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

NC Carty1, J Xu1, P Kurup1, J Brouillette1, SM Goebel-Goody1, DR Austin2, P Yuan2, G Chen2, PR Correa3, V Haroutunian4, C Pittenger1,3,5 and PJ Lombroso1,3,6

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. STEPβ1 is a brain-enriched protein tyrosine phosphatase that dephosphorylates a regulatory tyrosine on GluN2B, thereby promoting its internalization. Here, we report that STEPβ1 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 STEPβ1 after MK-801 treatment is due to a disruption in the ubiquitin proteasome system that normally degrades STEPβ1. 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 STEPβ1 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 STEPβ1, and, consequently, increased surface expression of GluN1/GluN2B receptors. Taken together, our findings suggest that STEPβ1 accumulation may contribute to the pathophysiology of SZ. Moreover, we show a mechanistic link between neuroleptic treatment, STEPβ1 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).1–13 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.1,5 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 STEPβ1 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 STEPβ1-mediated dephosphorylation of GluN2B and GluA2 promotes their internalization,29,30 while dephosphorylation of the kinases inactivates them.32,33,35–37 Elevated STEPβ1 reduces synaptic expression of GluN2B-containing NMDARs via two mechanisms: (1) dephosphorylation of GluN2B at Tyr1472 (refs 29, 30, 37) and (2) dephosphorylation and inactivation of Fyn Tyr420 (ref. 32), a kinase that phosphorylates GluN2B at Tyr1472 (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 STEPβ1 activity and consequently NMDAR trafficking. D1 receptor stimulation...
activates protein kinase A and leads to the phosphorylation of STEP61, at a regulatory serine (Ser221) within the substrate-binding KIM domain, thereby preventing STEP61 from interacting with and dephosphorylating its substrates.\(^{39,40}\) When STEP61 is phosphorylated at this site, or in STEP knockout (KO) mice, the tyrosine phosphorylation of STEP61 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 STEP61, dopamine signaling and NMDAR function, we hypothesized that dysregulation of STEP61 might contribute to the pathophysiology of SZ. We find elevated STEP61 levels in postmortem anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC) of two different cohorts of SZ patients, as well as in frontal cortex of mice treated with psychotomimetics. We also demonstrate that antipsychotics inactivate STEP61, leading to increased NMDAR phosphorylation and surface expression. These results suggest that the inactivation of STEP61 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 preparations 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 \(-80^\circ C\) 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\(\mu\)M; Tocris, Minneapolis, MN, USA) or PCP (100\(\mu\)M; Sigma, Ronkonkoma, NY, USA) for the time points indicated. 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 Tyr1472 (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\(\mu\)M acetic acid and diluted 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.

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 \(P \leq 0.05\) 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 described27 to determine whether a group spent more time with the novel object compared with the chance value of 15 sec.

Results

STEP61 is elevated in the cortex of SZ patients. We first examined STEP61 expression in brain homogenates prepared from anterior cingulate cortex of a well-characterized group of SZ patients from the Stanley Foundation, compared with ag-, sex- and PMI-matched control patients (SZ: n = 12; controls: n = 12; Supplementary Table S1).46 STEP61 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 STEP61 levels. We again observed a significant increase in STEP61 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.

MK-801 increases STEP61 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 STEP61 observed in human SZ tissue. We first examined STEP61 after stimulating cortical cultures with the NMDAR antagonist MK-801. STEP61 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 STEP61 phosphorylation (Ser221) (P<0.01), suggesting an overall increase in STEP61 activity. Consistent with this expectation, the tyrosine phosphorylation of STEP61-regulated sites on GluN2B (Tyr1472), Pyk2 (Tyr 402) and ERK1/2 (Tyr 204/187) were significantly increased at the NMDAR antagonist MK-801. STEP61 levels were significantly decreased 10 min post injection (P<0.01; Figure 2b). The tyrosine phosphorylation of GluN2B, Pyk2 and ERK1/2 was also significantly decreased (P<0.05; Figure 2d).

In contrast to the cultured neuron findings (although ERK1/2 phosphorylation remained low even 60 min post injection). These results likely reflect pharmacokinetic complexities in vivo in vivo. GLN2B 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 in vivo in vivo.
**Role of STEP in schizophrenia**

NC Carty et al

**Figure 2** MK-801 treatment increases STEP61 activity and decreases tyrosine phosphorylation of STEP61 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 STEP61, while also significantly reducing STEP61 phosphorylation at Ser221 (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 STEP61 substrates at sites that STEP is known to dephosphorylate, including GluN2B at Tyr1472 (pGluN2B), Pyk2 at Tyr402 (pPyk2) and ERK1/2 at Tyr204/187 (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 STEP61 levels and attenuated STEP61 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.

**Figure 2** MK-801 treatment increases STEP61 activity and decreases tyrosine phosphorylation of STEP61 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 STEP61, while also significantly reducing STEP61 phosphorylation at Ser221 (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 STEP61 substrates at sites that STEP is known to dephosphorylate, including GluN2B at Tyr1472 (pGluN2B), Pyk2 at Tyr402 (pPyk2) and ERK1/2 at Tyr204/187 (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 STEP61 levels and attenuated STEP61 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.

