron Culture Core facility (<http://www.med.upenn.edu/neuronsrus/>) and maintained *in vitro* for 14 days in Neurobasal media+B27 supplement. Cultures were infected with recombinant HSV at 1.5  $\mu$ l of virus/ml of culture medium. Lysates were prepared 2 days later and subjected to western blot for SAP97 or actin. Each experiment was performed on four

separate dishes of hippocampal neurons infected with either HSV-miRNA to SAP97 or HSV-miRNA scrambled and no fewer than three separate experimental determinations of miRNA efficacy were performed.

## RESULTS

### Blocking CP-AMPA in the Accumbens Core or Shell, but not Dorsolateral Striatum, Attenuated Cocaine Priming-Induced Reinstatement of Drug Seeking

To assess the role of CP-AMPA in the reinstatement of cocaine seeking, a CP-AMPA antagonist (Naspm) was microinjected into regions of the striatal complex that contribute to various aspects of cocaine-seeking behavior. Total active and inactive lever responding (mean  $\pm$  SEM) during the reinstatement of cocaine seeking following microinjection of saline or 40  $\mu$ g Naspm into the caudal lateral accumbens core, caudal medial accumbens shell, or DL striatum are shown Figure 1a–c, respectively. Reinstatement data were analyzed with three separate mixed factors analyses of variance (ANOVAs) with drug treatment as the between-subjects factor and lever as the within subjects factor. Analysis of the core data ( $n=12$ ) revealed significant main effects of treatment ( $F_{1,22}=5.544$ ,  $p<0.0279$ ) and lever ( $F_{1,22}=71.95$ ,  $p<0.0001$ ) as well as a significant treatment  $\times$  lever interaction ( $F_{1,22}=8.653$ ,  $p<0.0075$ ). *Post hoc* tests showed a significant difference in active lever responding between treatments (Bonferroni,  $p<0.05$ , Figure 1a). Analysis of the shell data ( $n=8$ ) showed significant main effects of treatment ( $F_{1,14}=16.51$ ,  $p<0.0012$ ) and lever ( $F_{1,14}=53.72$ ,  $p<0.0001$ ) as well as a significant treatment  $\times$  lever interaction ( $F_{1,14}=19.58$ ,  $p<0.0006$ ). Pairwise comparisons demonstrated a significant difference in active lever responding between treatments (Bonferroni,  $p<0.05$ , Figure 1b). Analysis of the DL striatum data ( $n=4$ ), which is shown in Figure 1c, revealed a significant main effect of lever ( $F_{1,6}=41.0$ ,  $p<0.0007$ ) but no significant main effect of treatment and no significant treatment  $\times$  lever interaction. The microinjection placements for the core, shell, and DL striatum are shown in Figure 1d–f, respectively. Collectively, these data indicate that antagonism of CP-AMPA in the caudal lateral accumbens core or caudal medial shell, but not the DL striatum, attenuates the reinstatement of cocaine seeking.

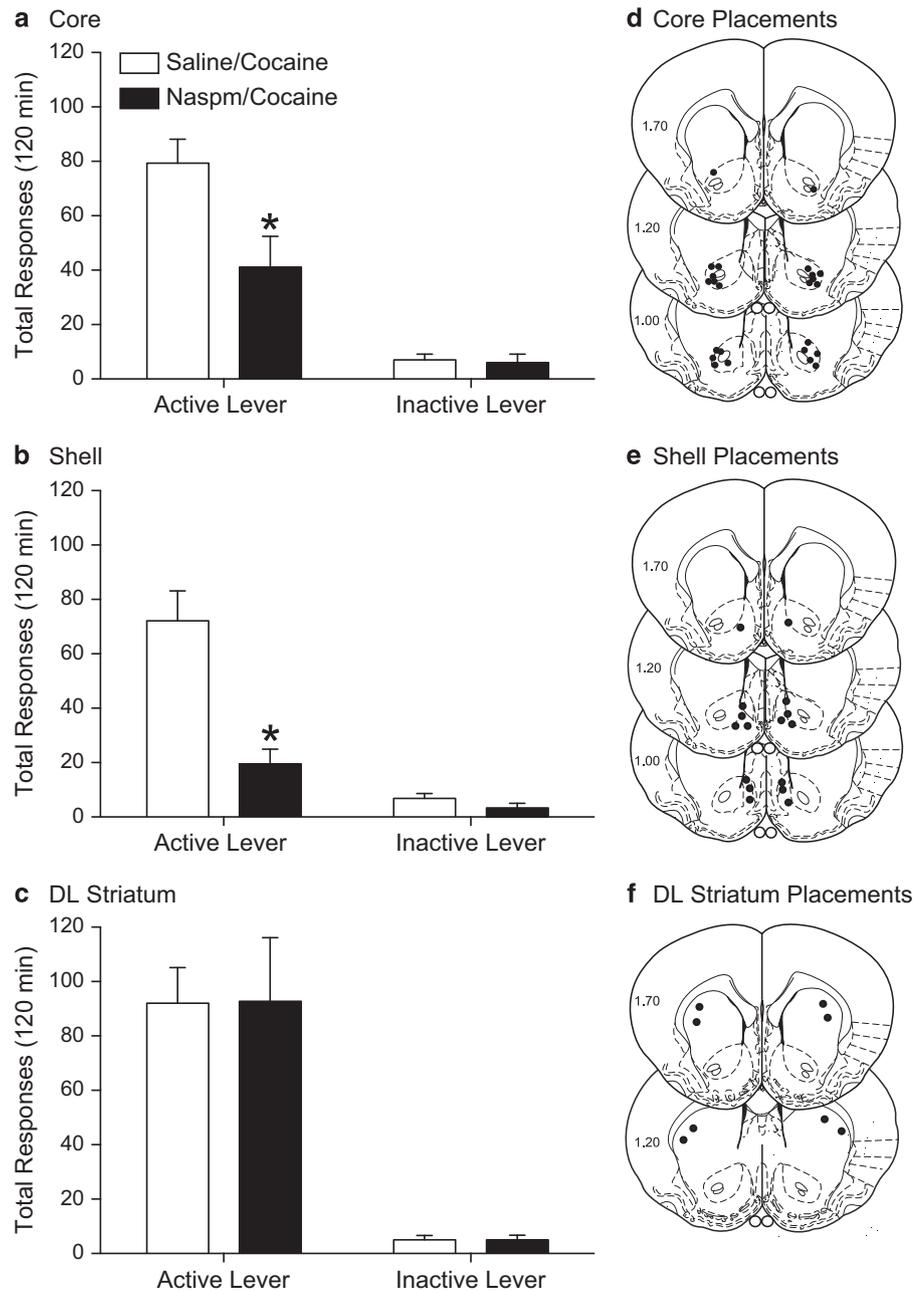
Decreases in responding, such as following intra-core or shell Naspm, could be due to generalized behavioral disruption. Although Naspm produced no effect on inactive lever responding, the low average number of inactive responses per session decreases the utility of this measure as a means to judge drug-induced behavioral suppression. Therefore, we also assessed the reinstatement of sucrose seeking. Total responses on the active lever (mean  $\pm$  SEM) during the sucrose reinstatement sessions are summarized in Table 1. Microinjection of Naspm into the medial shell had no influence on the reinstatement of sucrose seeking. However, intra-core Naspm significantly attenuated sucrose seeking, which indicates that CP-AMPA in the lateral core may have a more generalized influence on motivated behaviors. These data were analyzed with separate unpaired *t*-tests, the results of which are included in Table 1.

