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A Substrate Trapping Mutant Form of the Tyrosine Phosphatase STEP Prevents Amphetamine-induced Stereotypies and Long-term Potentiation in the Striatum

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Abstract

Background—Chronic, intermittent exposure to psychostimulants results in striatal neuroadaptations leading to an increase in an array of behavioral responses on subsequent challenge days. STEP, a brain-specific Striatum Enriched tyrosine Phosphatase, regulates synaptic strengthening by dephosphorylating and inactivating several key synaptic proteins. This study tests the hypothesis that a substrate-trapping form of STEP will prevent the development of amphetamine-induced stereotypies.

Methods—A substrate-trapping STEP protein, TAT-STEP (C-S), was infused into the ventrolateral striatum on each of five consecutive exposure days and 1 hour prior to amphetamine injection. Animals were challenged to see whether sensitization to the stereotypy-producing effects of amphetamine developed. The same TAT-STEP (C-S) protein was used on acute striatal slices to determine the impact on long-term potentiation and depression.

Results—Infusion of TAT-STEP (C-S) blocks the increase of amphetamine-induced stereotypies when given during the 5-day period of sensitization. TAT-STEP (C-S) has no effect if only infused on the challenge day. Treatment of acute striatal slices with TAT-STEP (C-S) blocks the induction of LTP and potentiates LTD.

Conclusions—A substrate trapping form of STEP blocks the induction of amphetamine-induced neuroplasticity within the ventrolateral striatum, and supports the hypothesis that STEP functions as a tonic break on synaptic strengthening.

Keywords

protein tyrosine phosphatase; STEP; amphetamine; ventral striatum; stereotypies; neuroplasticity

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INTRODUCTION

Repeated exposure to psychostimulants such as cocaine or amphetamine augment behavioral responses, a phenomenon termed sensitization. In rodents, behavioral sensitization can be characterized by progressive increases in stereotypies and locomotor activity following repeated intermittent exposure to psychostimulants (1,2). Behavioral sensitization is long lasting and can be observed after re-exposure to lower doses of the drug months later (3,4).

Addiction to psychostimulants is a neuroadaptive disorder characterized by dysregulation of the corticolimbic dopamine (DA) reward system that comprises the ventral tegmental area (VTA), amygdala, striatum, prefrontal and orbitofrontal cortex (5,6). Studies examining the molecular mechanisms underlying drug-induced behavioral sensitization indicate that psychostimulants have multifaceted effects on the corticolimbic brain regions and enhances the expression and/or activation of several proteins that require functional dopamine/D1 and NMDA receptors. Psychostimulant drugs such as cocaine, amphetamine, and morphine increase extracellular levels of dopamine, inducing immediate early gene expression that elicits stereotyped behavior (7–12). The NMDAR antagonist MK-801 blocks the development of sensitization to cocaine and amphetamine (13) and involves an enhancement of glutamatergic synaptic transmission in dopamine neurons (14–18).

The extracellular signal-regulated kinase 1/2 (ERK1/2) pathway also plays an important role in behavioral sensitization. Both acute and repeated cocaine administration activate ERK1/2 in corticolimbic areas, and inhibition of MEK, the upstream kinase that regulates ERK1/2 activity, prevents psychostimulant-induced locomotor sensitization (19–26). Acute amphetamine administration also activates ERK1/2 in the striatum, nucleus accumbens, ventral tegmental area and prefrontal cortex of mice and rats, increasing the expression of immediate early genes (8,10–12,27–29). These findings highlight the importance of both dopamine and glutamate/NMDAR-mediated synaptic transmission, subsequent activation of the ERK1/2 pathway, and the induction of immediate early genes in the development of stereotypies by psychostimulants.

Striatal-Enriched protein tyrosine Phosphatase (STEP) is a brain-specific tyrosine phosphatase that we hypothesized is involved in this process through the regulation of synaptic proteins required for the development of synaptic strengthening. Previous studies have shown that STEP limits the duration of ERK1/2 activation and downstream transcriptional responses following glutamate receptor stimulation (30,31). STEP also inhibits the activity of Fyn by dephosphorylating the regulatory tyr^{420} (32). Finally, STEP is involved in mediating glutamate receptor trafficking (33,34). STEP is present in the postsynaptic density (35) and associates with the NMDAR complex where it constitutively inhibits NMDA channels (36). Knock-down of STEP using RNAi increases NMDAR trafficking to neuronal membranes (37), whereas STEP activation increases NMDAR endocytosis by dephosphorylating NR2B at tyrosine (tyr^{1472}) (33). STEP also regulates mGluR-mediated AMPAR endocytosis by dephosphorylating GluR2 (38).

A recent study demonstrated that infusion of a substrate-trapping mutant of STEP into the lateral amygdala disrupted the consolidation of fear memories and long-term potentiation (LTP) (39). Taken together, these findings suggest that disrupting STEP pathways interfere with various proteins required for the development of synaptic strengthening.

