

ORIGINAL
ARTICLECasein kinase 1 enables nucleus accumbens
amphetamine-induced locomotion by regulating
AMPA receptor phosphorylationDongdong Li,* Stacy Herrera,* Nancy Bubula,* Elena Nikitina,†
Abraham A. Palmer,*‡§ Dorothy A. Hanck,†§ Jessica A. Loweth§ and
Paul Vezina*§*Department of Psychiatry and Behavioral Neuroscience, The University of Chicago, Chicago,
Illinois, USA

†Department of Medicine, The University of Chicago, Chicago, Illinois, USA

‡Department of Human Genetics, The University of Chicago, Chicago, Illinois, USA

§Committee on Neurobiology, The University of Chicago, Chicago, Illinois, USA

Abstract

The closely related δ and ϵ isoforms of the serine/threonine protein kinase casein kinase 1 (Csnk1) have been implicated in the generation of psychostimulant-induced behaviors. In this study, we show that Csnk1 δ/ϵ produces its effects on behavior by acting on the Darpp-32-PP1 signaling pathway to regulate AMPA receptor phosphorylation in the nucleus accumbens (NAcc). Inhibiting Csnk1 δ/ϵ in the NAcc with the selective inhibitor PF-670462 blocks amphetamine induced locomotion and its ability to increase phosphorylation of Darpp-32 at S137 and T34, decrease PP1 activity and increase phosphorylation of the AMPA receptor subunit at S845.

Consistent with these findings, preventing GluR1 phosphorylation with the alanine mutant GluR1(S845A) reduces glutamate-evoked currents in cultured medium spiny neurons and blocks the locomotor activity produced by NAcc amphetamine. Thus, Csnk1 enables the locomotor and likely the incentive motivational effects of amphetamine by regulating Darpp-32-PP1-GluR1(S845) signaling in the NAcc. As such, Csnk1 may be a critical target for intervention in the treatment of drug use disorders.

Keywords: AMPA glutamate receptors, amphetamine, casein kinase 1, Darpp-32, dopamine, GluR1.
J. Neurochem. (2011) **118**, 237–247.

Casein kinase 1 (Csnk1) is a serine/threonine protein kinase that phosphorylates a wide array of substrates, is involved in the regulation of diverse cellular processes, and is richly expressed in multiple tissues including brain (Knippschild *et al.* 2005). Recently, the closely related isoforms, Csnk1 δ and Csnk1 ϵ , both abundant in striatal forebrain regions (Löhler *et al.* 2009; Utz *et al.* 2010), were implicated in the generation of psychostimulant-induced locomotor behaviors. Mice genetically modified to over-express Csnk1 δ in forebrain show dopamine-dependent alterations in basal and amphetamine-induced locomotion (Zhou *et al.* 2010) while mice selectively bred for high sensitivity to methamphetamine-induced locomotion (Kamens *et al.* 2005) show a quantitative trait loci for increased Csnk1 ϵ expression with a close to 10-fold increase in *Csnk1 ϵ* gene expression in the nucleus accumbens (NAcc) (Palmer *et al.* 2005). A single-nucleotide polymorphism in the human *CSNK1 ϵ* gene has also

been associated with increased sensitivity to the subjective effects of amphetamine (Veenstra-VanderWeele *et al.* 2006). These findings indicate that Csnk1 can regulate dopamine signaling and psychostimulant-induced behaviors *in vivo* but little is known about the mechanisms by which it produces these effects.

Received November 30, 2010; revised manuscript received May 9, 2011; accepted May 10, 2011.

Address correspondence and reprint requests to Paul Vezina, PhD, Department of Psychiatry and Behavioral Neuroscience, 5841 S Maryland Avenue, MC3077, Chicago, IL 60637, USA.

E-mail: pvezina@yoda.bsd.uchicago.edu

Abbreviations used: AMPH, amphetamine; Csnk1, casein kinase 1; Darpp-32, cAMP-regulated phosphoprotein 32; GSK-3 β , glycogen synthase kinase 3 β ; NAcc, nucleus accumbens; PKA, protein kinase A; PP1, protein phosphatase 1.

Midbrain dopamine and cortical glutamate projections converge onto medium spiny output neurons in the NAcc where they interact to mediate the behavioral and incentive motivational effects of amphetamine and other psychostimulants (Wise and Bozarth 1987; Meredith *et al.* 1993; Pierce and Kalivas 1997; Sesack *et al.* 2003). Different dopamine and glutamate receptor initiated signaling pathways in these neurons, including those using calcium-calmodulin-dependent protein kinase II (Anderson *et al.* 2008) and dopamine and cAMP-regulated phosphoprotein 32 (Darpp-32) (Greengard 2001; Fernandez *et al.* 2006), have been proposed to bridge the contribution of these two neurotransmitters. The nature and extent of Darpp-32 activity is regulated by a number of kinases and protein phosphatases (Fernandez *et al.* 2006). In the rat, Csnk1 phosphorylates Darpp-32 at S137 to decrease the rate of pDarpp-32(T34) dephosphorylation by calcineurin (Desdouits *et al.* 1995a,b). Phosphorylation of Darpp-32 at T34 by protein kinase A (PKA) inhibits protein phosphatase 1 (PP1) activity (Hemmings *et al.* 1984), promoting the continued phosphorylation of a number of target proteins including the GluR1 subunit of the AMPA receptor at S845 (Snyder *et al.* 2000). Phosphorylation of GluR1 at S845 potentiates neuronal excitability by facilitating trafficking and synaptic insertion of GluR1-containing AMPA receptors (Ehlers 2000; Esteban *et al.* 2003; Mangiavacchi and Wolf 2004; Sun *et al.* 2005; Oh *et al.* 2006) and increasing peak current and channel open probability (Roche *et al.* 1996; Banke *et al.* 2000). As psychostimulant-induced locomotion requires NAcc AMPA receptor activation (Wolf 1998), we hypothesized that Csnk1 can enable the behavioral effects of drugs like amphetamine by enhancing the ability of Darpp-32 to inhibit PP1 activity and thus increase AMPA receptor phosphorylation at S845.

Materials and methods

Subjects

Male Sprague–Dawley rats weighing 250–275 g on arrival were obtained from Harlan Sprague–Dawley (Madison, WI, USA) and housed individually in a reverse cycle room (12-h light/12-h dark) with food and water freely available. Rats were given 4–5 days to acclimate and subsequently tested during the dark period of the light cycle. For experiments involving intra-NAcc infusions, rats were anesthetized with a mix of ketamine (100 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.), placed in a stereotaxic apparatus with the incisor bar positioned 5.0 mm above the interaural line (Pellegrino *et al.* 1979), and chronically implanted with bilateral guide cannulae (22 gauge, Plastics One, Roanoke, VA, USA) aimed at the NAcc core (A/P, +3.4 mm; L, \pm 1.5 mm; DV, –7.5 mm from bregma and skull). Cannulae were angled at 10° to the vertical and positioned either 1 mm (for biochemistry and behavioral testing with amphetamine and PF) or 4 mm (for Lentiviral infection and subsequent testing with amphetamine) above the final injection site. Guide cannulae were secured with dental acrylic anchored to stainless steel skull screws. After surgery, 28 gauge obturators were placed into the guide

cannulae (extending 1 mm for guide cannulae positioned 1 mm and flush for guide cannulae positioned 4 mm above the final injection site) and rats returned to their home cage for a 7 day recovery period. All surgical procedures were conducted using aseptic techniques according to approved Institutional Animal Care and Use Committee and Institutional Biosafety Committee protocols.

NAcc microinjections

In the behavioral and biochemistry experiments testing the effects of PF, microinjections were made in the freely moving rat in a volume of 0.5 μ L/side over 30 s through 28 gauge injection cannulae extending 1 mm beyond the guide cannula tips. In the experiment testing the effect of NAcc GluR1(S845A) expression, amphetamine microinjections were made using the same guide cannulae used to deliver the Lentiviral vectors. Thus, injection cannulae extended 4 mm beyond the guide cannula tips in this case. Injection cannulae were connected via polyethylene tubing (PE20) to Hamilton syringes and left in place for 1 min after the injection to allow for diffusion. Microinjection cannulae were then removed, the obturators replaced, and rats returned either to the activity boxes or their home cages.