### Extinction of Cocaine Self-Administration had no Effect on CP-AMPA Function in the Medial Accumbens Shell

In drug-naïve rats, CP-AMPA in the nucleus accumbens account for <10% of eEPSC amplitude (Conrad *et al*, 2008) and Naspm has a limited effect on eEPSCs in the caudal lateral accumbens core following short-access cocaine self-administration in the absence of extinction (Purgianto *et al*, 2013). Therefore, it seems unlikely that the influence of Naspm on the cocaine reinstatement as shown in Figure 1 is due to blocking CP-AMPA present in the nucleus accumbens prior to the reinstatement session. However, it is possible that the extinction of cocaine seeking behavior may increase CP-AMPA in the nucleus accumbens. To investigate this possibility, we recorded from medial accumbens shell neurons in slices obtained from rats whose cocaine self-administration behavior was extinguished or control rats that received passive yoked saline infusions. We found no differences in rectification of AMPA eEPSCs between these two groups. The rectification index ( $-70$  mV/ $+40$  mV) data were as follows (mean  $\pm$  SEM): yoked saline,  $2.366 \pm 0.189$  (12 cells from three animals); cocaine+extinction,  $2.508 \pm 0.155$  (13 cells from three animals). An unpaired *t*-test showed no difference between these treatments. These data suggest that extinction of cocaine seeking does not affect the relative abundance of CP-AMPA at excitatory synapses in the medial accumbens shell. As reviewed in more detail in the discussion section, it seems likely that Naspm is influencing accumbens CP-AMPA that are transported transiently to the cell surface during the reinstatement of cocaine seeking.

### Over-Expression of ‘Pore Dead’ GluA1-Q582E Subunits in the Core or Shell Reversibly Attenuated Cocaine Seeking

We initially characterized HSV GluA1-Q582E electrophysiologically in naive rats (ie, not exposed to cocaine) 3 days after microinjection of the virus into the nucleus accumbens. Representative examples of eEPSCs are shown in Figure 2. Quantification of this experiment is depicted in Figure 2b. These data were analyzed with a two-way ANOVA, which revealed significant main effects of treatment ( $F_{1,16}=11.51$ ,  $p<0.0037$ ) and stimulation intensity ( $F_{2,32}=15.45$ ,  $p<0.0001$ ) as well as a significant interaction between these factors ( $F_{2,32}=7.63$ ,  $p<0.0019$ ). Subsequent pairwise tests showed a significant difference between amplitudes measured from eGFP-negative and eGFP-positive cells at the strongest stimulation intensity (Bonferroni,  $p<0.0001$ , Figure 2b). The magnitude of the decrease in eEPSC amplitude in eGFP-positive cells was somewhat surprising as HSV GluA1-Q582E shouldn't influence GluA2A3 expression. The rectification index also was measured in accumbens eGFP-negative and eGFP-positive neurons, as summarized in Figure 2c. These data were analyzed with a *t*-test (unpaired, two-tailed), which revealed a significant effect of Q582E expression ( $t_{13}=2.907$ ,  $p<0.012$ ). It is important to emphasize that rectification index differences between eGFP- and eGFP+ cells were found despite the fact that these cells were frequently located close to each other, making it unlikely that rectification index differences arose from tissue damage imposed by virus injection. Together,



**Figure 1** Microinfusion of the CP-AMPA antagonist Naspam into the accumbens core or shell, but not the DL striatum, attenuates cocaine priming-induced reinstatement. Total active and inactive lever responding (mean  $\pm$  SEM) following microinfusions of saline or Naspam (40  $\mu$ g/site) into the accumbens core (a), accumbens shell (b), or dorsal lateral striatum (c) 10 minutes prior to a systemic injection of cocaine (10 mg/kg, i.p.). Note a significant effect of Naspam when administered into the core or shell but not the DL striatum. The asterisks represent significant differences between the saline and Naspam treatments ( $p < 0.05$ ). Cannula placements in the core, shell, and DL striatum are depicted in panels (d–f), respectively. Number of rats per experiment: core = 12, shell = 8, DL striatum = 4.

these data suggest that Q582E attenuates the overall strength of AMPAR-mediated signaling in the nucleus accumbens and increases the contribution of non-rectifying CI-AMPARs to eEPSCs.

Also included in Figure 2 is a comparison of the rectification indices of untreated animals as well as rats exposed to HSV eGFP or HSV GluA1-WT. A one-way ANOVA indicated no significant differences among these groups. These results indicate that eGFP alone likely does not

contribute to the observed differences between HSV GluA-Q582E eGFP+ and eGFP- cells. There are a number of reasons that may account for the lack of effect of HSV-GluA1-WT on rectification. For example, it is very likely that many of the overexpressed GluA1 subunits assembled with GluA2 subunits resulted in an increase in the number of synaptic AMPARs but have no effect on rectification if the ratio of GluA2-containing to GluA2-lacking AMPARs were preserved.

**Table 1** Effects of Naspnm, 'Pore Dead' GluA1-Q582E and miSAP97 Administered into the Core or the Shell on the Reinstatement of Sucrose Seeking

Treatment	N	Region administered	Reinstatement: active lever responding	Statistics
Saline	5	Core	85.80 ± 14.10	$t_8 = 3.327, p < 0.0104$
Naspnm	5	Core	28.60 ± 9.826	
Saline	8	Shell	77.5 ± 13.19	$t_{14} = 0.2291, p < 0.8221$
Naspnm	8	Shell	81.25 ± 9.689	
HSV eGFP	6	Core	77.67 ± 16.64	$t_{10} = 0.4970, p < 0.6299$
HSV GluA1-Q582E	6	Core	89.83 ± 17.96	
HSV eGFP	6	Shell	117.0 ± 24.46	$t_9 = 0.2370, p < 0.8180$
HSV GluA1-Q582E	5	Shell	125.2 ± 23.93	
HSV eGFP	6	Shell	117.0 ± 36.88	$t_{10} = 0.7485, p < 0.4714$
HSV miSAP97	6	Shell	158.0 ± 40.5	

The active lever responding is expressed as mean ± SEM. Note that intra-core Naspnm significantly decreased sucrose seeking relative to control.