Here we analyzed whether a substrate-trapping STEP-fusion protein could disrupt the development of amphetamine-induced stereotypies. Infusion of TAT-STEP (C-S) during chronic amphetamine exposure blocked the development of stereotypies on a subsequent amphetamine challenge one week later. Electrophysiological experiments showed that TAT-STEP (C-S) also disrupts the induction of LTP when added to corticostriatal slices, but induces

a low frequency stimulation (LFS)-dependent form of LTD. These findings support the hypothesis that STEP substrates play an important role in regulating the development of synaptic plasticity during amphetamine-induced stereotypies.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 250–350g, (Charles River Laboratories, Wilmington, MA) were group-housed with free access to food and water and a 12h light/12h dark cycle. Experiments were performed during the light phase of the cycle. Rats were handled for 3 days to habituate to the investigator. All procedures were in accordance to the NIH Guide for the Care and Use of Experimental Animals and approved by the Yale University Animal Care and Use Committee.

Surgery

Rats were anesthetized (Equithesin, 4.5 ml/kg i.p.) and placed in a stereotaxic frame. Bilateral 26-gauge guide cannulae were aimed at the ventrolateral striatum [(0.7 mm, anterior bregma, ± 4 mm lateral to midline, and 5.5 mm ventral from the skull surface (40)]. A control group was cannulated in cortex (0.7 mm anterior to bregma, ± 4 mm lateral to midline, and 0.5 mm ventral from the skull surface (40)). Twenty-two-gauge dummy cannulae were used to prevent obstruction. After surgery, rats were single-housed and allowed to recover for at least 6 days.

Drugs and STEP-fusion protein

D-amphetamine sulfate (Sigma-Aldrich) was dissolved in 0.9% saline. Rats were injected intraperitoneally (i.p.) with a single, daily dose (5 mg/kg) for five consecutive days. They were given 6 days of no injections before being challenged with a dose of 2 mg/kg (Fig 1).

TAT-STEP₄₆ was affinity purified as previously described (39); it has a point mutation in its catalytic domain (C300S) that renders it enzymatically inactive, and thus acts as a substrate-trapping mutant. A myelocytomatosis virus (myc) tag was added to the C-terminus. TAT-myc peptide was infused as a control (39). TAT-STEP (C-S) or TAT-myc peptide (4 μ M) was infused bilaterally using a 10 μ L Hamilton syringe at a constant rate of 0.25 μ L/min.

Behavioral procedure

Six days after surgery, rats were randomly assigned into two experimental groups. For the first experiment, 12 rats with cannulae in the VLS and 8 animals with cannulae in the cortex were infused with TAT-STEP (C-S) or TAT-myc bilaterally and left in their home cages. One hr later, rats were injected with D-amphetamine (5 mg/kg), transferred to a new cage for 1 hr before being transferred back to their home cage, and this procedure was repeatedly daily for 5 days (Figure 1A). Rats were challenged with a lower dose of D-amphetamine (2 mg/kg) 6 days later and transferred to a holding cage.

Next, we determined whether the animals previously infused with TAT-STEP (CS) could still be sensitized. The same rats tested in experiment 1 were injected again with D-amphetamine (5 mg/kg) for 5 days, but without infusions of TAT-STEP (C-S) or TAT-myc, and were again challenged 6 days later (Figure 1A).

Finally, we examined whether infusion of TAT-STEP (C-S) on the challenge day was effective in blocking stereotypies. Twelve rats were cannulated in VLS and injected with D-amphetamine (5 mg/kg) for 5 days, but without TAT-STEP (C-S) or TAT-myc infusions (Figure 1B). Six days later, animals were infused with TAT-STEP (C-S) or TAT-myc one hour before challenge with D-amphetamine (2 mg/kg).

Animals were scored using a slightly modified behavioral scale developed by Creese and Iversen (41,42), with additional counting for up and down head movements, while rearing (vertical movements) and crossovers (crossing the midline) counts were used as an indicator of locomotor activity. All sessions were videotaped, with the last 30 minutes being scored. The behavioral score index was as following: 1-asleep; 2-normal activity; 3-increased activity; 4-running with jerky movements; 5-slow repetitive exploration; 6-non-continuous stereotyped sniffing with fast repetitive exploration; 7-continuous stereotyped sniffing with rearing; 8-continuous focused stereotyped in one area (Figure 3a and 4a). Animals were given a behavioral score every 5 min during the 30 min of scoring. The behavioral score for “up and down head movements”, “crossovers” and “rearing” were counted during the whole period of the 30 min.

Behavioral data were analyzed with ANOVA. The first part of experiment 1 was analyzed with a two-way ANOVA with brain regions (VLS or cortex) and peptides infused [TAT-STEP (C-S) or TAT-myc] as factors. One-way ANOVA was used for the comparison of the second part of experiment 1 and for experiment 2. For the second part of experiment 1, previous treatment with TAT-STEP (C-S) or TAT-myc was used as a factor. Acute infusions of TAT-STEP (C-S) or TAT-myc before the challenge were the two variables on experiment 2. Post hoc Tukey test was used to determine the differences among factors, and differences were considered significant if $p < 0.05$.

Immunohistochemistry

Immunohistochemical analysis determined the length of time that the TAT-STEP (C-S)-fusion protein was detected after infusions and the extent of its diffusion. Rats were implanted with cannulae in the VLS, and infused with TAT-STEP (C-S). The rats were anesthetized at 30, 60 and 360 min following infusion and perfused intracardially with ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were processed for immunocytochemistry, and cryostat sections (10 μ m) were stained with anti-myc antibody to determine the diffusion pattern of TAT-STEP (C-S) as previously described (39). Tissue sections were incubated with Alexa Fluor 488 goat anti-mouse secondary antibody (1:250, Molecular Probes, Eugene, OR) for 1 hr at room temperature, before being cover-slipped for viewing using a fluorescent microscope (Zeiss Axiovert 2000). Cannula placements were analyzed using cresyl violet staining and cannula tips needed to be within 0.5 mm of the target site (39).

