A-Kinase Anchoring Protein 150 (AKAP150) Promotes Cocaine Reinstatement by Increasing AMPA Receptor Transmission in the Accumbens Shell

Leonardo A Guercio^{1,2}, Mackenzie E Hofmann², Sarah E Swinford-Jackson², Julia S Sigman², Mathieu E Wimmer³, Mark L Dell'Acqua⁴, Heath D Schmidt⁵ and R Christopher Pierce^{*2}

¹Neuroscience Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; ²Center for Neurobiology and Behavior, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; ³Department of Psychology, Temple University, Philadelphia, PA, USA; ⁴Department of Pharmacology, School of Medicine, University of Colorado, Anschutz Medical Campus, Aurora, CO, USA; ⁵Department for Biobehavioral Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, PA, USA

Previous work indicated that activation of D1-like dopamine receptors (D1DRs) in the nucleus accumbens shell promoted cocaine seeking through a process involving the activation of PKA and GluA1-containing AMPA receptors (AMPA). A-kinase anchoring proteins (AKAPs) localize PKA to AMPARs leading to enhanced phosphorylation of GluA1. AKAP150, the most well-characterized isoform, plays an important role in several forms of neuronal plasticity. However, its involvement in drug addiction has been minimally explored. Here we examine the role of AKAP150 in cocaine reinstatement, an animal model of relapse. We show that blockade of PKA binding to AKAPs in the nucleus accumbens shell of Sprague–Dawley rats attenuates reinstatement induced by either cocaine or a D1DR agonist. Moreover, this effect is specific to AKAP150, as viral overexpression of a PKA-binding deficient mutant of AKAP150 also impairs cocaine reinstatement. This viral-mediated attenuation of cocaine reinstatement was accompanied by decreased phosphorylation of GluA1-containing AMPARs and attenuated AMPAR eEPSCs. Collectively, these results suggest that AKAP150 facilitates the reinstatement of cocaine-seeking behavior by amplifying D1DR/PKA-dependent AMPA transmission in the nucleus accumbens.

Neuropsychopharmacology (2018) **43**, 1395–1404; doi:10.1038/npp.2017.297; published online 10 January 2018

INTRODUCTION

Neuroadaptations in both the dopamine and glutamate systems in the nucleus accumbens contribute significantly to the reinstatement of cocaine seeking, an animal model of relapse (Kauer and Malenka, 2007; Schmidt and Pierce, 2010; Shaham and Hope, 2005). In the dopamine system, it has been shown that stimulation of accumbens shell, but not core, D1DRs promoted the reinstatement of cocaine seeking (Anderson *et al*, 2003; Bachtell *et al*, 2005; Schmidt and Pierce, 2006b). D1DR stimulation leads to increased cyclic adenosine monophosphate (cAMP) production, ultimately activating PKA. Repeated exposure to cocaine leads to increased D1DR signaling via increased cAMP formation and PKA activity in the nucleus accumbens (Lu *et al*, 2003; Unterwald *et al*, 1996), which plays a critical role in cocaine reinstatement (Self *et al*, 1998). The intracellular targets of PKA include AMPARs, activation of which promotes

cocaine reinstatement (Schmidt and Pierce, 2010). PKA phosphorylation of the GluA1 subunit of AMPARs led to increased open probability of AMPARs as well as increased surface expression of GluA1-containing AMPARs (Banke *et al*, 2000; Man *et al*, 2007; Oh *et al*, 2006; Sun *et al*, 2005). In cultured accumbal neurons, D1DR stimulation increased both GluA1 phosphorylation by PKA and GluA1 surface expression (Chao *et al*, 2002a; Chao *et al*, 2002b). Moreover, cocaine reinstatement was attenuated by intra-accumbal shell administration of AAV10-GluA1-C99, which impairs the trafficking of GluA1-containing AMPARs to the cell surface (Anderson *et al*, 2008). Thus, one of the main mechanisms underlying cocaine priming-induced reinstatement of drug seeking is the activation of D1DRs in the accumbens shell, which increases PKA-dependent insertion of GluA1-containing AMPARs into synapses (Pierce and Wolf, 2013).

A-kinase anchoring proteins (AKAPs) are a family of proteins that bind and localize PKA to distinct subcellular compartments to facilitate second messenger signaling (Wong and Scott, 2004). While there are many different forms of AKAPs, AKAP79/150 (human79/rodent150; also known as AKAP5) is the best characterized (Wong and Scott, 2004). AKAP150 is highly expressed in the rodent brain, and binds numerous signaling, receptor, accessory, and ion channel proteins involved in long-term synaptic plasticity

*Correspondence: Dr C Pierce, Center for Neurobiology and Behavior, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, 125 South 31st Street, Philadelphia, PA 19104, USA; Tel: +215 746 8915; Fax: +215 573 7605; E-mail: rcpierce@penmedicine.upenn.edu

Received 7 July 2017; revised 29 November 2017; accepted 30 November 2017; accepted article preview online 12 December 2017

(Sanderson and Dell'Acqua, 2011). In particular, AKAP150 localizes PKA to AMPARs via interaction with the membrane-associated guanylate kinase (MAGUK) scaffolding proteins, PSD-95 and SAP97, leading to enhanced phosphorylation of GluA1 (Colledge *et al*, 2000; Tavalin *et al*, 2002). This process was shown to be critical in mediating synaptic plasticity and memory formation, as genetic deletion of AKAP150 or its PKA-anchoring domain, resulted in reduced GluA1 phosphorylation, alterations in hippocampal LTP and LTD as well as impaired operant learning and spatial memory (Lu *et al*, 2008; Sanderson *et al*, 2016; Tunquist *et al*, 2008; Weisenhaus *et al*, 2010). In addition, sleep-deprived mice exhibiting memory deficits showed reduced AKAP150 expression and reduced AMPAR phosphorylation (Hagewoud *et al*, 2010). Recent evidence also indicated that AKAP150 expression was increased in nucleus accumbens post-synaptic density of cocaine-treated rats and disruption of AKAP-dependent signaling in the accumbens attenuated cocaine reinstatement (Reissner *et al*, 2011).

In the current study, we further investigated the role of AKAP150 in the reinstatement of cocaine seeking. Using biochemical and electrophysiological techniques, we examined the underlying signaling mechanisms by which AKAP150 can affect cocaine reinstatement. Our results demonstrate that AKAP150 contributes to cocaine reinstatement by promoting the D1DR-mediated PKA phosphorylation of GluA1-containing AMPARs.