Although rectification index of AMPAR-mediated eEPSCs is a widely used measure of CP-AMPA content, AMPAR rectification also may depend on other factors such as receptor interactions with chaperone proteins (Soto *et al*, 2007). This distinction can be addressed by examining the magnitude of Naspnm effect on eEPSC amplitude. As Naspnm was not used in our electrophysiology experiments, we cannot exclude that factors other than virus-mediated reduction of GluA1-containing receptor signaling are responsible for changes in rectification observed in animals administered HSV GluA1-Q582E.

Next, we examined the effect of HSV GluA1-Q582E administered into the caudal lateral core or the caudal medial shell on the reinstatement of cocaine seeking. As summarized in the experimental design outlined in Figure 3a the first reinstatement session occurred 3 days after the virus microinjection (HSV eGFP or HSV GluA1-Q582E), which is when HSV expression peaks (see Figure 3b). Total active and inactive lever responding during the first reinstatement session for animals administered viruses into the core or shell are depicted in Figure 3c and d, respectively (the DL striatum was not examined due to the negative Naspnm results summarized previously). Reinstatement was prompted by administration of 10 mg/kg cocaine (i.p.). These data were analyzed with separate mixed factors ANOVAs with virus as the between-subjects factor and lever and the within subjects factor. Analysis of the core data at Reinstatement 1 ( $n = 8-9$ , Figure 3c) showed significant main effects of virus ( $F_{1,15} = 9.629, p < 0.0073$ ) and lever ( $F_{1,15} = 42.11, p < 0.0001$ ) as well as a significant virus × lever interaction ( $F_{1,15} = 10.72, p = 0.0051$ ). Subsequent pairwise analyses revealed a significant difference between virus treatments for active lever responses (Bonferroni,  $p < 0.05$ ). Analysis of the shell data at Reinstatement 1 (control  $n = 8$ , Pore Dead GluA  $n = 13$ , Figure 3d) revealed significant main effects of virus ( $F_{1,19} = 30.02, p < 0.0019$ ) and lever ( $F_{1,19} = 30.51, p < 0.0001$ ) as well as a significant virus × lever interaction ( $F_{1,19} = 10.72, p < 0.0039$ ). Subsequent pairwise analyses showed a significant difference between active lever virus effects (Bonferroni,  $p < 0.05$ ). Seven days after virus administration, when the second reinstatement session occurred, eGFP expression was negligible (see Figure 3e).

Separate ANOVAs of the caudal lateral core (Figure 3f) and caudal medial shell (Figure 3g) reinstatement data on Day 7 revealed only significant main effects of lever. Representative microinjection locations are depicted for the core and shell as insets of Figure 3f and g, respectively. These data indicate that when the 'pore dead' GluA1-Q582E virus was active (at Reinstatement 1) in either the caudal lateral core or the caudal medial shell reinstatement was suppressed. When the GluA1-Q582E virus in the core and shell was dormant (at Reinstatement 2) the level of cocaine reinstatement returned to control levels.

Sucrose reinstatement also was assessed after virus administration in a separate cohort of animals. The data, which are summarized in Table 1, indicated no significant differences between HSV GFP and HSV GluA1-Q582E active lever responding 3 days following virus administration into either the core or shell.

### Over-Expression of GluA1 Subunits in Shell, but not the Core, Promotes Cocaine Seeking

In these experiments, HSV eGFP or HSV GluA1-WT were microinjected into the caudal lateral core or caudal medial shell using the same paradigm as depicted in Figure 3 with the exception that a subthreshold dose of cocaine (5 mg/kg, i.p.) was used in the reinstatement sessions. The statistical analyses were the same as used in Figure 3. HSV GluA1-WT has been used previously in addiction-related experiments (Carlezon *et al*, 1997; Choi *et al*, 2011) and was shown to increase AMPAR rectification index in the ventral tegmental area (Choi *et al*, 2011). When the virus was maximally active (ie, Reinstatement 1), the analysis of the core reinstatement data ( $n = 7-8$ ) indicated no significant main effects or interaction (see Figure 4a). For the shell, analysis of the Reinstatement 1 data ( $n = 8-9$ ) indicated a significant main effect of treatment ( $F_{1,15} = 13.97, p < 0.002$ ), a significant main effect of lever ( $F_{1,15} = 46.25, p < 0.0001$ ), and a significant treatment × lever interaction ( $F_{1,15} = 8.452, p < 0.0108$ ). *Post hoc* tests revealed a significant active lever difference between treatments (Bonferroni,  $p < 0.05$ , Figure 4b). For the second reinstatement test, when the virus was dormant, the core data revealed no significant main effects or interaction

(see Figure 4c). Analysis of the Reinstatement 2 data when the virus was administered into the shell showed a significant main effect of lever ( $F_{1,14} = 8.446$   $p < 0.0115$ ), but no significant main effect of treatment and no significant interaction (see Figure 4d). Representative microinjection locations are depicted for the core and shell as insets of Figure 4c and d, respectively. These data indicate that a normally subthreshold dose of cocaine induced cocaine seeking when wild-type GluA1 subunits are overexpressed in the medial accumbens shell. There was a substantial, although not statistically significant, decrease in active lever responding on Reinstatement 1 when HSV GluA1-WT was

