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Role of GluR1 expression in nucleus accumbens neurons in cocaine sensitization and cocaine-seeking behavior

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Abstract

Chronic cocaine use reduces glutamate levels in the nucleus accumbens (NAc), and is associated with experience-dependent changes in (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) glutamate receptor membrane expression in NAc neurons. These changes accompany behavioral sensitization to cocaine and increased susceptibility to cocaine relapse. The functional relationship between neuroplasticity in AMPA receptors and the behavioral manifestation of cocaine addiction remains unclear. Thus, we examined the behavioral effects of up- and downregulating basal AMPA receptor function in the NAc core and shell using viral-mediated gene transfer of wild-type glutamate receptor 1 (wt-GluR1) or a dominant-negative pore-dead GluR1 (pd-GluR1), respectively. Transient increases in wt-GluR1 during or after cocaine treatments diminished the development of cocaine sensitization, while pd-GluR1 expression exacerbated cocaine sensitization. Parallel changes were found in D2, but not D1, receptor-mediated behavioral responses. As a correlate of the sensitization experiments, we overexpressed wt- or pd-GluR1 in the NAc core during cocaine self-administration, and tested the effects on subsequent drug-seeking behavior 3 weeks after overexpression declined. wt-GluR1 overexpression during self-administration had no effect on cocaine intake, but subsequently reduced cocaine seeking in extinction and cocaine-induced reinstatement, whereas pd-GluR1 facilitated cocaine-induced reinstatement. When overexpressed during reinstatement tests, wt-GluR1 directly attenuated cocaine- and D2 agonist-induced reinstatement, while pd-GluR1 enhanced reinstatement. In both experimental procedures, neither wt- nor pd-GluR1 expression affected cue-induced reinstatement. Together, these results suggest that degrading basal AMPA receptor function in NAc neurons is sufficient to facilitate relapse via sensitization in D2 receptor responses, whereas elevating basal AMPA receptor function attenuates these behaviors.

Introduction

Cocaine-induced adaptations in glutamate input to the nucleus accumbens (NAc) are thought to contribute to behavioral sensitization and a propensity for relapse (Kalivas *et al*., 2005; Kalivas & Hu, 2006). Recent work suggests an elaborate and complex regulation of excitatory input mediated by (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors in NAc neurons. Withdrawal from repeated cocaine treatments leads to increased surface expression of AMPA receptors, AMPA-mediated synaptic currents and enhanced long-term potentiation (LTP; Yao *et al*., 2004; Boudreau & Wolf, 2005; Kourrich *et al*., 2007). These changes parallel increased behavioral sensitivity to focal AMPA receptor stimulation in the NAc, and to a cocaine challenge (Pierce *et al*., 1996; Bell *et al*., 2000). Interestingly, increases in surface AMPA receptors appear to reverse upon renewed exposure to cocaine. Thus, after a period of withdrawal, a challenge injection of cocaine decreases surface AMPA receptors, AMPA-mediated synaptic currents and occludes further long-term depression (LTD) measured 1 day following drug challenge (Thomas *et al*., 2001; Boudreau *et al*., 2007). This reversal of withdrawal-induced elevations in surface AMPA receptors may be rapid and necessary for the expression of behavioral sensitization, as preventing AMPA receptor internalization and the resulting LTD during an amphetamine challenge prevents the expression of behavioral sensitization (Brebner *et al*., 2005).

Whether such dynamic AMPA receptor trafficking occurs after withdrawal from chronic self-administered cocaine is unknown. In contrast to sensitizing regimens of cocaine, recent studies found that withdrawal alone (without drug challenge) from chronic cocaine self-administration decreases excitability of NAc neurons and attenuates LTD at cortico-accumbens synapses (Martin *et al*., 2006; Schramm-Sapyta *et al*., 2006). These deficits in cellular excitability correlate with decreased extracellular glutamate levels (Keys *et al*., 1998; Baker *et al*., 2003), and experimental restoration of basal extracellular glutamate deficits reduces cocaine relapse behaviors (Baker *et al*., 2003; Madayag *et al*., 2007). However, acute AMPA receptor stimulation is sufficient to induce drug seeking in animals that extinguished from cocaine self-administration (Cornish *et al*., 1999; Cornish & Kalivas, 2000; Suto *et al*., 2004). Thus, the role of basal AMPA receptor expression in NAc neurons in cocaine sensitization and the propensity for cocaine-seeking behavior is unclear.

We studied the effects of increasing and decreasing basal AMPA receptor function in NAc neurons on cocaine sensitization and relapse behavior. We used viral-mediated expression of wild-type (wt) and dominant negative pore-dead (pd) glutamate receptor 1 (GluR1) subunits in NAc neurons. Exogenous overexpression of wt-GluR1 increases the excitability of infected neurons, consistent with

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membrane insertion of homomeric GluR1 AMPA receptors (Carlezon *et al*., 1997; Hayashi *et al*., 2000; Shi *et al*., 2001; Takahashi *et al*., 2003). Conversely, overexpression of pd-GluR1 containing a single point mutation (Q582E) in the pore region reduces synaptic AMPA currents through heteromeric interactions with endogenous AMPA subunits (Dingledine *et al*., 1999; Shi *et al*., 2001). Thus, these viral vectors were used to potentiate and depress basal AMPA receptor function in NAc neurons, and to study the effects on the development and expression of cocaine sensitization and reinstatement of cocaine-seeking behavior.

Materials and methods

Animals and housing conditions

Male Sprague–Dawley rats initially weighing 275–325 g (Charles River Laboratories, Kingston, NY, USA) were individually housed in wire cages with food and water available *ad libitum*. Most experiments were conducted during the light cycle of a 12 : 12 h light : dark cycle (lights on at 07.00 h). All experiments were in accordance with guidelines established by the National Institute of Health, and were approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. All surgery was conducted under sodium pentobarbital anesthesia (60 mg/kg), and animals were given the ketofen injections (5 mg/kg) daily for 3 days post-surgery to reduce pain, inflammation and discomfort.

Viral vector characterization in vitro

A herpes simplex virus (HSV) vector encoding wt-GluR1, pd-mutant GluR1^{Q582E}, protein kinase A mutant GluR1^{S845A} and LacZ were produced and administered as described previously (Carlezon *et al*., 1997; Kelz *et al*., 1999; Sutton *et al*., 2003). PC12 cells were grown to ~70% confluency in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells were infected with 2 μL/well of HSV vectors. After 24 h, cells were incubated for 30 min with either 5 μM forskolin or fresh medium. Cells were homogenized in buffer containing protease and phosphatase inhibitors. GluR1 and phosphoGluR1^{S845} levels were determined by Western blot of 20 μg samples labeled with rabbit anti-GluR1 (1 : 5000, Chemicon, Temecula, CA, USA) or rabbit anti-phosphoGluR1^{S845} (1 : 5000, Upstate Biotechnology, Lake Placid, NY, USA) followed by goat anti-rabbit peroxidase-conjugated secondary (1 : 25 000; Bio-Rad, Hercules, CA, USA) and chemiluminescence detection.

Intra-NAc cannulation and microinfusion

Guide cannulae were aimed at the either the NAc core (± 1.5 mm lateral) or shell (± 0.8 mm lateral) subregions positioned 1.7 mm anterior to bregma and −5.7 mm ventral to dura with level skull (Paxinos & Watson, 1998). An additional set of animals was implanted with guide cannulae aimed at the medial caudate putamen 2 mm dorsal to the NAc core site (−3.7 mm ventral to dura). Dummy stylets (33-gage), cut to extend 1 mm beyond the guide cannulae, were left in place throughout the experiment. Animals were allowed 5–7 days to recover before the onset of experimentation.

Vector titers were 4.0×10^7 infectious units/mL. Rats received bilateral (2.0 μL/side) microinfusions of the vectors into the NAc over 10 min through 33-gage injection cannulae extending 1 mm beyond the guide. The injectors were left in place for an additional 2 min to allow diffusion into the tissue. Behavioral testing began 24 h after the infusion and continued for a maximum of 5 days during overexpression.

Viral vector characterization in vivo

Quantitative RT-PCR of *in vivo* vector expression was performed on mRNA isolated from 14-gage NAc tissue punches surrounding the infusion site at 2 and 6 days post-infusion. Coronal brain slices (40 μm) were labeled with anti-GluR1 (1 : 1000), 2 or 6 days after HSV infusions, as described previously (Sutton *et al*., 2003). Briefly, tissue samples were homogenized in RNA-STAT60 (IsoTex Diagnostics, Friendswood, TX, USA) and RNA extracted according to manufacturer's instructions. DNAse-treated (Abion, Foster City, CA, USA) RNA samples were converted to cDNA using Superscript III (Invitrogen, Carlsbad, CA, USA). Cycle thresholds were calculated from triplicate reactions using the -ΔΔ cycle threshold method. Primer sequences are as follows; GluR1: 5′-GTCCGCCCTGAGAAATC-CAG-3′, 5′-CTCGCCCTTGTCGTACCAC-3′; GluR2: 5′-GCCGA-GGCGAAACGAATGA-3′, 5′-CACTCTCGATGCCATATACGTTG-3′; glyceraldehyde-3-phosphate dehydrogenase: 5′-AACGACCCCTTC-ATTGAC-3′, 5′-TCCACGACATACTCAGCAC-3′.

HSV-mediated wt- and pd-GluR1 protein expression *in vivo* was compared by immunohistochemical labeling for anti-GluR1 at 2 or 6 days after HSV infusions. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused with 4% paraformaldehyde. Serial 40-μm coronal sections through the striatum were collected and processed for GluR1 immunolabeling as previously described (Sutton *et al*., 2003). Briefly, sections were incubated with 1 : 1000 rabbit anti-GluR1 (Chemicon), and detection was performed using the avidin-biotin/diaminobenzidine visualization method (Vector Laboratories, Burlingame, CA, USA; Pierce, Rockford, IL, USA).