MATERIALS AND METHODS

Rats and Housing

Male Sprague–Dawley rats (*Rattus norvegicus*) weighing 250–300 g were obtained from Taconic Laboratories (Germantown, NY) and housed individually with food and water available *ad libitum*. A 12/12 h light/dark cycle was used with the lights on at 0700 hours. All experimental procedures were performed during the light cycle. 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.

Materials

All experiments used Med-Associates (East Fairfield, VT) instrumentation enclosed within ventilated, sound attenuating chambers. Each operant conditioning chamber was equipped with response levers, food pellet dispensers, and infusion pumps for injecting drugs intravenously.

Drugs and Viruses

Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD) and dissolved in bacteriostatic 0.9% saline. InCELlect AKAP inhibitor St-Ht31 and control peptide St-Ht31P were purchased from Promega (Madison, WI). The D1DR agonist R-(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF-81297) was purchased from Tocris Bioscience (Minneapolis, MN). SKF-81297 was dissolved in

bacteriostatic 0.9% saline. Herpes simplex virus (HSV) vectors were constructed and packaged at the Viral Gene Transfer Core at the McGovern Institute for Brain Research (MIT, Cambridge, MA, USA) as described previously (Neve *et al*, 2005). All viruses were designed to co-express enhanced green fluorescent protein (eGFP) driven by a separate CMV promoter. Control virus expressed an empty vector plus eGFP.

Surgery

Rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine before undergoing surgery as previously described (White *et al*, 2016).

Cocaine and Sucrose Reinstatement—St-Ht31 Microinjections

Cocaine and sucrose self-administration were performed as described previously (White *et al*, 2016). Following the extinction phase, reinstatement was assessed. The obturators were removed from the guide cannulae and 33-gauge stainless steel microinjectors were inserted. The microinjectors extended 2 mm below the ventral end of the guide cannulae into the nucleus accumbens shell. Rats received bilateral intra-accumbal shell microinfusions of St-Ht31 or control St-Ht31P peptide (0.5 μ l, 10 mM), which occurred over 120 s. This dose of St-Ht31 was previously shown to modulate cocaine-seeking behavior (Reissner *et al*, 2011) and a higher concentration (40 mM) administered into the amygdala was shown to modulate auditory fear memory (Moita *et al*, 2002). Following microinfusions, the microinjectors remained in place for an additional 60 s to allow the solution to diffuse away from the tips of the microinjectors before removal. For cocaine reinstatement, 30 min following intra-accumbal shell microinjections, a systemic priming injection of cocaine (10 mg/kg, i.p.) was administered immediately before a reinstatement test session. During reinstatement, satisfaction of the response requirements for each component resulted in a saline infusion rather than a cocaine infusion. For sucrose reinstatement, the experimenter remotely administered one sucrose pellet every 2 min for the first 10 min of the reinstatement test session. For sucrose reinstatement, active lever presses had no scheduled consequences. For both cocaine and sucrose reinstatement, each reinstatement session was followed by extinction sessions (typically only one or two) until responding was <15% of the response rate maintained by self-administration. The FR5 schedule was used throughout extinction and reinstatement. St-Ht31 or control (St-Ht31P) were administered in a counterbalanced manner. In a separate experiment, cocaine reinstatement was induced by intra-accumbal shell microinfusions of the selective D1-like dopamine receptor (D1DR) agonist SKF-81297 as described previously (Schmidt *et al*, 2006a). St-Ht31 or control St-Ht31P (0.5 μ l, 10 mM) was microinjected into the shell 30 min before intra-accumbal shell infusions of SKF-81297 (0.5 μ l).

Cocaine and Sucrose Reinstatement—Viral Vectors

In these experiments, rats underwent daily extinction sessions until active lever responding was ~20% of the responses averaged over the final 3 days of self-administration. Rats then received bilateral intra-accumbal

shell microinfusions of either HSV-GFP or HSV-AKAP79 Δ PKA over 10 min for a total volume of 2 μ l per hemisphere. Following the microinfusions, the microinjectors were left in place for an additional 120 s to allow the solution to diffuse away from the tips. Rats continued to undergo extinction for 3 days for peak viral expression before reinstatement testing. Cocaine and sucrose reinstatement were induced as described above.

Western Blotting

Rats underwent cocaine reinstatement with viral pretreatments as described above. Immediately before the reinstatement testing, rats randomly received a systemic priming injection of saline or cocaine (10 mg/kg, i.p.). Rats were placed immediately into the operant chambers following injection of saline or cocaine. Responding was recorded for 30 min, after which rats were removed from the operant chambers and immediately decapitated. Whole brains were extracted and flash-frozen in isopentane on dry ice, then stored at -80°C . Brains were sliced on a cryostat and the nucleus accumbens shell was dissected by tissue punch (2.0 mm Harris Unicore stainless steel punchers, Ted Pella). Tissue samples were stored at -80°C until processing for western blotting 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 μ g) were loaded and separated in 10% Tris-Glycine gels (Life Technologies, Grand Island, NY) by SDS-PAGE, then transferred to nitrocellulose membranes using the iBlot dry transfer system (Life Technologies), which were then preblocked with Tris-buffered saline containing 0.1% Tween 20 (TBST) and 5% bovine serum albumin for 1 h before overnight incubation with the following primary antibodies: pSer845-GluA1 (1:1000, Millipore #04-1073); total GluA1 (1:1000, Millipore #MAB2263); and PKA-RII (1:1000, Santa Cruz #sc-909). Membranes were concurrently incubated with mouse monoclonal anti-GAPDH (1:2000, Cell Signaling #2118) as a loading control. Primary antibody incubation was followed by three washes (10 min each with rocking, room temperature (RT)) in TBST. Membranes were then incubated for 1 h at RT with secondary antibodies (IRDye 800 goat anti-mouse and IRDye 680 goat anti-rabbit, 1:5000) in Odyssey blocking buffer 0.05% Tween 20 (LI-COR Biosciences). Antibody/protein complexes were visualized using the Odyssey IR imaging system (Li-Cor Biosciences). For pSer845 and GluA1 analysis, gels were transferred to nitrocellulose membranes for 1 h at 100V using SDS Running Buffer (Bio-Rad) and immunoprobed in TBST with 5% milk (Bio-Rad). HRP-conjugated secondary antibody dilutions were 1:10 000 (Bio-Rad). Bands were visualized using the myECL Imager (Thermo Fisher Scientific). Band intensities were quantified using either the Odyssey or the mECL Imaging software. For data analysis, all bands were normalized to GAPDH and divided by the mean of the control group. The ratio of phosphorylated to native protein was then calculated.