Locomotor testing

Rats were placed individually in locomotor monitoring chambers for 30–60 min, administered their respective NAcc infusion, and returned to the locomotor chambers for 60 min. In the experiments testing the effects of PF, PF (1.0, 5.0, or 10 μ g/side) was microinjected into the NAcc either alone or in cocktail with amphetamine (2.5 μ g/side). In each of three experiments, saline, amphetamine, and amphetamine + PF microinjections were made in counterbalanced order with at least three days between injections. In a fourth experiment, rats in different groups received microinjections into the NAcc of saline or PF alone (1.0, 5.0, or 10 μ g/side) again in counterbalanced order. In the experiment testing the effect of NAcc GluR1(S845A) expression, Lenti-GFP and Lenti-GluR1 (S845A) infected rats were administered amphetamine (2.5 μ g/side) into the NAcc 3 weeks following infection. Locomotor activity was measured using a bank of 12 activity monitoring boxes. Each box (22 \times 43 \times 33 cm) was constructed of opaque plastic (rear and two side walls), a plexiglas front-hinged door, and a tubular stainless steel ceiling and floor. Two photocell beams, positioned 2.5 cm above the floor and spaced evenly along the longitudinal axis of each box, estimated horizontal locomotion. Separate interruptions of photocell beams were detected and recorded via an electrical interface by a computer situated in an adjacent room using locally developed software.

NAcc signaling

Rats in different groups were infused intracranially with saline (0.5 μ L/side), amphetamine (2.5 μ g/side), PF (10 μ g/side) or amphetamine + PF into the NAcc and killed 20 min later. Brains were rapidly removed for subsequent assessment of protein levels and PP1 activity. For immunoblotting, brains were flash-frozen on dry ice, 2 mm thick sections obtained with a brain matrix, and 2 mm diameter punches of the NAcc taken around the injection cannula tips. Tissue punches were homogenized in ice-cold lysis buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate) containing protease and phosphatase inhibitor cocktails (1 and 2; Sigma-Aldrich,

St Louis, MO, USA). A total of 20 µg of protein was loaded per lane and separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Following transfer, membranes were incubated in blocking solution (5% milk in Tris-buffered saline containing Tween 20), incubated for 16 h at 4°C in primary antibody for Darpp32 (1 : 1000; Cell Signaling Technologies, Beverly, MA, USA), pDarpp32 (T34; 1 : 500; Millipore, Billerica, MA, USA), pDarpp32(S137; 1 : 5000; generously provided by Dr Paul Greengard, Rockefeller University, New York, NY, USA), GluR1 (1 : 1000; Millipore), pGluR1 (S831; 1 : 500; Millipore), pGluR1 (S845; 1 : 500; Millipore), Csnk1δ/ε (1 : 5000; BD Transduction Laboratories, Franklin Lake, NJ, USA), CopGFP (1 : 1000; Evrogen, Moscow, Russia), β-actin (1 : 2000; Sigma-Aldrich), or Tubulin (1 : 10 000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and washed in a mixture of Tris-buffered saline and Tween 20. Membranes were then incubated in a horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG and visualized using the ECL detection system (ECL Advanced; GE Healthcare, Waukesha, WI, USA). Blots were digitally imaged using the GeneSnap Bio Imaging System and quantified using Genetool software (Syngene, MD, USA). PP1 activity was assayed using the Protein Serine/Threonine Phosphatase assay system (BioLabs, Ipswich, MA, USA) by measuring the release of inorganic phosphate from labeled protein. Myelin basic protein (MyBP) labeled with Γ -³²P was used as a substrate. Fresh NAcc tissue was homogenized in lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM MnCl₂, 5 mM dithiothreitol, 5% glycerol) containing a protease inhibitor cocktail but without phosphatase inhibitors. Ten microgram of tissue lysate was added to 30 µL of assay buffer containing PP2A and PP2B inhibitors, 2 nM okadaic acid, and 2 µM FK-506. After 5 min of pre-incubation at 30°C, the reaction was started by adding 10 µL of substrate. Following 10 min of incubation at 30°C, the reaction was terminated by adding 200 µL of cold 20% trichloroacetic acid. After centrifugation at 12 000 g for 5 min, radioactivity was counted in 200 µL of supernatant by an LS6500 multi-purpose scintillation counter (Beckman, CA, USA). Data were expressed as pmol/min/µg protein.

Construct assembly and Lentiviral vectors

cDNAs for rat GluR1 (GenBank accession No. X17184) were constructed in a pcDNA1/Amp vector at the EcoRI and BamHI sites (Invitrogen, Carlsbad, CA, USA) to obtain a pcDNA1/GluR1 plasmid. Mutagenesis was performed using the Quickchange II XL site-Directed mutagenesis kit. The following primers were used to produce the GluR1 S845A mutants. Forward primer: 5'-G ACC CTC CCC CGG AAC GCT GGG GCA GGA GCC AGC-3'. Reverse primer: 5'-GCT GGC TCC TGC CCC AGC GTT CCG GGG GAG GGT C-3'. The full length of the GluR1(S845A) cDNA was subcloned into a pCDH1-CMV-MCS-EF1-copGFP vector (System Biosciences, Mountain View, CA, USA). Recombinant Lentivirus was packaged in HEK TN cells using a pPACKH Lentivector Packaging Kit as described by the manufacturer (System Biosciences). The Lentivirus was purified and concentrated by PEG-8000. The average titer of the recombinant viral stocks was 1×10^6 infectious units/mL. The following recombinant Lentiviral vectors were used: Lenti-GluR1(S845A) and Lenti-GFP as a control. The viability of the Lentiviral vectors was confirmed in HEK cells infected either with Lenti-GFP, Lenti-GluR1, or Lenti-GluR1

(S845A). GFP-positive cells were observed in all cases 48 h post-infection but GluR1 transgene expression was increased only in the Lenti-GluR1 and Lenti-GluR1(S845A) infected HEK cells (see Figure S1).

For the behavioral experiment, rats were transferred to a biosafety level 2 facility following 1 week of recovery from implant surgery and administered bilateral NAcc microinjections of the viral vectors in a fume hood (Loweth *et al.* 2010). Microinjections were made in freely moving rats in a volume of 2.0 µL/side at a rate of 0.1 µL/30 s through 28 gauge cannulae extending 4 mm beyond the guide cannulae tips. Microinjection cannulae loaded with Lentivirus were connected via polyethylene tubing (PE20) to Hamilton syringes and left in place for 5 min after the injection to allow for diffusion. Rats were returned to the housing room 72 h later.

For the electrophysiology experiments, dissociated cultures of medium spiny neurons were prepared from E18 Sprague–Dawley rats. Under isoflurane anesthesia, embryos were removed and the NAcc separated from diencephalon, dissected free of meninges, and diced. Tissues were digested in 0.03% (w/v) trypsin. After dissociation by trituration, cells were counted, suspended in culture medium consisting of Neurobasal supplemented with 2% B-27 (both from Gibco-Invitrogen Corp., Carlsbad, CA, USA), 0.5 mM Gluta-Max I, 10% deactivated fetal bovine serum, and 1% penicillin/Streptomycin. Cells were plated on poly L-lysine-coated 25-mm cover slips at a density of 8×10^4 cells/cm². Neurons were either left uninfected or infected with Lenti-GFP or Lenti-GluR1(S845A) at day 1 of culture. Recordings were performed 10–18 days following infection. Cells in these three groups were also assayed for evoked pGluR1(S845) following 15 min incubation with the PKA activating D₁ dopamine receptor agonist SKF81297 (10 µM).