Intra-NAc AMPA- and N-methyl-d-aspartic acid (NMDA)-mediated locomotion

Locomotor activity was recorded in the dark using circular test chambers with a 12-cm-wide runway, and equipped with four pairs of photocells located at 90-degree intervals around the 1.95 m perimeter. Animals with bilateral guide cannulae in the NAc core were initially habituated to the locomotor apparatus for 2 h the day prior to locomotor testing, and given intra-NAc infusions of the HSV vectors. Each subsequent test session incorporated a 2-h habituation phase followed by an intra-NAc core microinfusion of either a phosphate-buffered saline vehicle or AMPA (0.2 nmol/side) in a volume of 0.5 μL/side over 100 s through bilateral 33-gage injection cannulae. The injectors were left in place for 30 s to allow for diffusion into brain tissue, gently removed, and the animals were placed back into the locomotor apparatus where horizontal locomotion was recorded for 2 h. Each dose was administered in counterbalanced order across consecutive test days. A separate group of animals received intra-NAc core infusions of vehicle or NMDA (0.35 and 0.70 nmol/side) over three consecutive test days. A final set of animals was sensitized to cocaine with seven daily cocaine treatments (15 mg/kg/day, i.p.). Following 18 days of withdrawal, these animals were given intra-NAc infusions of the HSV vectors (LacZ, wt-GluR1 and pd-GluR1) 1–2 days prior to locomotor tests with intra-NAc core infusions of vehicle or AMPA (0.2 nmol/side).

Following behavioral testing, the localization of infusion sites was determined by infusing 0.5 mL of Cresyl violet dye through bilateral injection cannulae in chloral-hydrate-anesthetized animals as described above. Animals were immediately decapitated, brains

dissected, and 0.8-mm-thick coronal slices were collected throughout the forebrain and analysed under a dissecting microscope for the location of infusion sites according to the stereotaxic coordinates (Paxinos & Watson, 1998).

Cocaine locomotor sensitization procedure

A brief 4-day sensitizing regimen of cocaine was used to induce sensitization within the time frame of transient HSV overexpression (see Fig. 2). Animals were habituated to the locomotor testing apparatus for 2 h, and given intra-NAc infusions of the HSV vectors the day prior to initial cocaine administration. On the first test day, animals were habituated for 90 min, challenged with an i.p. injection of cocaine (10 mg/kg) and locomotor activity was assessed for 2 h. The following 3 days, animals were not habituated, but received daily injections of a higher dose of cocaine (20 mg/kg) followed by 2 h locomotor activity assessment. Animals were left in their home cage for 7 days of withdrawal, and locomotor responses were tested with a challenge dose of cocaine (10 mg/kg) on the eighth day, at least 6 days after HSV-mediated overexpression declines to low or undetectable levels. On the following 2 days, the locomotor response to D1 and D2 dopamine receptor challenge were measured with the D1 receptor agonist (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF 81297) or the D2 receptor agonist quinpirole in counterbalanced order as described previously (Edwards *et al*., 2007). Briefly, D1 and D2 agonist-induced locomotion was assessed in a 6-h within-session dose–response protocol as follows: 2-h habituation followed by hourly ascending doses of agonist (saline, 0.1, 0.3 and 1.0 mg/kg, s.c.). To determine whether modulating AMPA receptor function could alter the expression of sensitization after induction had occurred, animals were given HSV infusions the day after repeated cocaine treatment (or no treatment), and challenged with cocaine, SKF 81297 and quinpirole as described above beginning 8 days after the HSV infusion (2 days after HSV expression declined).

Quinpirole locomotor tests

In a separate experiment, the direct effect of AMPA receptor modulation on D2-mediated locomotor responses was tested in cocaine-naïve animals using the dose–response procedure described above. Locomotor activity was assessed in a pre-test 2 days before animals were given HSV infusions, and tested again 1–2 days after the HSV infusion during HSV-mediated overexpression.

Acquisition of cocaine self-administration

Self-administration and reinstatement testing were performed in operant test chambers (Medical-Associates, Georgia, VT, USA) equipped with two response levers and an infusion pump system as described (Edwards *et al*., 2007). Most animals were initially trained to lever press for sucrose pellets to facilitate acquisition of cocaine self-administration. These rats were food-restricted to prevent weight gain, and trained to lever-press for sucrose pellets on a fixed ratio 1 (FR1) reinforcement schedule until acquisition criteria had been achieved (100 sucrose pellets in three consecutive sessions). After lever-press training, animals were fed *ad libitum* for at least 1 day prior to being surgically implanted with both a chronic intrajugular catheter and 26-gage bilateral intracerebral guide cannulae aimed at the NAc core or caudate-putamen, as previously described (Self *et al*., 1998).

Following at least 5–7 days recovery from surgery, animals were allowed to self-administer intravenous cocaine (0.5 mg/kg/50 mL injection) on a FR1 reinforcement schedule in daily 4-h sessions for 5–6 days/week. Cocaine injections were delivered over 2.5 s concurrent with the illumination of a cue light above the active lever, and followed by an additional 12.5-s time out period (time out 15 s) when the house light remained off and responding was without consequence. Inactive lever responses produced no consequence throughout testing. A subset of animals was allowed to acquire cocaine self-administration during viral-mediated overexpression of wt- or pd-GluR1. In this experiment, animals did not receive prior lever-press training with sucrose pellets to ascertain potential effects on the acquisition of cocaine self-administration and operant learning. These animals received intra-NAc core infusions of viral vectors 1 day prior to acquisition of cocaine self-administration (0.5 mg/kg/25 mL injection delivered in 1.25 s) in daily 10-h sessions for the first 5 days during the dark cycle. In the following 2 weeks, animals self-administered cocaine in daily 4-h sessions for 5 days/week as described above (during the light cycle), but received intra-NAc infusions of viral vectors 1 day prior to each weekly test.

Extinction/reinstatement testing

After a minimum of 15 cocaine self-administration sessions, animals remained in their home cages for 7 days of withdrawal. On Days 8–13 of withdrawal, animals returned to the operant test chambers for extinction training in the absence of cocaine reinforcement in 4-h test sessions. Responses on the lever that previously delivered cocaine injections during self-administration (drug-paired lever) and on the inactive lever were recorded, but had no programmed drug or cue delivery. Cocaine seeking during extinction served as a dependent experimental variable in animals that previously acquired cocaine self-administration during wt- and pd-GluR1 overexpression.

Cue and cocaine-primed reinstatement

We tested the ability of prior wt- or pd-GluR1 overexpression during the self-administration phase to subsequently modulate the propensity for relapse after overexpression declined in cocaine withdrawal (indirect effects). We also tested the ability of wt- or pd-GluR1 overexpression to directly modulate relapse behaviors in animals receiving viral vector infusions 1 day prior to reinstatement testing. Cue- and cocaine-primed reinstatement of cocaine-seeking behavior was measured the week after extinction training across 4 days of testing (Days 15–18 of cocaine withdrawal). Each 1-h reinstatement test was preceded by an additional 3 h of extinction conditions. Cue-induced reinstatement was measured in the first reinstatement test by initial non-contingent (priming) with the cocaine injection cues delivered every 2 min for the first 10 min, while responding at the drug-paired lever resulted in response-contingent cue delivery throughout the 1-h test (2.5 s illumination of cue light and infusion pump, 15 s termination of house light). Cocaine-primed reinstatement was tested on the next 3 days by experimenter-administered vehicle or cocaine (5 and 15 mg/kg, i.p.) immediately prior to the 1-h reinstatement test in counterbalanced order. Responses at both drug-paired and inactive levers were recorded, but produced no cue or drug delivery during testing.

Quinpirole-induced reinstatement

In separate study groups, animals received intra-NAc viral vector infusions 1 day prior to reinstatement testing, with the D2-receptor agonist quinpirole following self-administration and extinction

training as described above. Given the longer duration of quinpirole action relative to cocaine, priming injections of quinpirole (0.1, 0.3, 1.0 and 3.0 mg/kg, s.c.) were given before the final 2 h of the session immediately after 2 h of extinction conditions. Drug doses were administered in counterbalanced order across test days, with the exception of the highest dose (3.0 mg/kg), which was always administered during the final session. Responses at both levers were recorded, but resulted in no cue or cocaine delivery.

Sucrose reinstatement

Animals were implanted with guide cannulae aimed at the NAc core, and trained to self-administer sucrose pellets on an FR1 schedule as described above. After 15 daily sessions (100 pellets/session), animals remained in their home cages for 7 days of withdrawal, and were then subjected to extinction training in six daily 4-h sessions. Following extinction training, animals received intra-NAc infusions of viral vectors, and were tested for reinstatement of sucrose seeking the next day using non-contingent sucrose pellet delivery in a single 1-h test immediately following an initial 1 h of extinction conditions. During the reinstatement phase, animals were presented with the non-contingent delivery of a sucrose pellet every 2 min for 1 h (30 pellets/h). Responding at both levers was recorded, but resulted in no cues or sucrose pellet delivery.

Data analysis

Locomotor data (beam breaks) were analysed by two-factor analysis of variance (ANOVA) on viral vector groups with repeated-measures on dose (AMPA, NMDA, SKF 81297, quinpirole) or test phase (acute vs challenge cocaine injections). Self-administration (# of injections) and extinction (drug-paired lever responses) data were analysed by two-factor ANOVA with repeated-measures on test session. Cue-induced reinstatement data (drug-paired lever responses) were analysed by two-factor ANOVA with repeated-measures on extinction baseline (third hour) vs cue presentation. Cocaine- and quinpirole-primed reinstatement data also were analysed with a two-factor ANOVA with repeated-measures on dose. Responding on the inactive lever was analysed similarly in reinstatement tests. In all cases, interactive effects were followed by simple main effects analyses (one-way ANOVA) and *post hoc* tests [Fisher's least significant difference (LSD)]. Statistical significance was preset at $P < 0.05$.

Drugs

SKF 81297, quinpirole [(–)-quinpirole hydrochloride], AMPA and NMDA were purchased from Sigma (St Louis, MO, USA). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC, USA). All drugs were dissolved in sterile-filtered physiological saline or phosphate-buffered saline (pH 7.4) for brain infusions.