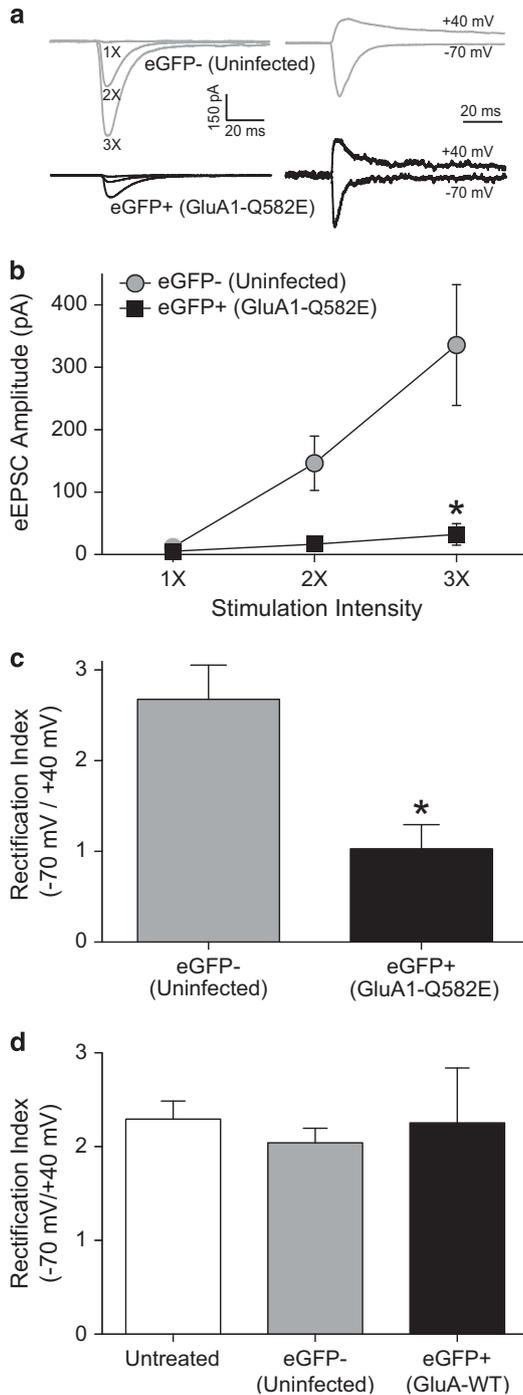
administered into the caudal lateral core, which is noteworthy as HSV GluA1-WT administered more rostrally in the core attenuates cocaine reinstatement (Bachtell *et al.*, 2008).

### Inhibiting the Interaction between 4.1N and GluA1 in the Shell has no Effect on Cocaine Seeking

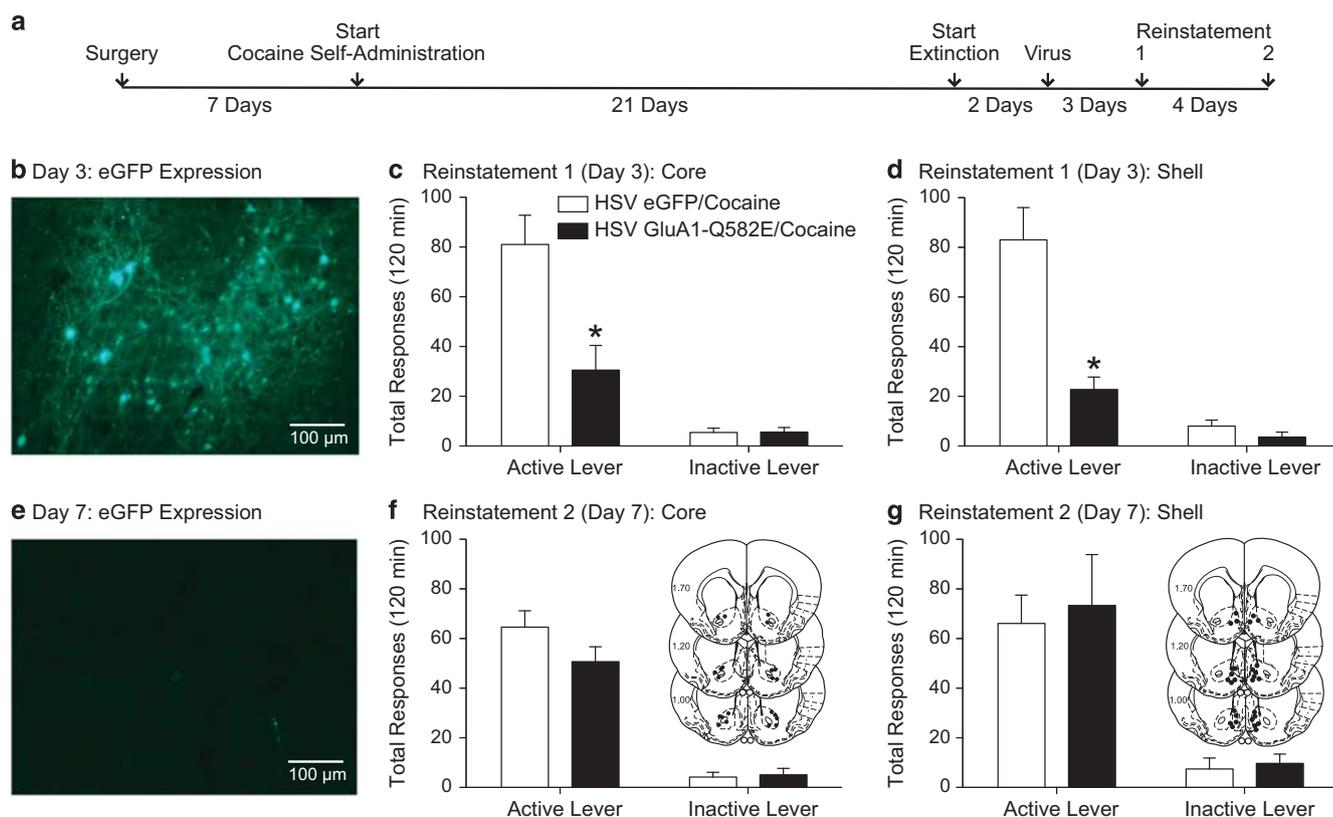
The next experiment examined potential mechanisms underlying the role of GluA1 subunits in the reinstatement of cocaine seeking. As the most consistent results of the previously described experiments were obtained with medial shell manipulations, we focused our attention on this accumbal subregion. We first tested the virus in drug-naïve rats and confirmed that eGFP expression in the nucleus accumbens peaked 3 days following administration (see Figure 5a) and was dormant 7 days after microinjection (data not shown). As shown in Figure 5b, 4.1N protein expression (native 4.1N plus 4.1N-CTD) was significantly increased 3 days after HSV 4.1N-CTD ( $t_{18} = 2.694$ ,  $p < 0.0148$ ). Analysis of the reinstatement data revealed only significant main effects of lever responding at Reinstatement 1 ( $F_{1,19} = 48.02$ ,  $p < 0.0001$ , Figure 5c) and Reinstatement 2 ( $F_{1,10} = 20.37$   $p = 0.0011$ , Figure 5d). There were 5–13 subjects per treatment. Representative microinjection locations in the accumbens shell are shown in the inset of Figure 5d. These results show that overexpression of 4.1N-CTD in the medial accumbens shell had no influence on the reinstatement of cocaine seeking.