Electrophysiology

Cultured medium spiny neurons were plated on coverslips, placed in an experimental chamber, and visualized using an inverted microscope under phase-contrast illumination (Nikon, Tokyo, Japan). All experiments were performed at room temperature (~20°C). The chamber was flushed with bath solution for 5 min before recording began. During experiments, solution flow was ~0.5 mL/min. Bath solution contained (in mM/L) 140 NaCl, 1 MgCl₂, 5 KCl, 10 Glucose, 10 Hepes, 2 CaCl₂·2H₂O at pH = 7.4. Patch electrodes were pulled from borosilicate glass capillary tubing (TW150-4; World Precision Instruments Inc., Sarasota, FL, USA) on a micropipette puller (Sutter P-97; Sutter Instrument Co., Novato, CA, USA). They had a resistance of 2–4 MΩ when filled with solution containing (in mM/L) 135 CsCl, 1MgCl₂, 10 HEPES, 10 EGTA, 3.6 Mg-ATP, 0.1 Na-GTP, and 14 Na-Creatinephosphate. Whole-cell voltage-clamp was performed using an Axopatch 200B, 16-bit data Digidata 1332A acquisition system driven by Clampex 8.2 (Axon Instruments, Foster City, CA, USA). Cells were held at –60 mV, and gap-free recordings were acquired for 2 min each. Multiple recordings were possible in most cells. Puffs of 0.5 mM Na-glutamate dissolved in bath solution were applied for 50ms with a Picospritzer II (General Valve Corp., Fairfield, NJ, USA). The tip of the infusion pipette was placed within 80–100 µm of the recorded cell. Current traces were filtered at 1 kHz and digitized at 2 kHz. For each cell, a representative glutamate response was chosen for group analysis and background activity was analyzed from 30 s of recording prior to glutamate application.

Histology and immunofluorescence

After the completion of the behavioral experiments testing the effects of NAcc PF, rats were deeply anesthetized and perfused transcardially with 0.9% saline and 10% formalin. Brains were removed and stored in the formalin solution for at least 24 h. Forty-micrometer sections were then prepared, mounted on gelatin-coated slides, and stained with cresyl violet to identify rats with injection cannula tips located bilaterally in the NAcc core. Only rats with both cannula tips located in the NAcc core were retained for statistical analyses. Four rats failed to meet this criterion.

After completion of the behavioral experiment testing the effects of NAcc GluR1(S845A), rats were perfused with 0.9% saline and 4% paraformaldehyde and brains transferred to a 25% sucrose solution. To detect GFP fluorescence 40 μ m coronal sections were prepared, mounted on gelatin-coated slides with Fluoromount-G (Southern Biotech, Birmingham, AL, USA), and analyzed using a fluorescence microscope. Injection cannula tips located in the NAcc core were identified and the distribution pattern of GFP-positive cells around the cannula tips assessed. Entire brains (from midbrain to prefrontal cortex) were also examined for GFP-positive cells to determine the extent of anterograde and retrograde transport of the virus from the NAcc core microinjection site. GFP-positive cells were observed only in close proximity to the injection cannula tips in the NAcc and were not detected in any other brain region. Again, only rats with both cannula tips located in the NAcc core were retained for statistical analysis. Five rats failed to meet this criterion.

Drugs

PF-670462 (4-(3-cyclohexyl-5-(4-fluoro-phenyl)-3H-imidazol-4-yl)pyrimidin-2-ylamine) was generously provided by Pfizer (Groton, CT, USA). S(+)-amphetamine sulfate was purchased from Sigma-Aldrich. Both drugs were dissolved in physiological saline. SKF81297 was purchased from Tocris (Ellisville, MO, USA) and dissolved in dimethylsulfoxide. Doses refer to the weight of the salt.

Data analyses

The PF-amphetamine locomotor and electrophysiology peak current data were analyzed with one way ANOVA. The immunoblot data were normalized to β -actin or tubulin and ratios analyzed with two way between ANOVA with amphetamine and PF as the factors. The GluR1(S845A) expression pGluR1(S845) and GluR1 protein data were analyzed with two way between ANOVA with infection and drug challenge as the factors. The NAcc GluR1(S845A) expression locomotor data were analyzed with between-within ANOVA with infection as the between factor and time as the within factor. In all cases, *post hoc* comparisons after ANOVA were made with the Scheffe test.

Results

Inhibiting Csnk1 blocks NAcc amphetamine-induced locomotion

Using the selective Csnk1 δ/ϵ inhibitor, PF-670462 (PF) (Badura *et al.* 2007; Walton *et al.* 2009), we first assessed whether inhibiting Csnk1 in the NAcc core could prevent the increased locomotion normally observed in rats when amphetamine is infused bilaterally into this site. In each of

three experiments, NAcc amphetamine alone (2.5 μ g/side), produced a close to twofold increase in locomotion compared with saline (Fig. 1a; 1.0 PF: $F_{2,10} = 8.84$, $p < 0.01$; 5.0 PF: $F_{2,14} = 6.01$, $p < 0.05$; 10.0 PF: $F_{2,10} = 20.17$, $p < 0.001$; revealed by analyses of variance, ANOVA). Consistent with results previously reported for methamphetamine in mice (Bryant *et al.* 2009), co-infusing PF with amphetamine into the NAcc dose-dependently reduced its ability to increase locomotion, with no effect observed at the lowest dose of PF (1.0 μ g/side) and a significant attenuation observed at the highest (10.0 μ g/side; $p < 0.01$, revealed by *post hoc* Scheffe comparisons). None of the doses of PF tested produced an effect on locomotion that differed from saline when infused by themselves into the NAcc (Fig. 1b).

Inhibiting Csnk1 blocks NAcc amphetamine-induced alterations in pDarpp-32

To confirm the Csnk1 inhibiting effects of PF, tissue punches were obtained in the NAcc of separate rats 20-min following the local infusion of amphetamine, amphetamine + PF, saline, or PF alone and subjected to Western blot analyses. As reported previously following systemic injections in mice (Svenningsson *et al.* 2003), NAcc amphetamine (2.5 μ g/side) significantly increased pDarpp-32(S137) relative to saline controls (Fig. 2b; $p < 0.05$, by *post hoc* Scheffe comparison following ANOVA showing a significant effect of PF, $F_{1,20} = 10.15$, $p < 0.01$, and a significant amphetamine-PF interaction, $F_{1,20} = 6.12$, $p < 0.05$). PF at the behaviorally effective dose (10.0 μ g/side) completely blocked this effect when infused with amphetamine into the NAcc ($p < 0.01$). As expected (Svenningsson *et al.* 2003), NAcc amphetamine also robustly increased PKA phosphorylation at T34 of Darpp-32 (Fig. 2e; $p < 0.05$, by *post hoc* Scheffe comparison following ANOVA showing a significant amphetamine-PF interaction, $F_{1,20} = 13.01$, $p < 0.01$). Again, this effect was completely blocked by PF ($p < 0.05$). This dose of PF produced no significant effects on pDarpp-32 relative to saline when administered alone. In addition, neither NAcc amphetamine nor PF altered Csnk1 δ/ϵ or Darpp-32 protein levels relative to saline (Fig. 2c and f).

Inhibiting Csnk1 blocks NAcc amphetamine-induced inhibition of PP1 and phosphorylation of GluR1(S845)

The NAcc amphetamine-induced increase in pDarpp-32(T34) led to a significant decrease in PP1 activity relative to saline (Fig. 3a; $p < 0.01$, by *post hoc* Scheffe comparison following ANOVA showing significant effects of amphetamine, $F_{1,20} = 7.73$, $p < 0.05$, PF, $F_{1,20} = 4.42$, $p < 0.05$, and a significant amphetamine-PF interaction, $F_{1,20} = 13.54$, $p < 0.01$). PF (10.0 μ g/side) blocked this effect when infused with amphetamine into the NAcc ($p < 0.01$). As predicted (Snyder *et al.* 2000), the observed NAcc amphetamine induced decrease in PP1 activity led to a significant increase