Electrophysiology

A separate cohort of rats underwent cocaine reinstatement with viral pretreatments as described above. Immediately

after a 30 min reinstatement session, rats were decapitated and brain slices prepared as described previously (Ortinski *et al*, 2013). To avoid recording from damaged cells, neurons within 50 μ m of the injection cannula track were excluded from the analysis. There were no differences on any of the measures between GFP- negative cells in slices exposed to HSV-AKAP79 Δ PKA and slices exposed to HSV-GFP. Therefore, these cells were pooled for analysis.

Verification of Cannula Placements

After the completion of all experiments, rats were given an overdose of pentobarbital (100 mg/kg, i.p.), brains were removed and stored in 10% formalin for at least 1 week. Coronal sections (100 μ m) were taken at the level of the nucleus accumbens with a vibratome (Technical Products International; St. Louis, MO, USA). The sections were mounted on gelatin-coated slides. Rats with cannula placements located outside of the accumbens shell, or with excessive mechanical damage, were excluded from subsequent data analysis.

Viral Expression

To ascertain viral expression, we injected (2 μ l/hemisphere) HSV-AKAP79 Δ PKA into the nucleus accumbens shell of separate, drug-naïve rats. At 3 days after injection, rats 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 (30 μ m) were taken using a vibratome (Technical Products International; St. Louis, MO, USA) 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, 1:500, Millipore #MAB3580 and anti-AKAP79, 1:500, Santa Cruz #sc-17772) diluted 1:1000 in 0.1% Triton+3% donkey serum in PBS to the slides and incubated overnight at 4°C . The next day, the slides were washed in $1\times$ PBS before incubation in secondary fluorescent antibody at RT for 2 h (Alexa Fluor 488, 1:500; Alexa Fluor 562, 1:500; Jackson ImmunoResearch, West Grove, PA). After 2 h, slides were washed in $1\times$ PBS before being coverslipped using Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and imaged for GFP expression using fluorescent confocal microscopy. Z-stacks were captured at $\times 20$ and $\times 40$ magnification and images were stacked using Z projection in ImageJ (NIH).

RESULTS

AKAP Signaling Is Required for the Reinstatement of Cocaine, but not Sucrose Seeking

AKAP signaling in the nucleus accumbens core has been implicated in the reinstatement of cocaine seeking (Reissner *et al*, 2011). To determine whether this effect is also seen in the accumbens shell, we microinjected a cell-permeable inhibitory peptide (St-Ht31), which disrupts the binding of PKA-RII subunits to all AKAP isoforms, into the nucleus

Figure 1 Intra-accumbal shell microinjections of St-Ht31 attenuates cocaine, but not sucrose, reinstatement. Mean ($\pm$ SEM) active and inactive lever responses from (a) cocaine reinstatement session and (b) sucrose reinstatement session. (c) Cannula placements from the nucleus accumbens shell (dark circles). The values are in millimeters, relative to bregma. * $p < 0.001$ St-Ht31 compared to St-Ht31P Control. There were 8–9 rats per group.

accumbens shell of rats that had previously self-administered cocaine and responding was reinstated with an acute priming injection of cocaine (10 mg/kg, i.p.). As a control, we used the inactive St-Ht31P peptide, which does not interact with PKA-RII due to the substitution of two proline residues in the conserved AKAP-RII binding α -helical motif. Total active and inactive lever responses (mean $\pm$ SEM) are shown in Figure 1a ($n = 9$). These data were analyzed using a two-way ANOVA (treatment between subjects and lever within subjects), which revealed significant main effects of treatment ($F_{(1,8)} = 34.46$, $p < 0.001$), lever responding ($F_{(1,8)} = 49.62$, $p < 0.001$), and a significant interaction between these variables ($F_{(1,8)} = 26.05$, $p < 0.001$). Subsequent pairwise analyses indicated that the total active lever responses between the St-Ht31 and control treatments were significantly different (Bonferroni, $p < 0.0001$).

Previous evidence suggests that intra-accumbal core infusions of St-Ht31 do not affect cocaine-induced locomotor activity (Reissner *et al*, 2011). However, AKAP150 mutant mice lacking the PKA-binding domain exhibit, among other things, deficits in operant learning behavior (Weisenhaus *et al*, 2010). To determine whether the effects of St-Ht31 impair operant learning generally, we tested its effects on sucrose reinstatement. Total active and inactive lever responses (mean $\pm$ SEM) for rats pretreated with intra-accumbal shell microinfusions of either St-Ht31 or control (St-Ht31P) before reinstatement of sucrose-seeking behavior are shown in Figure 1b ($n = 8$). These data were analyzed using a two-way ANOVA, which revealed no effect of treatment ($F_{(1,7)} = 1.726$, $p = 0.23$), a significant effect of lever responding ($F_{(1,7)} = 50.74$, $p < 0.001$), and no interaction between these variables ($F_{(1,7)} = 1.472$, $p = 0.26$). The cannula placements in the nucleus accumbens shell are shown in Figure 1c. These data indicate that St-Ht31 impairs the reinstatement of cocaine, but not sucrose reinstatement.

AKAP Signaling Is Required for D1DR Agonist-Induced Reinstatement of Cocaine Seeking

Stimulation of D1DRs in the accumbens shell promotes the reinstatement of cocaine seeking (Bachtell *et al*, 2005; Schmidt *et al*, 2006a), an effect likely mediated by PKA activation (Anderson *et al*, 2008; Self *et al*, 1998). To determine whether AKAPs are required for D1DR-stimulated reinstatement of cocaine seeking, rats were pretreated with microinfusions of either St-Ht31 or its control before intra-accumbal shell injections of the D1DR agonist, SKF-81297. Total active and inactive lever responses (mean $\pm$ SEM) are shown in Figure 2a ($n = 5$). These data were analyzed using a two-way ANOVA, which revealed significant effects of treatment ($F_{(1,4)} = 31.4$, $p < 0.01$), lever responding ($F_{(1,4)} = 13.48$, $p < 0.05$), and a significant interaction between these variables ($F_{(1,4)} = 33.57$, $p < 0.01$). Subsequent pairwise analyses indicated that the total active lever responses between the St-Ht31 and control treatments were significantly different (Bonferroni, $p < 0.01$). Cannula placements are shown in Figure 2b.