### Reduced Expression of SAP97 in the Accumbens Shell Attenuates Cocaine Priming-Induced Reinstatement

The experimental design, shown in Figure 6a, was slightly different in this experiment as reinstatement was assessed 4 and 8 days after virus administration. HSV-miSAP97 was initially tested in drug-naïve rats. With this virus, the peak effect was observed 4 days after administration of HSV miSAP97 into the nucleus accumbens (see Figure 6b). Knockdown of SAP97 protein by overexpression of miSAP97 was assessed via western blot 2 days following virus



**Figure 2** The overexpression of 'pore dead' GluA1-Q582E in accumbens shell MSNs attenuates synaptic strength and decreases inward rectification of AMPAR eEPSCs. (a) Left, representative eEPSC traces at multiples of minimal stimulation intensity in neurons expressing GluA1-Q582E (eGFP+) relative to those not expressing eGFP (eGFP-) recorded from the same slice. Right, representative eEPSC traces at +40 mV and -70 mV for eGFP+ and eGFP- neurons, used for rectification index analyses. For ease of visualization, stimulus artifacts were removed and the amplitudes were normalized to the -70 mV eEPSC in eGFP-neuron. (b) The input-output relationship of AMPAR eEPSCs illustrates decreased synaptic strength in MSNs expressing GluA1-Q582E ( $n = 6$  cells from four animals) for peak eEPSC amplitude compared with non-GFP controls ( $n = 12$  cells from six animals) at  $3 \times$  stimulation. (c) Inward rectification of AMPA eEPSCs is more prominent in eGFP- than in eGFP+ cells (ie, peak current is smaller at +40 mV relative to -70 mV). (d) Inward rectification is similar between cells from naïve animals, not exposed to virus injections (untreated), cells that were not transduced by GluA1-WT injections (eGFP-), and cells overexpressing GluA1-WT (eGFP+). Untreated, nine cells from two animals; eGFP-, six cells from two animals; eGFP+, five cells from one animal.



**Figure 3** Overexpression of 'pore dead' GluA1-Q582E in the nucleus accumbens core or shell reversibly attenuated cocaine priming-induced reinstatement. (a) Schematic of experimental paradigm. (b) Representative image of peak eGFP fluorescence in the nucleus accumbens 3 days following HSV GluA1-Q582E administration. Total active and inactive lever responding (mean  $\pm$  SEM) 3 days after virus microinjection into the core or shell are shown in panels c and d, respectively. Note the significant attenuation of cocaine reinstatement when GluA1-Q-582E was overexpressed in the core or shell. The asterisks represent significant differences between the HSV eGFP and HSV GluA1-Q582E treatments ( $p < 0.05$ ). (e) Representative image of the relative absence of eGFP expression in the nucleus accumbens 7 days following HSV GluA1-Q582E administration. Total active and inactive responding (mean  $\pm$  SEM) 7 days following virus administration into the core or shell are shown in panels f and g, respectively. Note that the active lever responding does not differ between treatments. The insets in panels f and g depict cannula placements for the core and shell microinjections, respectively. There were 8–9 rats per treatment.

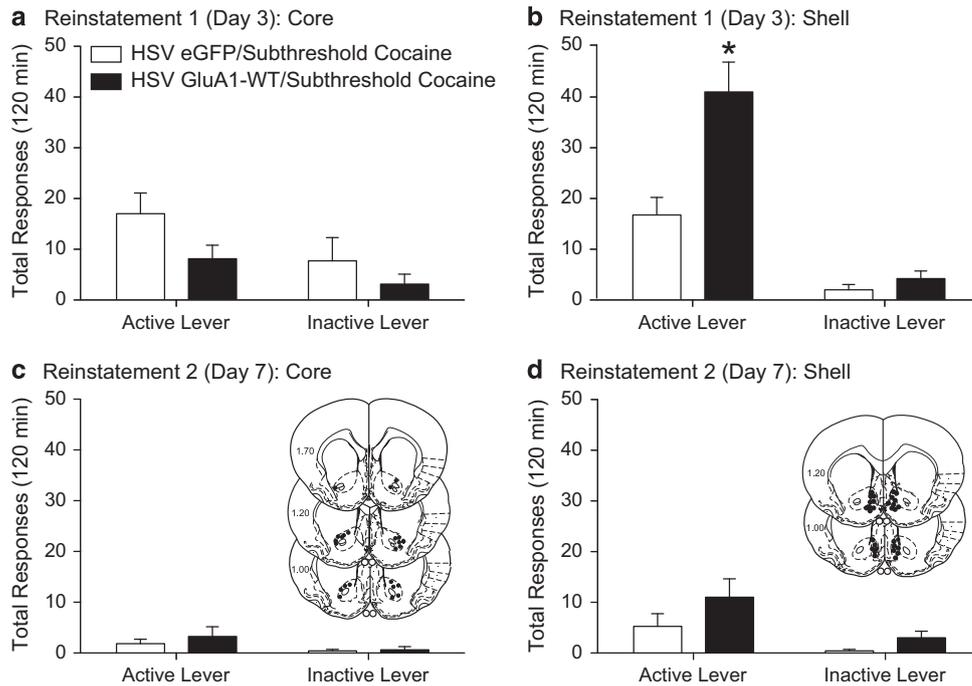
administration onto cultured hippocampal neurons. Analysis of these data (one-way ANOVA) revealed a significant main effect ( $F_{2,9} = 60.36$ ,  $p < 0.0001$ ). Subsequent *post hoc* comparisons (Dunnett's) indicated that SAP97 miRNA significantly decreased SAP97 expression relative to control ( $p < 0.05$ ), whereas the miRNA with a scrambled sequence had no significant effect (see Figure 6c). Analysis of the Reinstatement 1 data ( $n = 9$  per treatment) revealed significant main effects of treatment ( $F_{1,16} = 8.225$ ,  $p < 0.0112$ ) and lever ( $F_{1,16} = 57.19$ ,  $p < 0.0001$ ) and a significant virus  $\times$  lever interaction ( $F_{1,16} = 7.107$ ,  $p < 0.0169$ ). *Post hoc* tests indicated a significant active lever difference between treatments (Bonferroni,  $p < 0.05$ , Figure 6d). The virus was dormant 7 days after microinjection. Analysis of the second reinstatement session showed only a significant main effect of lever ( $F_{1,11} = 90.56$ ,  $p < 0.0001$ ) with no other significant main effect or interaction (see Figure 6e). Representative microinjection locations in the accumbens shell are shown in the inset of Figure 6e. The reinstatement of sucrose seeking also was assessed. HSV miSAP97 had no effect on sucrose seeking (see Table 1). These findings indicate that SAP97 has an important role in the reinstatement of cocaine seeking.