Electrophysiology

Adult male rats (150–250 g) were used in accordance with the European Communities Council Directive (86/609/EEC) and with approval of the Institutional Animal Care and Use Committee of the University of Rome Tor Vergata. Corticostriatal slices were prepared for electrophysiological recordings as described (43,44). Vibratome-cut coronal slices (200–300 μ m) were first incubated (1 hr) in the presence of TAT-STEP (C-S) (300 nM) or TAT-myc (300 nM), and then transferred to a recording chamber submerged in a continuously flowing artificial cerebrospinal fluid (ACSF, 33°C, 2–3 ml/min) gassed with 95% O₂/5% CO₂. The composition of the bathing solution was (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 2.4 CaCl₂, 11 Glucose, 25 NaHCO₃. In some experiments, TAT-STEP (C-S) or TAT-myc was applied during the electrophysiological recordings for 30–40 min after the induction of corticostriatal LTP. In other experiments, amphetamine (10 μ M; 10 min) (gift from Dr. NB Mercuri) was applied to striatal slices previously treated with TAT-myc or TAT-STEP (C-S).

Intracellular recording electrodes were filled with 2 M KCl (tip resistance of 30–60 M Ohms). Signals were recorded with the use of an Axoclamp 2A amplifier, displayed on a separate oscilloscope and stored and analyzed on a digital system (pClamp 8, Axon Instruments, Union

City, CA). For synaptic stimulation, bipolar electrodes were used. They were located in the white matter between the cortex and the striatum to activate corticostriatal fibers. EPSPs were evoked at 0.1 Hz in the presence of 10 μ M bicuculline (Sigma, Italy) to block GABAA-mediated transmission.

High-frequency stimulation (HFS; 3 trains, 3 s duration, 100 Hz frequency, 20 s interval) and low-frequency stimulation (LFS; 10 min 2Hz) of corticostriatal fibers were used to induce corticostriatal synaptic plasticity. LTP was induced in response to HFS following the removal of magnesium ions from the bathing solution, while long-term depression (LTD) was induced by HFS in the presence of physiological concentrations of magnesium (1.2 mM; 43–45). An alternative form of LTD by LFS was examined in magnesium-free solution (43,46).

Quantitative data on modifications of EPSPs are expressed as a percentage of the controls, the latter representing the mean of responses recorded during a stable period (5–10 min) before the repetitive (HFS or LFS) synaptic stimulation. Values represent means $\pm$ SEM. Only one cell per slice and less than five neurons per animal were recorded. Student's *t* test for unpaired and paired observations was used to compare two groups of means and ANOVA was used for multiple comparisons.

RESULTS

TAT-STEP infusion into the VLS reduces the induction of behavioral stereotypes

To investigate the role of STEP as a regulator of amphetamine-induced stereotypes, we generated a substrate-trapping mutant of TAT-STEP₄₆ in which an essential cysteine residue in the catalytic domain was converted to a serine (36) (Figure 2A). We first established that TAT-STEP (C-S) entered cells surrounding the site of injection. Staining with anti-myc antibody was detectable only in TAT-STEP (C-S) infused animals, and not in non-infused animals, and lasted up to 6 hours after the infusion (Figure 2B).

We next examined whether infusion of TAT-STEP (C-S) into the VLS would reduce the induction of stereotypes following repeated, intermittent amphetamine injection. TAT-STEP (C-S) or TAT-myc (control) was infused into the VLS 1 hour prior to the injection of amphetamine (5 mg/kg). This procedure was repeated for five consecutive days. Six days later, rats were challenged with a single dose of amphetamine (2 mg/kg) and stereotyped behaviors and locomotor activity were recorded. Post hoc analysis of the total behavioral scores demonstrated that rats infused with TAT-STEP (CS) showed a significant decrease in stereotypic behavior as compared with those receiving TAT-myc infusions (two-way ANOVA, brain region versus peptide effects, $F_{1,12} = 26.150$; $p < 0.0001$, post hoc Tukey test) (Figure 3A). In a control experiment, rats infused with TAT-STEP (C-S) into the cortex showed no decrease in stereotypic behavior compared with the TAT-myc control (Figure 3A).

We further examined up and down head movements as indirect measurements of stereotypic activity, as well as cross-overs and rearing behaviors as measures of locomotor activity. Animals infused with TAT-STEP (C-S) in the VLS showed a significant decrease in up and down head movements as compared to the TAT-myc-infused animals (two-way ANOVA, brain region vs. peptide effect, $F_{1,12} = 10.396$, $p < 0.005$) (Figure 3B). Crossovers and rearing were not influenced by infusion of TAT-STEP (C-S) or TAT-myc (Figure 3B). Histological examination of all cannulae placements are shown in Figure 3C and D, and no animals were removed from analyses due to misplacement of cannulae.