Results

Bidirectional modulation of AMPA receptor responses

Competent viral-mediated expression was initially verified in PC12 cells infected with wt-GluR1 and pd-GluR1 *in vitro* (Fig. 1A). Forskolin-mediated activation of adenylate cyclase increased GluR1 phosphorylation at serine 845 in both wt- and pd-GluR1, a necessary event for membrane insertion (Derkach *et al*., 2007), but not when the serine residue was mutated to alanine. Previous work has shown that HSV-mediated expression of wt-GluR1 protein peaks 2–3 days following HSV infusions and diminishes after 6 days (Kelz *et al*., 1999; Sutton *et al*., 2003; Todtenkopf *et al*., 2006). We confirmed this transient expression profile for both wt- and pd-GluR1 following infusions into the NAc core of rats. Figure 1B shows that both wt- and pd-GluR1 vectors selectively increased GluR1, but not GluR2, expression by ~twofold 2 days after intra-NAc infusions relative to LacZ-expressing controls, and GluR1 mRNA declined completely to control levels after 6 days. Immunolabeling for GluR1 protein also shows that wt- and pd-GluR1 expression remained highly localized near the infusion site and was undetectable after 6 days (Fig. 1C–G). Importantly, the functional effects of wt- and pd-GluR1 were confirmed based on the locomotor response to intra-NAc AMPA infusions (Fig. 1G). In cocaine-naïve animals, overexpression of wt-GluR1 in the NAc core nearly doubled the locomotor response to intra-NAc AMPA, but not NMDA infusions, whereas pd-GluR1 expression attenuated AMPA-mediated locomotion (treatment–dose interaction; $F_{2,21} = 13.09$, $P < 0.001$). In animals treated with cocaine for 7 days followed by 18 days of withdrawal, a similar profile was observed (treatment–dose interaction; $F_{2,21} = 3.92$, $P < 0.05$), although baseline AMPA responses (LacZ) were almost twofold higher. These findings indicate that wt- and pd-GluR1 expression in NAc neurons functionally up- and downregulate AMPA-mediated behavioral responses, respectively. Thus, we used these vectors to experimentally increase and decrease basal AMPA receptor function in NAc neurons in studies on cocaine sensitization, self-administration and reinstatement described below.

NAc GluR1 modulates the development of cocaine and D2 receptor sensitization

We exploited the transient HSV expression profile to study the effects of modulating basal AMPA receptor input to NAc neurons on the development of cocaine sensitization. Figure 2A depicts the experimental design to limit GluR1 overexpression during only the induction of cocaine sensitization. Wt- and pd-GluR1 expression differentially altered the induction of cocaine sensitization based on the change in locomotor responses from acute to post-induction cocaine challenge (group × test; $F_{2,49} = 3.61$, $P < 0.05$). Thus, overexpression of pd-GluR1 in NAc core neurons was sufficient to enhance initial sensitivity to cocaine-induced locomotion compared with LacZ controls (group; $F_{2,49} = 7.15$, $P < 0.005$), but wt-GluR1 expression had no direct effect on initial cocaine sensitivity (Fig. 2B). While all treatment groups developed locomotor sensitization to a cocaine challenge compared with the acute cocaine response (test; $F_{1,49} = 33.30$, $P < 0.001$), wt-GluR1 expression during cocaine treatments reduced, and pd-GluR1 enhanced, the development of cocaine sensitization when animals were challenged 7 days later after overexpression declined (group; $F_{2,49} = 13.53$, $P < 0.001$).

To investigate potential changes in dopamine receptor sensitivity after induction of cocaine sensitization, we measured locomotor responses to the dopamine agonists SKF 81297 (D1) and quinpirole (D2) using a within-session dose–response procedure. While there were no differences in responses to D1-induced locomotion (Fig. 2C), locomotor responses to D2 receptor challenge paralleled changes in cocaine sensitivity with wt- and pd-GluR1 ($F_{2,49} = 10.82$, $P < 0.001$), indicating that expression of cocaine sensitization is related selectively to enhanced D2 receptor responsiveness. We also determined whether wt-GluR1 and pd-GluR1 would directly modulate D2-mediated locomotor responses in cocaine-naïve animals tested with quinpirole before and during HSV-mediated overexpression

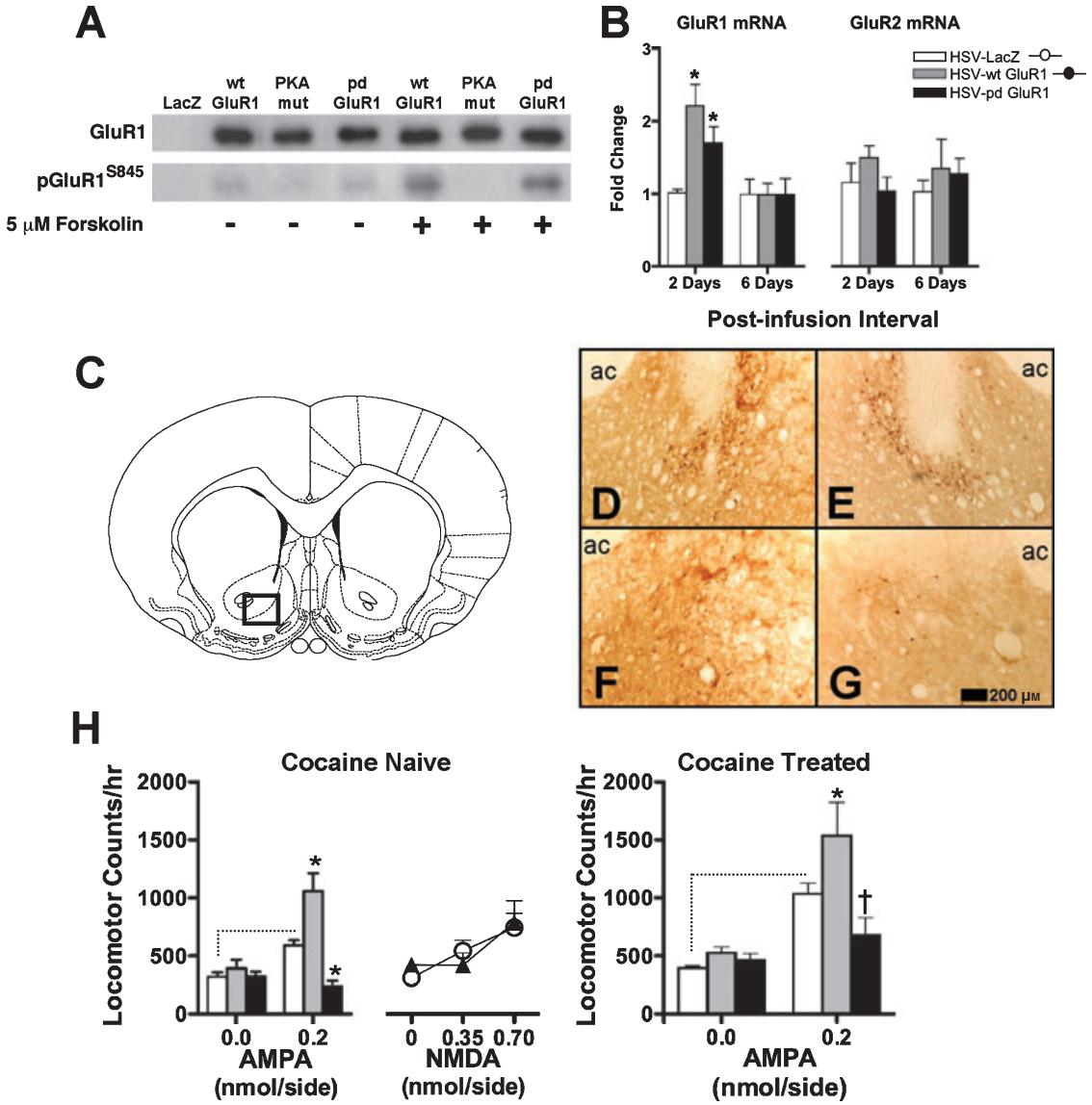

FIG. 1. Bidirectional modulation of (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-mediated behavioral responses following herpes simplex virus (HSV)-mediated overexpression of wild-type (wt)- and pore-dead (pd)-glutamate receptor 1 (GluR1) in the NAc core. (A) Infection of PC12 cells with HSV vectors shows equivalent expression of wt- and pd-GluR1 and protein kinase A (PKA)-mediated GluR1^{S845} phosphorylation with forskolin treatment (5 μM), but not in a PKA-resistant GluR1^{S845A} mutant. (B) Following HSV infusions *in vivo*, quantitative RT-PCR of GluR1 and GluR2 mRNA levels in the NAc punches confirms selective and transient expression of wt- and pd-GluR1. Data are mean (± SEM) mRNA levels normalized to GAPDH and expressed as fold change from LacZ-expressing controls. (C) Schematic illustrating the NAc core region (box) where HSV-mediated overexpression is depicted in (D–G). Overexpression of wt- and pd-GluR1 shows localized and transient immunohistochemical labeling of GluR1 protein 2 days (D: wt; E: pd) but not 6 days (F: wt; G: pd) after intra-NAc infusions (ac, anterior commissure). (H) Overexpression of wt- and pd-GluR1 in the NAc core of cocaine-naïve animals bidirectionally alters locomotor responses to intra-NAc AMPA, but not *N*-methyl-D-aspartate (NMDA), infusions (left). Similar bidirectional regulation of AMPA-mediated locomotion is observed in animals 18 days after receiving seven daily cocaine treatments (15 mg/kg; right). Data are mean (± SEM) photocell beam breaks/h. The dotted line indicates a significant increase from vehicle-treated LacZ controls ($P < 0.05$). *$P < 0.05$ compared with HSV-LacZ by Fisher's LSD test; $^{+}P < 0.05$ compared with AMPA-treated LacZ by *t*-test ($n = 5$–12/group).

