Citation: Transl Psychiatry (2012) 2, e137, doi:10.1038/tp.2012.63

www.nature.com/tp

The tyrosine phosphatase STEP: implications in schizophrenia and the molecular mechanism underlying antipsychotic medications

NC Carty[1], J Xu[1], P Kurup[1], J Brouillette[1], SM Goebel-Goody[1], DR Austin[2], P Yuan[2], G Chen[2], PR Correa[3], V Haroutunian[4], C Pittenger[1,3,5] and PJ Lombroso[1,3,6]

Glutamatergic signaling through *N*-methyl-D-aspartate receptors (NMDARs) is required for synaptic plasticity. Disruptions in glutamatergic signaling are proposed to contribute to the behavioral and cognitive deficits observed in schizophrenia (SZ). One possible source of compromised glutamatergic function in SZ is decreased surface expression of GluN2B-containing NMDARs. $STEP_{61}$ is a brain-enriched protein tyrosine phosphatase that dephosphorylates a regulatory tyrosine on GluN2B, thereby promoting its internalization. Here, we report that $STEP_{61}$ levels are significantly higher in the postmortem anterior cingulate cortex and dorsolateral prefrontal cortex of SZ patients, as well as in mice treated with the psychotomimetics MK-801 and phencyclidine (PCP). Accumulation of $STEP_{61}$ after MK-801 treatment is due to a disruption in the ubiquitin proteasome system that normally degrades $STEP_{61}$. STEP knockout mice are less sensitive to both the locomotor and cognitive effects of acute and chronic administration of PCP, supporting the functional relevance of increased $STEP_{61}$ levels in SZ. In addition, chronic treatment of mice with both typical and atypical antipsychotic medications results in a protein kinase A-mediated phosphorylation and inactivation of $STEP_{61}$ and, consequently, increased surface expression of GluN1/GluN2B receptors. Taken together, our findings suggest that $STEP_{61}$ accumulation may contribute to the pathophysiology of SZ. Moreover, we show a mechanistic link between neuroleptic treatment, $STEP_{61}$ inactivation and increased surface expression of NMDARs, consistent with the glutamate hypothesis of SZ.
Translational Psychiatry (2012) **2,** e137; doi:10.1038/tp.2012.63; published online 10 July 2012

Introduction

Disrupted glutamatergic signaling, and specifically hypofunction of *N*-methyl-D-aspartate receptors (NMDARs), is proposed as a contributing factor in the etiology of schizophrenia (SZ).[1–3] Several neuropathological studies show abnormal glutamate receptor densities in the prefrontal cortex, thalamus and temporal lobe, as well as decreased receptor function in SZ brains.[4,5] Noncompetitive NMDAR antagonists such as phencyclidine (PCP) and ketamine elicit SZ-like symptoms in normal individuals and exacerbate psychotic episodes in SZ patients;[2,3,6,7] and administration of these psychotomimetics, or the more selective NMDAR antagonist MK-801, is used to model SZ in rodents and primates. These drugs produce cognitive deficits and behavioral phenotypes reminiscent of SZ symptoms, as well as induce biochemical modifications and disrupted glutamatergic transmission thought to be present in SZ brains.[2,8–18] Altogether, these findings link aberrant NMDAR function to behavioral deficits in SZ;[19–21] however, the mechanism(s) underlying NMDAR hypofunction remain unclear.

The brain-enriched protein tyrosine phosphatase STEP is a critical regulator of NMDAR function.[22] The *STEP* gene is alternatively spliced to produce several isoforms with domains that regulate their localization, substrate specificity and activity.[23–25] $STEP_{61}$ is associated with postsynaptic densities in the striatum, cortex, hippocampus and related regions.[26–28] Substrates include the NMDAR subunit GluN2B,[29,30] the AMPA receptor subunit GluA2,[31] Fyn,[32] Pyk2,[33,34] and the MAPK proteins ERK1/2 and p38.[35,36] $STEP_{61}$-mediated dephosphorylation of GluN2B and GluA2 promotes their internalization,[29,30] while dephosphorylation of the kinases inactivates them.[32,33,35] Thus, elevated $STEP_{61}$ reduces synaptic expression of GluN2B-containing NMDARs via two mechanisms: (1) dephosphorylation of GluN2B Tyr^{1472} (refs 29, 30, 37) and (2) dephosphorylation and inactivation of Fyn Tyr^{420} (ref. 32), a kinase that phosphorylates GluN2B at Tyr^{1472} (ref. 38). A model has therefore emerged whereby STEP opposes synaptic strengthening by negatively regulating kinase activity and NMDAR surface expression.[22]

Dopamine signaling also regulates $STEP_{61}$ activity and consequently NMDAR trafficking. D1 receptor stimulation

[1]Child Study Center, Yale University School of Medicine, New Haven, CT, USA; [2]Laboratory of Molecular Pathophysiology, Mood and Anxiety Disorders Research Program, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, Bethesda, MD, USA; [3]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; [4]Department of Psychiatry, The Mount Sinai School of Medicine, New York, NY, USA; [5]Department of Psychology, Yale University School of Medicine, New Haven, CT, USA and [6]Department of Neurobiology, Yale University School of Medicine, New Haven, CT, USA
Correspondence: Dr PJ Lombroso, Child Study Center, Yale University School of Medicine, P.O. Box 207900, New Haven, CT 06520-7900, USA.
E-mail: paul.lombroso@yale.edu
Keywords: glutamate hypothesis; neuroleptics; NMDA receptor trafficking; schizophrenia; STEP; tyrosine phosphatase
Received 14 Feburary 2012; revised 13 June 2012; accepted 14 June 2012

activates protein kinase A and leads to the phosphorylation of $STEP_{61}$ at a regulatory serine (Ser^{221}) within the substrate-binding KIM domain, thereby preventing $STEP_{61}$ from interacting with and dephosphorylating its substrates.[39,40] When $STEP_{61}$ is phosphorylated at this site, or in STEP knockout (KO) mice, the tyrosine phosphorylation of $STEP_{61}$ substrates and surface expression of GluN1/GluN2B-containing receptors are increased.[33,37,39,41] Protein kinase A also activates dopamine and cAMP-regulated phosphoprotein-32 that inhibits PP1-mediated dephosphorylation of STEP.[40,42,43]

Because of the relationship between $STEP_{61}$, dopamine signaling and NMDAR function, we hypothesized that dysregulation of $STEP_{61}$ might contribute to the pathophysiology of SZ. We find elevated $STEP_{61}$ levels in postmortem anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) of two different cohorts of SZ patients, as well in frontal cortex of mice treated with psychotomimetics. We also demonstrate that antipsychotics inactivate $STEP_{61}$, leading to increased NMDAR phosphorylation and surface expression. These results suggest that the inactivation of $STEP_{61}$ may contribute to the beneficial effects of medications used to treat SZ.

Materials and methods

Postmortem brain tissue. Postmortem anterior cingulate cortex from SZ patients and nonpsychotic controls was obtained from the Stanley Foundation Brain Bank. A second cohort of postmortem samples was obtained from the Mount Sinai Brain Bank and consisted of DLPFC. Subject and tissue parameters for both cohorts are shown in Supplementary Tables S1 and S2. Tissue collection[44,45] and sample preparation were performed as described.[46] Samples were stored at −80 °C until processing by quantitative immunoblotting. Lactate dehydrogenase was used for normalization.

Primary cortical cultures and stimulation. All procedures were approved by the Yale University Institutional Animal Care and Use Committee and strictly adhered to the NIH Guide for the Care and Use of Laboratory Animals. Primary cortical neurons were isolated from rat E18 embryos.[30] Neurons (14–21 DIV) were treated with MK-801 (50 μM; Tocris, Minneapolis, MN, USA) or PCP (100 μM; Sigma, Ronkonkoma, NY, USA) for the time points indicated. The D2 antagonist sulpiride (25–50 μM; Sigma) or D1 agonist SKF-82958 (25–50 μM; Sigma) were applied to neurons for 15 min. In some cases, neurons were pretreated for 30 min before MK-801 application with anisomycin (40 μM; EMD Biosciences, Billerica, MA, USA), actinomycin D (25 μM; Sigma), LY294001 (10 μM; Tocris) or U0126 (10 μM; EMD Biosciences). After treatments, cells were lysed in 1 × RIPA buffer supplemented with NaF (5 mM), Na_3VO_4 (2 mM), MG-132 (10 μM, EMD Biosciences) and complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA), and spun at 1000 *g* for 10 min, and supernatants were subjected to SDS-PAGE and western blotting.

Ubiquitinated protein pull-down. MK-801-treated cultured neurons or cortical tissue were homogenized as described.[30] Lysates were incubated with 20 μl of Agarose-TUBE2 (Tandem Ubiquitin Binding Entity, LifeSensors, Malvern, PA, USA) beads overnight at 4 °C, bound proteins eluted and processed by western blots.

Surface biotinylation and phosphatase activity. After stimulations, cortical cultures were labeled with EZ-Link Sulfo-NHS-SS-Biotin (Pierce, Rockford, IL, USA) as described.[31] Neurons were lysed and incubated with NeutrAvidin Biotin-binding Protein immobilized to agarose beads. For phosphatase activity, the GST-GluN2B C-terminal was phosphorylated by Fyn, mixed with immunoprecipitated STEP and a phospho-specific antibody was used to assess phosphorylation of GluN2B at Tyr^{1472} (ref. 30).