We next examined whether repeated infusions into the VLS might caused lasting damage. Rats with earlier TAT-STEP (C-S) infusions were reexposed to amphetamine (5 mg/kg) for 5 consecutive days. Six days after the last injection, animals were challenged with a single dose

of amphetamine (2 mg/kg). Animals previously infused with either TAT-STEP (C-S) or TAT-myc showed no significant differences in their behavioral scores, up and down head movements and rearing behavior (one-way ANOVA, $p = 0.73$, $p = 0.30$ and $p = 0.93$, respectively; Figure 4A and B). Together, the findings indicate that the reduction in behavioral stereotypies following TAT-STEP (C-S) infusion into the VLS was not due to damage of the VLS.

TAT-STEP infusions after amphetamine treatment did not block induction of stereotypies

We next examined whether infusion of TAT-STEP (C-S) on the challenge day only could block the expression of stereotypies. Rats were injected with amphetamine (5 mg/kg) for five days and then received no injections for six days. On the challenge day, they were infused with TAT-STEP (C-S) or TAT-myc one hour prior to the amphetamine (2 mg/kg) challenge. There was no significant difference in the behavioral scores, up and down head movements, rearing and crossover scores (one-way ANOVA, $p = 0.75$; $p = 0.18$; $p = 0.87$; $p = 0.60$, respectively) between the two groups of animals (Figure 5A and B). Histological examinations of cannulae placements are shown in Figure 5C. Infusions of TAT-STEP (C-S) into the VLS on the challenge day, unlike infusions during the five days of amphetamine treatments, did not block amphetamine-induced sensitization.

TAT-STEP blocks increases in EPSP amplitude after high frequency stimulation in corticostriatal slices

It is known that chronic exposure to stimulants alters synaptic transmission at corticostriatal synapses, raising the possibility that abnormal synaptic plasticity underlies some of the behavioral effects of psychostimulant addiction (44). Therefore, we next examined the effect of TAT-STEP (C-S) on electrophysiological responses in corticostriatal slices *in vitro*. We first established that TAT-STEP (C-S) and TAT-myc (control) were able to enter cells in slices and found that the TAT-peptides were detected within 5 minutes of addition to the aCSF (data not shown), as demonstrated in a previous study (39). HFS stimulation of the control slices pre-incubated with TAT-myc peptide produced a sustained increase in EPSP amplitude (long-term potentiation, LTP) for the entire period of the study ($p < 0.01$ at 5, 10, 20 and 30 min post-HFS) in the majority of the striatal neurons studied (9 out of 11). On the other hand, application of TAT-STEP (C-S) prior to HFS stimulation completely blocked the increase in EPSP amplitude as observed with the TAT-myc controls ($p < 0.01$ at 5, 10, 20 and 30 min post-HFS) in all the neurons tested (12 out of 12) (Figure 6A).

TAT-STEP (C-S) was also able to prevent long-term potentiation (LTP) of corticostriatal EPSP amplitude when HFS was delivered in slices bathed in the presence of amphetamine (10 μ M, 10 min application prior HFS). Accordingly, EPSP amplitude recorded 30 min post-HFS was $152 \pm 4\%$ of pre-HFS values ($p < 0.01$) in slices treated with TAT-myc plus amphetamine, and $103 \pm 4\%$ in slices treated with TAT-STEP (C-S) plus amphetamine ($p > 0.05$) ($n = 4$ for both conditions). In 3 neurons, we applied TAT-STEP (C-S) 10 min after the HFS protocol, to see whether this treatment was able to interfere with the maintenance phase of corticostriatal LTP. Application of TAT-STEP (C-S) after HFS did not reverse corticostriatal LTP, as EPSP amplitude was about 150% of pre-HFS values throughout the entire period of the experiments ($p < 0.01$ at 20, 30 and 40 min post-HFS).

We also tested the effects of both TAT-myc ($n = 4$) and TAT-STEP (C-S) ($n = 4$) on HFS-induced corticostriatal long-term depression (HFS-LTD), an alternative form of synaptic plasticity independent of dopamine D1 receptor stimulation (45). Neither treatment interfered with the induction of HFS-LTD. EPSP amplitude recorded 30 min after HFS was in fact $72 \pm 5\%$ of control in slices treated with TAT-myc ($p < 0.01$), and $68 \pm 4\%$ in slices incubated with TAT-STEP (C-S) ($p < 0.01$).

A low frequency stimulation (LFS)-dependent form of LTD has also been described at corticostriatal synapses (46). Furthermore, LFS is particularly effective in depressing striatal glutamate transmission when delivered after the induction of LTP (43,44). Moreover, striatal synapses become refractory to the depressant action of this stimulation in chronic psychostimulant-treated rats (44). Thus, we examined whether TAT-STEP (C-S) might attenuate psychostimulant effects by favoring LFS-dependent LTD. To test this possibility, we applied LFS to both TAT-STEP (C-S) and TAT-myc-bathed slices. In the control slices, TAT-myc-bathed neurons, LFS failed to induce a persistent depression of corticostriatal EPSPs (n=8 out of 8). In contrast, LFS induced a stable LTD in TAT-STEP (C-S)-exposed neurons (n=8 out of 8) (Student's t test: $p < 0.05$ at 5, 10, 20 and 30 min post-LFS) (Figure 6B). Resting membrane potential, input resistance, firing discharge, and current-voltage relationship were comparable in neurons from TAT-STEP (C-S) and TAT-myc-treated striatal slices, and remarkably similar to those described previously in control rats (47).