(Fig. 2D). All groups developed sensitization to quinpirole after the pre-test (test; $F_{1,68} = 13.17$, $P < 0.001$), but wt-GluR1 and pd-GluR1 overexpression produced opposite effects on locomotor responses to quinpirole (test × group; $F_{2,68} = 7.93$, $P < 0.001$). Thus, wt-GluR1 directly attenuated, and pd-GluR1 directly enhanced, locomotor responses to quinpirole during overexpression ($F_{2,68} = 11.71$, $P < 0.001$). These results suggest that AMPA receptor-mediated input to NAc neurons directly opposes D2-mediated behavioral responses. Wt- and pd-GluR1 produced nearly identical effects on both cocaine sensitization and D2-mediated locomotion when overexpressed in the NAc shell (Fig. 3).

While pd-GluR1 produced profound effects on initial cocaine sensitivity, wt-GluR1 reduced the expression of sensitization through less obvious interactions with cocaine. Therefore, we tested whether overexpressing wt- and pd-GluR1 after the cocaine treatments (Fig. 4A) would alter the expression of cocaine sensitization independent of initial induction mechanisms. Figure 4B shows that wt-GluR1 overexpression during cocaine withdrawal lessened, while pd-GluR1 enhanced, the expression of cocaine sensitization (group × test; $F_{2,27} = 7.04$, $P < 0.005$), although all groups developed some degree of locomotor sensitization (Fig. 4B; test; $F_{1,27} = 63.41$, $P < 0.001$). These changes were accompanied by

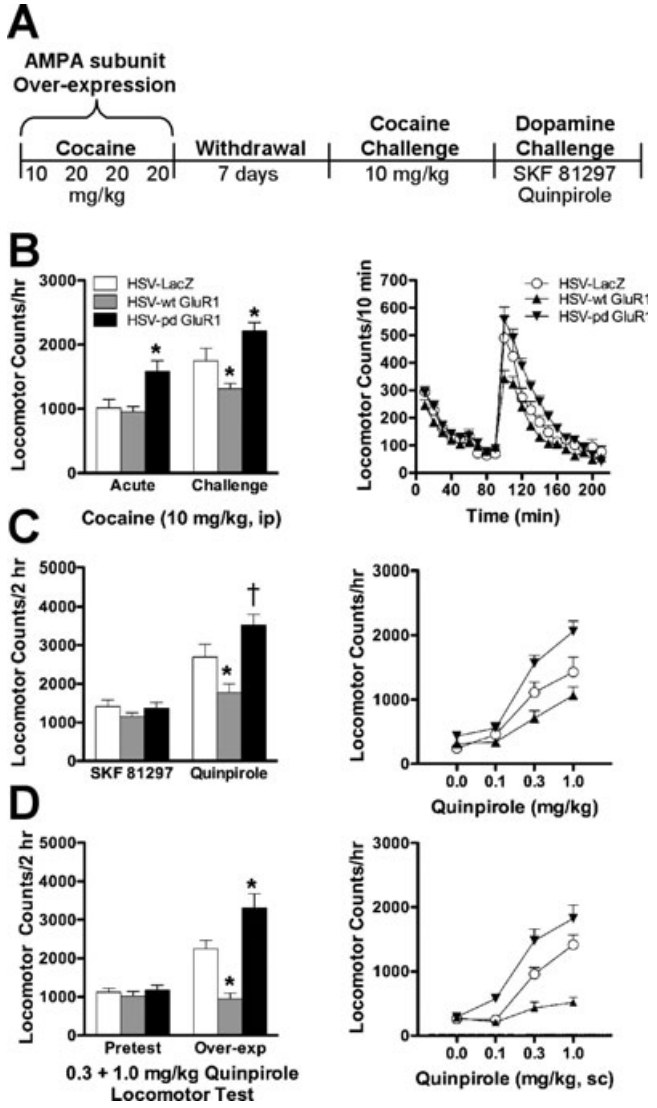

FIG. 2. Wild-type (wt)- and pore-dead (pd)-glutamate receptor 1 (GluR1) overexpression in the NAc core produces opposite effects on the development of cocaine sensitization. (A) Experimental time line restricting wt- and pd-GluR1 expression to cocaine treatment followed by post-expression drug challenge. (B) Overexpression of pd-GluR1 in the NAc core enhances the initial locomotor response to cocaine, and facilitates the induction of sensitization. Overexpression of wt-GluR1 has no direct effect on initial cocaine responses, but weakens the subsequent expression of sensitization. All groups develop significant sensitization (*post hoc* comparisons with acute all $P < 0.05$). Data are mean (± SEM) photobeam breaks in the first hour after cocaine (bar graphs) or per 10-min bin (line graph). (C) Post-expression locomotor responses to the D1 agonist SKF 81297 fail to differ, but locomotor responses to the D2 agonist quinpirole show bidirectional changes that parallel cocaine responses. (D) In cocaine-naïve animals, overexpression of wt- and pd-GluR1 produces direct bidirectional changes in locomotor responses to quinpirole. Bar graphs (left) show cumulative locomotor responses following 0.3 mg/kg and 1.0 mg/kg of SKF 81297 or quinpirole in a within-session dose–response procedure (right). $^{*}P < 0.05$, $^{+}P < 0.10$, Fisher's LSD test compared with herpes simplex virus (HSV)-LacZ ($n = 9$–20/group). AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid.

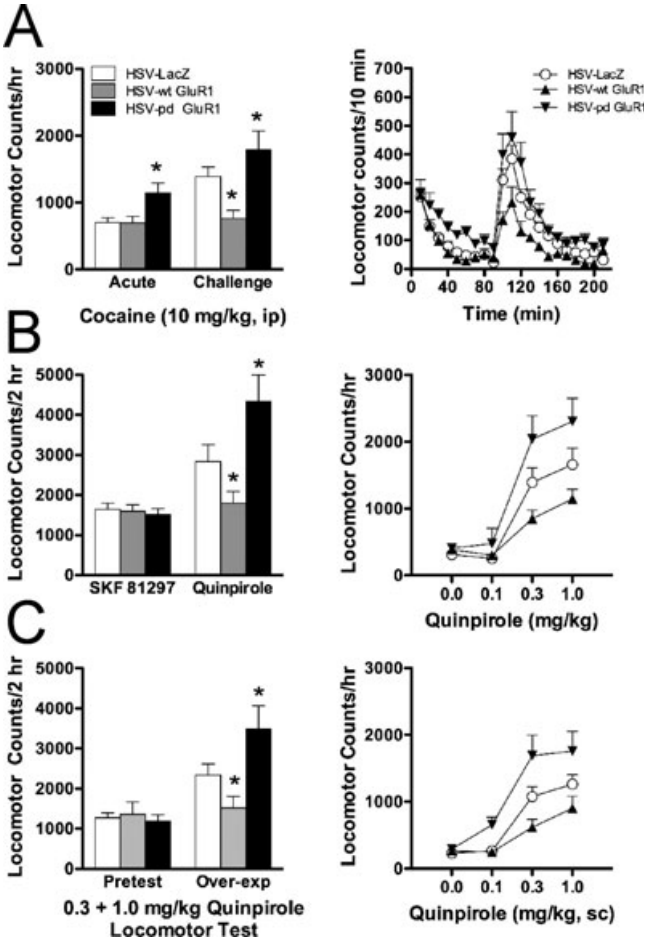

FIG. 3. Wild-type (wt)- and pore-dead (pd)-glutamate receptor 1 (GluR1) overexpression in the NAc shell also produces opposite effects on the development of cocaine sensitization. (A) Wt- and pd-GluR1 overexpression during cocaine treatments differentially alters the expression of cocaine sensitization (group × test; $F_{2,33} = 3.52$, $P < 0.05$). Thus, pd-GluR1 enhances the initial locomotor response to cocaine in drug-naïve animals (acute; $F_{2,33} = 5.93$, $P < 0.01$), while wt-GluR1 reverses the subsequent expression of sensitization based on post-expression cocaine challenge (challenge; $F_{2,33} = 7.396$, $P < 0.01$). All groups except wt-GluR1 developed significant sensitization (test: $F_{1,33} = 18.12$, $P < 0.001$, *post hoc* comparisons; LacZ and pd-GluR1, $P < 0.05$). Data are expressed as mean (± SEM) photobeam breaks per hour. (B) Post-expression locomotion to SKF 81297 failed to differ, but locomotion to quinpirole shows bidirectional changes that parallel cocaine responses ($F_{2,33} = 6.041$, $P < 0.01$). Bar graphs (left) show cumulative locomotor responses following 0.3 mg/kg and 1.0 mg/kg of SKF 81297 or quinpirole in a within-session dose–response procedure (right). (C) In cocaine-naïve animals, overexpression of wt- and pd-GluR1 produces direct bidirectional changes in locomotor responses to quinpirole ($F_{2,56} = 10.24$, $P < 0.001$). $^{*}P < 0.05$, Fisher's LSD test compared with herpes simplex virus (HSV)-LacZ ($n = 9$–20/group).

similar changes in D2 ($F_{2,27} = 12.18$, $P < 0.001$), but not D1, receptor sensitivity (Fig. 4C). These effects are not attributable to lasting expression of wt- or pd-GluR1 during the challenge tests, as similar tests performed after overexpression declined failed to alter locomotor responses to cocaine or dopamine receptor agonists in cocaine-naïve animals (Fig. 4D and E). Thus, bidirectional regulation of cocaine sensitization requires prior cocaine exposure, presumably reflecting necessary plasticity in this system induced by repeated cocaine treatments.