Expression of HSV-AKAP79 Δ PKA in The Accumbens Shell Attenuates The Reinstatement of Cocaine, But Not Sucrose Seeking

Although there are over 50 different isoforms of AKAPs, AKAP150 is perhaps the best characterized (Wong and Scott, 2004). AKAP150 is highly expressed in the striatum and nucleus accumbens and plays a critical role in learning (Ostroveanu *et al*, 2007; Tunquist *et al*, 2008; Weisenhaus *et al*, 2010). In addition, recent evidence suggests that AKAP150 expression is upregulated in the nucleus accumbens core after cocaine self-administration and extinction (Reissner *et al*, 2011).

We sought to examine the specific role of AKAP150 in the reinstatement of cocaine-seeking behavior. Following

Figure 2 Intra-accumbal shell microinjections of St-Ht31 attenuates D1DR-agonist-induced reinstatement of cocaine seeking. (a) Mean ($\pm$ SEM) active and inactive lever responses from reinstatement session. (b) Cannula placements from the nucleus accumbens shell (dark circles). The values are in millimeters, relative to bregma. * $p < 0.05$ St-Ht31 compared to St-Ht-31P control. There were 5 rats per group.

self-administration and extinction, we virally expressed GFP with or without co-expression of a PKA-binding-deficient mutant of AKAP79, the human ortholog of AKAP150 (HSV-AKAP79 Δ PKA), in the accumbens shell. As outlined in experimental timeline illustrated in Figure 3a, the reinstatement session occurred 3 days following viral microinjections (HSV-eGFP *vs* HSV-AKAP79 Δ PKA), at which point HSV expression peaks (White *et al*, 2016) (Figures 3b and d). Cannula placements for rats evaluated for cocaine or sucrose reinstatement are shown in Figure 3c. Total active and inactive lever responses (mean $\pm$ SEM) from the cocaine reinstatement session are presented in Figure 3e ($n = 10$ –13). These data were analyzed using a two-way ANOVA (virus treatment between subjects factor and lever within subjects), which revealed no main effect of virus treatment ($F_{(1,20)} = 3.166$, $p = 0.09$), a significant effect of lever responding ($F_{(1,20)} = 111.7$, $p < 0.0001$), and a significant interaction between these variables ($F_{(1,20)} = 8.423$, $p < 0.01$). Subsequent pairwise analyses indicated that the total active lever responses between the HSV-eGFP and HSV-AKAP79 Δ PKA treatments were significantly different (Bonferroni, $p < 0.01$).

To determine whether the disruption of PKA binding to AKAP150 affects general operant behavior, we tested the effects intra-accumbal shell injections of HSV-AKAP79 Δ PKA on sucrose reinstatement. Total active and inactive lever responding (mean $\pm$ SEM) from the sucrose reinstatement sessions are presented in Figure 3f ($n = 7$). These data were analyzed using a two-way ANOVA, which revealed no effect of virus treatment ($F_{(1,12)} = 0.01814$, $p = 0.89$), a significant main effect of lever responding ($F_{(1,12)} = 26.99$, $p < 0.001$), and no interaction between these variables ($F_{(1,12)} = 0.01413$, $p = 0.91$). These data indicate that HSV-AKAP79 Δ PKA attenuates cocaine, but not sucrose, reinstatement. These results strongly suggest that AKAP150 is the primary AKAP isoform mediating cocaine reinstatement.

Expression of HSV-AKAP79 Δ PKA in the Accumbens Shell Attenuates PKA-Mediated Phosphorylation of GluA1 Ser845

Previous work showed that intra-accumbal core injections of St-Ht31 attenuated GluA1 surface expression (Reissner *et al*,

2011). Since it is well established that open probability and surface expression of GluA1 are regulated by PKA phosphorylation (Man *et al*, 2007; Oh *et al*, 2006; Sun *et al*, 2005), we examined the phosphorylation state of Ser845, a PKA phosphorylation site on GluA1 AMPAR subunits, following cocaine reinstatement after intra-accumbal shell administration of HSV-AKAP79 Δ PKA. All rats in this experiment were killed after a 30 min cocaine-primed reinstatement session, which corresponds to the peak of drug seeking. Total active lever responses (mean $\pm$ SEM) from the 30 min reinstatement session are shown in Figure 4a. These data were analyzed using a two-way ANOVA, which revealed significant main effects of virus ($F_{(1,36)} = 16.29$, $p < 0.001$), treatment ($F_{(1,36)} = 47.31$, $p < 0.0001$), and a significant interaction between these variables ($F_{(1,36)} = 22.06$, $p < 0.0001$). *Post hoc* analyses showed that total active lever responses were significantly different between GFP/cocaine groups and all other groups ($p < 0.0001$; $n = 9$ –11).

The average intensity for pSer845 in the nucleus accumbens shell was normalized to total GluA1 levels and expressed as percent change from control and is shown in Figure 4b. Percentages were analyzed by two-way ANOVA, which revealed no effect of virus ($F_{(1,36)} = 2.397$, $p = 0.13$), no effect of treatment ($F_{(1,36)} = 3.384$, $p = 0.07$) but a significant interaction between these variables ($F_{(1,36)} = 4.332$, $p < 0.05$). *Post hoc* analyses showed that the GFP/Coc group was significantly different from GFP/Sal and AKAP79 Δ PKA/Coc groups ($p < 0.05$; $n = 9$ –11). Since the AKAP79/150 α -helical motif binds to the PKA-RII regulatory subunit dimer near the AKAP C terminus (Sanderson and Dell'Acqua, 2011), we wanted to be sure that deletion of this anchoring domain did not affect overall expression of the PKA-RII subunit in the accumbens shell. The average intensity for PKA-RII in the nucleus accumbens shell was expressed as percent change from control and is shown in Figure 4c ($n = 7$). Percentages were analyzed by two-way ANOVA, which revealed no effects of virus ($F_{(1,24)} = 0.3518$, $p = 0.56$), treatment ($F_{(1,24)} = 0.1112$, $p = 0.74$), or interaction between these variables ($F_{(1,24)} = 0.00374$, $p = 0.95$).