Subcellular fractionations and western blot analyses. Subcellular fractionation was performed and synaptosomal fractions (P2) were prepared for western blot analysis or all experiments where glutamate receptor subunits or STEP substrates were investigated *in vivo* from cortical tissue.[31] Antibodies used are shown in Supplementary Table S3. Bands were visualized with a G:BOX with a GeneSnap image program (Syngene, Fredrick, MD, USA) and quantified with Image J 1.33 (NIH). Levels of phosphoproteins were normalized first to total protein levels and then normalized again with glyceraldehyde-3-phosphate dehydrogenase.

***In vivo* treatments.** Male wild-type (WT) C57BL/6 mice (6–8 months) received subchronic injections haloperidol (2 mg kg^{-1}), clozapine (5 mg kg^{-1}) or risperidone (2 mg kg^{-1}) administered intraperitoneal (i.p.) daily for 3 weeks. These drugs were dissolved in 100 mM acetic acid and titrated to pH 6.5 and diluted in 0.9% normal saline to desired concentration. MK-801 (0.6 mg kg^{-1}, i.p.) and PCP (5 mg kg^{-1}, i.p.) were dissolved in 0.9% saline and administered to male WT mice. Control animals received 0.9% saline injections. Mice receiving subchronic antipsychotic treatment were killed 24 h following the last injection or at indicated time points following MK-801 administration.

Frontal cortex (anterior to motor cortex) was dissected out and synaptosomal fractions (P2) were prepared.[31]

Behavioral assessments. For locomotor activity, male WT and STEP KO mice ($n = 8$–10; 6–10 months) were assessed for 2 days in an open field.[37] On day 1, mice were drug naïve; on day 2, they were administered PCP (2.5–20 mg kg^{-1} dose) or vehicle by i.p. injection. Separate mice were used for each dose of PCP. Total distance traveled in 60 min was determined by AnyMaze software (Stoelting, Wood Dale, IL, USA). For object recognition, PCP was administered subchronically (5 mg kg^{-1}, i.p. twice daily for 7 days)[47,48] to male WT and STEP KO mice ($n = 10$–11; 6–8 months). At 1 week after the last injection, preference to a novel object after a 2-hr delay was determined as described previously.[37]

Data analysis. All data were presented as means ± s.e.m., and $P \leqslant 0.05$ was considered significant. For the postmortem data, ANCOVA (IBM SPSS Statistics v19, Poughkeepsie,

NY, USA) was used to determine differences between groups (Control and SZ) using sex, age and postmortem interval (PMI) as covariates. For all other biochemical analyses, either Student's unpaired two-tailed *t*-test or one-way analysis of variances were performed with Fisher's *post-hoc* tests. For locomotor activity, a repeated-measures analysis of variance with Fisher's *post-hoc* was used. For object recognition, a one-sample *t*-test was used as previously described[37] to determine whether a group spent more time with the novel object compared with the chance value of 15 sec.

Results

$STEP_{61}$ is elevated in the cortex of SZ patients. We first examined $STEP_{61}$ expression in brain homogenates prepared from anterior cingulate cortex of a well-characterized group of SZ patients from the Stanley Foundation, compared with age-, sex- and PMI-matched control patients (SZ: $n=12$; controls: $n=12$; Supplementary Table S1).[46] $STEP_{61}$ was significantly elevated in SZ subjects compared with controls using sex, age and PMI as covariates ($P<0.05$; Figures 1a and b), with no interactions between those covariates and the main effect. As most SZ patients from the Stanley Foundation were under antipsychotic medication at the time of death (Supplementary Table S1), we wanted to confirm our findings in a second SZ cohort from the Mount Sinai Brain Bank where few patients where under antipsychotic treatment (Supplementary Table S2). DLPFC homogenates from SZ ($n=14$) and controls ($n=12$) were assessed for $STEP_{61}$ levels. We again observed a significant increase in $STEP_{61}$ in the DLPFC of SZ subjects using sex, age and PMI as covariates ($P<0.05$; Figures 1c and d), and there was no influence of the covariates on the main effect.

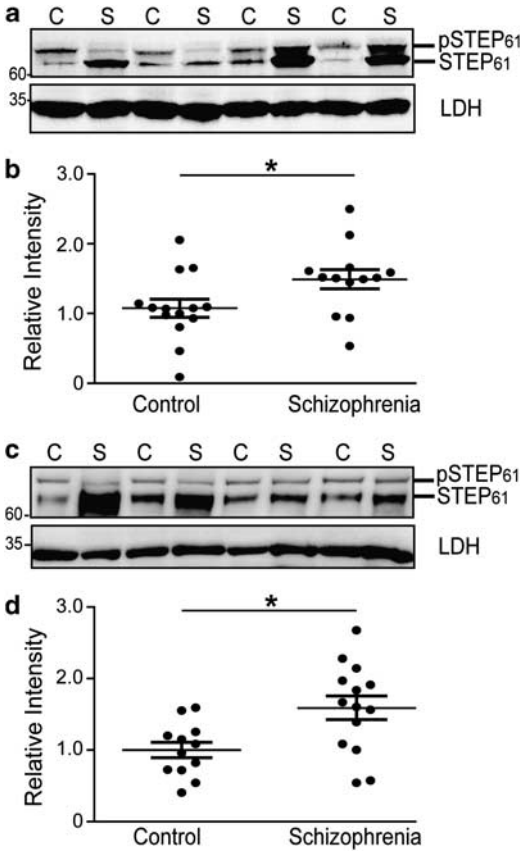

Figure 1 $STEP_{61}$ levels are increased in human postmortem brains with SZ. $STEP_{61}$ was measured in protein homogenates from the anterior cingulate cortex of controls and patients with SZ ($n=12$) (**a**, **b**) or the DLPFC of controls ($n=12$) and patients with SZ ($n=14$) (**c**, **d**). (**a**, **c**) Representative western blots of subjects demonstrating an overall increase in $STEP_{61}$ expression in SZ patients compared with controls. The top band is $pSTEP_{61}$ while the bottom band is the nonphosphorylated, active form of $STEP_{61}$, which was quantified. Lactate dehydrogenase (LDH) levels were not significantly different between control and SZ samples, and were used for normalization. (**b**, **d**) analysis of covariance model with sex, age and PMI as covariates was used to demonstrate that $STEP_{61}$ was significantly higher in SZ subjects (*$P<0.05$).

MK-801 increases $STEP_{61}$ activity and promotes NMDAR internalization. Noncompetitive NMDAR antagonists have psychotomimetic properties in humans and produce SZ-like effects in animal models. We therefore determined whether treatment with NMDAR antagonists would mimic the increase in $STEP_{61}$ observed in human SZ tissue. We first examined $STEP_{61}$ after stimulating cortical cultures with the NMDAR antagonist MK-801. $STEP_{61}$ levels were significantly increased in cultured neuronal homogenates at all time points examined (10, 30 and 60 min; $P<0.01$; Figure 2a). MK-801 treatment concomitantly decreased $STEP_{61}$ phosphorylation (Ser^{221}) ($P<0.01$), suggesting an increase in $STEP_{61}$ activity. Consistent with this expectation, the tyrosine phosphorylation of $STEP_{61}$-regulated sites on GluN2B (Tyr^{1472}), Pyk2 (Tyr^{402}) and ERK1/2 ($Tyr^{204/187}$) were significantly decreased ($P<0.01$; Figure 2b). Total levels of GluN2B, Pyk2 and ERK1/2 did not change following MK-801 treatment in neuronal homogenates (data not shown), suggesting that the overall phosphorylation state and not the total level of these proteins were affected by MK-801.

To more accurately determine whether MK-801 increased $STEP_{61}$ levels and altered $STEP_{61}$ substrates *in vivo* at the synapse, WT mice were injected acutely with MK-801, and synaptosomal (P2) fractions were prepared from frontal cortex at various time points post injection. Consistent with the cultured neuron findings, $STEP_{61}$ levels were increased, while phosphorylation of $STEP_{61}$ was decreased 10 min post injection ($P<0.05$; Figure 2c). The tyrosine phosphorylation of GluN2B, Pyk2 and ERK1/2 was also significantly decreased ($P<0.05$; Figure 2d).