NAc GluR1 during cocaine self-administration attenuates subsequent relapse behaviors

We used an analogous approach to study the effect of GluR1 on the development of addictive behavior in animals self-administering cocaine. We chose to specifically analyse the NAc core in these

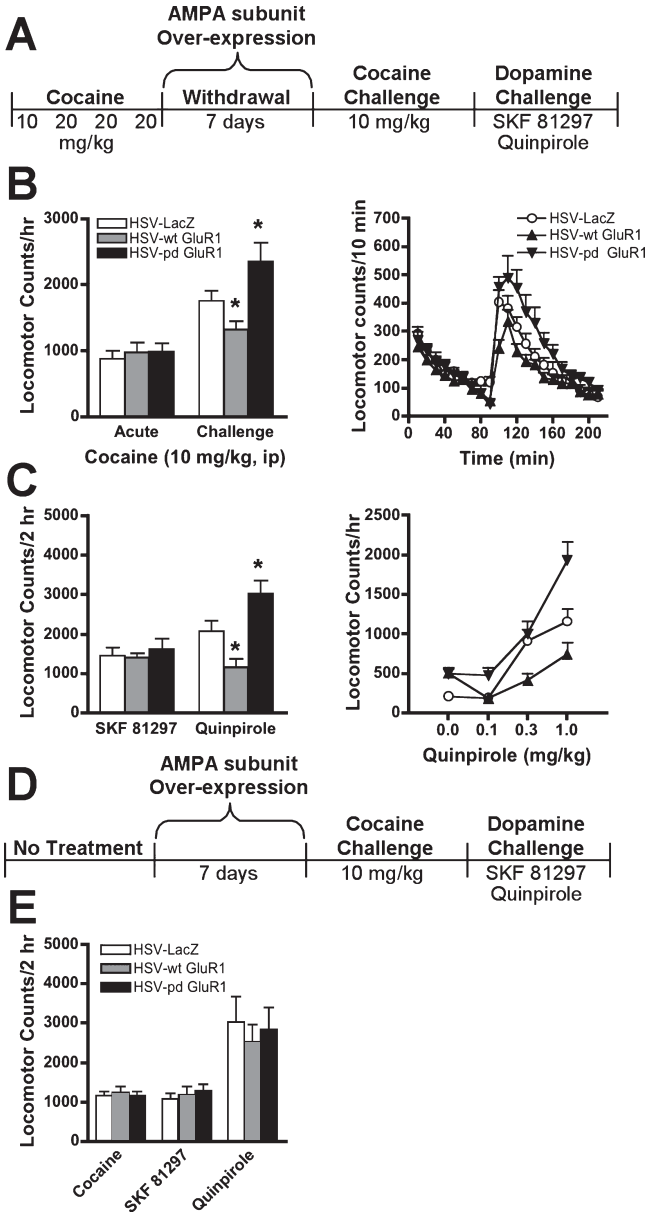

FIG. 4. Wild-type (wt)-glutamate receptor 1 (GluR1) reduces and pore-dead (pd)-GluR1 enhances cocaine sensitization when overexpressed after cocaine treatments in withdrawal. (A) Experimental time line restricting wt- and pd-GluR1 expression to a cocaine withdrawal period followed by post-expression drug challenge. (B) Overexpression of wt-GluR1 in the NAc core during withdrawal weakens expression of cocaine sensitization, while pd-GluR1 enhances expression of sensitization. All groups develop significant sensitization (*post hoc* comparisons with acute all $P < 0.05$). (C) Similar changes are observed in locomotor responses to quinpirole, but not SKF 81297. (D) Experimental time line for transient wt- and pd-GluR1 expression in cocaine-naïve animals. (E) Prior wt- or pd-GluR1 overexpression in cocaine-naïve animals has no effect on post-expression locomotor responses to cocaine, SKF 81297 or quinpirole. Data are expressed as described in Fig. 2. *$P < 0.05$, Fishers LSD test compared with herpes simplex virus (HSV)-LacZ ($n = 9$–11/group). AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid.

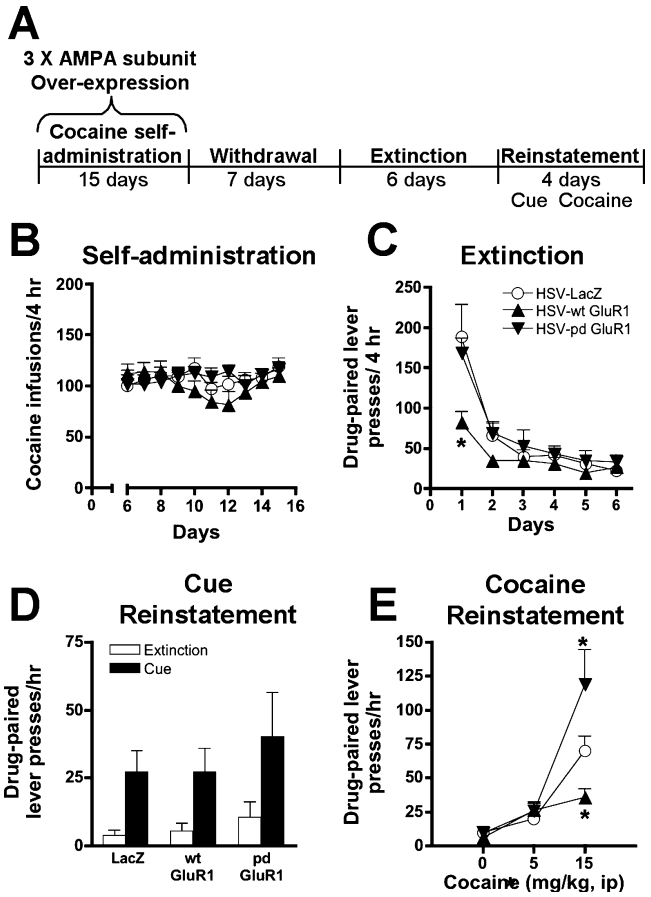

FIG. 5. Prolonged modulation of relapse behaviors following transient wild-type (wt)- and pore-dead (pd)-glutamate receptor 1 (GluR1) overexpression during cocaine self-administration. (A) Experimental time line restricting wt- and pd-GluR1 expression to cocaine self-administration (0.5 mg/kg/injection) followed by post-expression extinction and reinstatement testing. (B) Overexpression of wt- and pd-GluR1 has no effect on cocaine intake. (C) Prior wt-GluR1 overexpression reduces cocaine-seeking responses on the drug-paired lever in the initial extinction test after 1 week withdrawal. (D) There are no group differences in cue-induced reinstatement (compared with extinction responding in the 1 h prior to reinstatement), (E) but prior wt- and pd-GluR1 overexpression during cocaine self-administration produces bidirectional changes in cocaine-primed reinstatement of drug-paired lever responding. *$P < 0.05$, Fishers LSD test compared with herpes simplex virus (HSV)-LacZ ($n = 8$–13/group). AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid.

studies because of the importance of NAc core glutamate signaling for reinstatement behaviors (e.g. McFarland *et al.*, 2003). As illustrated in Fig. 5A, three weekly intra-NAc core infusions of HSV were given when animals acquired and maintained intravenous cocaine self-administration without prior lever-press training. Wt- and pd-GluR1 failed to affect the acquisition of self-administration during the first week of testing (data not shown), and cocaine intake stabilized at similar levels in all groups during the second and third week of self-administration (Fig. 5B). Following 7 days of withdrawal in their home cages, and after wt- and pd-GluR1 expression declined, animals were returned to the test chambers for extinction tests in the absence of cocaine availability. Animals previously overexpressing wt-GluR1 during cocaine self-administration exhibited nearly 50% less drug-paired lever responding than LacZ controls during the first extinction test (Fig. 5C; group × session; $F_{10,135} = 3.19$, $P < 0.001$). These data suggest that increasing basal AMPA receptor input to NAc neurons during cocaine use subsequently dampens the motivational salience of the cocaine-paired environment. Prior pd-GluR1 overexpression failed to alter initial extinction responding, and all groups extinguished to equivalent levels by the final extinction session.

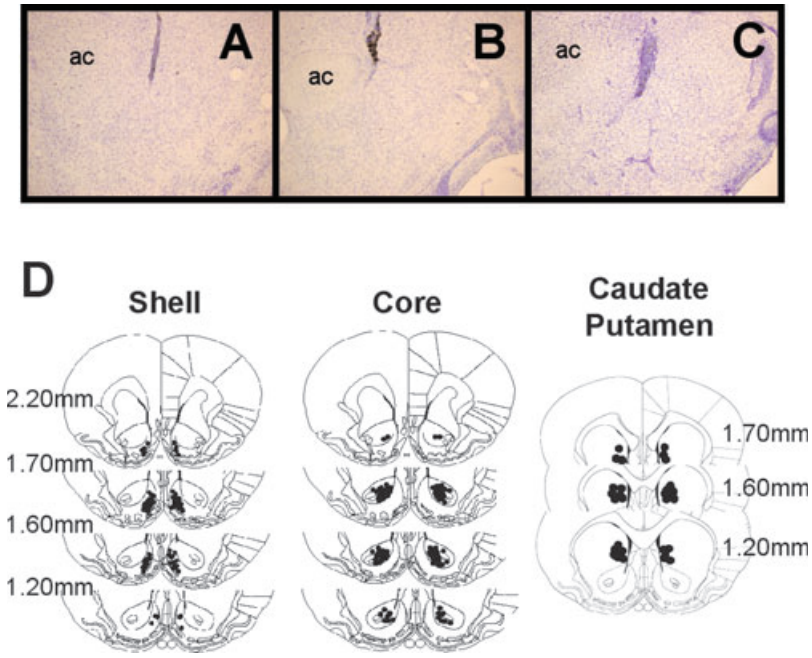

FIG. 6. Lack of gliosis or scarring with three weekly HSV infusions in animals self-administering cocaine and tested in extinction/reinstatement infusions. Nissl-stained sections around infusion sites from representative animals in the LacZ (A), wt-GluR1 (B) and pd-GluR1 (C) groups (collected following the final reinstatement test in Fig. 5). (D) Localization of infusion sites in NAc core, shell and caudate-putamen from all animals used in the study.

Following extinction, discrete cues that previously were paired with cocaine injections showed similar abilities to reinstate cocaine-seeking behavior in all HSV treatment groups (Fig. 5D). However, wt-GluR1 overexpression during prior self-administration led to a prolonged (~3 week) reduction in drug-paired lever responding elicited by a 15 mg/kg priming injection of cocaine, while prior pd-GluR1 overexpression enhanced cocaine-primed reinstatement (Fig. 5E; group × dose; $F_{4,54} = 3.91$, $P < 0.01$). There was no significant responding at the inactive lever in any group with cocaine priming (not shown). These reinstatement data parallel the effects of wt- and pd-GluR1 during cocaine treatments on the subsequent expression of locomotor sensitization, and suggest that increasing and decreasing basal AMPA receptor function in NAc neurons during cocaine self-administration reduces and exacerbates, respectively, neuroadaptive changes that contribute to cocaine relapse in withdrawal.