Figure 3 Intra-accumbal shell expression of HSV-AKAP79ΔPKA attenuates cocaine, but not sucrose, reinstatement. (a) Schematic of experimental paradigm. (b) Representative expression of HSV-AKAP79ΔPKA in the nucleus accumbens shell. Three days before the reinstatement test session, all rats received either HSV-AKAP79ΔPKA or HSV-GFP injections to the nucleus accumbens shell. The boxed region shows localization of images in d. (c) Cannula placements from the nucleus accumbens shell (dark circles). The values are in millimeters, relative to bregma. (d) Immunofluorescence images showing GFP and AKAP79ΔPKA expression in the nucleus accumbens 3 days following HSV-AKAP79ΔPKA injection. Arrows highlight examples of colocalization between GFP and AKAP79ΔPKA. (e) Mean (± SEM) active and inactive lever responses from cocaine reinstatement session and (f) sucrose reinstatement session. * $p < 0.05$ HSV-AKAP79ΔPKA compared to HSV-GFP. There were 10–13 rats per group.

Expression of HSV-AKAP79ΔPKA in the Accumbens Shell Attenuates AMPAR Currents

Given our biochemical findings that intra-accumbal shell administration of HSV-AKAP79ΔPKA attenuated phosphorylation of GluA1-Ser845, we sought to examine the

effects of this virus on AMPAR eEPSCs in a separate cohort of rats that underwent cocaine reinstatement. Representative traces of eEPSCs from AKAP79ΔPKA-infected and non-transduced neurons are shown in Figure 5a. Quantification of this experiment is depicted in Figure 5b. These data were

Figure 4 Intra-accumbal shell expression of HSV-AKAP79ΔPKA attenuates GluA1-Ser845 phosphorylation. All rats received either HSV-AKAP79ΔPKA or HSV-GFP injections to the nucleus accumbens shell 3 days before the reinstatement test session. At the reinstatement test session, all rats received either a saline or cocaine priming injection. (a) Mean ($\pm$ SEM) active and inactive lever responses from 30 min reinstatement session. (b) Decreases in GluA1-Ser845 phosphorylation are measured by western blot (see insets). (c) No change in PKA-RII expression is observed by western blot (see insets). * $p < 0.05$ HSV-AKAP79ΔPKA compared to HSV-GFP. There were 9–11 rats per group.

Figure 5 HSV-AKAP79ΔPKA reduces the recruitment of AMPA receptors following cocaine reinstatement. (a) Representative traces from an AKAP79ΔPKA-positive neuron (left) and a nontransduced neuron (right). A minimal intensity for eEPSC recruitment was obtained (1 $\times$) and then increased to 2 $\times$ and 3 $\times$ this intensity. Arrowheads represent stimulation and the stimulation artifacts have been removed for visual clarity. (b) Summary of the recruitment curves for AKAP79ΔPKA-positive (black circles, $n=9$) and nontransduced (red circles, $n=9$) neurons. * $p < 0.05$.

analyzed with a two-sample t -test, which revealed a significant difference in the size of the AMPA eEPSCs between the two groups at 2 $\times$ intensity (AKAP+: $n=9$; AKAP-: $n=9$; $p < 0.008$) and 3 $\times$ intensity ($p < 0.03$). The rectification index, a measure of GluA1 insertion into the

synaptic membrane, was also assessed in both AKAP+ and nontransduced (AKAP-) cells, which revealed no significant differences between the two groups ($p=0.18$, two-sample t -test). In addition, we analyzed the AMPAR eEPSCs of rats treated with HSV-GFP. A one-way ANOVA indicated no significant differences between AKAP- ($n=9$), GFP+ ($n=7$), and GFP- ($n=8$) neurons at 2 $\times$ ($F_{(2,21)}=0.92$, $p < 0.41$) or 3 $\times$ ($F_{(2,21)}=1.23$, $p < 0.31$) intensities. Finally, to assess whether AKAP79ΔPKA blocks the cocaine-induced potentiation of the eEPSCs or reduces the AMPA currents in general, we injected the virus into drug-naïve rats and recorded from AKAP+ and nontransduced cells (AKAP-). The data were as follows (mean $\pm$ SEM): 1 $\times$ AKAP+ (-116.2 ± 20.1); 1 $\times$ AKAP- (-75.4 ± 1.3); 2 $\times$ AKAP+ (-206 ± 12.2); 2 $\times$ AKAP- (-236 ± 121.3); 3 $\times$ AKAP+ (-192.8 ± 13.9); and 3 $\times$ AKAP- (-397.2 ± 161.6). Two-sample t -tests revealed a significant difference between AKAP+ and AKAP- neurons at 3 $\times$ (AKAP+: $n=4$; AKAP-: $n=3$; $p=0.05$) but not at 1 $\times$ ($p=0.15$) or 2 $\times$ ($p=0.63$).

DISCUSSION

Our results demonstrate that AKAP150 in the accumbens shell contributes to cocaine reinstatement by facilitating

PKA-dependent phosphorylation of GluA1-containing AMPARs. These results suggest that AKAP150 is necessary to bridge the dopamine and glutamate systems in the nucleus accumbens during cocaine seeking. The current findings are consistent with a previous study showing that nonspecific disruption of PKA binding to all AKAP isoforms in the nucleus accumbens core attenuates cocaine priming-induced reinstatement (Reissner *et al*, 2011).