Increased $STEP_{61}$ activity has been shown in other contexts to promote internalization of NMDARs.[29,30,37] Thus, we examined GluN2B levels in P2 fractions following MK-801 treatment. There was a trend for GluN2B levels to decrease after 10 min post injection (-27.3 ± 12.6; $P=0.09$), consistent with increased internalization of GluN2B NMDARs. In most cases, substrate phosphorylation and GluN2B levels returned to baseline at later time points *in vivo* in P2 fractions, in contrast to the cultured neuron findings (although ERK1/2 phosphorylation remained low even 60 min post injection). These results likely reflect pharmacokinetic complexities

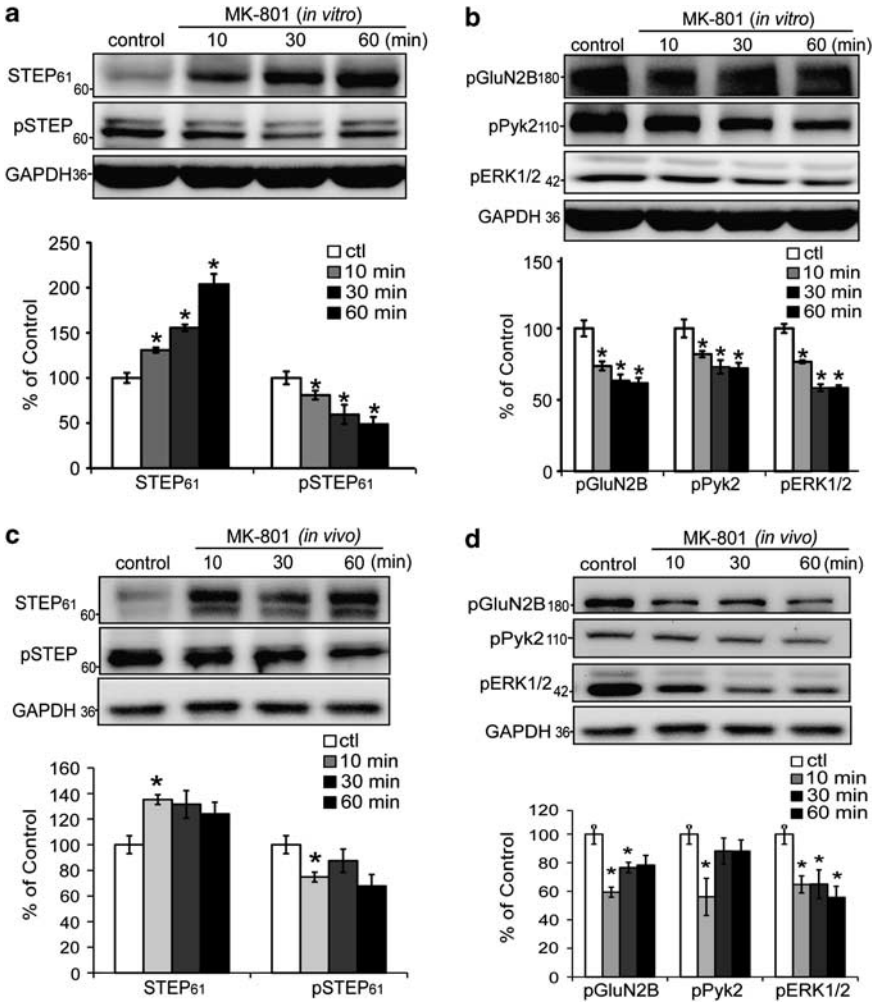

Figure 2 MK-801 treatment increases $STEP_{61}$ activity and decreases tyrosine phosphorylation of $STEP_{61}$ substrates. (**a**, **b**) Primary cortical cultures (*in vitro*) were treated with MK-801 (50 μM) for 10, 30 or 60 min. (**a**) MK-801 treatment significantly increased total levels of $STEP_{61}$, while also significantly reducing $STEP_{61}$ phosphorylation at Ser^{221} (pSTEP) (one-way analysis of variance (ANOVA); $^*P<0.01$ different from control, Tukey's *post-hoc*; $n=4$). (**b**) Administration of MK-801 significantly reduced the Tyr phosphorylation of $STEP_{61}$ substrates at sites that STEP is known to dephosphorylate, including GluN2B at Tyr^{1472} (pGluN2B), Pyk2 at Tyr^{402} (pPyk2) and ERK1/2 at $Tyr^{204/187}$ (pERK1/2) (one-way ANOVA; $^*P<0.01$ different from control, Tukey's *post-hoc*; $n=4$). (**c**, **d**) WT mice were acutely injected with MK-801 (0.6 mg kg^{-1}, i.p.) or saline (control). Frontal cortex was dissected at 10, 30 or 60 min post injection. (**c**) MK-801 treatment *in vivo* significantly increased $STEP_{61}$ levels and attenuated $STEP_{61}$ phosphorylation in synaptosomal (P2) fractions prepared from frontal cortex 10 min post injection (one-way ANOVA; $^*P<0.05$ different from control, Tukey's *post-hoc*; $n=6$). (**d**) Tyr phosphorylation of GluN2B, Pyk2 and ERK1/2 was also significantly reduced (one-way ANOVA; $^*P<0.05$ different from control, Tukey's *post-hoc*; $n=6$). In all panels, each phosphoprotein was normalized to the total protein level for that protein and then to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data represent the mean percentage of control ± s.e.m.

in vivo not captured in culture. Similar results were observed in the hippocampus (data not shown).

To further confirm that $STEP_{61}$ activity, and not just its quantity, was increased by MK-801, we conducted a phosphatase assay using a phospho–GluN2B fusion protein as a substrate of $STEP_{61}$ immunoprecipitated from MK-801-treated and control cultured neurons. Immunoprecipitated $STEP_{61}$ from MK-801-treated neurons significantly decreased GluN2B phosphorylation compared with control ($P<0.01$; Figure 3a).

As a more direct approach to determine whether surface levels of NMDARs were altered by MK-801, we investigated NMDAR surface expression using a surface biotinylation assay in cultured neurons. MK-801 significantly reduced GluN2B and GluN1 surface expression ($P<0.05$), but not GluN2A ($P=0.28$) or $GABA_A\beta2/3$ ($P=0.79$) that are not $STEP_{61}$ substrates (Figure 3b). As observed previously (Figure 2), MK-801 significantly increased $STEP_{61}$ in total neuronal homogenates and concomitantly decreased phosphorylation of GluN2B ($P<0.05$) and ERK1/2 ($P<0.01$; Figure 3c). Total levels of NMDARs, ERK1/2 and $GABA_A\beta2/3$ remained unchanged ($P>0.05$; Figure 3c). Taken together, our findings indicate that MK-801 increased $STEP_{61}$ levels and activity, decreased the tyrosine phosphorylation of $STEP_{61}$ substrates and reduced the surface expression of GluN2B-containing NMDARs.