There was no evidence for aberrant gliosis in Nissl-stained sections from animals receiving repeated HSV treatments (Fig. 6A–C). In addition, the fact that cue reinstatement was not altered rules out the potential for excitotoxic effects with prolonged wt-GluR1 expression, as functional or excitotoxic lesions of the NAc core attenuate responding maintained by cocaine cues (Di Ciano & Everitt, 2004; Ito *et al*., 2004). Finally, as discussed above, there were no enduring effects of wt- and pd-GluR1 on locomotor responses in cocaine-naïve rats (Fig. 4E), suggesting that these treatments do not cause prolonged generalized effects on behavior.

NAc GluR1 directly modulates relapse behaviors

We also tested the effects of wt- and pd-GluR1 overexpression during withdrawal on reinstatement of cocaine-seeking behavior (Fig. 7A). Cocaine self-administration and extinction responding were balanced across treatment groups (data not shown), and animals were infused with HSV-LacZ, wt- or pd-GluR1 in the NAc core 1 day prior to the onset of reinstatement testing. Neither wt- nor pd-GluR1 directly altered cue-induced reinstatement of drug-paired lever responding (Fig. 7B). However, cocaine-primed reinstatement was directly attenuated by wt-GluR1, and markedly enhanced by pd-GluR1 to almost threefold of responding in LacZ-expressing controls (Fig. 7B; group × dose; $F_{4,68} = 2.76$, $P < 0.05$), with no significant increase in responding on the inactive lever (not shown). These findings indicate that upregulating basal AMPA receptor function in the NAc directly opposes cocaine-induced relapse to drug-seeking behavior, while deficits in AMPA input to NAc neurons exacerbate cocaine relapse.

Because the expression of cocaine sensitization was associated with increased D2 receptor sensitivity, we tested the ability of wt- and pd-GluR1 overexpression to modulate cocaine seeking induced by D2 stimulation. Figure 7B shows that wt-GluR1 overexpression in the NAc core attenuated reinstatement induced by systemic quinpirole, while overexpression of pd-GluR1 significantly enhanced quinpirole-induced reinstatement (group × dose; $F_{8,168} = 2.43$, $P < 0.05$). Thus, up- and downregulating AMPA receptor function produces opposing effects on D2 receptor-mediated relapse consistent with effects on cocaine-induced relapse behavior.

These effects specifically involved mesolimbic dopamine terminal regions as similar HSV infusions in more dorsal caudate-putamen failed to alter cue, cocaine or quinpirole-induced reinstatement compared with LacZ controls (Fig. 8A and B). Moreover, overexpression of wt- and pd-GluR1 in the NAc core had no effect on sucrose-primed reinstatement in animals trained to self-administer sucrose pellets (Fig. 8C), suggesting that NAc AMPA receptor function does not alter incentive motivational responses to natural rewards.

Discussion

Our findings suggest that increasing and decreasing basal AMPA receptor function in NAc neurons produces bidirectional effects on sensitized locomotor responses and drug-seeking behaviors. Thus,

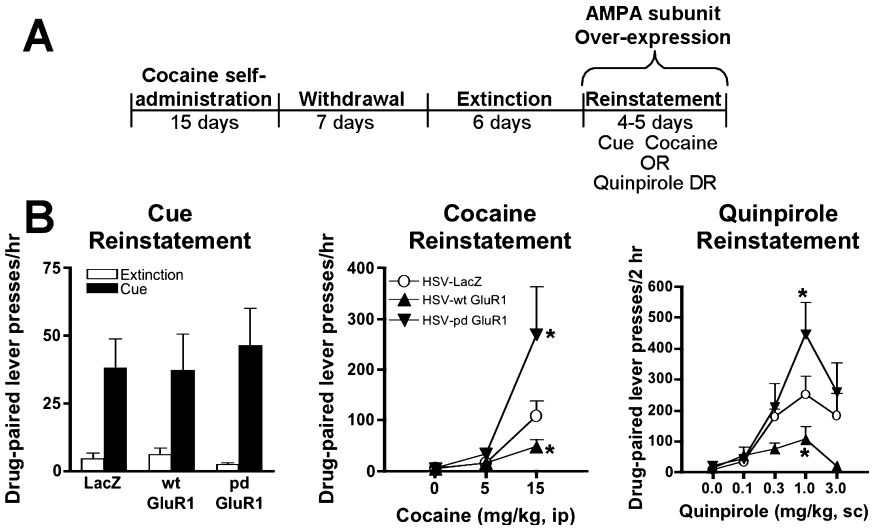

FIG. 7. Opposite and direct modulation of relapse behavior by wild-type (wt)- and pore-dead (pd)-glutamate receptor 1 (GluR1) overexpression during reinstatement testing. (A) Experimental time line for self-administration, extinction training and reinstatement testing during overexpression. (B) Overexpression of wt- or pd-GluR1 has no direct effect on cue reinstatement, but produces bidirectional changes in cocaine- and quinpirole-primed reinstatement of drug-paired lever responding. $^*P < 0.05$, Fishers LSD tests compared with herpes simplex virus (HSV)-LacZ ($n = 11–17$/group). AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid.

exogenous overexpression of GluR1 during or after cocaine use reduces several preclinical measures of addictive behavior. Conversely, overexpression of a dominant-negative that interferes with endogenous AMPA receptor function augments these behaviors. These changes are associated with similar bidirectional modulation of D2 (but not D1) dopamine receptor-mediated behavioral responses. From these data, it is hypothesized that increasing basal AMPA receptor function, either during cocaine use or during cocaine withdrawal, can partially prevent or reverse some of the neurobiological changes underlying sensitization to the pharmacological and incentive properties of cocaine that are thought to increase the propensity for cocaine relapse (Robinson & Berridge, 2001). On the other hand, decreasing basal AMPA receptor function may amplify these neurobiological and behavioral changes associated with sensitization.

The ability of wt-GluR1 overexpression to directly attenuate drug-induced relapse is consistent with its ability to reduce cocaine reward in pavlovian place conditioning (Kelz *et al*., 1999) and increase brain stimulation reward thresholds (Todtenkopf *et al*., 2006), suggesting that increases in basal NAc GluR1 levels directly reduce the motivational impact of cocaine on endogenous reward substrates. Similarly, overexpression of wt-GluR1 in the NAc directly reduces relapse behavior elicited by exposure to a cocaine-associated environment in the absence of pharmacological stimulation (Sutton *et al*., 2003). These effects do not appear to be an artifact of exogenous increases in the GluR1 subunit, as increases in endogenous NAc GluR1 levels induced by: (1) repeated extinction training (Sutton *et al*., 2003); or (2) repeated electrical stimulation of cortical afferents (Levy *et al*., 2007) are related to a reduction in cocaine-seeking behavior. These results also agree with the ability of elevated basal extracellular glutamate levels to reduce cocaine relapse behaviors (Baker *et al*., 2003). However, it is important to note that overexpression of GluR1 may alter cocaine-induced plasticity, potentially involving differences in synaptic vs extra-synaptic AMPA receptor expression, the relative abundance of heteromeric and homomeric GluR1-containing AMPA receptors, or saturation of proteins that regulate AMPA receptor trafficking.

We also found that overexpressing GluR1 selectively during cocaine use can produce lasting effects on both locomotor sensitization and subsequent relapse behavior. In both situations, no direct effects of the GluR1 upregulation were observed on cocaine intake or initial locomotor activity. However, when animals were subsequently tested in the absence of GluR1 overexpression, sensitized locomotor responses and drug seeking elicited by either cocaine or D2 receptor stimulation were reduced. The reduction of sensitization and relapse by increasing GluR1 levels restricted to periods of repeated cocaine exposure is remarkably similar to the effects of elevating basal extracellular glutamate during repeated cocaine use on the subsequent development of cocaine sensitization and drug-seeking behavior in a recent study (Madayag *et al*., 2007). Taken together, it appears that increasing basal glutamate transmission in the NAc either during cocaine use or during periods of relapse vulnerability is sufficient to prevent or reverse drug-induced adaptations that contribute to addictive behavior.

Interestingly, modulating AMPA receptor function in our study did not affect drug seeking induced by response-contingent delivery of cocaine-associated injection cues. This is surprising given recent evidence that intra-accumbens infusions of AMPA receptor antagonists block cue-induced cocaine-seeking behavior (Di Ciano & Everitt, 2001; Backstrom & Hyytia, 2007). Genetic deletion of GluR1, however, has no impact on either cocaine self-administration or cocaine seeking elicited by cues (Mead *et al*., 2007). This could reflect developmental compensation to receptor deletion, or that the role of AMPA receptor transmission in cue reinstatement involves GluR1-lacking AMPA receptors. Further studies will certainly aid in elucidating these discrepancies.