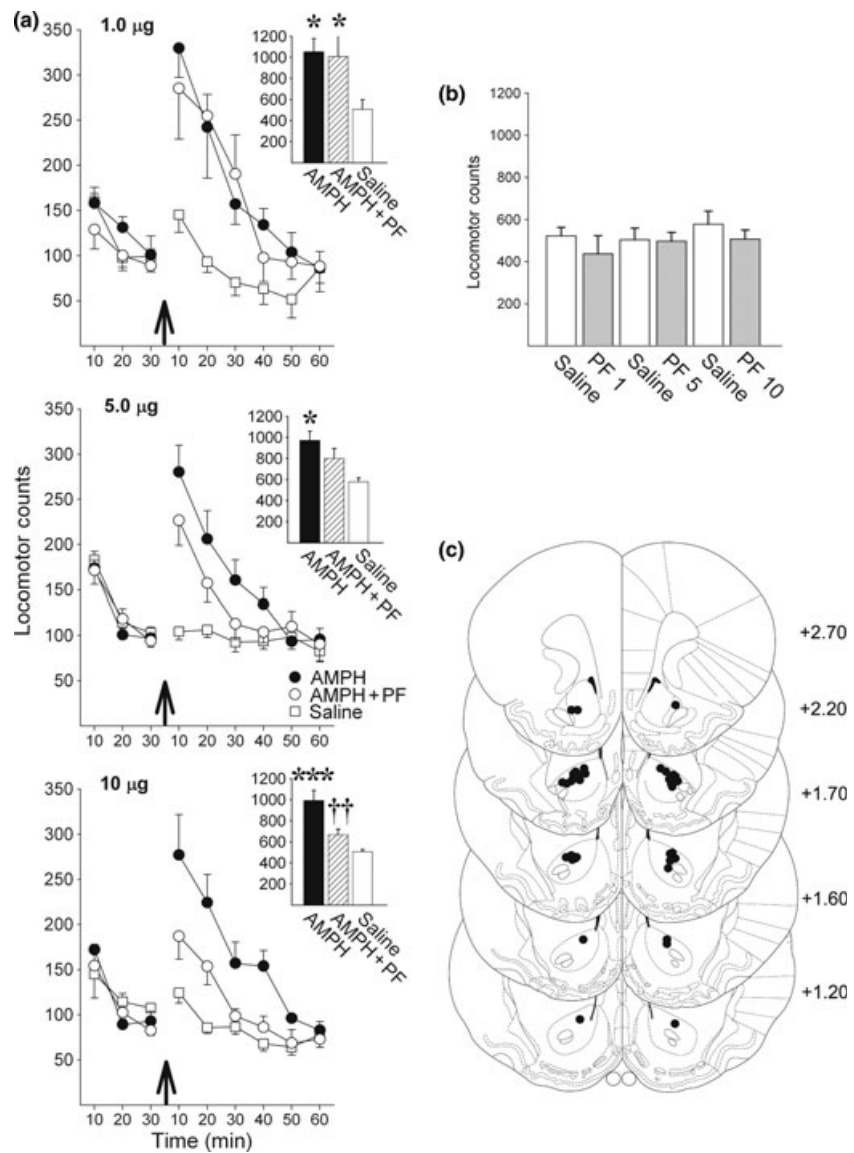


Fig. 1 The Csnk1 δ/ϵ inhibitor PF-670462 (PF) dose-dependently blocks NAcc amphetamine (AMPH)-induced locomotion. (a) Locomotor activity counts before and after NAcc AMPH (arrows) in three experiments each testing the indicated dose of PF ($n/\text{group} = 6\text{--}8$). Insets show 60-min total locomotor counts following AMPH ($*p < 0.05$, $***p < 0.001$ vs. Saline; $^{\dagger\dagger}p < 0.01$ vs. AMPH; Scheffé). (b) 60-min total locomotor counts following NAcc saline or PF alone ($n/\text{group} = 6\text{--}7$). (c) Injection cannula placements in the NAcc core. The numbers to the right indicate millimeters from bregma (Paxinos and Watson 1997). Data are shown as mean \pm SEM.

in phosphorylation of GluR1 at S845 (Fig. 3c; $p < 0.001$, by *post hoc* Scheffé comparison following ANOVA showing significant effects of amphetamine, $F_{1,20} = 22.26$, $p < 0.001$, PF, $F_{1,20} = 9.96$, $p < 0.01$, and a significant amphetamine–PF interaction, $F_{1,20} = 19.12$, $p < 0.001$). Again, PF blocked this effect when infused with amphetamine into the NAcc ($p < 0.001$). PF produced no significant effects on PP1 activity or pGluR1(S845) relative to saline when administered alone. Consistent with previous reports (Snyder *et al.* 2000; Nelson *et al.* 2009), phosphorylation of GluR1 at S831 and total NAcc GluR1 protein levels were unaltered by either amphetamine or PF (Fig. 3d–f).

AMPA receptor phosphorylation at S845 is necessary for NAcc amphetamine-induced locomotion

The above findings indicate that inhibiting Csnk1 in the NAcc blocks the ability of amphetamine in this site to

increase locomotion possibly by preventing amphetamine-initiated phosphorylation of Darpp-32, inhibition of PP1 and phosphorylation of GluR1(S845). To directly assess this possibility, site-directed mutagenesis was used to generate a mutant form of GluR1 with the S845 residue replaced by an alanine [GluR1(S845A)] to prevent phosphorylation at this site and the construct inserted into an HIV-1-derived lentiviral vector containing GFP. In a first experiment, this vector was used to express the mutant construct [Lenti-GluR1(S845A)] in cultured medium spiny neurons (Fig. 4a). Incubating cultures with the D₁ dopamine receptor agonist SKF81297 (10 μM) to activate PKA increased pGluR1(S845) as expected in neurons that were not infected (Control) or neurons infected with a vector containing GFP alone (Lenti-GFP) but not in neurons infected with Lenti-GluR1(S845A) 10–18 days earlier (Fig. 4c; $p < 0.001$, by *post hoc* Scheffé comparisons following ANOVA showing a

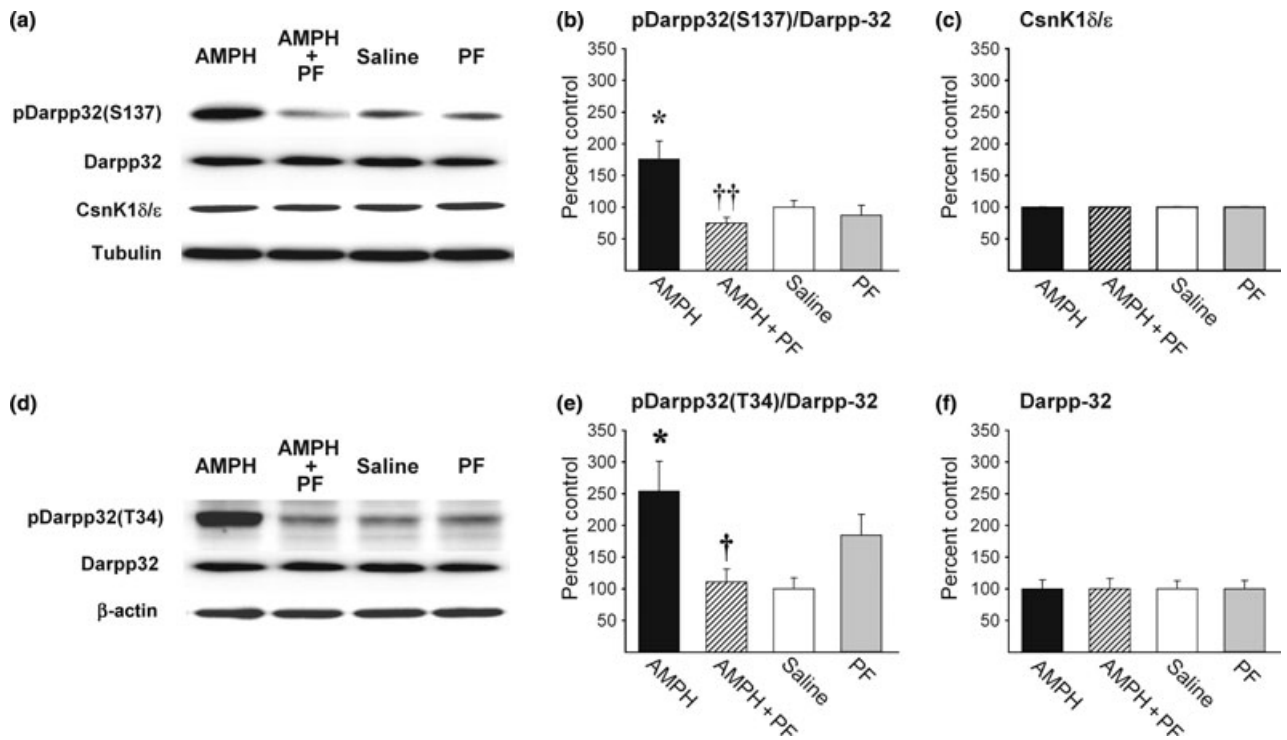


Fig. 2 PF blocks NAcc AMPH induced phosphorylation of Darpp-32. Representative immunoblots of (a) pDarpp-32(S137), Darpp-32 and Csnk1δ/ε and (d) pDarpp-32(T34) in the NAcc of rats in the four treatment groups. (b) pDarpp-32(S137)/Darpp-32 ratios (* $p < 0.05$ vs. Saline; †† $p < 0.01$ vs. AMPH; Scheffé), (c) Csnk1δ/ε protein levels, (e)

pDarpp-32(T34)/Darpp-32 ratios (* $p < 0.05$ vs. Saline; † $p < 0.05$ vs. AMPH; Scheffé), and (f) Darpp-32 protein levels obtained in the NAcc for the four treatment groups ($n/\text{group} = 6$). Data are shown as mean \pm SEM.