## DISCUSSION

The current results indicate that inhibition of CP-AMPA receptors in the nucleus accumbens blocks the reinstatement of cocaine seeking. This was demonstrated both pharmacologically and by overexpressing a 'pore dead' GluA1 subunit in the nucleus accumbens. Not only are CP-AMPA receptors assembled with GluA1 required for cocaine reinstatement, they appear to be sufficient for its expression. Thus, administration of a subthreshold cocaine dose reinstated cocaine seeking when GluA1 subunits were overexpressed in the nucleus accumbens. Although the precise role of CP-AMPA receptors in cocaine-induced changes in accumbens excitatory synaptic transmission needs to be explored in greater detail, our observations indicate that SAP97 is critically involved in this process.

### CP-AMPA receptors and Cocaine Reinstatement

The present results revealed that administration of the CP-AMPA receptor antagonist, Naspam, into the nucleus accumbens attenuated the reinstatement of cocaine seeking. In the nucleus accumbens, CP-AMPA receptors are predominantly GluA1



**Figure 4** Overexpression of GluA1-WT in the nucleus accumbens shell, but not the core, transiently promotes cocaine-seeking behavior. Total active and inactive lever responding (mean  $\pm$  SEM) 3 days following the intra-core or intra-shell microinjection of HSV GFP or HSV GluA1-WT (when expression is maximal) are shown in panels a and b, respectively. Note the robust reinstatement response to a subthreshold dose (5 mg/kg, i.p.) of cocaine only when GluA1 subunits are overexpressed in the shell. (b) The asterisk represents a significant difference between the HSV eGFP and GluA1-WT treatments ( $p < 0.05$ ). Total active and inactive lever responding (mean  $\pm$  SEM) 1 week following intra-core or intra-shell administration of HSV GFP or HSV GluA1-WT, when the viruses are dormant, are depicted in panels c and d, respectively. Note that a subthreshold injection of cocaine (5 mg/kg, i.p.) failed to produce reinstatement in either viral treatment condition in either the core or shell subregion of the nucleus accumbens. There were 7–9 rats per treatment.

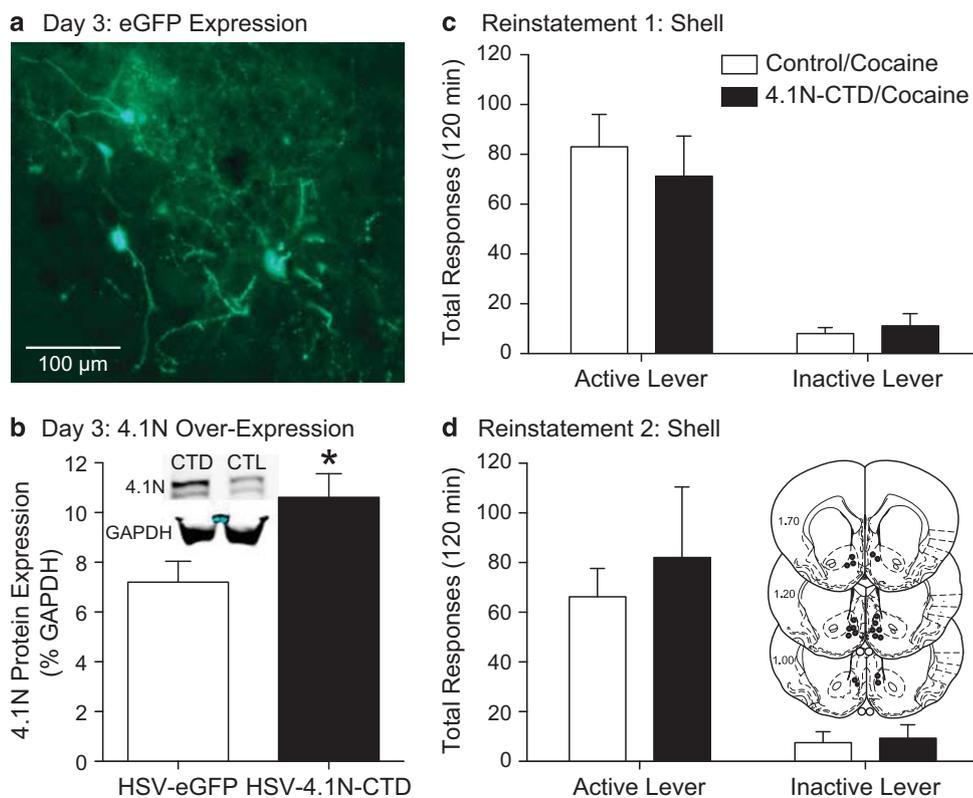
homomers or GluA1/GluA3 heteromers (Boudreau *et al*, 2007; Reimers *et al*, 2011). It was previously shown that administration of Nasp into the accumbens core blocked ‘incubated’ cocaine seeking (Conrad *et al*, 2008). Consistent with this finding, we showed that Nasp administered into either subregion of the accumbens, the core or the shell, attenuated the reinstatement of cocaine seeking induced by a cocaine priming injection. Administration of Nasp into the core also impaired sucrose seeking, suggesting that the core has a broader role in the modulation of reinforced behaviors, as noted previously (Bari and Pierce, 2005). Nasp administered into the DL striatum had no effect on cocaine seeking. Previous work indicated no changes in GluA1 phosphorylation in the DL striatum following cocaine self-administration (White *et al*, 2013). Collectively, these results indicated that GluA1-containing AMPARs in the accumbens core and shell contribute to the reinstatement of cocaine seeking during extinction, with the effects in the shell being relatively cocaine-specific.

In rats, CP-AMPA in the nucleus accumbens typically account for <10% of eEPSC amplitude (Conrad *et al*, 2008). However, extended-access cocaine self-administration followed by protracted forced abstinence increased synaptic expression of CP-AMPA in the nucleus accumbens such that these AMPARs were responsible for ~30% of eEPSC amplitude (Conrad *et al*, 2008; Mameli *et al*, 2009). Extended-access cocaine self-administration enhanced the functional influence of CP-AMPA in both the accumbens core (Ferrario *et al*, 2011; McCutcheon *et al*, 2011a, c) and

shell (Mameli *et al*, 2009; McCutcheon *et al*, 2011c). In contrast, the ability of Nasp to attenuate eEPSCs in the accumbens core during abstinence following short-access cocaine self-administration did not differ from saline-treated controls (Purgianto *et al*, 2013). It is questionable, therefore, that blocking CP-AMPA present in the nucleus accumbens prior to the reinstatement session is responsible for the ability of Nasp to attenuate the reinstatement of cocaine seeking using the current short-access cocaine self-administration paradigm. An important difference between the current work and previous studies of extended- and short-access cocaine self-administration is that our experiments included the extinction of cocaine seeking. However, we failed to observe differences in the inward rectification of AMPAR eEPSCs between animals that did or did not undergo extinction following short-access cocaine self-administration. It seems most likely, therefore, that the reinstatement of cocaine seeking using the current paradigm is associated with transient increases in surface CP-AMPA accumulation in the nucleus accumbens, which is consistent with previous work (Anderson *et al*, 2008; Schierberl *et al*, 2011).