DISCUSSION

We have shown that infusing a substrate-trapping isoform of STEP into the VLS blocks the induction of amphetamine-induced stereotypies. Infusion of TAT-STEP (C-S) in the VLS on the challenge day alone or into cortical regions did not block stereotypies. Therefore, these effects are not likely the result of a non-specific disruption of TAT-STEP (C-S) on brain function.

In addition to the behavioral effects, TAT-STEP protein altered corticostriatal synaptic plasticity. Notably, HFS-LTP, but not HFS-LTD, is absent in mice lacking the D1 dopamine receptor, indicating a crucial role for D1 receptor-initiated intracellular signaling in this form of synaptic plasticity (45). Accordingly, HFS of corticostriatal fibers causes a dramatic and transient increase of endogenous dopamine content in slices (48), and the resulting stimulation of D1Rs leads to LTP induction (45). Corticostriatal LTP, however, is particularly unstable immediately after its induction, and can be erased experimentally with the LFS protocol. The stabilization of LTP requires the activation of D1Rs persisting *after* the HFS, explaining why non-physiological dopamine release in the striatum, as obtained following psychostimulant administration, is able to prevent corticostriatal depotentiation (44). In accordance with the idea that STEP is an important downstream effector of D1 receptor stimulation, here we found that TAT-STEP (C-S) mimicked the effects of D1 receptor ablation on corticostriatal synaptic plasticity, because it selectively prevented LTP and spared HFS-LTD. Contrary to the hippocampus (49,50), in the striatum dopamine/D1 receptors (45) and ERK1/2 signaling (51) are probably necessary in the early phase of striatal LTP, and this might explain why TAT-STEP (C-S) was able to block this form of synaptic plasticity since its induction phase.

Direct bath application of D1 receptor agonist or cocaine, as well as chronic psychostimulant or L-DOPA injections in rats (43,44) prevent LFS-induced depotentiation of corticostriatal LTP. Therefore, we also investigated whether TAT-STEP (C-S) could favor the emergence of an LFS-dependent form of LTD. We found that TAT-STEP (C-S) unmasked an LFS-dependent form of corticostriatal LTD, suggesting that STEP plays an important role in lowering the threshold of synaptic stimulation required for LTD induction. In control condition, in fact, 2 Hz LFS is not sufficient to induce a stable LTD, and a stronger protocol of HFS is required to induce this form of synaptic plasticity (43,45). Conversely, LFS is able to depress corticostriatal EPSPs when delivered after LTP induction, a process prevented by dopamine D1 receptor activation (43,45), and favored by TAT-STEP (C-S) (present study). TAT-STEP (C-S), however, was unable to alter *per se* (i.e., in the absence of a stimulation protocol) corticostriatal synaptic transmission, even when applied after the induction of LTP (present study).

The disruptive effects of TAT-STEP (C-S) on corticostriatal LTP and its favorable influence on LFS-induced depression of EPSPs are both important for the blockade of amphetamine-induced stereotypies, because the behavioral effects of chronic psychostimulant treatment relies, at least in part, on normal induction of corticostriatal LTP by HFS but reduced sensitivity of corticostriatal synapses to LFS-induced depotentiation (44). Further confirmation is provided by results showing that TAT-STEP (C-S) abolished LTP in slices treated with amphetamine, because both chronic intraperitoneal cocaine injections and bath application in slices favor LTP over LTD in the striatum (44).

Our electrophysiological findings are consistent with a previous study showing that bilateral infusion of the same substrate-trapping protein into the lateral amygdala disrupted long-term fear conditioning memories (39). The acquisition of fear conditioning was normal as determined by freezing behaviors at 1 hour after training; however, the consolidation of these memories was significantly disrupted when tested at 24 hours. As with the present study, the application of TAT-STEP (C-S) to acute amygdala slices blocked HFS induction of LTP.

There are several possible mechanisms that might explain these results. TAT-STEP (C-S) binds constitutively to ERK1/2, and blocks nuclear translocation and ERK1/2-mediated transcriptional activation (30). Indeed, long-lasting behavioral alterations induced by amphetamine involves activation of ERK1/2 through stimulation of dopamine/D1Rs in the dorsal striatum (10,20,21). Dopamine/D1R stimulation also leads to inactivation of endogenous STEP through the activation of PKA and phosphorylation of a regulatory serine residue within the KIM domain of STEP. Phosphorylation of that site prevents STEP from binding to ERK1/2 and prolongs ERK1/2 activity (30,52).