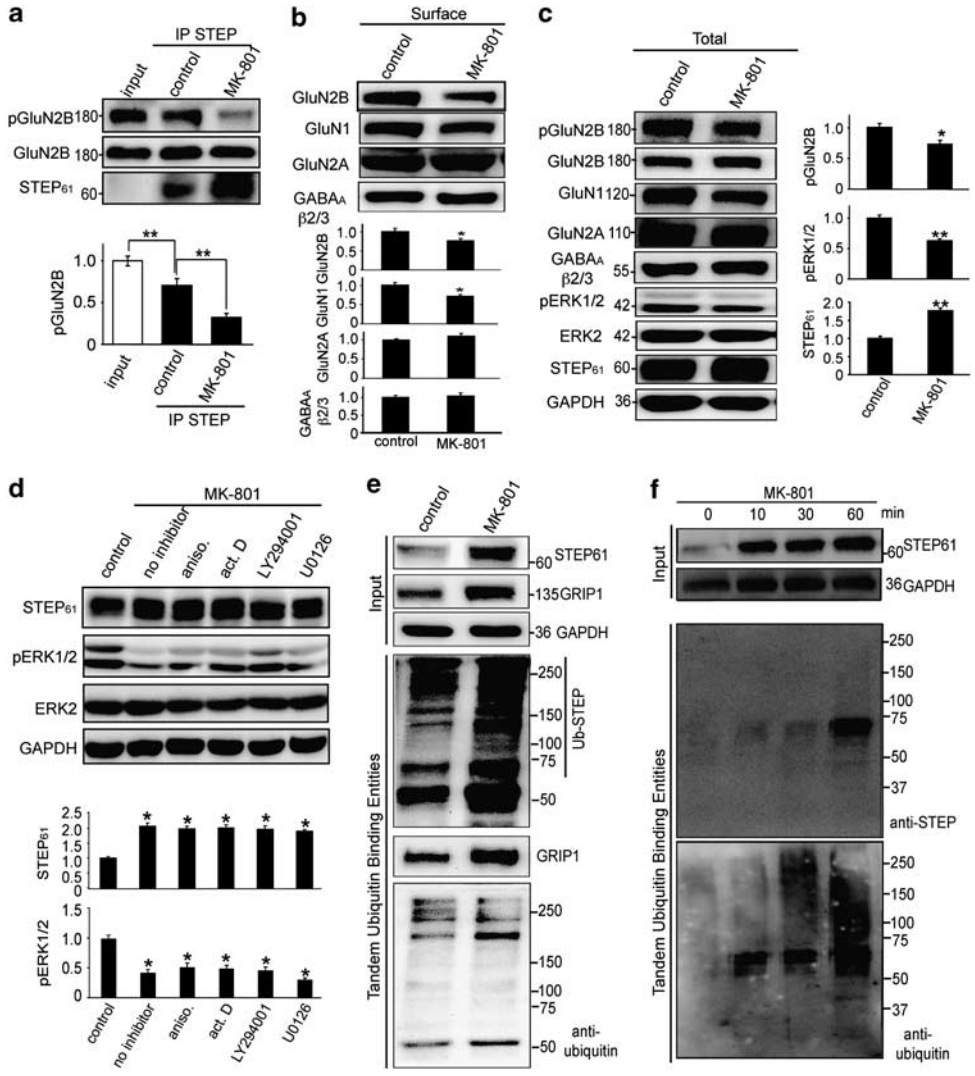

Figure 3 The MK-801-induced accumulation of $STEP_{61}$ occurs via blockade of the UPS and promotes internalization of NMDARs. (**a**) Primary cortical cultures were treated with MK-801 (50 μM) for 2 h, and the activity of immunoprecipitated $STEP_{61}$ from control and treated neurons was measured against phospho–GluN2B fusion protein. Phosphorylation of GluN2B at Tyr^{1472} (pGluN2B) was significantly lower in MK-801-treated immunoprecipitates than in controls (Student's *t*-test; $^{**}P<0.01$; $n=4$). (**b**, **c**) Surface proteins were biotinylated after MK-801 treatment. (**b**) MK-801 treatment significantly decreased surface levels of GluN2B and the obligatory subunit GluN1, but not GluN2A or $GABA_A\beta2/3$. (**c**) Total levels of subunits were unaltered by MK-801 treatment (quantification not shown), while an overall significant decrease in the phosphorylation of GluN2B at Tyr^{1472} and ERK1/2 at $Tyr^{204/187}$ (pERK1/2) was observed again alongside increased $STEP_{61}$ levels. For (**b**, **c**), $^{*}P<0.05$ and $^{**}P<0.01$ (Student's *t*-test; $n=4$). (**d**) Cortical cultures were pretreated with various inhibitors for 20–30 min before applying MK-801; none prevented the MK-801-induced increase in $STEP_{61}$ or the decrease in ERK1/2 phosphorylation (one-way analysis of variance; $^{*}P<0.01$ different from control, Tukey's *post-hoc*; $n=4$). For (**a**–**d**), each phosphoprotein was normalized to the total protein level for that protein and then to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data were normalized to control and represent the mean normalized value ± s.e.m. (**e**) Ubiquitinated proteins were isolated from control and MK-801-treated cultures, and probed with anti-STEP and anti-Ub antibodies. An increase in ubiquitinated $STEP_{61}$ was observed following MK-801 treatment. Glutamate receptor-interacting protein 1 (GRIP1) served as a positive control, as it is degraded by the UPS ($n=4$). (**f**) Ubiquitinated proteins were also more abundant in mouse cortical lysates isolated following an acute injection of MK-801 (0.6 mg kg^{-1}, i.p.) ($n=4$).

Accumulation of $STEP_{61}$ occurs via blockade of the ubiquitin proteasome system. We tested several mechanisms that might explain the MK-801-induced increase in $STEP_{61}$. Translation of STEP is regulated in part by the PI3K-Akt and ERK pathways.[31,49] Therefore, neuronal cultures were pretreated with either the PI3K inhibitor (LY294001) or MEK inhibitor (U0126) before MK-801 administration (Figure 3d). Neither inhibitor blocked the MK-801-induced increase in $STEP_{61}$, demonstrating that the PI3K-Akt and ERK pathways were not involved. Likewise, pretreatment with inhibitors of transcription (actinomycin D) and translation (anisomycin) failed to block the elevation in $STEP_{61}$ levels (Figure 3d). These results indicate that the observed increase in $STEP_{61}$ levels following MK-801 treatment was not due to increased transcription or translation.

$STEP_{61}$ is normally ubiquitinated and degraded by the ubiquitin proteasome system (UPS), and disruption of the

UPS increases $STEP_{61}$ levels.[30] The UPS can be activated by NMDAR signaling, and blocking NMDARs leads to rapid accumulation of several proteins normally degraded by the UPS.[50,51] To determine whether disruptions in the UPS mediate the MK-801-induced increase in $STEP_{61}$, we measured polyubiquitinated $STEP_{61}$ following MK-801 treatment in cultured neurons, as well as in brain homogenates from MK-801-treated WT mice. Polyubiquitinated proteins were enriched and probed with an anti-STEP antibody or anti-glutamate receptor-interacting protein 1 antibody as a positive control (Figures 3e and f). Acute injections of MK-801 increased polyubiquitinated $STEP_{61}$ (MK-801: 10 min = 113 ± 1.8%; 30 min = + 117 ± 0.6%; 60 min = 126 ± 2.6% (*Ps* < 0.01), suggesting that blocking NMDARs with MK-801 disrupts the UPS and leads to the accumulation of polyubiquitinated $STEP_{61}$.

Neuroleptics inactivate $STEP_{61}$ and increase the phosphorylation of its substrates. Our findings thus far demonstrate that MK-801 increased both $STEP_{61}$ expression and activity in synaptosomal fractions. As increased $STEP_{61}$ activity leads to loss of GluN2B-containing NMDARs from neuronal membrane surfaces, we next investigated whether antipsychotic medications exert their beneficial effects by inactivating $STEP_{61}$. We first examined the phosphorylation of $STEP_{61}$ and its substrates in cultured neurons treated with the D1R agonist, SKF-82958, or the D2R antagonist, sulpiride. Both treatments significantly increased the phosphorylation of $STEP_{61}$, and tyrosine phosphorylation of GluN2B and ERK1/2 at the two doses examined (*Ps* < 0.05; Figures 4a and b), consistent with an inactivation of $STEP_{61}$ and activation of its substrates. No significant changes were observed in total levels of GluN2B, ERK1/2 or $STEP_{61}$ in cultured neuronal homogenates (data not shown).

To more closely recapitulate the clinical use of antipsychotics, WT mice were subchronically treated with three different antipsychotic medications (haloperidol, clozapine or risperidone) for 21 days, and phosphorylation of $STEP_{61}$ and its substrates were examined in synaptosomal (P2) fractions from frontal cortex (Figures 4c–h).

Haloperidol significantly increased $STEP_{61}$ phosphorylation ($P < 0.05$) and concomitantly enhanced the tyrosine phosphorylation of GluN2B, Pyk2 and ERK1/2 (GluN2B and ERK1/2: $P < 0.005$; Pyk2: $P < 0.05$; Figures 4c–f). The atypical antipsychotics, clozapine and risperidone, similarly increased $STEP_{61}$ phosphorylation ($P < 0.05$ and $P < 0.005$, respectively; Figure 4c), as well as the tyrosine phosphorylation of Pyk2 and ERK1/2 (*Ps* < 0.005; Figures 4e and f).

Consistent with increased surface expression of GluN2B, clozapine and risperidone significantly enhanced GluN2B levels in the P2 fraction (*Ps* < 0.005) and a similar trend was observed with haloperidol ($P = 0.06$) (Figure 4g). Similar to the results using SKF-82958 and sulpiride, subchronic treatment of haloperidol did not alter total $STEP_{61}$ levels in P2 fractions (+0.8 ± 5.4%; $P > 0.05$), while clozapine and risperidone did (+135.1 ± 30.5% and +74.9 + 15.4%, respectively; $P < 0.05$). As a positive control, antipsychotic treatment significantly increased the phosphorylation of dopamine and cAMP-regulated phosphoprotein-32 (Thr^{34}) (clozapine: $P < 0.05$; Figure 4h), which is in agreement with prior studies.[43] Comparable results were observed for these measures in the striatum and hippocampus (data not shown). No significant changes were detected in GluN2A ($P = 0.907$) or $GABA_A\beta$ 2/3 ($P = 0.431$) in P2 fractions (data not shown). Altogether, these findings indicate that different classes of neuroleptics increase $STEP_{61}$ phosphorylation, increase the tyrosine phosphorylation of $STEP_{61}$ substrates and enhance NMDAR surface expression.

STEP KO mice are less sensitive to behavioral abnormalities elicited by the psychotomimetic PCP. If increased activity of $STEP_{61}$ and consequent downstream changes are mechanistically important for the psychotomimetic effects of NMDAR antagonists, then STEP KO mice should be less sensitive behaviorally than WT controls to these effects. Consistent with previous reports,[52,53] we found that even low doses of acute MK-801 injections (0.2 mg kg^{-1}) resulted in convulsive jumping and wild running fits that severely affected motor coordination and prevented accurate baseline locomotor measurements and analysis of object recognition (data not shown). Therefore, we utilized another well-characterized psychotomimetic and NMDAR antagonist, PCP, for behavioral analyses. To confirm that PCP altered $STEP_{61}$ and its substrates in a similar manner as MK-801 (Figure 2), we administered PCP acutely to both in cortical neurons and *in vivo* (Supplementary Figure 1). In cortical neurons, PCP significantly decreased $STEP_{61}$ phosphorylation ($P < 0.01$) and increased $STEP_{61}$ levels ($P < 0.05$) following a 60 min treatment. PCP also decreased the tyrosine phosphorylation of GluN2B, Pyk2 and ERK1/2 (*Ps* < 0.05) (Supplementary Figure 1a).

WT mice were subsequently injected with an acute dose of PCP (5 mg kg^{-1}; i.p.) and synaptosomal (P2) fractions prepared from the frontal cortex 60-min post injection (Supplementary Figure 1b). $STEP_{61}$ phosphorylation was significantly decreased in P2 fractions ($P < 0.01$), while total $STEP_{61}$ levels were increased ($P < 0.05$). Tyrosine phosphorylation of GluN2B, Pyk2 and ERK1/2 was significantly reduced following an acute injection of PCP (*Ps* < 0.05). These findings indicate that PCP, like MK-801, increases $STEP_{61}$ activity and levels, leading to decreased tyrosine phosphorylation of $STEP_{61}$ substrates.