significant effect of infection, $F_{2,24} = 15.53$, $p < 0.001$, incubation, $F_{1,24} = 26.46$, $p < 0.001$, and a significant infection–incubation interaction, $F_{2,24} = 6.98$, $p < 0.01$. Relative to non-infected cells, Lenti-GluR1(S845A) also increased levels of GFP (Fig. 4a) but not total GluR1 protein levels (Fig. 4d). These findings, together with those showing increased GluR1 protein levels in HEK cells 48 h post-infection (Figure S1), suggest that post-transcriptional or post-translational homeostatic mechanisms reduced native GluR1 protein to maintain overall GluR1 protein levels constant in the medium spiny neurons 10–18 days post-infection. Electrophysiological recordings were then obtained in these three groups of cells before and after application of 0.5 mM glutamate. Before application of glutamate, spontaneous transient currents with amplitudes between 5 and 200 pA, with occasional larger amplitude responses, were variously present in all preparations but no statistically significant differences between groups were detected in number of transient spikes, spike amplitude distributions or frequencies. In response to glutamate, both Control and Lenti-GFP infected medium spiny neurons exhibited larger currents with group mean amplitudes approaching 1000 pA. Paralleling the lack of SKF81297-evoked GluR1 phosphorylation, glutamate-evoked currents

were significantly smaller in the Lenti-GluR1(S845A) infected cells indicating that phosphorylation of GluR1 at S845 is necessary for medium spiny neurons to respond to glutamate (Fig. 4f; $p < 0.01$, by *post hoc* Scheffé comparison following ANOVA showing a significant effect of infection, $F_{2,20} = 6.57$, $p < 0.01$).

To directly test whether phosphorylation of GluR1 at S845 is necessary for NAcc amphetamine-induced locomotion, rats were administered bilateral microinjections into the NAcc of Lenti-GluR1(S845A) or Lenti-GFP. Immunohistochemical examination of brain sections three weeks later showed strong GFP expression limited to cells in close proximity to the NAcc injection site in both conditions (Fig. 5d). Administration of amphetamine (2.5 $\mu\text{g}/\text{side}$) into the NAcc at this time increased pGluR1(S845) as expected in non-infected (Control) and Lenti-GFP-infected rats but not in rats infected with Lenti-GluR1(S845A) (Fig. 5b; $p < 0.001$, by *post hoc* Scheffé comparisons following ANOVA showing a significant effect of infection, $F_{2,28} = 18.13$, $p < 0.001$, challenge, $F_{1,28} = 62.49$, $p < 0.001$, and a significant infection–challenge interaction, $F_{2,28} = 5.88$, $p < 0.01$). As with the NAcc cultured neurons, relative to non-infected rats, Lenti-GluR1(S845A) also increased levels of GFP (Fig. 5a) but not total GluR1 protein levels (Fig. 5c), again suggesting

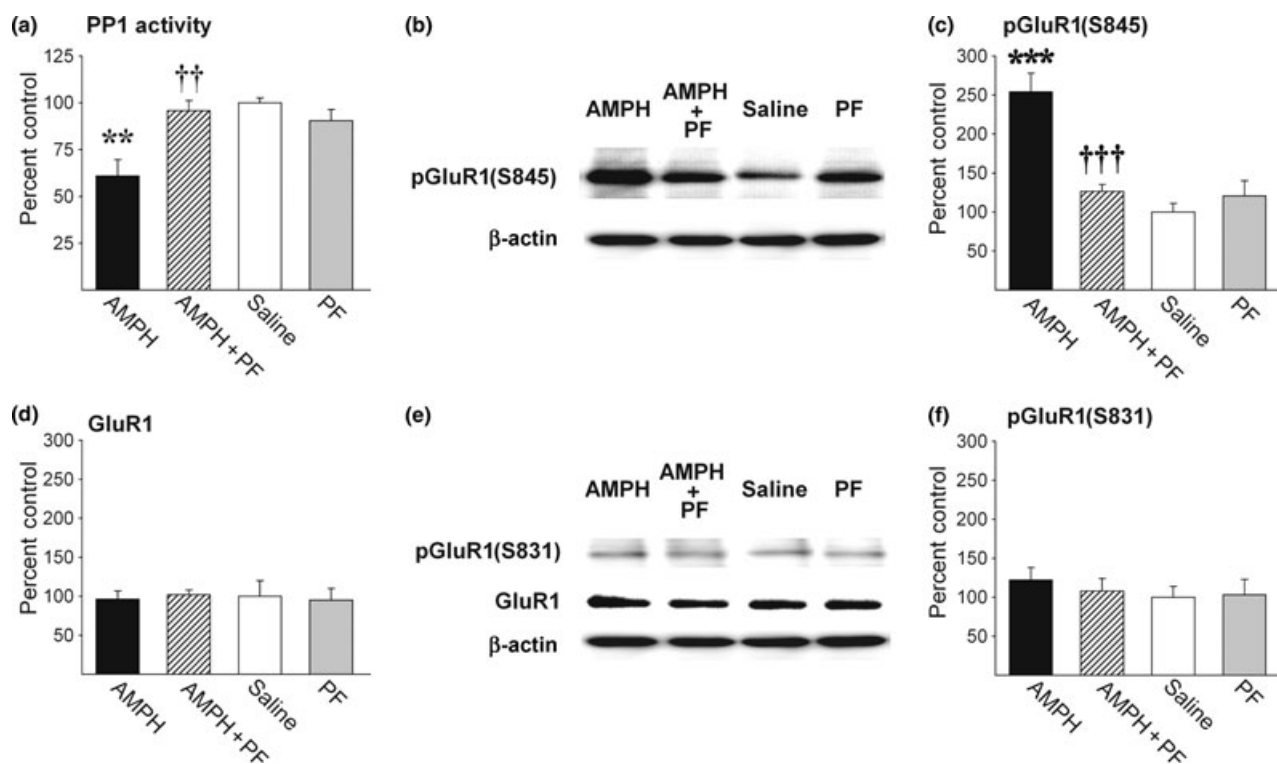
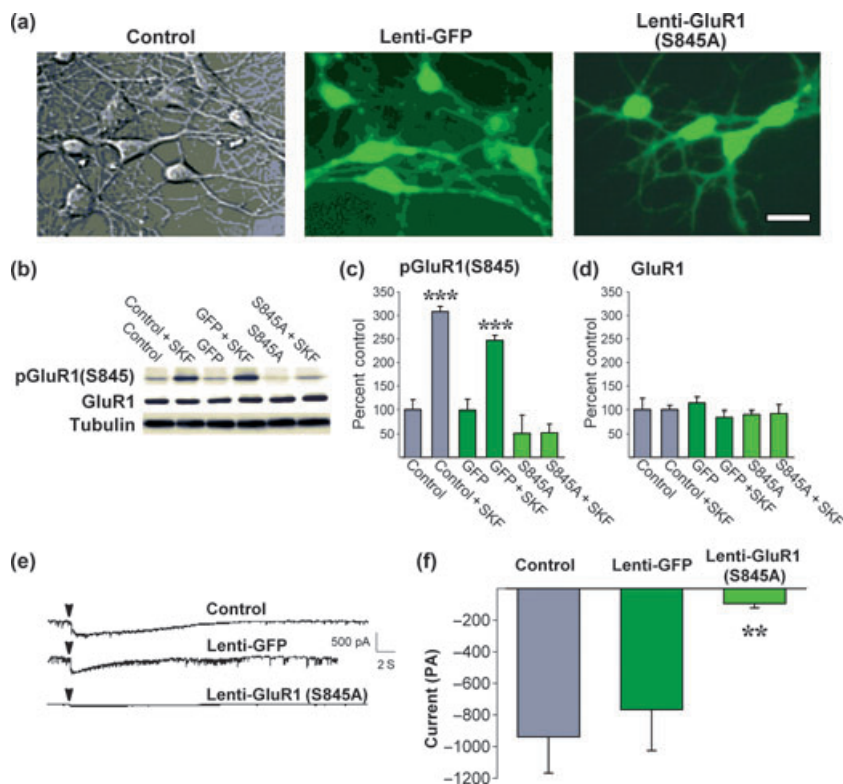