NMDAR stimulation is also required for amphetamine-induced activation of ERK1/2 (10). Previous studies have demonstrated that STEP attenuates NMDAR activity by dephosphorylation of a regulatory tyrosine (tyr¹⁴⁷²) on the NR2B subunit of the NMDAR (33). In this study, beta amyloid led to the activation of STEP, which in turn dephosphorylated tyr¹⁴⁷² on the NR2B subunit and promoted internalization of the NMDAR complex (33). Therefore, it is possible that besides regulation of the ERK1/2 pathway, TAT-STEP (C-S) also disrupts NMDAR trafficking. Finally, STEP dephosphorylates and inactivates the tyrosine kinase Fyn (32). Because Fyn phosphorylates tyr¹⁴⁷² on the NR2B subunit that promotes exocytosis of the NMDAR complex, TAT-STEP (C-S) can act on two pathways to modulate NMDAR trafficking. Thus, it is likely that TAT-STEP (C-S) blocks amphetamine-induced stereotypies by regulating ERK1/2, NMDAR, Fyn, or another unidentified substrate of STEP. We have demonstrated that a substrate-trapping mutant of STEP blocks the development of amphetamine-induced stereotypies in the ventrolateral striatum. These results are in agreement with a previous study in which the same TAT-STEP protein blocked the consolidation of fear memories (39). STEP is present in other brain regions implicated in experience-induced plasticity, including the striatum, hippocampus, cortex and amygdala, and may participate in other forms of learning that occur within these regions. The results of these studies are consistent with the hypothesis that STEP normally opposes the development of synaptic plasticity through its ability to induce glutamate receptor internalization and inactivate key signaling proteins present at synapses. However, a limitation of the paper is that the use of a substrate-trapping mutant does not definitively clarify the role of endogenous STEP. Future investigations are required in the STEP KO mouse to address whether the absence of STEP potentiates synaptic strengthening. If so, then decreasing STEP activity with specific STEP inhibitors may prove therapeutic by promoting cognitive function.

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References

1. Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev* 1991;16:223–244. [PubMed: 1665095]
2. Robinson TE, Becker JB. Enduring changes in the brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res Rev* 1996;11:157–198.
3. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 1993;18:247–291. [PubMed: 8401595]
4. Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacol (Berl)* 2000;151:99–120.
5. Nestler EJ, Berhow MT, Brodtkin ES. Molecular mechanisms of drug addiction: adaptations in signal transduction pathways. *Mol Psychiatry* 1996;1:190–199. [PubMed: 9118343]
6. Camí J, Farré M. Drug addiction. *N Engl J Med* 2003;349:975–986. [PubMed: 12954747]
7. Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. *J Neurosci* 2002;22:3312–3320. [PubMed: 11978805]
8. Choe ES, Chung KT, Mao L, Wang JQ. Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors. *Neuropsychopharmacology* 2002;27:565–575.
9. Rothman RB, Baumann MH. Monoamine transporters and psychostimulant drugs. *Eur J Pharmacol* 2003;479:23–40. [PubMed: 14612135]
10. Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol J-C, et al. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc Natl Acad Sci U S A* 2005;102:491–496. [PubMed: 15608059]
11. Shi X, McGinty. Extracellular signal-regulated mitogen-activated protein kinase inhibitors decrease amphetamine-induced behavior and neuropeptide gene expression in the striatum. *Neuroscience* 2006;138:1289–1296. [PubMed: 16459022]
12. Shi X, McGinty JF. Repeated amphetamine treatment increases phosphorylation of extracellular signal-regulated kinase, protein kinase B, and cyclase response element-binding protein in the rat striatum. *J Neurochem* 2007;103:706–713. [PubMed: 17635666]
13. Karler R, Calder LD, Chaudhry IA, Turkanis SA. Blockade of “reverse tolerance” to cocaine and amphetamine by MK-801. *Life Sci* 1989;45:599–606. [PubMed: 2671566]
14. Stewart J, Druhan JP. Development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the non-competitive NMDA receptor antagonist, MK-801. *Psychopharmacology* 1993;110:125–132. [PubMed: 7870871]
15. Wolf ME, Jeziorski M. Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. *Brain Res* 1993;613:291–294. [PubMed: 8186979]
16. Giorgetti M, Hotsenpiller G, Ward P, Teppen T, Wolf ME. Amphetamine-induced plasticity of AMPA receptors in the ventral tegmental area: effects on extracellular levels of dopamine and glutamate in freely moving rats. *J Neurosci* 2001;21:6362–6369. [PubMed: 11487659]
17. White FJ, Hu XT, Zhang XF, Wolf ME. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J Pharmacol Exp Ther* 1995;273:445–454. [PubMed: 7714800]
18. Zhang XF, Hu XT, White FJ, Wolf ME. Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and