We next investigated the effect of PCP on behavior in WT versus STEP KO mice. As expected, WT mice became increasingly hyperactive with acute injections of low and intermediated doses of PCP (2.5–15 mg kg^{-1}) and were sedated by the highest dose (20 mg kg^{-1}; Figure 5a). There was no difference between genotypes in baseline locomotor activity (data not shown). While there was a within-genotype dose effect for both genotypes ($P < 0.001$), STEP KO mice were significantly less hyperactive than WT mice in response to PCP (significant dose by genotype interaction; $P < 0.02$). These results confirm our hypothesis that STEP KO mice are less sensitive behaviorally to PCP-induced hyperactivity.

Subchronic administration of PCP induces long-term cognitive impairments and mimics aspects of the positive and negative symptomatology of SZ in both rodents and primates.[54–59] To assess whether STEP KO mice were less sensitive to PCP-induced cognitive deficits, WT and STEP KO mice were treated for 7 days with PCP (5 mg kg^{-1}, i.p. twice

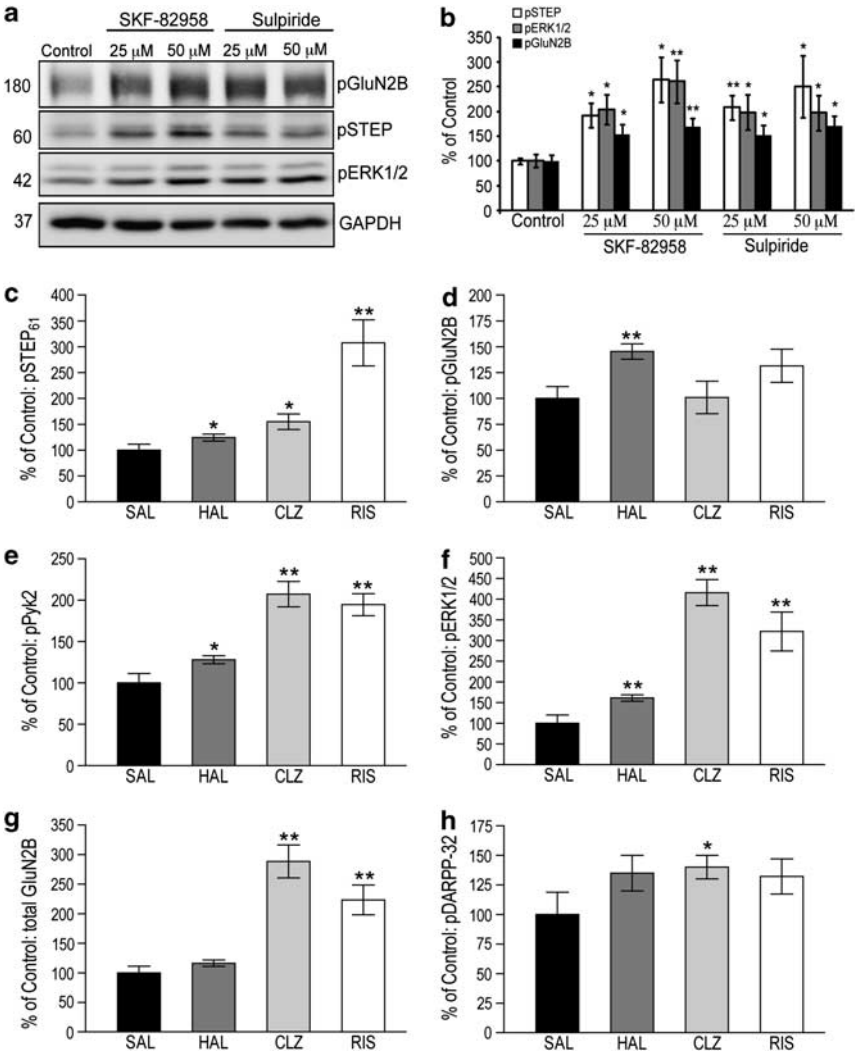

Figure 4 Neuroleptics increase the phosphorylation of $STEP_{61}$, $STEP_{61}$ substrates and dopamine and cAMP-regulated phosphoprotein-32. (**a**, **b**) Primary cortical cultures were treated with the DA D1R agonist SKF-82958 (25 or 50 μM) or D2R antagonist sulpiride (25 or 50 μM). Both drugs at either concentration significantly increased phosphorylation of $STEP_{61}$ at Ser^{221} (pSTEP), GluN2B at Tyr^{1472} (pGluN2B) and pERK1/2 at $Tyr^{204/187}$ (pERK1/2) (one-way analysis of variance; $^{*}P<0.05$ and $^{**}P<0.01$ different from control, Tukey's *post-hoc*; $n=4$). (**c**–**h**) WT mice were injected with saline (SAL; control), haloperidol (HAL; 2 mg kg^{-1}), clozapine (CLZ; 5 mg kg^{-1}) and risperidone (RIS; 2 mg kg^{-1}) daily for 3 weeks, and synaptosomal (P2) fractions were prepared from frontal cortex. Phosphorylation of (**c**) $STEP_{61}$, (**d**) GluN2B, (**e**) Pyk2 and (**f**) ERK1/2 were significantly increased by neuroleptics (Student's *t*-test, saline versus drug; $^{*}P<0.05$ and $^{**}P<0.005$; $n=4$–6) (**g**) Total GluN2B in P2 fractions was also significantly increased following neuroleptic treatment (Student's *t*-test, saline versus drug; $^{**}P<0.005$; $n=4$–6). (**h**) Increased phosphorylation of dopamine and cAMP-regulated phosphoprotein-32 at Thr^{34} (pDARPP-32) following neuroleptic treatment was used as a positive control (Student's *t*-test, saline versus drug; $^{*}P<0.05$; $n=4$–6). In all panels, phosphoproteins were first normalized to the total protein and then to GADPDH. Data represent the mean percentage of control ± s.e.m.

daily) and tested in a novel object recognition task (Figure 5b). No genotypic differences were observed in exploration time of two identical objects during the acquisition phase (data not shown), and both saline-treated WT and STEP KO mice spent significantly more time with the novel object than chance (15 s) during the 24-hr delay retention trial ($P<0.05$, Figure 5b). After PCP treatment, however, WT mice did not explore the novel object significantly more than chance. STEP KOs treated with PCP were protected from this impairment and continued to show a clear preference for the novel object despite PCP treatment ($P<0.05$). Taken together, the behavioral findings indicate that STEP KOs are less sensitive to PCP-induced hyperactivity and cognitive impairment compared with WT mice.

To determine the molecular alterations induced by subchronic PCP treatment that could account for these long-term behavioral effects, we analyzed whether there were changes in $STEP_{61}$ or its substrates that occurred in a sustained fashion 24 h after the last of the subchronic PCP injections. No significant differences were observed for $STEP_{61}$ phosphorylation ($+8.9\pm9.8\%$, $P\geqslant0.05$) or $STEP_{61}$ levels ($+6.0\pm6.9\%$, $P\geqslant0.05$) in PCP- versus saline-injected WT mice. In line with previous studies,[60] we found that ERK1/2, a direct substrate of $STEP_{61}$,[35] was activated 24 h after the last PCP injection in WT mice but not in STEP KOs (Figure 6). Altogether, these findings suggest that $STEP_{61}$ is transiently activated after the first PCP injection and that other proteins downstream of $STEP_{61}$ are altered more sustainably. We also

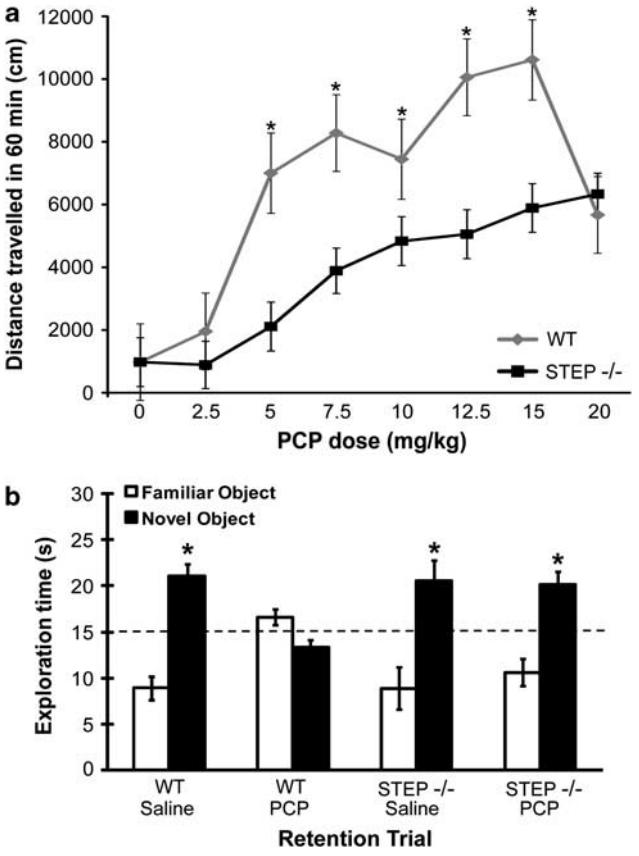

Figure 5 STEP KO mice are less sensitive to PCP-induced behavioral and cognitive deficits. (**a**) WT ($n=10$) and STEP KO ($n=8$) were acutely injected with PCP (2.5–20 mg kg^{-1}, i.p.), and analyzed for locomotor activity in an open field task. A repeated-measures analysis of variance indicated a main effect of dose ($P<0.001$) and a significant dose by genotype interaction ($P<0.02$). Tukey's *post-hoc* indicated that WT was significantly different from STEP KO at the PCP doses indicated (*$P<0.05$). (**b**) Exploration time of the novel and familiar objects during the 24-h delay object recognition retention trial ($n=12$ for all groups). Both saline-treated WT and STEP KO mice spent significantly more time with the novel object than the chance value of 15 s (one-sample *t*-test; *$P<0.05$). WT mice treated subchronically with PCP (5 mg kg^{-1}, i.p. twice daily for 7 consecutive days) did not spend more time than chance with the novel object. In contrast, STEP KOs spent significantly more time with the novel object (one-sample *t*-test; *$P<0.05$).