D1DR Signaling, PKA, and AKAP150

Our results demonstrate that AKAP signaling is required for D1DR agonist-induced reinstatement of cocaine seeking. D1DR stimulation in the nucleus accumbens, particularly the shell subregion, promotes cocaine reinstatement by increasing transmission through GluA1-containing AMPARs (Anderson *et al*, 2008; Schmidt and Pierce, 2010). The C-terminal region of the GluA1 subunit of AMPARs can be phosphorylated by PKA, PKC, and calcium/calmodulin-dependent kinase II (CaMKII) (Anggono and Huganir, 2012) all of which contribute to the reinstatement of cocaine seeking (Schmidt and Pierce, 2010; Schmidt *et al*, 2013). In part, D1DR stimulation reinstates cocaine seeking via serial activation of L-type calcium channels and CaMKII (Anderson *et al*, 2008). Cocaine reinstatement also is associated with D1DR-dependent increases in GluA1-pSer831, a PKC/CaMKII phosphorylation site, as well as increased surface expression of GluA1-containing AMPARs (Anderson *et al*, 2008). However, D1DR stimulation leads to cAMP and subsequently PKA activation, which is linked to cocaine seeking (Self *et al*, 1998). Surprisingly, intra-accumbal administration of a PKA inhibitor, Rp-cAMPs, promotes the reinstatement of cocaine seeking (Self *et al*, 1998). A potential explanation for this unexpected result is that Rp-cAMPs can also inhibit other cAMP-activated targets, such as exchange factors directly activated by cAMP (Epacs) (Bos, 2006). Epac activation leads to increased levels of the GTPase Rap, which can interact with the Ras/ERK cascade to modulate ERK-dependent processes (Lin *et al*, 2003) that contribute to the reinstatement of cocaine seeking (Thomas *et al*, 2008).

The present results demonstrate that AKAP signaling is required for the appropriate subcellular targeting of PKA during D1DR-mediated reinstatement of cocaine seeking, consistent with the postsynaptic localization of AKAP proteins (Bhattacharyya *et al*, 2009; Robertson *et al*, 2009; Sanderson and Dell'Acqua, 2011; Smith *et al*, 2006). Our findings also indicate that disruption of PKA binding specifically to the AKAP150 isoform in the nucleus accumbens shell attenuates cocaine reinstatement. It was not determined whether AKAP150 specifically was required for D1DR-mediated reinstatement of cocaine seeking; however, previous findings revealed that AKAP150 is the most highly expressed isoform in the brain (Sanderson and Dell'Acqua, 2011). In addition, the striatum, which includes the nucleus accumbens, exhibits the highest neuronal levels of AKAP150 expression (Ostroveanu *et al*, 2007). These findings, coupled with our observations of the effects of St-Ht31 peptide or AKAP79ΔPKA virus on cocaine priming-induced reinstatement, suggest that AKAP150 is likely the specific isoform involved in D1DR-mediated cocaine reinstatement. It is important to note that the duration of the

effects of St-Ht31 and the AKAP79ΔPKA virus differ in that the virus effect lasted several days, which could result in compensatory adaptations.

In addition to the C-terminal PKA-binding domain, AKAP150 also contains an internal binding site for the MAGUK scaffolding proteins, PSD-95 and SAP97, that promote its interaction with AMPARs (Colledge *et al*, 2000; Sanderson and Dell'Acqua, 2011). Furthermore, AKAP150 enhances PKA-mediated phosphorylation of AMPARs, especially Ser845 on GluA1 subunits (Colledge *et al*, 2000; Tavalin *et al*, 2002). The current results show that disrupting the binding of PKA to AKAP150 attenuates GluA1-Ser845 phosphorylation. PKA phosphorylation of GluA1 at Ser845 leads to increased open probability of AMPARs and increases surface expression of GluA1-containing AMPARs (Man *et al*, 2007). In cultured accumbal neurons, D1DR stimulation increases both Ser845 phosphorylation and GluA1 surface expression (Chao *et al*, 2002a; Chao *et al*, 2002b). Consistent with these findings, cocaine reinstatement is attenuated by intra-accumbal shell administration of AAV10-GluA1-C99, which impairs the trafficking of GluA1-containing AMPA receptors to the cell surface (Anderson *et al*, 2008). Moreover, forced abstinence following cocaine self-administration leads to both increased GluA1 surface expression, Ser845 phosphorylation, and increased rectification, which suggests an increase in calcium permeable AMPARs (CP-AMPA) (Conrad *et al*, 2008; McCutcheon *et al*, 2011).

AKAPs and GluA1-Containing AMPARs

Our findings are consistent with previous work demonstrating that disrupting AKAP signaling in the nucleus accumbens reduces GluA1 surface expression (Reissner *et al*, 2011). Surprisingly, Reissner *et al* (2011) showed no change in the phosphorylation status of GluA1-Ser845, although notably these measurements were made in drug-naïve rats (Reissner *et al*, 2011). Stimulus-driven changes in GluA1-Ser845 phosphorylation can enhance AMPA-mediated excitatory synaptic transmission and increase synaptic localization of GluA1, partially through AKAP150-PKA binding, whereas unstimulated changes in GluA1 surface expression and AMPA transmission can be independent of basal Ser845 phosphorylation (Lu *et al*, 2008; Man *et al*, 2007; Sanderson *et al*, 2016).

The current findings also reveal decreases in AMPAR eEPSCs after disruption of PKA binding to AKAP150. This is consistent with previous work showing that synaptic plasticity is impaired in GluA1 KO mice and S845A mutant mice that have impaired Ser845 phosphorylation, as well as in PKA-deficient AKAP150-D36 mice (Lee *et al*, 2003; Lu *et al*, 2008). In addition, disruption of PKA binding to AKAPs leads to downregulation of AMPAR currents (Tavalin *et al*, 2002). It is well known that the nucleus accumbens consists primarily (90–95%) of medium spiny neurons. Therefore, it is most likely that our observed decreases to evoked AMPAR amplitudes occur in these neurons. However, cocaine reinstatement can be modulated through accumbal interneurons (Smith *et al*, 2017). Therefore, we cannot fully eliminate the possibility that viral expression of AKAP79ΔPKA in these interneurons may play a role in the observed behavioral and electrophysiological

results. It also has been shown that PKA binding to AKAP150 and phosphorylation of GluA1-Ser845 can lead to increased surface expression of GluA1-containing AMPARs, particularly CP-AMPARs (Sanderson *et al*, 2016). However, we did not observe any significant reduction in rectification index. Typically, CP-AMPA accumulation in the nucleus accumbens core is observed only after a period of abstinence following cocaine self-administration (Conrad *et al*, 2008; McCutcheon *et al*, 2011; Purgianto *et al*, 2013). However, a recent study illustrated that blocking CP-AMPARs in the nucleus accumbens attenuates the reinstatement of cocaine seeking using a paradigm identical to the one used here (White *et al*, 2016); this effect was likely mediated through transient increases in GluA1 surface expression as seen previously (Anderson *et al*, 2008; Schierberl *et al*, 2011). The present results suggest that AKAP150 facilitates PKA-dependent GluA1-Ser845 phosphorylation and increased AMPAR transmission, contributing to cocaine reinstatement.