- selectively involves AMPA receptors. *J Pharmacol Exp Ther* 1997;281:699–706. [PubMed: 9152375]
19. Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J Neurosci* 1996;16:4707–4715. [PubMed: 8764658]
 20. Corbille AG, Valjent E, Marsicano G, Ledent C, Lutz B, Herve D, et al. Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. *J Neurosci* 2007;27:6937–6947. [PubMed: 17596442]
 21. Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *Eur J Neurosci* 2004;19:1826–1836. [PubMed: 15078556]
 22. Valjent E, Corbillé AG, Bertran-Gonzalez J, Hervé D, Girault JA. Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proc Natl Acad Sci U S A* 2006;103:2932–2937. [PubMed: 16473939]
 23. Yoon HS, Kim S, Park HK, Kim JH. Microinjection of CART peptide 55–102 into the nucleus accumbens blocks both the expression of behavioral sensitization and ERK phosphorylation by cocaine. *Neuropharmacol* 2007;53:344–351.
 24. Zhang J, Xu M. Opposite regulation of cocaine-induced intracellular signaling and gene expression by dopamine D1 and D3 receptors. *Ann N Y Acad Sci* 2006;1074:1–12. [PubMed: 17105899]
 25. Radwanska K, Caboche J, Kaczmarek L. Extracellular signal-regulated kinases (ERKs) modulate cocaine-induced gene expression in the mouse amygdala. *Eur J Neurosci* 2005;22:939–948. [PubMed: 16115217]
 26. Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, et al. Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J Neurosci* 2004;24:3344–3354. [PubMed: 15056714]
 27. Rajadhyaksha A, Husson I, Satpute SS, Kuppenbender KD, Ren JQ, Guerriero RM, et al. L-type Ca^{2+} channels mediate adaptation of extracellular signal-regulated kinase 1/2 phosphorylation in the ventral tegmental area after chronic amphetamine treatment. *J Neurosci* 2004;24:7464–7476. [PubMed: 15329393]
 28. Pascoli V, Valjent E, Corbille AG, Corvol JC, Tassin JP, Girault JA, et al. cAMP and extracellular signal-regulated kinase signaling in response to d-amphetamine and methylphenidate in the prefrontal cortex in vivo: role of beta 1-adrenoceptors. *Mol Pharmacol* 2005;68:421–429. [PubMed: 15890841]
 29. Choe ES, Wang JQ. CaMKII regulates amphetamine-induced ERK1/2 phosphorylation in striatal neurons. *Neuroreport* 2002;13:1013–1016. [PubMed: 12060798]
 30. Paul S, Nairn A, Wang P, Lombroso PJ. NMDA-mediated activation of the protein tyrosine phosphatase, STEP, regulates the duration of ERK signaling. *Nature Neurosci* 2003;6:34–42. [PubMed: 12483215]
 31. Muñoz JJ, Tárrega C, Blanco-Aparicio C, Pulido R. Differential interaction of the tyrosine phosphatases PTP-SL, STEP and HePTP with the mitogen-activated protein kinases ERK1/2 and p38alpha is determined by a kinase specificity sequence and influenced by reducing agents. *Biochem J* 2003;372:193–201. [PubMed: 12583813]
 32. Nguyen TH, Liu J, Lombroso PJ. Striatal enriched phosphatase 61 (STEP₆₁) dephosphorylates Fyn at phosphotyrosine 420. *J Biol Chem* 2002;277:24274–24279. [PubMed: 11983687]
 33. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al. Regulation of NMDA receptor trafficking by beta-amyloid. *Nature Neurosci* 2005;8:1051–1058. [PubMed: 16025111]
 34. Braithwaite S, Paul S, Nairn A, Lombroso PJ. Learning and Memory: One STEP at Time. *Trend Neurosci* 2006;29:452–458. [PubMed: 16806510]
 35. Oyama T, Goto S, Nishi T, Sato K, Yamada K, Yoshikawa M, Ushio Y. Immunocytochemical localization of the striatal enriched protein tyrosine phosphatase in the rat striatum: a light and electron microscopic study with a complementary DNA-generated polyclonal antibody. *Neuroscience* 1995;69:869–880. [PubMed: 8596655]
 36. Pelkey K, Askalan R, Paul S, Kalia LV, Nguyen T-H, Pitcher GM, et al. Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. *Neuron* 2002;34:127–138. [PubMed: 11931747]

37. Braithwaite SP, Adkisson M, Leung J, Nava A, Masterson B, Urfer R, Oksenberg D, Nikolic K. Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *Eur J Neurosci* 2006b;23:2847–2856. [PubMed: 16819973]
38. Zhang Y, Vankitaramani DV, Gladding CM, Zhang YF, Kurup P, Molnar E, Collingridge GL, Lombroso PJ. The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. *J Neurosci.* (in press)
39. Paul S, Olausson P, Venkitaramani D, Ruchkina I, Moran TD, Mills E, et al. The protein tyrosine phosphatase STEP gates long-term potentiation and fear memory in the lateral amygdala. *Biol Psych* 2007;61:1049–1061.
40. Swanson, LW. *Brain Maps: Structure of the Rat Brain*. Amsterdam: Elsevier Science Publishers; 1992.
41. Creese I, Iversen SD. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res* 1975;83:419–436. [PubMed: 234270]
42. Moore H, Grace AA. A role for electrotonic coupling in the striatum in the expression of dopamine receptor-mediated stereotypies. *Neuropsychopharmacology* 2002;27:980–992. [PubMed: 12464455]
43. Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, et al. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat Neurosci* 2003;6:501–506. [PubMed: 12665799]
44. Centonze D, Costa C, Rossi S, Prosperetti C, Pisani A, Usiello A, et al. Chronic Cocaine Prevents Depotentiation at Corticostriatal Synapses. *Biol Psych* 2006;60:436–443.
45. Centonze D, Grande C, Saulle E, Martin AB, Gubellini P, Pavon N, et al. Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. *J Neurosci* 2003;23:8506–8512. [PubMed: 13679419]
46. Roseni J, Lovinger DM. Induction of Striatal long-term synaptic depression by moderate frequency activation of cortical afferents in rats. *J Physiol* 2005;562:245–256. [PubMed: 15498813]
47. Kawaguchi Y, Wilson CJ, Emson PC. Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. *J Neurophysiol* 1989;62:1052–1068. [PubMed: 2585039]
48. Calabresi P, Fedele E, Pisani A, Fontana G, Mercuri NB, Bernardi G, et al. Transmitter release associated with long-term synaptic depression in rat corticostriatal slices. *Eur J Neurosci* 1995;7:1889–1894. [PubMed: 8528463]
49. Huang YY, Kandel ER. D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *PNAS* 1005;92:2446–2450. [PubMed: 7708662]
50. Kelleher RJ III, Govindarajan A, Tonegawa S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* 2004;44:59–73. [PubMed: 15450160] Calabresi P, Saulle E, Centonze D, Pisani A, Marfia GA, Bernardi G. Post-ischaemic long-term synaptic potentiation in the striatum: a putative mechanism for cell type-specific vulnerability. *Brain* 2002;125:844–60. [PubMed: 11912117]
51. Paul S, Hisayuki Y, Snider G, Picciotto M, Nairn A, Lombroso PJ. Dopamine/D1 receptor mediates the phosphorylation and inactivation of the protein tyrosine phosphatase, STEP, through a PKA-mediated pathway. *J Neurosci* 2000;20:5630–5638. [PubMed: 10908600]
52. Venkitaramani DV, Chin J, Netzer WJ, Gouras GK, Lesne S, Malinow R, et al. Beta amyloid modulation of synaptic transmission and plasticity. *J Neurosci* 2007;27:11832–11837. [PubMed: 17978019]