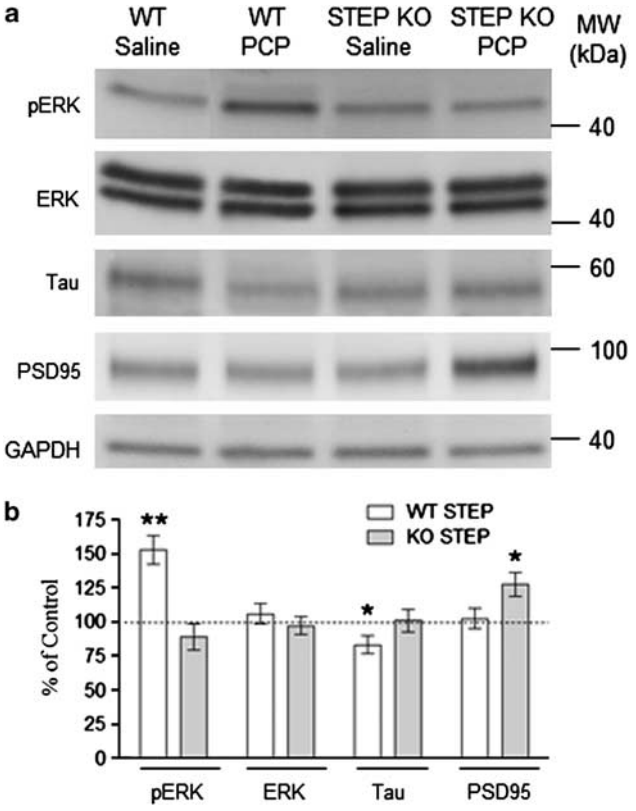

Figure 6 Subchronic PCP treatment differentially affects ERK1/2 activation and total levels of Tau and PSD-95 in WT and STEP KO mice. (**a**, **b**) WT and STEP KO mice were subchronically injected with PCP (5 mg kg^{-1}, i.p. twice daily for 7 days) or an equal volume of saline in control mice, and tissue was extracted 24 h following the last injection. PCP treatment significantly increased the phosphorylation of ERK1/2 and decreased protein levels of Tau in cortical synaptosomal (P2) fractions of WT mice, but not STEP KOs (*$P<0.05$, **$P<0.01$; one-sample *t*-test). Conversely, PSD-95 was significantly increased after subchronic PCP injections in P2 fractions of STEP KO mice and not WT mice (*$P<0.05$; one-sample *t*-test). All protein levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and pERK1/2 was normalized to the total ERK1/2 levels. Data represent the mean percentage of control ± s.e.m.

observed that the level of Tau, a direct substrate of ERK1/2 that is required for synaptic plasticity,[61] was decreased in WT mice after subchronic PCP treatment, but not in STEP KOs (Figure 6). Furthermore, PSD-95 levels were significantly increased in STEP KOs, and not in WT mice, after subchronic PCP treatment (Figure 6). These findings demonstrate that the expression of signaling molecules required for synaptic plasticity and cognitive function are differentially affected by subchronic PCP treatment in WT and STEP KO mice, providing a potential explanation for why STEP KOs are less sensitive behaviorally to PCP.

Discussion

Several converging lines of evidence point to reduced NMDAR function as an integral part of SZ pathophysiology. Abnormalities in NMDAR density are observed in postmortem studies of SZ brains, specifically in regions exhibiting impaired activation patterns during cognitive tasks.[4,5,62–65] Moreover, positron emission tomography scans suggest that hippocampal NMDAR binding is significantly reduced in untreated, but not antipsychotic-treated, SZ patients.[66] Genetic association studies have identified several candidate genes implicated in NMDAR stability or trafficking, including neuregulin 1,[67] disrupted-in-schizophrenia 1[68] and calcineurin.[69] Given that NMDARs have an integral role in cognitive function and synaptic plasticity,[70] these studies suggest that cognitive impairments in SZ are mediated, at least in part, by NMDAR hypofunction.

We present data establishing a link between high levels of $STEP_{61}$ and aberrant NMDAR signaling in SZ. $STEP_{61}$ levels are increased in two independent patient populations and two different brain regions (DLPFC and anterior cingulate cortex). It has been suggested that medication status might affect gene expression in SZ.[71,72] Given that $STEP_{61}$ levels are elevated in SZ patient populations both on (Stanley

Foundation cohort; Supplementary Table S1) or off (Mt. Sinai cohort; Supplementary Table S2) antipsychotic medication, it seems unlikely that antipsychotic treatment was responsible for the observed elevation of $STEP_{61}$ in human postmortem tissue.

We propose that increased $STEP_{61}$ levels promote the dephosphorylation of $STEP_{61}$ substrates, including GluN2B, leading to internalization and hypofunction of NMDARs. In support of this, acute treatment with the psychotomimetics MK-801 and PCP increase $STEP_{61}$ levels and activity, decrease tyrosine phosphorylation of $STEP_{61}$ substrates and reduce the surface expression of GluN2B-containing NMDARs in cultured neurons and *in vivo*. While we did not find significant changes in $STEP_{61}$ levels or phosphorylation after subchronic PCP treatment, both short- and long-term changes in NMDAR binding are found in mouse brain following chronic PCP treatment.[18] Further studies will be needed to determine whether acute disruption of $STEP_{61}$ activity and its downstream substrates (for example, GluN2B, ERK1/2 and Fyn) are sufficient to lead to longer-term PCP-induced changes in synaptic plasticity.

Recent evidence supports a link between NMDAR-mediated signaling and the UPS. UPS activation occurs through NMDAR- and Ca^{2+}-dependent mechanisms, and degradation of glutamate receptor-interacting protein 1 is NMDAR-dependent.[51] Moreover, Ca^{2+} influx through NMDARs and L-type voltage-gated Ca^{2+} channels activates CaMKII, a necessary component for proteasomal trafficking into dendritic spines.[73] We found that MK-801 treatment impairs the UPS and led to an increase in $STEP_{61}$. Previous studies establish that disruption of the UPS by β-amyloid promotes an elevation of $STEP_{61}$.[30] However, it is likely that impairment of the UPS leads to accumulation of additional proteins as well. The fact that STEP KOs are less sensitive behaviorally to the acute and chronic effects of PCP suggest that $STEP_{61}$ accumulation is an important contributor to the observed findings. Although we provide evidence for UPS involvement in the psychotomimetic-induced increase in $STEP_{61}$ expression in cultured neurons and mice, whether a similar mechanism occurs in humans with SZ remains to be determined.

We tested the causal importance of the changes in $STEP_{61}$ expression and its substrates by examining the behavioral effects of PCP in WT and STEP KO mice. Acute use of PCP in humans induces positive symptoms of SZ, while PCP abuse can lead to persistent SZ-like symptoms.[2,74,75] Unlike other psychotomimetic drugs, PCP elicits positive and negative symptoms, and cognitive deficits in humans.[76,77] Our data are consistent with STEP KOs being less sensitive than WT to PCP-induced hyperactivity and cognitive deficits. As STEP KOs exhibit increased cortical NMDAR surface expression and ERK1/2 phosphorylation,[37,41] it is possible that the enhanced tyrosine phosphorylation of $STEP_{61}$ substrates and increased NMDAR surface expression contribute to the findings that STEP KOs are less sensitive to the behavioral effects of PCP. Moreover, the activation and expression of signaling molecules required for synaptic plasticity and cognitive function, including ERK1/2, Tau and PSD-95, are differentially affected by subchronic PCP treatment in WT and STEP KO mice, pointing to potential molecular underpinnings that may explain why STEP KOs are less sensitive behaviorally to PCP than WT.

Another important finding was that neuroleptic treatments regulate $STEP_{61}$ phosphorylation. Rodent and human studies demonstrate that first-generation neuroleptics are more effective at treating positive symptoms in SZ, compared with their efficacy on negative symptoms or cognitive deficits.[78,79] Second-generation medications, including clozapine and risperidone, appear to be more effective at reducing the cognitive and behavioral deficits induced by PCP, ketamine and MK-801.[12,47,48] While the underlying mechanism(s) mediating the beneficial effects of neuroleptics are still under debate, our data suggest that neuroleptics function, at least in part, through the inactivation of $STEP_{61}$.