#### GluA1-Containing AMPARs and Cocaine Reinstatement

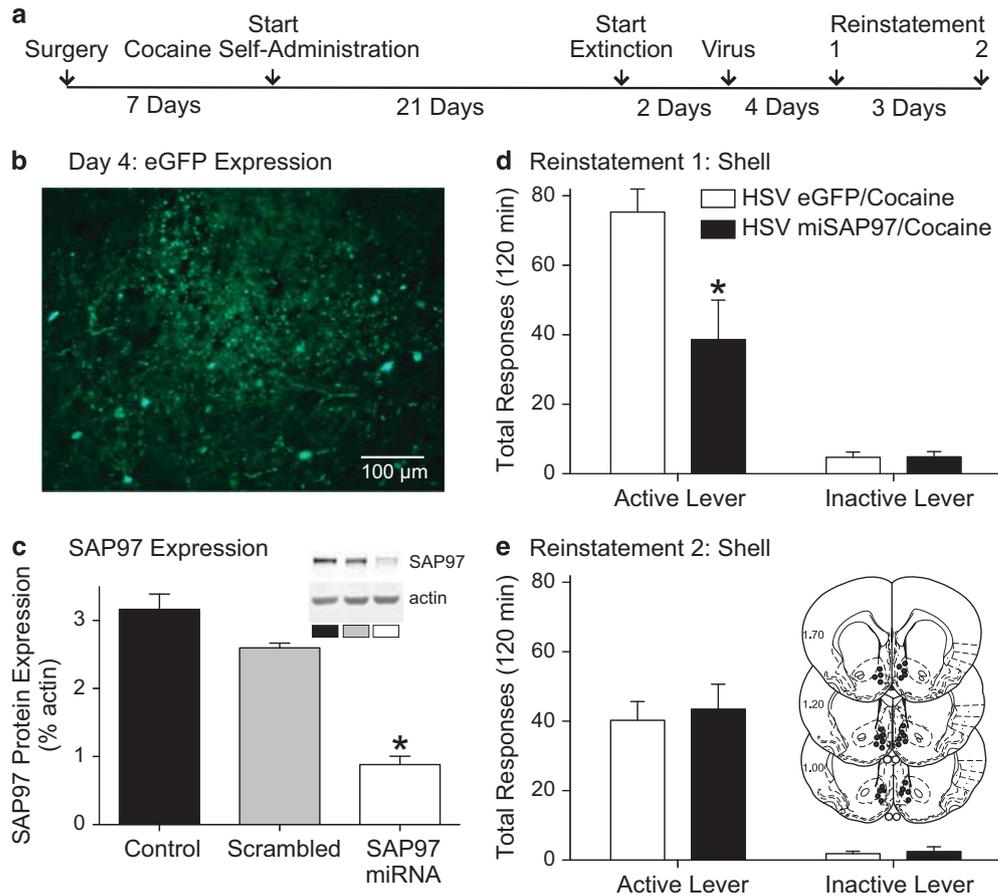
As noted previously, there is extensive evidence that cocaine self-administration influences nucleus accumbens GluA1-containing AMPARs. In the present experiments, overexpressing pore dead GluA1-Q582E in either the caudal



**Figure 5** Viral-mediated overexpression of 4.1N-CTD in the nucleus accumbens shell has no effect on the reinstatement of cocaine seeking. (a) Representative image of peak eGFP fluorescence in the nucleus accumbens 3 days following HSV 4.1N-CTD infusion. (b) Increases in 4.1N protein expression induced by HSV 4.1N-CTD as assessed with western blot (see inset). Total active and inactive lever responding (mean  $\pm$  SEM) 3 days or 1 week following intra-shell microinfusion of HSV eGFP or HSV 4.1N-CTD is shown in panels c and d, respectively. Note that the active lever responding to a systemic injection of cocaine (10 mg/kg, i.p.) does not differ between treatments at either time point. The inset in panel d depicts cannula placements for all shell microinjections. There were 5–13 rats per treatment.

lateral core or caudal medial shell subregion of the nucleus accumbens attenuated cocaine priming-induced reinstatement, without altering sucrose-seeking behavior. Correspondingly, the present results showed that viral-mediated overexpression of wild-type GluA1 in the shell facilitated drug seeking when combined with a normally subthreshold dose of cocaine. Taken together, these findings suggest that synaptic incorporation of accumbens GluA1-containing AMPARs, some of which may form CP-AMPARs, facilitates cocaine seeking. However, previous work showed that overexpression of ‘pore dead’ GluA1 in the accumbens core actually facilitated the reinstatement of cocaine seeking (Bachtell *et al*, 2008). There are several methodological differences between the previous and current studies, but perhaps most important are the respective delineations of the accumbens core. It has been shown that the disrupting AMPAR transmission in the rostral accumbens core promotes appetitive behaviors, with this effect reversing when AMPAR antagonists are administered more caudally (Reynolds and Berridge, 2008). Similarly, impairing GluA1-mediated AMPAR transmission in the caudal core in the present experiments impaired cocaine seeking, whereas this identical manipulation in the rostral core enhanced cocaine reinstatement (Bachtell *et al*, 2008). These results highlight critical differences in the role GluA1-mediated AMPARs in subregions of the accumbens core.

Approximately 95% of nucleus accumbens neurons are GABAergic medium spiny outputs that are differentiated through their expression of D1 and D2 dopamine receptors (Lobo and Nestler, 2011). Accumulating evidence indicates that activation of D1 dopamine receptors (D1DRs) in the nucleus accumbens shell promotes cocaine seeking by influencing transmission through GluA1-containing AMPARs. Thus, stimulation of D1DRs in the nucleus accumbens shell reinstates cocaine seeking, in part, by serially activating L-type calcium channels and calcium/calmodulin-dependent protein kinase II (CaMKII) (Anderson *et al*, 2008; Schmidt *et al*, 2006a; Schmidt and Pierce, 2006d). Cocaine reinstatement also was associated with D1DR-dependent increases in GluA1-pSer831, a CaMKII phosphorylation site, as well as increased cell surface expression of GluA1-containing AMPARs in the shell (Anderson *et al*, 2008). Notably, administration of a D1DR agonist into the medial, but not the lateral, core reinstated cocaine seeking (Bachtell *et al*, 2005; Schmidt *et al*, 2006a), which emphasizes medial-lateral in addition to rostral-caudal (see above) differences in accumbens modulation of drug seeking. Viral-mediated expression of GluA1-Q582E in the medial core blocked intra-accumbal D1DR agonist-induced reinstatement of cocaine seeking (Hobson *et al*, 2013). Taken together, these results indicate that the reinstatement of cocaine seeking is facilitated by enhanced transmission through GluA1-containing AMPARs in D1DR-expressing neurons