We found that decreasing basal AMPA function in NAc neurons directly increases the initial sensitivity to cocaine-induced locomotor activity, and augments the development of sensitization. Complementary work has shown that AMPA receptor internalization is necessary for expression of behavioral sensitization to amphetamine (Brebner *et al*., 2005). Re-exposure to cocaine reverses the increase in surface and synaptic AMPA receptor expression following a period of cocaine withdrawal in sensitized animals (Thomas *et al*., 2001; Boudreau &

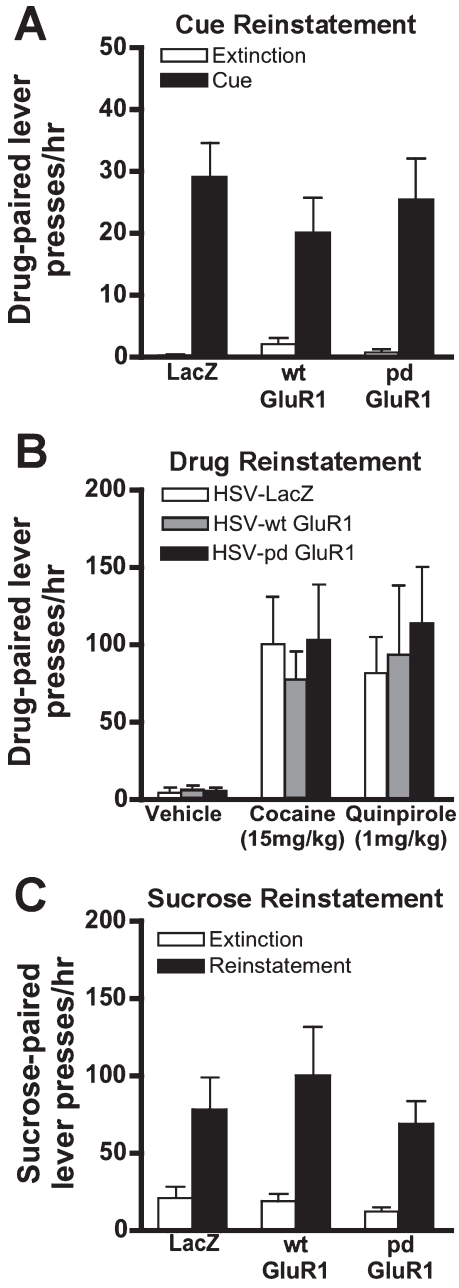

FIG. 8. Anatomical and behavioral controls for wild-type (wt)- and pore-dead (pd)-glutamate receptor 1 (GluR1) effects in reinstatement tests. Following cocaine self-administration and extinction, overexpression of wt- or pd-GluR1 in the caudate-putamen has no effect on (A) cue- or (B) cocaine- and quinpirole-induced reinstatement of drug-pared lever responding compared with LacZ-expressing controls. (C) Animals trained to self-administer sucrose pellets were tested for reinstatement induced by non-contingent sucrose pellet delivery for 1 h during herpes simplex virus (HSV)-mediated overexpression in the NAc core (compared with extinction responding in the 1 h prior to reinstatement). There is no effect of wt- or pd-GluR1 overexpression on sucrose-primed reinstatement of responding at the sucrose-paired lever (n = 8–10/group).

Wolf, 2005; Boudreau *et al*., 2007; Kourrich *et al*., 2007). Because the dominant-negative pd-GluR1 interferes with endogenous AMPA receptor function, it is hypothesized that pd-GluR1 would mimic the internalization-induced loss of membrane AMPA receptor function that has been implicated in the expression of amphetamine sensitization (Brebner *et al*., 2005) and that may contribute to the expression of cocaine sensitization (Boudreau *et al*., 2007; Kourrich *et al*., 2007). Supporting this notion, decreasing endogenous AMPA receptor activity by pd-GluR1 overexpression in NAc neurons appears sufficient to induce behavioral sensitization without prior cocaine exposure. Together, these findings suggest that the expression of sensitization to a psychostimulant challenge may involve an initial and rapid AMPA receptor internalization that consequently decreases AMPA receptor function and, thus, facilitates D2 receptor responsiveness with concomitant elevations in dopamine levels.

One potential mechanism for AMPA receptor internalization in sensitized animals is a transient surge in cocaine-induced glutamate levels that occurs only after repeated cocaine exposure (Pierce *et al*., 1996; Reid & Berger, 1996). Such excessive stimulation of AMPA and NMDA receptors can rapidly internalize AMPA receptors (Carroll *et al*., 1999a; Lissin *et al*., 1999; Beattie *et al*., 2000; Snyder *et al*., 2003; Mangiavacchi & Wolf, 2004), and lead to LTD-like decreases in AMPA-mediated synaptic currents (Carroll *et al*., 1999b; Heynen *et al*., 2000). Importantly, however, intra-accumbens infusions of AMPA receptor antagonists block the expression of cocaine sensitization (Pierce *et al*., 1996; Bell *et al*., 2000) and cocaine-induced drug seeking (Cornish & Kalivas, 2000; Park *et al*., 2002), suggesting that this surge in AMPA receptor activity acutely mediates a major component of addictive behavior. In addition, acute stimulation of AMPA receptors in the NAc elicits drug seeking in extinguished animals (Cornish *et al*., 1999; Cornish & Kalivas, 2000; Suto *et al*., 2004). Thus, phasic AMPA-mediated transmission in the NAc is both necessary and sufficient for the expression of sensitization and cocaine seeking behavior, apparently contradicting results when basal AMPA receptor function is modulated through molecular approaches.

One possible explanation for this discrepancy is that antagonist blockade of AMPA receptors prevents AMPA receptor internalization resulting from excessive glutamate, as shown in cultured accumbens neurons, although AMPA blockade can produce a modest internalization in the absence of glutamate (Mangiavacchi & Wolf, 2004). Another possibility is that molecular approaches to alter the amount of functional AMPA receptors change the intrinsic excitability of NAc neurons in a generalized manner to affect cocaine-induced behavior. Decreasing intrinsic excitability of NAc neurons by overexpressing a voltage-gated potassium channel is sufficient to increase behavioral sensitivity to cocaine (Dong *et al*., 2006), similar to the effects of dominant-negative pd-GluR1 in our study. On the other hand, generalized increases in background AMPA receptor function with wt-GluR1 could blunt phasic excitatory signals that mediate cocaine-seeking behavior, consistent with the ability of elevated basal extracellular glutamate levels to attenuate cocaine-induced relapse (Baker *et al*., 2003; Madayag *et al*., 2007). In this sense, deficits in basal extracellular glutamate levels in the NAc during cocaine withdrawal have been suggested to enhance the signal to noise ratio of cortical glutamate inputs acutely activated during drug-seeking behavior (Kalivas & Hu, 2006). A similar enhancement in phasic glutamate signals may result from generalized decreases in basal excitability of NAc neurons with pd-GluR1 expression.

We found similar effects of GluR1 modulation in both core and shell NAc subregions on cocaine/D2 sensitization in locomotor tests, and these results agree with the effects of GluR1 modulation in the NAc core in tests for relapse behaviors. Similarly, we previously found that overexpressing wt-GluR1 in the NAc shell reduces cocaine seeking in extinction conditions as discussed above (Sutton *et al*., 2003). In contrast, pharmacological blockade of AMPA receptors in the core (Pierce *et al*., 1996; Bell *et al*., 2000; Cornish & Kalivas, 2000; Park *et al*., 2002), but not shell, is effective in attenuating

cocaine-induced relapse mediated by acute stimulation of cortical glutamatergic afferents to the NAc (McFarland & Kalivas, 2001; Park *et al*., 2002; McFarland *et al*., 2003). Our results indicate that changes in D2 dopamine receptor-mediated responses are a major consequence of modulating basal AMPA receptor function, and D2 receptors mediate relapse to cocaine seeking in both medial core (Bachtell *et al*., 2005) and shell NAc subregions (Bachtell *et al*., 2005; Schmidt & Pierce, 2006; Schmidt *et al*., 2006). Therefore, we hypothesize that decreases in AMPA receptor function in either core or shell, whether through rapid AMPA receptor internalization under drug challenge conditions (Brebner *et al*., 2005; Boudreau *et al*., 2007; Kourrich *et al*., 2007) or via deficits in basal extracellular glutamate (Keys *et al*., 1998; Baker *et al*., 2003), would contribute to sensitization in D2-mediated locomotor and relapse behavior associated with more addicted biological states in animal models (De Vries *et al*., 1999, 2002; Edwards *et al*., 2007).

In summary, our findings suggest that decreases in basal AMPA receptor function contribute to cocaine sensitization and relapse, while increases in basal AMPA receptor function are sufficient to diminish sensitization and restore inhibitory control over drug seeking. The ability of transient upregulation in AMPA receptor function to modulate the subsequent expression of cocaine sensitization requires a prior history of cocaine exposure, suggesting that repeated cocaine use induces a state of neuroplasticity amenable to such manipulations. Several adaptations associated with neuroplasticity have been reported following repeated cocaine treatments or chronic cocaine self-administration, including increased dendritic spine formation (Robinson & Kolb, 1999; Robinson *et al*., 2001), increased surface expression of endogenous AMPA receptors (Boudreau & Wolf, 2005), attenuated ability to induce LTD (Thomas *et al*., 2001; Martin *et al*., 2006) or enhanced ability to induce LTP (Yao *et al*., 2004). Many of these changes may be driven by homeostatic mechanisms to restore deficits in extracellular or synaptic glutamatergic input to NAc neurons in cocaine withdrawal (Boudreau & Wolf, 2005). Our findings suggest that exogenous upregulation of basal AMPA receptor function restores these glutamatergic deficits sufficient to reduce susceptibility to relapse. On the other hand, downregulating endogenous basal AMPA receptor function exacerbates these deficits and worsens the behavioral plasticity associated with cocaine exposure. These studies underscore the complex and important role of AMPA-mediated input to NAc neurons in the development and persistence of cocaine addiction.

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Abbreviations

AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; FR1, fixed ratio 1; GluR1, glutamate receptor 1; HSV, herpes simplex virus; LSD, least significant difference; LTD, long-term depression; LTP, long-term potentiation; NAc, nucleus accumbens; NMDA, *N*-methyl-D-aspartic acid; pd-GluR1, pore-dead glutamate receptor 1; SKF 81297, (±)-6-chloro-7, 8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide; wt-GluR1, wild-type glutamate receptor 1.

References

Bachtell, R.K., Whisler, K., Karanian, D. & Self, D.W. (2005) Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. *Psychopharmacology (Berl.)*, **183**, 41–53.

Backstrom, P. & Hyytia, P. (2007) Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl.)*, **192**, 571–580.

Baker, D.A., McFarland, K., Lake, R.W., Shen, H., Tang, X.C., Toda, S. & Kalivas, P.W. (2003) Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat. Neurosci.*, **6**, 743–749.

Beattie, E.C., Carroll, R.C., Yu, X., Morishita, W., Yasuda, H., von Zastrow, M. & Malenka, R.C. (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat. Neurosci.*, **3**, 1291–1300.