Administration of D2 antagonists to cortical cultures led to the phosphorylation of $STEP_{61}$, a modification that prevents $STEP_{61}$ from interacting with its substrates. Subchronic haloperidol treatment in mice led to a similar increase in $STEP_{61}$ phosphorylation with no change in total $STEP_{61}$ levels. Subchronic risperidone and clozapine treatment significantly increased the phosphorylation of $STEP_{61}$. However, both of these neuroleptics also increased $STEP_{61}$ levels in synaptosomal (P2) fractions. While the mechanism by which these two drugs increase $STEP_{61}$ expression remains to be determined, it is possible that subchronic neuroleptic treatment promotes trafficking of $STEP_{61}$ to the synapse as a homeostatic mechanism. As $STEP_{61}$ phosphorylation is increased by antipsychotics to a greater magnitude than its expression level, it appears that increased $STEP_{61}$ in the P2 fraction is insufficient to reverse the decreased $STEP_{61}$ activity and enhanced NMDAR surface expression elicited by neuroleptic treatment.

In summary, our results implicate $STEP_{61}$ as one likely mediator of NMDAR hypofunction in SZ. Increased $STEP_{61}$ levels are present both in postmortem SZ brains and mice exposed to psychotomimetics, and genetically eliminating $STEP_{61}$ results in progeny less sensitive behaviorally to these drugs. One possible explanation for increased $STEP_{61}$ levels is impaired UPS function in SZ. It is likely that other mechanisms affect $STEP_{61}$ expression, such as mutations in specific UPS enzymes or disruption of microRNAs that regulate $STEP_{61}$ expression. The present findings establish that neuroleptics used to treat SZ act, at least in part, via $STEP_{61}$ inactivation. Dysregulation of $STEP_{61}$ represents a previously unappreciated pathophysiological contributor to SZ and implicates $STEP_{61}$ as a novel pharmacological target in SZ.

Conflict of interest

The authors declare no competing financial interests. Dr Chen is now at Johnson & Johnson Pharmaceutical Research and Development; the work under his supervision was initiated while he was at the NIMH.

Acknowledgements. We thank Ms Veronica Galvin and Mr Evan Wilson-Wallis for technical assistance, as well as laboratory members for helpful discussions and critical reading of the manuscript. The work was funded by NIH grants MH091037 and MH052711 (PJL). Postmortem brain study was supported by

the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH).

Author contributions: NCC performed animal injections, tissue processing and biochemistry; NCC performed the majority of behavioral tests with assistance from PRC and CP; PK and JB assisted in tissue and cell culture preparation and biochemistry; JX assisted in cell culture preparation, stimulations and biochemistry: DRA and PY performed the postmortem analyses with supervision from GC; SMG-G performed statistical analyses; NCC, SMG-G, CP and PJL wrote the manuscript. All authors contributed to the experimental design.

1. Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* 2001; **158**: 1367–1377.
2. Krystal JH, Anand A, Moghaddam B. Effects of NMDA receptor antagonists: implications for the pathophysiology of schizophrenia. *Arch Gen Psychiatry* 2002; **59**: 663–664.
3. Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1999; **20**: 201–225.
4. Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology* 2007; **32**: 1888–1902.
5. Meador-Woodruff JH, Clinton SM, Beneyto M, McCullumsmith RE. Molecular abnormalities of the glutamate synapse in the thalamus in schizophrenia. *Ann N Y Acad Sci* 2003; **1003**: 75–93.
6. Stone JM, Erlandsson K, Arstad E, Squassante L, Teneggi V, Bressan RA *et al.* Relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy: a [(123)I]CNS-1261 SPET study. *Psychopharmacology* 2008; **197**: 401–408.
7. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD *et al.* Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 1994; **51**: 199–214.
8. Jentsch JD, Elsworth JD, Taylor JR, Redmond Jr DE, Roth RH. Dysregulation of mesoprefrontal dopamine neurons induced by acute and repeated phencyclidine administration in the nonhuman primate: implications for schizophrenia. *Adv Pharmacol* 1998; **42**: 810–814.
9. Stoet G, Snyder LH. Effects of the NMDA antagonist ketamine on task-switching performance: evidence for specific impairments of executive control. *Neuropsychopharmacology* 2006; **31**: 1675–1681.
10. Vales K, Bubenikova-Valesova V, Klement D, Stuchlik A. Analysis of sensitivity to MK-801 treatment in a novel active allothetic place avoidance task and in the working memory version of the Morris water maze reveals differences between Long-Evans and Wistar rats. *Neurosci Res* 2006; **55**: 383–388.
11. Zhang WN, Bast T, Feldon J. Microinfusion of the non-competitive N-methyl-D-aspartate receptor antagonist MK-801 (dizocilpine) into the dorsal hippocampus of wistar rats does not affect latent inhibition and prepulse inhibition, but increases startle reaction and locomotor activity. *Neuroscience* 2000; **101**: 589–599.
12. Ishii D, Matsuzawa D, Kanahara N, Matsuda S, Sutoh C, Ohtsuka H *et al.* Effects of aripiprazole on MK-801-induced prepulse inhibition deficits and mitogen-activated protein kinase signal transduction pathway. *Neurosci Lett* 2010; **471**: 53–57.
13. Bast T, Zhang W, Feldon J, White IM. Effects of MK801 and neuroleptics on prepulse inhibition: re-examination in two strains of rats. *Pharmacol Biochem Behav* 2000; **67**: 647–658.
14. Harris LW, Sharp T, Gartlon J, Jones DN, Harrison PJ. Long-term behavioural, molecular and morphological effects of neonatal NMDA receptor antagonism. *Eur J Neurosci* 2003; **18**: 1706–1710.
15. Jentsch JD, Elsworth JD, Redmond Jr DE, Roth RH. Phencyclidine increases forebrain monoamine metabolism in rats and monkeys: modulation by the isomers of HA966. *J Neurosci* 1997; **17**: 1769–1775.
16. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 1997; **17**: 2921–2927.
17. Bubenikova-Valesova V, Horacek J, Vrajova M, Hoschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev* 2008; **32**: 1014–1023.
18. Newell KA, Zavitsanou K, Huang XF. Short and long term changes in NMDA receptor binding in mouse brain following chronic phencyclidine treatment. *J Neural Transm* 2007; **114**: 995–1001.
19. Stephan KE, Friston KJ, Frith CD. Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. *Schizophr Bull* 2009; **35**: 509–527.
20. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995; **52**: 998–1007.
21. Abi-Saab WM, D'Souza DC, Moghaddam B, Krystal JH. The NMDA antagonist model for schizophrenia: promise and pitfalls. *Pharmacopsychiatry* 1998; **31**(Suppl 2): 104–109.
22. Goebel-Goody SM, Baum M, Paspalas CD, Fernandez SM, Carty NC, Kurup P *et al.* Therapeutic implications for striatal-enriched protein tyrosine phosphatase (STEP) in neuropsychiatric disorders. *Pharmacol Rev* 2012; **64**: 65–87.
23. Sharma E, Zhao F, Bult A, Lombroso PJ. Identification of two alternatively spliced transcripts of STEP: a subfamily of brain-enriched protein tyrosine phosphatases. *Brain Res Mol Brain Res* 1995; **32**: 87–93.
24. Bult A, Zhao F, Dirkx Jr R, Raghunathan A, Solimena M, Lombroso PJ. STEP: a family of brain-enriched PTPs. Alternative splicing produces transmembrane, cytosolic and truncated isoforms. *Eur J Cell Biol* 1997; **72**: 337–344.
25. Bult A, Zhao F, Dirkx Jr R, Sharma E, Lukacsi E, Solimena M *et al.* STEP61: a member of a family of brain-enriched PTPs is localized to the endoplasmic reticulum. *J Neurosci* 1996; **16**: 7821–7831.
26. Lombroso PJ, Naegele JR, Sharma E, Lerner M. A protein tyrosine phosphatase expressed within dopaminoceptive neurons of the basal ganglia and related structures. *J Neurosci* 1993; **13**: 3064–3074.
27. Boulanger LM, Lombroso PJ, Raghunathan A, During MJ, Wahle P, Naegele JR. Cellular and molecular characterization of a brain-enriched protein tyrosine phosphatase. *J Neurosci* 1995; **15**: 1532–1544.
28. Goebel-Goody SM, Davies KD, Alvestad Linger RM, Freund RK, Browning MD. Phospho-regulation of synaptic and extrasynaptic N-methyl-D-aspartate receptors in adult hippocampal slices. *Neuroscience* 2009; **158**: 1446–1459.
29. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY *et al.* Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 2005; **8**: 1051–1058.
30. Kurup P, Zhang Y, Xu J, Venkitaramani DV, Haroutunian V, Greengard P *et al.* Abeta-mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. *J Neurosci* 2010; **30**: 5948–5957.
31. Zhang Y, Venkitaramani DV, Gladding CM, Zhang Y, Kurup P, Molnar E *et al.* The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. *J Neurosci* 2008; **28**: 10561–10566.
32. Nguyen TH, Liu J, Lombroso PJ. Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. *J Biol Chem* 2002; **277**: 24274–24279.
33. Venkitaramani DV, Moura PJ, Picciotto MR, Lombroso PJ. Striatal-enriched protein tyrosine phosphatase (STEP) knockout mice have enhanced hippocampal memory. *Eur J Neurosci* 2011; **33**: 2288–2298.
34. Xu J, Kurup P, Bartos JA, Patriarchi T, Hell JW, Lombroso PJ. STriatal-enriched protein tyrosine phosphatase (STEP) regulates Pyk2 activity. *J Biol Chem* 2012; **287**: 20942–20956.
35. Paul S, Nairn AC, Wang P, Lombroso PJ. NMDA-mediated activation of the tyrosine phosphatase STEP regulates the duration of ERK signaling. *Nat Neurosci* 2003; **6**: 34–42.
36. Xu J, Kurup P, Zhang Y, Goebel-Goody SM, Wu PH, Hawasli AH *et al.* Extrasynaptic NMDA receptors couple preferentially to excitotoxicity via calpain-mediated cleavage of STEP. *J Neurosci* 2009; **29**: 9330–9343.
37. Zhang Y, Kurup P, Xu J, Carty N, Fernandez SM, Nygaard HB *et al.* Genetic reduction of striatal-enriched tyrosine phosphatase (STEP) reverses cognitive and cellular deficits in an Alzheimer's disease mouse model. *Proc Natl Acad Sci USA* 2010; **107**: 19014–19019.
38. Nakazawa T, Komai S, Tezuka T, Hisatsune C, Umemori H, Semba K *et al.* Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. *J Biol Chem* 2001; **276**: 693–699.
39. Paul S, Snyder GL, Yokakura H, Picciotto MR, Nairn AC, Lombroso PJ. The dopamine/D1 receptor mediates the phosphorylation and inactivation of the protein tyrosine phosphatase STEP via a PKA-dependent pathway. *J Neurosci* 2000; **20**: 5630–5638.
40. Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC *et al.* Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc Natl Acad Sci USA* 2005; **102**: 491–496.
41. Venkitaramani DV, Paul S, Zhang Y, Kurup P, Ding L, Tressler L *et al.* Knockout of striatal enriched protein tyrosine phosphatase in mice results in increased ERK1/2 phosphorylation. *Synapse* 2009; **63**: 69–81.
42. Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, Calvert CR, Jokel ES, Fienberg AA *et al.* Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. *J Neurophysiol* 2002; **88**: 3010–3020.
43. Bateup HS, Svenningsson P, Kuroiwa M, Gong S, Nishi A, Heintz N *et al.* Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. *Nat Neurosci* 2008; **11**: 932–939.
44. Jarskog LF, Gilmore JH, Selinger ES, Lieberman JA. Cortical bcl-2 protein expression and apoptotic regulation in schizophrenia. *Biol Psychiatry* 2000; **48**: 641–650.
45. Torrey EF, Webster M, Knable M, Johnston N, Yolken RH. The stanley foundation brain collection and neuropathology consortium. *Schizophr Res* 2000; **44**: 151–155.
46. Yuan P, Zhou R, Wang Y, Li X, Li J, Chen G *et al.* Altered levels of extracellular signal-regulated kinase signaling proteins in postmortem frontal cortex of individuals with mood disorders and schizophrenia. *J Affect Disord* 2010; **124**: 164–169.
47. Grayson B, Idris NF, Neill JC. Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav Brain Res* 2007; **184**: 31–38.
48. Beraki S, Kuzmin A, Tai F, Ogren SO. Repeated low dose of phencyclidine administration impairs spatial learning in mice: blockade by clozapine but not by haloperidol. *Eur Neuropsychopharmacol* 2008; **18**: 486–497.