**Figure 6** HSV miSAP97-mediated reduction of SAP97 protein expression in the accumbens shell reversibly attenuated cocaine priming-induced reinstatement. (a) Schematic of the experimental paradigm. (b) Representative image of peak eGFP fluorescence in the nucleus accumbens 4 days following HSV miSAP97 infusion. (c) Decreased SAP97 expression is seen in hippocampal neuron cultures measured by western blot (see inset) following administration of HSV SAP97 miRNA. Total active and inactive lever responding (mean  $\pm$  SEM) 4 days following intra-shell microinfusion of HSV scramble or HSV miSAP97 are depicted in panel d ( $n=9$ ). Note the significant reduction in cocaine reinstatement in the HSV miSAP97 group. The asterisk represents a significant difference between the HSV scramble and HSV miSAP97 treatments ( $p < 0.05$ ). Total active and inactive lever responding (mean  $\pm$  SEM) 7 days following intra-shell microinfusion of HSV scramble or HSV miSAP97 is depicted in panel e. Note that the active lever responding following a systemic injection of cocaine (10 mg/kg, i.p.) did not differ between treatments. The inset in panel e depicts cannula placements for all shell microinjections.

in the medial core and medial shell of the nucleus accumbens.

Similar to D1DR agonists, administration of D2 dopamine receptor (D2DR) agonists into the medial core or medial shell promoted the reinstatement of cocaine seeking (Bachtell et al, 2005; Schmidt et al, 2006a; Schmidt and Pierce, 2006d). D2DRs signal through G-proteins including  $G\beta\gamma$  and Gi. Recent evidence indicates that D2DR-induced activation of  $G\beta\gamma$ -PLC- $PIP_2$ -PKC promotes cocaine seeking by internalizing GluA2-containing AMPARs, thereby decreasing overall AMPAR-mediated synaptic strength in D2DR-expressing neurons (Famous et al, 2008; Schmidt et al, 2013, 2014; Wiggins et al, 2011). Collectively, these results indicate that enhanced AMPAR-mediated neurotransmission in D2DR-containing accumbens output neurons suppresses cocaine reinstatement. Consistent with these results, strengthening of glutamatergic inputs onto D2DR-containing accumbens neurons restrains cocaine self-administration (Bock et al, 2013). In contrast, as noted above, increased AMPA transmission in accumbens neurons that express D1DRs promotes cocaine seeking. Differential

effects of cocaine on the two populations of accumbens output neurons also may help to explain discrepancies in the literature in terms of the precise role of accumbens AMPAR subunits in the reinstatement of cocaine seeking (Anderson et al, 2008; Conrad et al, 2008; Famous et al, 2008; Hobson et al, 2013; Ping et al, 2008; Sutton et al, 2003). That said, studies have shown increased AMPAR rectification in the nucleus accumbens following cocaine self-administration when neurons were selected in an unbiased manner, which suggests increased accumulation of CP-AMPA in both populations of accumbens efferent neurons (Ma et al, 2014; McCutcheon et al, 2011c; Scheyer et al, 2014).

#### GluA1 Accessory Proteins and Cocaine Reinstatement

The current results showed that reducing the abundance of SAP97 suppressed cocaine priming-induced reinstatement of drug seeking. However, impairing the interaction between 4.1N and GluA1 had no influence on cocaine seeking. This latter effect was somewhat surprising given that 4.1N

interacts with PKC and GluA1 subunits (Lin *et al*, 2009), both of which contribute to the reinstatement of cocaine seeking (Pierce and Wolf, 2013; Schmidt *et al*, 2014).

CaMKII, which has a critical role in psychostimulant-mediated behavioral plasticity (Anderson *et al*, 2008; Loweth *et al*, 2010; Pierce *et al*, 1998), phosphorylates SAP97 (at Ser39), which promotes the transport of SAP97 to synapses (Mauceri *et al*, 2004). Initial studies suggested that CaMKII phosphorylated GluA1 (at Ser831) to promote the trafficking of GluA1-containing AMPARs to the cell surface (Malinow and Malenka, 2002). However, it was subsequently shown that this effect was not influenced by mutating the GluA1 Ser831 residue (Hayashi *et al*, 2000). These results suggested that CaMKII might influence AMPAR trafficking indirectly, perhaps via interactions with accessory proteins such as SAP97. Indeed, impairing the interaction between SAP97 and GluA1 impairs surface insertion of GluA1 (Passafaro *et al*, 2001) in a CaMKII-dependent manner (Nikandrova *et al*, 2010; Schluter *et al*, 2006). Contrary to these findings, a knock-in mouse lacking the final seven GluA1 C-terminal amino acids (the SAP97-binding site) had normal GluA1 synaptic localization, trafficking, and basal synaptic transmission (Kim *et al*, 2005; Zhou *et al*, 2008). However, these results may be due to compensation by other MAGUKs, which are functionally redundant (Howard *et al*, 2010). Interactions between SAP97 and GluA1 also promote dendritic branching in an activity-dependent manner (Jablonski and Kalb, 2013; Zhou *et al*, 2008). Given that structural plasticity is observed in the nucleus accumbens following exposure to cocaine (Robinson and Kolb, 2004; Shen *et al*, 2009), it is intriguing to speculate that SAP97-GluA1 may have a role in this process.

SAP97 interacts with many other targets in addition to GluA1. For example, CaMKII phosphorylation of SAP97 at Ser232 regulates the synaptic targeting of NMDA receptors containing NR2A subunits (Gardoni *et al*, 2003; Mauceri *et al*, 2007). Although there is substantially less evidence for a role of NMDA receptors in cocaine-induced neuronal plasticity relative to AMPARs, there is evidence that repeated cocaine alters NR2A expression in the nucleus accumbens (Ary and Szumlinski, 2007; Ortinski *et al*, 2013; Schumann and Yaka, 2009). We also recently showed an increase in extrasynaptic NMDA receptor signaling in cocaine-experienced rats (Ortinski *et al*, 2013).

## CONCLUSIONS

These present results add to the growing literature indicating that increased transmission through GluA1-containing AMPARs, some of which are CP-AMPARs, in both subregions of the nucleus accumbens promotes the reinstatement of cocaine seeking. Moreover, viral-mediated expression of a miRNA, which reduces the expression of the GluA1 chaperone protein, SAP97, attenuated cocaine seeking. These data suggest that the AMPAR accessory protein SAP97 may be a potential novel target for the development of cocaine addiction pharmacotherapies.

## FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

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