Bell, K., Duffy, P. & Kalivas, P.W. (2000) Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology*, **23**, 335–344.

Boudreau, A.C., Reimers, J.M., Milovanovic, M. & Wolf, M.E. (2007) Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J. Neurosci.*, **27**, 10621–10635.

Boudreau, A.C. & Wolf, M.E. (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J. Neurosci.*, **25**, 9144–9151.

Brebner, K., Wong, T.P., Liu, L., Liu, Y., Campsall, P., Gray, S., Phelps, L., Phillips, A.G. & Wang, Y.T. (2005) Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science*, **310**, 1340–1343.

Carlezon, W.A. Jr, Boundy, V.A., Haile, C.N., Lane, S.B., Kalb, R.G., Neve, R.L. & Nestler, E.J. (1997) Sensitization to morphine induced by viral-mediated gene transfer. *Science*, **277**, 812–814.

Carroll, R.C., Beattie, E.C., Xia, H., Luscher, C., Altschuler, Y., Nicoll, R.A., Malenka, R.C. & von Zastrow, M. (1999a) Dynamin-dependent endocytosis of ionotropic glutamate receptors. *Proc. Natl Acad. Sci. USA*, **96**, 14112–14117.

Carroll, R.C., Lissin, D.V., von Zastrow, M., Nicoll, R.A. & Malenka, R.C. (1999b) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nat. Neurosci.*, **2**, 454–460.

Cornish, J.L., Duffy, P. & Kalivas, P.W. (1999) A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. *Neuroscience*, **93**, 1359–1367.

Cornish, J.L. & Kalivas, P.W. (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *J Neurosci.*, **20**, RC89.

De Vries, T.J., Schoffelmeer, A.N., Binnekade, R., Raaso, H. & Vanderschuren, L.J. (2002) Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology*, **26**, 18–26.

De Vries, T.J., Schoffelmeer, A.N., Binnekade, R. & Vanderschuren, L.J. (1999) Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. *Psychopharmacology (Berl.)*, **143**, 254–260.

Derkach, V.A., Oh, M.C., Guire, E.S. & Soderling, T.R. (2007) Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat. Rev. Neurosci.*, **8**, 101–113.

Di Ciano, P. & Everitt, B.J. (2001) Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. *Neuropsychopharmacology*, **25**, 341–360.

Di Ciano, P. & Everitt, B.J. (2004) Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. *J. Neurosci.*, **24**, 7167–7173.

Dingledine, R., Borges, K., Bowie, D. & Traynelis, S.F. (1999) The glutamate receptor ion channels. *Pharmacol. Rev.*, **51**, 7–61.

Dong, Y., Green, T., Saal, D., Marie, H., Neve, R., Nestler, E.J. & Malenka, R.C. (2006) CREB modulates excitability of nucleus accumbens neurons. *Nat. Neurosci.*, **9**, 475–477.

Edwards, S., Whisler, K.N., Fuller, D.C., Orsulak, P.J. & Self, D.W. (2007) Addiction-related alterations in D1 and D2 dopamine receptor behavioral responses following chronic cocaine self-administration. *Neuropsychopharmacology*, **32**, 354–366.

Hayashi, Y., Shi, S.H., Esteban, J.A., Piccini, A., Poncer, J.C. & Malinow, R. (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ. domain interaction. *Science*, **287**, 2262–2267.

Heynen, A.J., Quinlan, E.M., Bae, D.C. & Bear, M.F. (2000) Bidirectional, activity-dependent regulation of glutamate receptors in the adult hippocampus in vivo. *Neuron*, **28**, 527–536.

Ito, R., Robbins, T.W. & Everitt, B.J. (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nat. Neurosci.*, **7**, 389–397.

Kalivas, P.W. & Hu, X.T. (2006) Exciting inhibition in psychostimulant addiction. *Trends Neurosci.*, **29**, 610–616.

Kalivas, P.W., Volkow, N. & Seamans, J. (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron*, **45**, 647–650.

Kelz, M.B., Chen, J., Carlezon, W.A. Jr, Whisler, K., Gilden, L., Beckmann, A.M., Steffen, C., Zhang, Y.J., Marotti, L., Self, D.W., Tkatch, T., Baranauskas, G., Surmeier, D.J., Neve, R.L., Duman, R.S., Picciotto, M.R. & Nestler, E.J. (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature*, **401**, 272–276.

Keys, A.S., Mark, G.P., Emre, N. & Meshul, C.K. (1998) Reduced glutamate immunolabeling in the nucleus accumbens following extended withdrawal from self-administered cocaine. *Synapse*, **30**, 393–401.

Kourrich, S., Rothwell, P.E., Klug, J.R. & Thomas, M.J. (2007) Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *J. Neurosci.*, **27**, 7921–7928.

Levy, D., Shabat-Simon, M., Shalev, U., Barnea-Ygael, N., Cooper, A. & Zangen, A. (2007) Repeated electrical stimulation of reward-related brain regions affects cocaine but not 'natural' reinforcement. *J. Neurosci.*, **27**, 14179–14189.

Lissin, D.V., Carroll, R.C., Nicoll, R.A., Malenka, R.C. & von Zastrow, M. (1999) Rapid, activation-induced redistribution of ionotropic glutamate receptors in cultured hippocampal neurons. *J. Neurosci.*, **19**, 1263–1272.

Madayag, A., Lobner, D., Kau, K.S., Mantsch, J.R., Abdulhameed, O., Hearing, M., Grier, M.D. & Baker, D.A. (2007) Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *J. Neurosci.*, **27**, 13968–13976.

Mangiavacchi, S. & Wolf, M.E. (2004) Stimulation of N-methyl-D-aspartate receptors, AMPA receptors or metabotropic glutamate receptors leads to rapid internalization of AMPA receptors in cultured nucleus accumbens neurons. *Eur. J. Neurosci.*, **20**, 649–657.

Martin, M., Chen, B.T., Hopf, F.W., Bowers, M.S. & Bonci, A. (2006) Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nat. Neurosci.*, **9**, 868–869.

McFarland, K. & Kalivas, P.W. (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.*, **21**, 8655–8663.

McFarland, K., Lapish, C.C. & Kalivas, P.W. (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.*, **23**, 3531–3537.

Mead, A.N., Zamanillo, D., Becker, N. & Stephens, D.N. (2007) AMPA-receptor GluR1 subunits are involved in the control over behavior by cocaine-paired cues. *Neuropsychopharmacology*, **32**, 343–353.

Park, W.K., Bari, A.A., Jey, A.R., Anderson, S.M., Spealman, R.D., Rowlett, J.K. & Pierce, R.C. (2002) Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J. Neurosci.*, **22**, 2916–2925.

Paxinos, G. & Watson, C. (1998) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

Pierce, R.C., Bell, K., Duffy, P. & Kalivas, P.W. (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J. Neurosci.*, **16**, 1550–1560.

Reid, M.S. & Berger, S.P. (1996) Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. *Neuroreport*, **7**, 1325–1329.

Robinson, T.E. & Berridge, K.C. (2001) Incentive-sensitization and addiction. *Addiction*, **96**, 103–114.

Robinson, T.E., Gorny, G., Mitton, E. & Kolb, B. (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse*, **39**, 257–266.

Robinson, T.E. & Kolb, B. (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur. J. Neurosci.*, **11**, 1598–1604.

Schmidt, H.D., Anderson, S.M. & Pierce, R.C. (2006) Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat. *Eur. J. Neurosci.*, **23**, 219–228.

Schmidt, H.D. & Pierce, R.C. (2006) Cooperative activation of D1-like and D2-like dopamine receptors in the nucleus accumbens shell is required for the reinstatement of cocaine-seeking behavior in the rat. *Neuroscience*, **142**, 451–461.

Schramm-Sapyta, N.L., Olsen, C.M. & Winder, D.G. (2006) Cocaine self-administration reduces excitatory responses in the mouse nucleus accumbens shell. *Neuropsychopharmacology*, **31**, 1444–1451.

Self, D.W., Genova, L., Hope, B.T., Barnhart, W.J., Spencer, J.J. & Nestler, E.J. (1998) Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. *J. Neurosci.*, **18**, 1848–1859.

Shi, S., Hayashi, Y., Esteban, J.A. & Malinow, R. (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell*, **105**, 331–343.

Snyder, G.L., Galdi, S., Fienberg, A.A., Allen, P., Nairn, A.C. & Greengard, P. (2003) Regulation of AMPA receptor dephosphorylation by glutamate receptor agonists. *Neuropharmacology*, **45**, 703–713.

Suto, N., Tanabe, L.M., Austin, J.D., Creekmore, E., Pham, C.T. & Vezina, P. (2004) Previous exposure to psychostimulants enhances the reinstatement of cocaine seeking by nucleus accumbens AMPA. *Neuropsychopharmacology*, **29**, 2149–2159.

Sutton, M.A., Schmidt, E.F., Choi, K.H., Schad, C.A., Whisler, K., Simmons, D., Karanian, D.A., Monteggia, L.M., Neve, R.L. & Self, D.W. (2003) Extinction-induced upregulation in AMPA receptors reduces cocaine-seeking behaviour. *Nature*, **421**, 70–75.

Takahashi, T., Svoboda, K. & Malinow, R. (2003) Experience strengthening transmission by driving AMPA receptors into synapses. *Science*, **299**, 1585–1588.

Thomas, M., Beurrier, C., Bonci, A. & Malenka, R. (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat. Neurosci.*, **4**, 1217–1223.

Todtenkopf, M.S., Parsegian, A., Naydenov, A., Neve, R.L., Konradi, C. & Carlezon, W.A. Jr (2006) Brain reward regulated by AMPA receptor subunits in nucleus accumbens shell. *J. Neurosci.*, **26**, 11665–11669.

Yao, W.D., Gainetdinov, R.R., Arbuckle, M.I., Sotnikova, T.D., Cyr, M., Beaulieu, J.M., Torres, G.E., Grant, S.G. & Caron, M.G. (2004) Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. *Neuron.*, **41**, 625–638.