49. Hu Y, Zhang Y, Venkitaramani DV, Lombroso PJ. Translation of striatal-enriched protein tyrosine phosphatase (STEP) after beta1-adrenergic receptor stimulation. *J Neurochem* 2007; **103**: 531–541.
50. Colledge M, Snyder EM, Crozier RA, Soderling JA, Jin Y, Langeberg LK *et al.* Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron* 2003; **40**: 595–607.
51. Guo L, Wang Y. Glutamate stimulates glutamate receptor interacting protein 1 degradation by ubiquitin-proteasome system to regulate surface expression of GluR2. *Neuroscience* 2007; **145**: 100–109.
52. Deutsch SI, Hitri A. Measurement of an explosive behavior in the mouse, induced by MK-801, a PCP analogue. *Clin Neuropharmacol* 1993; **16**: 251–257.
53. Deutsch SI, Rosse RB, Riggs RL, Koetzner L, Mastropaolo J. The competitive NMDA antagonist CPP blocks MK-801-elicited popping behavior in mice. *Neuropsychopharmacology* 1996; **15**: 329–331.
54. Elsworth JD, Jentsch JD, Morrow BA, Redmond Jr DE, Roth RH. Clozapine normalizes prefrontal cortex dopamine transmission in monkeys subchronically exposed to phencyclidine. *Neuropsychopharmacology* 2008; **33**: 491–496.
55. Jentsch JD, Redmond Jr DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH. Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. *Science* 1997; **277**: 953–955.
56. Jentsch JD, Tran A, Le D, Youngren KD, Roth RH. Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. *Neuropsychopharmacology* 1997; **17**: 92–99.
57. Hashimoto K, Fujita Y, Shimizu E, Iyo M. Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol. *Eur J Pharmacol* 2005; **519**: 114–117.
58. Noda Y, Yamada K, Furukawa H, Nabeshima T. Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. *Br J Pharmacol* 1995; **116**: 2531–2537.
59. Nabeshima T, Hiramatsu M, Furukawa H, Kameyama T. Effects of acute and chronic administrations of phencyclidine on the levels of serotonin and 5-hydroxyindoleacetic acid in discrete brain areas of mouse. *Life Sci* 1985; **36**: 939–946.
60. Kyosseva SV, Owens SM, Elbein AD, Karson CN. Differential and region-specific activation of mitogen-activated protein kinases following chronic administration of phencyclidine in rat brain. *Neuropsychopharmacology* 2001; **24**: 267–277.
61. Chen Q, Zhou Z, Zhang L, Wang Y, Zhang YW, Zhong M *et al.* Tau protein is involved in morphological plasticity in hippocampal neurons in response to BDNF. *Neurochem Int* 2012; **60**: 233–242.
62. Gao XM, Sakai K, Roberts RC, Conley RR, Dean B, Tamminga CA. Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am J Psychiatry* 2000; **157**: 1141–1149.
63. Heckers S, Konradi C. Hippocampal neurons in schizophrenia. *J Neural Transm* 2002; **109**: 891–905.
64. Meador-Woodruff JH, Hogg Jr AJ, Smith RE. Striatal ionotropic glutamate receptor expression in schizophrenia, bipolar disorder, and major depressive disorder. *Brain Res Bull* 2001; **55**: 631–640.
65. Meador-Woodruff JH, Watson SJ. Postmortem studies in schizophrenic brain. *J Psychiatr Res* 1997; **31**: 157–158.
66. Pilowsky LS. Probing targets for antipsychotic drug action with PET and SPET receptor imaging. *Nucl Med Commun* 2001; **22**: 829–833.
67. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S *et al.* Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 2002; **71**: 877–892.
68. Johnstone M, Thomson PA, Hall J, McIntosh AM, Lawrie SM, Porteous DJ. DISC1 in schizophrenia: genetic mouse models and human genomic imaging. *Schizophr Bull* 2011; **37**: 14–20.
69. Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H *et al.* Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proc Natl Acad Sci USA* 2003; **100**: 8987–8992.
70. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993; **361**: 31–39.
71. Fatemi SH, Folsom TD, Reutiman TJ, Novak J, Engel RH. Comparative gene expression study of the chronic exposure to clozapine and haloperidol in rat frontal cortex. *Schizophr Res* 2012; **134**: 211–218.
72. Girgenti MJ, Nisenbaum LK, Bymaster F, Terwilliger R, Duman RS, Newton SS. Antipsychotic-induced gene regulation in multiple brain regions. *J Neurochem* 2010; **113**: 175–187.
73. Bingol B, Wang CF, Arnott D, Cheng D, Peng J, Sheng M. Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. *Cell* 2010; **140**: 567–578.
74. Cohen BD, Rosenbaum G, Luby ED, Gottlieb JS. Comparison of phencyclidine hydrochloride (Sernyl) with other drugs. Simulation of schizophrenic performance with phencyclidine hydrochloride (Sernyl), lysergic acid diethylamide (LSD-25), and amobarbital (Amytal) sodium; II. Symbolic and sequential thinking. *Arch Gen Psychiatry* 1962; **6**: 395–401.
75. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 1991; **148**: 1301–1308.
76. Ellison G, Keys A, Noguchi K. Long-term changes in brain following continuous phencyclidine administration: an autoradiographic study using flunitrazepam, ketanserin, mazindol, quinuclidinyl benzilate, piperidyl-3,4-3H(N)-TCP, and AMPA receptor ligands. *Pharmacol Toxicol* 1999; **84**: 9–17.
77. Ellison GD, Keys AS. Persisting changes in brain glucose uptake following neurotoxic doses of phencyclidine which mirror the acute effects of the drug. *Psychopharmacology* 1996; **126**: 271–274.
78. Molteni R, Calabrese F, Racagni G, Fumagalli F, Riva MA. Antipsychotic drug actions on gene modulation and signaling mechanisms. *Pharmacol Ther* 2009; **124**: 74–85.
79. Shin JK, Malone DT, Crosby IT, Capuano B. Schizophrenia: a systematic review of the disease state, current therapeutics and their molecular mechanisms of action. *Curr Med Chem* 2011; **18**: 1380–1404.

Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)