Figure 1. Schematic design of experiments

(A) Six days after bilateral cannula implantation into ventrolateral striatum (VLS) or cortex, rats were infused with TAT-STEP (C-S) or TAT-myc one hour before an injection of amphetamine (5 mg/kg), and this was repeated for 5 consecutive days. The first drug challenge took place after 6 days of no injections and consisted of a single injection of amphetamine (2 mg/kg) (Day 12). In the second part of this experiment, rats were again injected with d-amphetamine (5 mg/kg) for 5 days, but without any infusion of TAT-STEP (C-S) or TAT-myc. Six days later, animals received a challenge dose of amphetamine (2 mg/kg). (B) Six days after bilateral cannula implantation, animals were injected with amphetamine (5 mg/kg) for 5 consecutive days. On day 12, rats were injected with amphetamine (2 mg/kg) 1 hour after being infused with TAT-STEP (C-S) or TAT-myc.

Figure 2. TAT-STEP (C-S) infusions into VLS

(A) Schematic diagram of TAT-STEP (C-S) indicating the positions of the TAT-peptide, myc and six histidine (His) tags, the KIM domain, and the C-S mutation site within the phosphatase domain. (B) Immunocytochemical staining was performed with anti-myc antibody at different time points (30 min, 60 min and 6 hours).

Figure 3. TAT-STEP (C-S) infusion into VLS blocks the development of amphetamine-induced stereotypies

(A) Challenge day 1 (behavioral score): rats were infused with TAT-STEP (C-S) (n=6) or TAT-myc (control; n=6) in VLS 1 hr before intraperitoneal injection of d-amphetamine (5 mg/kg) for 5 days. After 6 days of no injections, rats received a challenge dose of d-amphetamine (2 mg/kg). Rats infused with TAT-STEP (C-S) showed significant differences in the behavioral score. Rats infused into the cortex showed no significant differences (B) Up and down head movements were significantly different as well. Crossovers and rearings were not different. (A) and (B) Mean $\pm$ S.E. *** $p < 0.0001$, ** $p < 0.005$ (C) Histological verification of cannula tip placements in rats infused with either TAT-STEP (C-S) (triangles) or TAT-myc into VLS (circles). (D) Cannula tip placement analyses in rats infused with either TAT-STEP (C-S) (triangles) or TAT-myc (circles) into cortex.

Figure 4. TAT-STEP infusion did not cause permanent damage in VLS

(A) Rats that had had a previous infusion with the indicated compound were injected with amphetamine (5 mg/kg) for 5 days, but without infusion of TAT-STEP (C-S) or TAT-myc. There were now no significant differences between the two groups with the challenge dose of amphetamine (2 mg/kg). (B) There were no significant differences in the other behavioral measures.

Figure 5. TAT-STEP infusion only on the challenge day does not block amphetamine-induced stereotypies

(A) Animals received 5 days of d-amphetamine injections (5 mg/kg). Infusions occurred only on the challenge day; TAT-STEP (C-S) (n=6) or TAT-myc (control; n=6). No significant differences were found in behavioral scores between groups. (B) No significant differences were found for up and down head movements, crossover, and rearing scores. (C) Histological verification of cannula placements in rats infused with either TAT-STEP (C-S) (triangles) or TAT-myc (circles).

Figure 6. Effects of TAT-STEP on corticostriatal plasticity

(A) Preincubation of striatal slices with TAT-STEP (C-S) (300 nM) blocked HFS-induced LTP in striatal spiny neurons. LTP induction was unaffected by TAT-myc (300 nM). Traces on the right are examples of EPSPs recorded before (upper) and 10 minutes after HFS (lower) from neurons treated with TAT-myc and TAT-STEP (C-S). (B) LFS protocol fails to induce long-lasting changes of corticostriatal transmission in TAT-myc-bathed slices, while it causes LTD in slices preincubated with TAT-STEP (C-S). Traces on the right are examples of EPSPs recorded before and 10 minutes after the end of LFS protocol.