Fig. 3 PF blocks NAcc AMPH induced inhibition of PP1 activity and phosphorylation of GluR1 at S845. (a) PP1 activity (** $p < 0.01$ vs. Saline; †† $p < 0.01$, vs. AMPH; Scheffé) and (c) pGluR1(S845) (** $p < 0.001$ vs. Saline; ††† $p < 0.001$ vs. AMPH; Scheffé), (d) GluR1 and (f)

pGluR1(S831) protein levels in the NAcc of rats in the four treatment groups ($n/\text{group} = 6$). Data are shown as mean \pm SEM. Representative immunoblots of (b) pGluR1(S845) and (e) pGluR1(S831) and GluR1 in the NAcc of rats in the four treatment groups.

Fig. 4 Viral-mediated expression of GluR1(S845A) in cultured medium spiny neurons blocks SKF81297-evoked phosphorylation of GluR1 and glutamate-evoked currents. (a) Photomicrographs of neurons that were (left) not infected, (middle) infected with Lenti-GFP or (right) infected with Lenti-GluR1(S845A). Scale bar = 20 μm . (b) Representative immunoblots of pGluR1(S845) and GluR1, (c) pGluR1(S845) (** $p < 0.001$ vs. remaining groups; Scheffé), and (d) GluR1 protein levels in the three groups of neurons either incubated or not incubated with SKF81297. $n/\text{group} = 4-7$. (e) Representative current traces obtained in the three groups of neurons before and after focal application of glutamate (arrowheads). (f) Mean peak currents obtained following glutamate in the three groups of neurons (** $p < 0.01$ vs. Control non-infected cells; Scheffé). $n/\text{group} = 5-12$. Data are shown as mean \pm SEM.



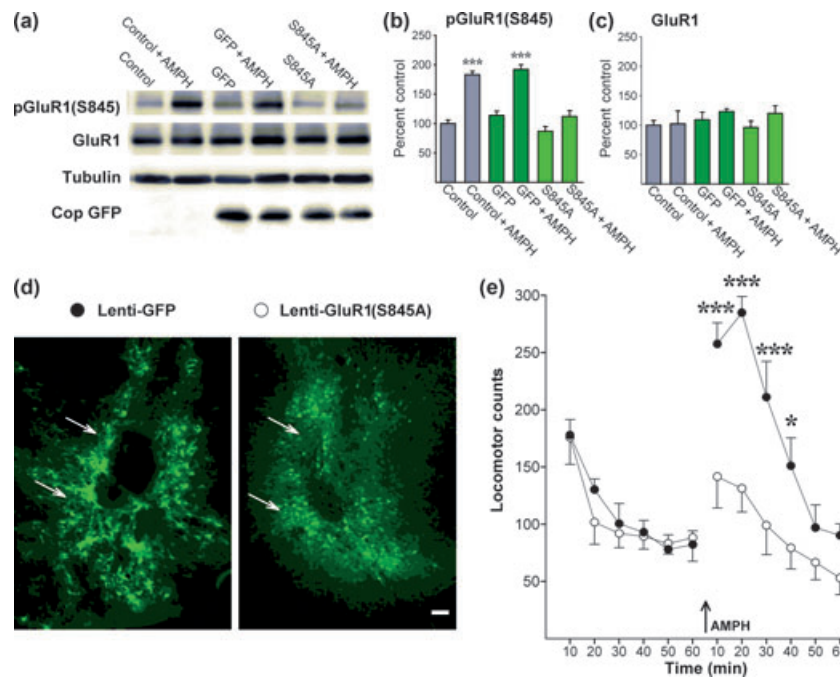


Fig. 5 Viral-mediated expression of GluR1(S845A) in the NAcc blocks AMPH-induced phosphorylation of GluR1 and AMPH-induced locomotion. (a) Representative immunoblots of GluR1(S845), GluR1 and Cop GFP, (b) pGluR1(S845) (** $p < 0.001$ vs. remaining groups; Scheffé) and (c) GluR1 protein levels in rats not infected (Control), infected with Lenti-GFP, or infected with Lenti-GluR1(S845A) either challenged or not challenged with NAcc AMPH. $n/\text{group} = 5\text{--}6$. (d)

Photomicrographs of the NAcc obtained 3 weeks following infection with Lenti-GFP or Lenti-GluR1(S845A) illustrating GFP-positive neurons in close proximity to the injection cannula tips (arrows). Scale bar = 50 μm . (e) Locomotor activity counts obtained before and after (arrow) NAcc AMPH in the two groups (* $p < 0.05$, *** $p < 0.001$ vs. Lenti-GFP; Scheffé). $n/\text{group} = 5\text{--}6$. Data are shown as mean \pm SEM.

homeostatic regulation of overall GluR1 protein levels to accommodate the Lenti-mediated increase in exogenous GluR1 3 weeks post-infection. Paralleling these findings, Lenti-GFP infected rats administered NAcc amphetamine (2.5 $\mu\text{g}/\text{side}$) showed the expected close to twofold increase in locomotion but this effect was absent in Lenti-GluR1(S845A) infected rats. These rats showed significantly lower locomotor counts compared with rats infected with Lenti-GFP (Fig. 5e; $P_s < 0.05\text{--}0.001$, by *post hoc* Scheffé comparisons following ANOVA showing a significant effect of infection, $F_{1,9} = 13.47$, $p < 0.01$, time, $F_{5,45} = 33.49$, $p < 0.001$, and a significant infection-time interaction, $F_{5,45} = 5.79$, $p < 0.001$). No significant differences were observed between the two groups before the NAcc amphetamine infusion.

Discussion

In the present study, we show that inhibiting Csnk1 in the NAcc blocks amphetamine-induced locomotion as well as amphetamine's ability in this site to increase phosphorylation of Darpp-32 at S137 and T34, decrease PP1 activity, and increase phosphorylation of GluR1 at S845. Preventing GluR1 phosphorylation with the alanine mutant GluR1(S845A) reduces glutamate-evoked currents in cultured

medium spiny neurons and mimics the effect of Csnk1 inhibition in the NAcc to block amphetamine-induced locomotion. These results indicate that Csnk1 is essential to amphetamine's ability to increase locomotion and that it enables this stimulant effect by acting on the Darpp-32-PP1 signaling pathway to regulate AMPA receptor phosphorylation in the NAcc.

Increased *Csnk1 δ/ϵ* gene expression in mouse forebrain has been associated with increased basal locomotor activity and increased sensitivity to the locomotor activating effects of psychostimulant drugs (Palmer *et al.* 2005; Zhou *et al.* 2010). Similarly in humans, subjects with more copies of the rs135745C allele of the *CSNK1 ϵ* gene are more sensitive to the subjective effects of amphetamine (Veenstra-VanderWeele *et al.* 2006). An association between a different SNP in *CSNK1 ϵ* (rs1534891) and heroin addiction has also been reported (Levrán *et al.* 2008). Our findings provide a mechanism by which Csnk1 can promote these effects. Dopamine and glutamate interact in the NAcc to mediate the locomotor and incentive motivational effects of psychostimulant drugs (Wise and Bozarth 1987; Meredith *et al.* 1993; Pierce and Kalivas 1997; Sesack *et al.* 2003) and Darpp-32 integrates the intracellular signaling initiated by these neurotransmitters (Greengard 2001; Fernandez *et al.* 2006). Darpp-32 knockout (Fienberg *et al.* 1998; Zachariou *et al.* 2002) and alanine

Darpp-32(T34A) mutant (Zachariou *et al.* 2006) mice show reduced sensitivity to the locomotor activating and rewarding effects of cocaine. By phosphorylating Darpp-32 at S137, Csnk1 can regulate these pathways and the behaviors they mediate. The activation of Csnk1 and the subsequent increase in pDarpp-32(S137) by amphetamine in the NAcc is in fact critical as inhibition of Csnk1 in this site blocks amphetamine's ability to increase pDarpp-32(S137), maintain increased pDarpp-32(T34), and increase locomotion (Figs 1 and 2). As shown for metabotropic glutamate receptors (Liu *et al.* 2001, 2002), amphetamine may activate Csnk1 δ/ϵ via calcium-dependent stimulation of calcineurin and dephosphorylation of Csnk1 from its inhibited autophosphorylated state, but unlike metabotropic glutamate receptors, recruit calcium through a pathway initiated by activation of dopamine receptors leading to phosphorylation of L-type calcium channels by PKA (Surmeier *et al.* 1995). Our findings show that once initiated, the phosphorylation of Darpp-32 by Csnk1 supersedes up- and downstream actions of PKA in the NAcc. pDarpp-32(S137) is necessary to maintain amphetamine-induced phosphorylation of Darpp-32(T34) and GluR1(S845), both PKA residues (Roche *et al.* 1996; Banke *et al.* 2000; Esteban *et al.* 2003).