AKAP150 Interactions with PKC

In addition to associating with AMPARs via its MAGUK domain, AKAP150 can also bind PKC (Klauck *et al*, 1996), which plays a critical role in cocaine reinstatement. Cocaine reinstatement is associated with increased PKC activation and can be attenuated by intra-accumbal administration of PKC inhibitors (Schmidt *et al*, 2015; Schmidt *et al*, 2013). Moreover, cocaine reinstatement is associated with increased GluA1-Ser831 phosphorylation (Anderson *et al*, 2008). PKC phosphorylates GluA1 subunits at Ser831, facilitating GluA1 insertion into the membrane (Song and Hugarir, 2002). AKAP150 can also interact with L-type calcium channels via interaction with a leucine zipper domain at its C terminus (Oliveria *et al*, 2007). AKAP150 facilitates PKA phosphorylation of L-type calcium channels at Ser1700 and Ser1928, thereby increasing channel activity (Gao *et al*, 1997; Murphy *et al*, 2014). This channel plays a major role in cocaine-induced synaptic plasticity and cocaine reinstatement. Specifically, intra-accumbal shell administration of diltiazem, an L-type calcium channel antagonist, attenuates the reinstatement of cocaine seeking precipitated either by systemic cocaine injection or intra-accumbal shell administration of a D1DR agonist (Anderson *et al*, 2008). Though we did not investigate the interactions between PKC, L-type calcium channels, and AKAP150 in this study, these findings underscore multiple ways in which AKAP150 may regulate cocaine-induced behavioral and neuronal plasticity.

SUMMARY AND CONCLUSIONS

The present results contribute to a growing literature indicating that increased transmission through D1DRs and GluA1-containing AMPARs in the nucleus accumbens shell promotes the reinstatement of cocaine seeking. Moreover, the present findings demonstrate a compelling role for AKAP150/PKA as a critical link between dopamine and glutamate systems in the nucleus accumbens during cocaine reinstatement, which suggests that AKAP150 may be a potential target for the development of cocaine addiction pharmacotherapies.

FUNDING AND DISCLOSURE

This work was supported by the following grants from the National Institutes of Health: T32 DA28874 and F31 DA037748 (LAG); K01 DA39308 (MEW); R01 NS040701 and R01 MH102338 (MLD); K01 DA030445 and R01 DA037897 (HDS); as well as R01 DA15214 and R01 DA22339 (RCP). The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Rachael Neve (MIT) for her invaluable assistance in packaging our construct into an HSV vector. We also thank Adrian Arreola, Duncan Van Nest, John Maurer, and Daria Lukasz for their technical assistance as well as Drs John Dani and Mariella De Biasi for use of equipment and reagents.

REFERENCES

- Anderson SM, Bari AA, Pierce RC (2003). Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. *Psychopharmacology (Berl)* **168**: 132–138.
- Anderson SM, Famous KR, Sadri-Vakili G, Kumaresan V, Schmidt HD, Bass CE *et al* (2008). CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nat Neurosci* **11**: 344–353.
- Anggono V, Hugarir RL (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. *Curr Opin Neurobiol*, (2012) **22**: 461–469.
- Bachtell RK, Whisler K, Karanian D, Self DW (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.
- Banke TG, Bowie D, Lee H, Hugarir RL, Schousboe A, Traynelis SF (2000). Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J Neurosci* **20**: 89–102.
- Bhattacharyya S, Biou V, Xu W, Schluter O, Malenka RC (2009). A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. *Nat Neurosci* **12**: 172–181.
- Bos JL (2006). Epac proteins: multi-purpose cAMP targets. *Trends Biochem Sci* **31**: 680–686.
- Chao SZ, Ariano MA, Peterson DA, Wolf ME (2002a). D1 dopamine receptor stimulation increases GluR1 surface expression in nucleus accumbens neurons. *J Neurochem* **83**: 704–712.
- Chao SZ, Lu W, Lee HK, Hugarir RL, Wolf ME (2002b). D(1) dopamine receptor stimulation increases GluR1 phosphorylation in postnatal nucleus accumbens cultures. *J Neurochem* **81**: 984–992.
- Colledge M, Dean RA, Scott GK, Langeberg LK, Hugarir RL, Scott JD (2000). Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* **27**: 107–119.
- Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y *et al* (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* **454**: 118–121.
- Gao T, Yatani A, Dell'Acqua ML, Sako H, Green SA, Dascal N *et al* (1997). cAMP-dependent regulation of cardiac L-type Ca²⁺ channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron* **19**: 185–196.
- Hagewoud R, Havekes R, Novati A, Keijsers JN, Van der Zee EA, Meerlo P (2010). Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. *J Sleep Res* **19**: 280–288.
- Kauer JA, Malenka RC (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**: 844–858.

- Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, Scott JD (1996). Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. *Science* **271**: 1589–1592.
- Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G et al (2003). Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* **112**: 631–643.
- Lin SL, Johnson-Farley NN, Lubinsky DR, Cowen DS (2003). Coupling of neuronal 5-HT₇ receptors to activation of extracellular-regulated kinase through a protein kinase A-independent pathway that can utilize Epac. *J Neurochem* **87**: 1076–1085.
- Lu L, Grimm JW, Shaham Y, Hope BT (2003). Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. *J Neurochem* **85**: 1604–1613.
- Lu Y, Zhang M, Lim IA, Hall DD, Allen M, Medvedeva Y et al (2008). AKAP150-anchored PKA activity is important for LTD during its induction phase. *J Physiol* **586**: 4155–4164.
- Man HY, Sekine-Aizawa Y, Haganir RL (2007). Regulation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1 subunit. *Proc Natl Acad Sci USA* **104**: 3579–3584.
- McCutcheon JE, Wang X, Tseng KY, Wolf ME, Marinelli M (2011). Calcium-permeable AMPA receptors are present in nucleus accumbens synapses after prolonged withdrawal from cocaine self-administration but not experimenter-administered cocaine. *J Neurosci* **31**: 5737–5743.
- Moita MA, Lamprecht R, Nader K, LeDoux JE (2002). A-kinase anchoring proteins in amygdala are involved in auditory fear memory. *Nat Neurosci* **5**: 837–838.
- Murphy JG, Sanderson JL, Gorski JA, Scott JD, Catterall WA, Sather WA et al (2014). AKAP-anchored PKA maintains neuronal L-type calcium channel activity and NFAT transcriptional signaling. *Cell Rep* **7**: 1577–1588.
- Neve RL, Neve KA, Nestler EJ, Carlezon WA Jr. (2005). Use of herpes virus amplicon vectors to study brain disorders. *Biotechniques* **39**: 381–391.
- Oh MC, Derkach VA, Guire ES, Soderling TR (2006). Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. *J Biol Chem* **281**: 752–758.
- Oliveria SF, Dell'Acqua ML, Sather WA (2007). AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca²⁺ channel activity and nuclear signaling. *Neuron* **55**: 261–275.
- Ortinski PI, Turner JR, Pierce RC (2013). Extrasynaptic targeting of NMDA receptors following D1 dopamine receptor activation and cocaine self-administration. *J Neurosci* **33**: 9451–9461.
- Ostroveanu A, Van der Zee EA, Dolga AM, Luiten PG, Eisel UL, Nijholt IM (2007). A-kinase anchoring protein 150 in the mouse brain is concentrated in areas involved in learning and memory. *Brain Res* **1145**: 97–107.
- Pierce RC, Wolf ME (2013). Psychostimulant-induced neuroadaptations in nucleus accumbens AMPA receptor transmission. In: Pierce RC, Kenny PJ (eds) *Addiction*. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY. pp 121–134.
- Purgianto A, Scheyer AF, Loweth JA, Ford KA, Tseng KY, Wolf ME (2013). Different adaptations in AMPA receptor transmission in the nucleus accumbens after short vs long access cocaine self-administration regimens. *Neuropsychopharmacology* **38**: 1789–1797.
- Reissner KJ, Uys JD, Schwacke JH, Comte-Walters S, Rutherford-Bethard JL, Dunn TE et al (2011). AKAP signaling in reinstated cocaine seeking revealed by iTRAQ proteomic analysis. *J Neurosci* **31**: 5648–5658.
- Robertson HR, Gibson ES, Benke TA, Dell'Acqua ML (2009). Regulation of postsynaptic structure and function by an A-kinase anchoring protein-membrane-associated guanylate kinase scaffolding complex. *J Neurosci* **29**: 7929–7943.
- Sanderson JL, Dell'Acqua ML (2011). AKAP signaling complexes in regulation of excitatory synaptic plasticity. *Neuroscientist* **17**: 321–336.
- Sanderson JL, Gorski JA, Dell'Acqua ML (2016). NMDA receptor-dependent LTD requires transient synaptic incorporation of Ca²⁺-permeable AMPARs mediated by AKAP150-anchored PKA and calcineurin. *Neuron* **89**: 1000–1015.
- Schierberl K, Hao J, Tropea TF, Ra S, Giordano TP, Xu Q et al (2011). Ca_v1.2L-type Ca²⁺ channels mediate cocaine-induced GluA1 trafficking in the nucleus accumbens, a long-term adaptation dependent on ventral tegmental area Ca_v1.3 channels. *J Neurosci* **31**: 13562–13575.
- Schmidt HD, Anderson SM, Pierce RC (2006a). 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 HD, Kimmey BA, Arreola AC, Pierce RC (2015). Group I metabotropic glutamate receptor-mediated activation of PKC gamma in the nucleus accumbens core promotes the reinstatement of cocaine seeking. *Addict Biol* **20**: 285–296.
- Schmidt HD, Pierce RC (2006b). 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.
- Schmidt HD, Pierce RC (2010). Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. *Ann N Y Acad Sci* **1187**: 35–75.
- Schmidt HD, Schassburger RL, Guercio LA, Pierce RC (2013). Stimulation of mGluR5 in the accumbens shell promotes cocaine seeking by activating PKC gamma. *J Neurosci* **33**: 14160–14169.
- Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ (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.
- Shaham Y, Hope BT (2005). The role of neuroadaptations in relapse to drug seeking. *Nat Rev Neurosci* **8**: 1437–1439.
- Smith ACW, Scofield MD, Heinsbroek JA, Gipson CD, Neuhofer D, Roberts-Wolfe DJ et al (2017). Accumbens nNOS interneurons regulate cocaine relapse. *J Neurosci* **37**: 742–756.
- Smith KE, Gibson ES, Dell'Acqua ML (2006). cAMP-dependent protein kinase postsynaptic localization regulated by NMDA receptor activation through translocation of an A-kinase anchoring protein scaffold protein. *J Neurosci* **26**: 2391–2402.
- Song I, Haganir RL (2002). Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci* **25**: 578–588.
- Sun X, Zhao Y, Wolf ME (2005). Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J Neurosci* **25**: 7342–7351.
- Tavalin SJ, Colledge M, Hell JW, Langeberg LK, Haganir RL, Scott JD (2002). Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. *J Neurosci* **22**: 3044–3051.
- Thomas MJ, Kalivas PW, Shaham Y (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *Br J Pharmacol* **154**: 327–342.
- Tunquist BJ, Hoshi N, Guire ES, Zhang F, Mullendorff K, Langeberg LK et al (2008). Loss of AKAP150 perturbs distinct neuronal processes in mice. *Proc Natl Acad Sci USA* **105**: 12557–12562.
- Unterwald EM, Fillmore J, Kreek MJ (1996). Chronic repeated cocaine administration increases dopamine D1 receptor-mediated signal transduction. *Eur J Pharmacol* **318**: 31–35.
- Weisenhaus M, Allen ML, Yang L, Lu Y, Nichols CB, Su T et al (2010). Mutations in AKAP5 disrupt dendritic signaling complexes and lead to electrophysiological and behavioral phenotypes in mice. *PLoS ONE* **5**: e10325.
- White SL, Ortinski PI, Friedman SH, Zhang L, Neve RL, Kalb RG et al (2016). A critical role for the GluA1 accessory protein, SAP97, in cocaine seeking. *Neuropsychopharmacology* **41**: 736–750.
- Wong W, Scott JD (2004). AKAP signalling complexes: focal points in space and time. *Nat Rev Mol Cell Biol* **5**: 959–970.