The phosphorylation of GluR1 at S845 promotes trafficking and synaptic insertion of GluR1-containing AMPA receptors (Ehlers 2000; Esteban *et al.* 2003; Mangiavacchi and Wolf 2004; Sun *et al.* 2005; Oh *et al.* 2006) and increases peak current and channel open probability (Roche *et al.* 1996; Banke *et al.* 2000) thereby potentiating neuronal excitability. We show that phosphorylation of this residue is indeed necessary for NAcc medium spiny neurons to respond to glutamate and for NAcc amphetamine to produce increased locomotion (Figs 4 and 5). Thus, by regulating Darpp-32 and PP1 activity, Csnk1 regulates the phosphorylation state of GluR1(S845) and amphetamine-induced locomotor activity. These findings are consistent with a number of reports showing that AMPA receptor blockade in the NAcc blocks the acute locomotor stimulant effects of amphetamine and cocaine (Wolf 1998) and show that conditions that increase medium spiny neuron activity also increase NAcc amphetamine-induced locomotion. In apparent contrast to these results, expression in the NAcc core of pore-dead GluR1, a Q582E GluR1 mutant designed to reduce synaptic AMPA currents, has been reported to decrease the locomotion induced by NAcc AMPA but increase the locomotion induced by cocaine (Bachtell *et al.* 2008). The finding of reduced NAcc AMPA-induced locomotion is consistent with the expected reduction in AMPA currents, although the subcellular distribution of these mutant subunits was not examined in this report and they are not targeted to synapses under all conditions (Shi *et al.* 2001). In addition, cocaine was administered systemically in these experiments (Bachtell *et al.* 2008), raising the possibility that AMPA receptors in other brain regions contributed to the

observed increase in cocaine-induced locomotion. Our findings show that glutamate-evoked currents are blocked in Lenti-GluR1(S845A) infected medium spiny neurons and that amphetamine is unable to increase locomotion when infused specifically into the site of expression of GluR1(S845A) in the NAcc. In the dorsal striatum, it has also been reported that Csnk1 does not regulate AMPA receptor activity but inhibits other ionotropic receptor currents by directly activating PP1 in this site (Chergui *et al.* 2005). No evidence was obtained for activation of NAcc PP1 by Csnk1 in the present study. Rather, inhibition of Csnk1 produced no effects on PP1 activity by itself and prevented the decrease in PP1 activity and subsequent increase in pGluR1(S845) produced by amphetamine in the NAcc (Fig. 3). These results indicate either that Csnk1 does not directly activate PP1 in the NAcc or that this action is overwhelmed by Csnk1 regulated Darpp-32 inhibition. These findings may reflect differences in Csnk1-associated signaling in dorsal and ventral striatum. For example, D₁ dopamine receptor and Darpp-32 dependent activation of ERK1/2 by amphetamine is considerably more pronounced in the NAcc than the dorsal striatum (Gerfen *et al.* 2008). Our findings showing that Csnk1 regulates the Darpp-32-PP1 signaling pathway to promote AMPA receptor phosphorylation in the NAcc are consistent with a similar striatal organization.

The behaviors produced by psychostimulant drugs are complex. As such, their expression can be modulated by a number of signaling pathways. Csnk1 interacts with some of these pathways in addition to those involving Darpp-32 and PP1 and these interactions may also potentially influence psychostimulant-induced behaviors. For example, Csnk1 δ/ϵ phosphorylates PERIOD proteins to influence the circadian rhythm (Knippschild *et al.* 2005; Badura *et al.* 2007; Walton *et al.* 2009) which is known to influence responding to psychostimulant drugs (Falcón and McClung 2009). Csnk1 also regulates amphetamine-induced changes in glycogen synthase kinase 3 β (GSK-3 β) phosphorylation (Svenningsson *et al.* 2003) which contribute to the locomotion produced by this drug (Beaulieu *et al.* 2004). Consistent with a modulating role, effects in these pathways are observed either in other sites but not the NAcc, as in methamphetamine-induced increases in PERIOD mRNA (Nikaido *et al.* 2001), or at time points following initiation of locomotor responding to the drug, as with PERIOD mRNA levels (Nikaido *et al.* 2001), amphetamine-induced decreases in pGSK-3 β , and the decreased locomotor response to amphetamine exhibited by GSK-3 β +/- mice (Beaulieu *et al.* 2004). In the present study, we show that inhibition of Csnk1 blocks the initiation of NAcc amphetamine-induced locomotion as well as those changes in Darpp-32-PP1-GluR1(S845) signaling that are necessary for its expression. These findings point to a pivotal role for NAcc Csnk1 in the generation of psychostimulant-induced behaviors. Considering that medium spiny neurons in the NAcc also integrate the contribution of dopamine and glutamate to the

incentive motivational effects of a number of abused drugs (Wise and Bozarth 1987; Meredith *et al.* 1993; Pierce and Kalivas 1997; Sesack *et al.* 2003), Csnk1 is likely also a critical target for intervention in the treatment of drug craving and addiction.

Acknowledgements

This study was supported by grants R01-DA-09397 (PV) and F31-DA-022834 (JAL) from the NIH. The authors declare no conflicts of interest. The authors are also grateful to Dr Sangram S. Sisodia for facilitating the PPI assays.

Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1. HEK cells were infected either with Lenti GFP, Lenti GluR1, or Lenti GluR1(S845A).

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

References

- Anderson S. M., Famous K. R., Sadri-Vakili G., Kumaresan V., Schmidt H. D., Bass C. E., Terwilliger E. F., Cha J.-H. J. and Pierce R. C. (2008) CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nature Neurosci.* **11**, 344–353.
- Bachtell R. K., Choi K.-H., Simmons D. L., Falcon E., Monteggia L. M., Neve R. L. and Self D. W. (2008) Role of GluR1 expression in the nucleus accumbens neurons in cocaine sensitization and cocaine-seeking behavior. *Eur. J. Neurosci.* **27**, 2229–2240.
- Badura L., Swanson T., Adamowicz W. *et al.* (2007) An inhibitor of casein kinase I epsilon induces phase delays in circadian rhythms under free-running and entrained conditions. *J. Pharmacol. Exp. Ther.* **322**, 730–738.
- Banke T. G., Bowie D., Lee H.-K., Haganir R. L., Schousboe A. and Traynelis S. F. (2000) Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J. Neurosci.* **20**, 89–102.
- Beaulieu J.-M., Sotnikova T. D., Yao W.-D., Kockeritz L., Woodgett J. R., Gainetdinov R. R. and Caron M. G. (2004) Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl Acad. Sci. USA* **101**, 5099–5104.
- Bryant C. D., Graham M. E., Distler M. G., Munoz M. B., Li D., Vezina P., Sokoloff G. and Palmer A. A. (2009) A role for casein kinase 1 epsilon in the locomotor stimulant response to methamphetamine. *Psychopharmacology* **203**, 703–711.
- Chergui K., Svenningsson P. and Greengard P. (2005) Physiological role for casein kinase I in glutamatergic synaptic transmission. *J. Neurosci.* **25**, 6601–6609.
- Desdouts F., Cohen D., Nairn A. C., Greengard P. and Girault J.-A. (1995a) Phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, by casein kinase I in vitro and in vivo. *J. Biol. Chem.* **270**, 8772–8778.
- Desdouts F., Siciliano J. C., Greengard P. and Girault J.-A. (1995b) Dopamine- and cAMP-regulated phosphoprotein DARPP-32: Phosphorylation of Ser-137 by casein kinase I inhibits dephosphorylation of Thr-34 by calcineurin. *Proc. Natl Acad. Sci. USA* **92**, 2682–2685.
- Ehlers M. D. (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* **28**, 511–525.
- Esteban J. A., Shi S.-H., Wilson C., Nuriya M., Haganir R. L. and Malinow R. (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nature Neurosci.* **6**, 136–143.
- Falcón E. and McClung C. A. (2009) A role for circadian genes in drug addiction. *Neuropharmacology* **56**, 91–96.
- Fernandez E., Schiappa R., Girault J.-A. and Le Novère N. (2006) DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLoS Comp. Biol.* **2**, 1619–1633.
- Fienberg A. A., Hiroi N., Mermelstein P. G. *et al.* (1998) Darpp-32: regulator of the efficacy of dopaminergic neurotransmission. *Science* **281**, 838–842.
- Gerfen C. R., Paletzki R. and Worley P. (2008) Differences between dorsal and ventral striatum in Drd1a dopamine receptor coupling of dopamine- and cAMP-regulated phosphoprotein-32 to activation of extracellular signal-regulated kinase. *J. Neurosci.* **28**, 7113–7120.
- Greengard P. (2001) The neurobiology of slow synaptic transmission. *Science* **294**, 1024–1030.
- Hemmings H. C. Jr, Greengard P., Tung H. Y. L. and Cohen P. (1984) DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* **310**, 503–505.
- Kamens H. M., Burkhart-Kasch S., McKinnon C. S., Li N., Reed C. and Phillips T. J. (2005) Sensitivity to psychostimulants in mice bred for high and low stimulation to methamphetamine. *Genes Brain Behav.* **4**, 110–125.
- Knippschild U., Gocht A., Wolff S., Huber N., Löhler J. and Stöter M. (2005) The casein kinase 1 family: participation in multiple cellular processes in eukaryotes. *Cell. Signal.* **17**, 675–689.
- Levrin O., Londono D., O'Hara K. *et al.* (2008) Genetic susceptibility to heroin addiction: a candidate gene association study. *Genes Brain Behav.* **7**, 720–729.
- Liu F., Ma X.-H., Ule J., Bibb J. A., Nishi A., DeMaggio A. J., Yan Z., Nairn A. C. and Greengard P. (2001) Regulation of cyclin-dependent kinase 5 and casein kinase 1 by metabotropic glutamate receptors. *Proc. Natl Acad. Sci. USA* **98**, 11062–11068.
- Liu F., Virshup D. M., Nairn A. C. and Greengard P. (2002) Mechanism of regulation of casein kinase 1 activity by group 1 metabotropic glutamate receptors. *J. Biol. Chem.* **277**, 45393–45399.
- Löhler J., Hirner H., Schmidt B., Kramer K., Fischer D., Thal D. R., Leithäuser F. and Knippschild U. (2009) Immunohistochemical characterization of cell-type specific expression of CK1δ in various tissues of young adult BALB/c mice. *PLoS ONE* **4**, 1–12.
- Loweth J. A., Singer B. F., Baker L. K. *et al.* (2010) Transient overexpression of α -Ca²⁺/calmodulin-dependent protein kinase II in the nucleus accumbens shell enhances behavioral responding to amphetamine. *J. Neurosci.* **30**, 939–949.
- Mangiavacchi S. and Wolf M. E. (2004) D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. *J. Neurochem.* **88**, 1261–1271.
- Meredith G. E., Pennartz C. M. and Groenewegen H. J. (1993) The cellular framework for chemical signaling in the nucleus accumbens. *Prog. Brain Res.* **99**, 3–24.

- Nelson C. L., Milovanovic M., Wetter J. B., Ford K. A. and Wolf M. E. (2009) Behavioral sensitization to amphetamine is not accompanied by changes in glutamate receptor surface expression in the rat nucleus accumbens. *J. Neurochem.* **109**, 35–51.
- Nikaido T., Akiyama M., Moriya T. and Shibata S. (2001) Sensitized increase of Period gene expression in the mouse caudate/putamen caused by repeated injection of methamphetamine. *Mol. Pharmacol.* **59**, 894–900.
- Oh M. C., Derkack V. A., Guire E. S. and Soderling T. R. (2006) Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. *J. Biol. Chem.* **281**, 752–758.
- Palmer A. A., Verbitsky M., Suresh R. *et al.* (2005) Gene expression differences in mice divergently selected for methamphetamine sensitivity. *Mamm. Genome* **16**, 291–305.
- Paxinos G. and Watson C. (1997) *The Rat Brain in Stereotaxic Coordinates*, 3rd edn. Academic Press, New York.
- Pellegrino L. L., Pellegrino A. S. and Cushman A. J. (1979) *A Stereotaxic Atlas of the Rat Brain*. Plenum Press, New York.
- Pierce R. C. and Kalivas P. W. (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res. Rev.* **25**, 192–216.
- Roche K. W., O'Brien R. J., Mammen A. L., Bernhardt J. and Haganir R. L. (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* **16**, 1179–1188.
- Sesack S. R., Carr D. B., Omelchenko N. and Pinto A. (2003) Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. *Ann. N Y Acad. Sci.* **1003**, 36–52.
- Shi S.-H., Hayashi Y., Esteban J. A. and Malinow R. (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **105**, 331–343.
- Snyder G. L., Allen P. B., Fienberg A. A., Valle C. G., Haganir R. L., Nairn A. C. and Greengard P. (2000) Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *J. Neurosci.* **20**, 4480–4488.
- Sun X., Zhao Y. and Wolf M. E. (2005) Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *J. Neurosci.* **25**, 7342–7351.
- Surmeier D. J., Bargas J., Hemmings H. C. Jr, Nairn A. C. and Greengard P. (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* **14**, 385–397.
- Svenningsson P., Tzavara E. T., Carruthers R. *et al.* (2003) Diverse psychotomimetics act through a common signaling pathway. *Science* **302**, 1412–1415.
- Utz A. C., Hirner H., Blatz A. *et al.* (2010) Analysis of cell type-specific expression of CK1 ϵ in various tissues of young adult BALB/c mice and in mammary tumors of SV40 T-Ag-transgenic mice. *J. Histochem. Cytochem.* **58**, 1–15.
- Veenstra-VanderWeele J., Qaadir A., Palmer A. A., Cook E. H. Jr and de Wit H. (2006) Association between the casein kinase 1 epsilon gene region and subjective response to D-amphetamine. *Neuropsychopharmacology* **31**, 1056–1063.
- Walton K. M., Fisher K., Rubitski D. *et al.* (2009) Selective inhibition of casein kinase 1 ϵ minimally alters circadian clock period. *J. Pharmacol. Exp. Ther.* **330**, 430–439.
- Wise R. A. and Bozarth M. A. (1987) A psychomotor stimulant theory of addiction. *Psychol. Rev.* **94**, 469–492.
- Wolf M. E. (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog. Neurobiol.* **54**, 679–720.
- Zachariou V., Benoit-Marand M., Allen P. B., Ingrassia P., Fienberg A. A., Ganon F., Greengard P. and Picciotto M. R. (2002) Reduction of cocaine place preference in mice lacking the protein phosphatase 1 inhibitors Darpp-32 or Inhibitor 1. *Biol. Psychiatry* **51**, 612–620.
- Zachariou V., Sgambato-Faure V., Sasaki T., Svenningsson P., Berton O., Fienberg A. A., Nairn A. C., Greengard P. and Nestler E. J. (2006) Phosphorylation of Darpp-32 at Threonine-34 is required for cocaine action. *Neuropsychopharmacology* **31**, 555–562.
- Zhou M., Rebbholz H., Brocia C., Warner-Schmidt J. L., Fienberg A. A., Nairn A. C., Greengard P. and Flajolet M. (2010) Forebrain overexpression of CK1 δ leads to down-regulation of dopamine receptors and altered locomotor activity reminiscent of ADHD. *Proc. Natl Acad. Sci. USA* **107**, 4401–4406.