**Figure 2** MK-801 treatment increases STEP61 activity and decreases tyrosine phosphorylation of STEP61 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 STEP61, while also significantly reducing STEP61 phosphorylation at Ser221 (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 STEP61 substrates at sites that STEP is known to dephosphorylate, including GluN2B at Tyr1472 (pGluN2B), Pyk2 at Tyr402 (pPyk2) and ERK1/2 at Tyr204/187 (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 STEP61 levels and attenuated STEP61 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.

**Figure 2** MK-801 treatment increases STEP61 activity and decreases tyrosine phosphorylation of STEP61 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 STEP61, while also significantly reducing STEP61 phosphorylation at Ser221 (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 STEP61 substrates at sites that STEP is known to dephosphorylate, including GluN2B at Tyr1472 (pGluN2B), Pyk2 at Tyr402 (pPyk2) and ERK1/2 at Tyr204/187 (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 STEP61 levels and attenuated STEP61 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 STEP61 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 STEP61 immunoprecipitated from MK-801-treated and control cultured neurons. Immunoprecipitated STEP61 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 GABAβ2/3 (*P* = 0.79) that are not STEP61 substrates (Figure 3b). As observed previously (Figure 2), MK-801 significantly increased STEP61 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 GABAβ2/3 remained unchanged (*P* > 0.05; Figure 3c). Taken together, our findings indicate that MK-801 increased STEP61 levels and activity, decreased the tyrosine phosphorylation of STEP61 substrates and reduced the surface expression of GluN2B-containing NMDARs.
Accumulation of STEP61 occurs via blockade of the ubiquitin proteasome system. We tested several mechanisms that might explain the MK-801-induced increase in STEP61. Translation of STEP is regulated in part by the PI3K-Akt and ERK pathways. 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 STEP61, 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 STEP61 levels (Figure 3d). These results indicate that the observed increase in STEP61 levels following MK-801 treatment was not due to increased transcription or translation.

STEP61 is normally ubiquitinated and degraded by the ubiquitin proteasome system (UPS), and disruption of the UPS leads to accumulation of ubiquitinated proteins. An increase in ubiquitinated STEP61 was observed following MK-801 treatment. Glutamate receptor-interacting protein 1 (GRIP1) served as a positive control, as it is degraded by the UPS. An increase in ubiquitinated STEP61 was observed following MK-801 treatment. Glutamate receptor-interacting protein 1 (GRIP1) served as a positive control, as it is degraded by the UPS. An increase in ubiquitinated STEP61 was observed following MK-801 treatment. Glutamate receptor-interacting protein 1 (GRIP1) served as a positive control, as it is degraded by the UPS.
UPS increases STEP61 levels. The UPS can be activated by NMDAR signaling, and blocking NMDARs leads to rapid accumulation of several proteins normally degraded by the UPS. To determine whether disruptions in the UPS mediate the MK-801-induced increase in STEP61, we measured polyubiquitinated STEP61 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 STEP61 (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 STEP61.

Neuroleptics inactivate STEP61 and increase the phosphorylation of its substrates. Our findings thus far demonstrate that MK-801 increased both STEP61 expression and activity in synaptosomal fractions. As increased STEP61 activity leads to loss of GluN2B-containing NMDARs from neuronal membrane surfaces, we next investigated whether antipsychotic medications exert their beneficial effects by inactivating STEP61. We first examined the phosphorylation of STEP61 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 STEP61, 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 STEP61 and activation of its substrates. No significant changes were observed in total levels of GluN2B, ERK1/2 or STEP61 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 STEP61 and its substrates were examined in synaptosomal (P2) fractions from frontal cortex (Figures 4c–h).

Haloperidol significantly increased STEP61 phosphorylation (Ps < 0.05) and concomitantly enhanced the tyrosine phosphorylation of GluN2B, Pyk2 and ERK1/2 (GluN2B and ERK1/2: Ps < 0.005; Pyk2: Ps < 0.05; Figures 4c–f). The atypical antipsychotics, clozapine and risperidone, similarly increased STEP61 phosphorylation (Ps < 0.05 and Ps < 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 (Ps = 0.06) (Figure 4g). Similar to the results using SKF-82958 and sulpiride, subchronic treatment of haloperidol did not alter total STEP61 levels in P2 fractions (+ 0.8 ± 5.4%; Ps > 0.05), while clozapine and risperidone did (+ 135.1 ± 30.5% and + 74.9 ± 15.4%, respectively; Ps < 0.05). As a positive control, antipsychotic treatment significantly increased the phosphorylation of dopamine and cAMP-regulated phosphoprotein-32 (Thr32) (clozapine: Ps < 0.05; Figure 4h), which is in agreement with prior studies. Comparable results were observed for these measures in the striatum and hippocampus (data not shown).

STEP KO mice are less sensitive to behavioral abnormalities elicited by the psychotomimetic PCP. If increased activity of STEP61, 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, 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 STEP61 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 STEP61 phosphorylation (Ps < 0.01) and increased STEP61 levels (Ps < 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). STEP61 phosphorylation was significantly decreased in P2 fractions (Ps < 0.01), while total STEP61 levels were increased (Ps < 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 STEP61 activity and levels, leading to decreased tyrosine phosphorylation of STEP61 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 intermediate 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 (Ps < 0.001), STEP KO mice were significantly less hyperactive than WT mice in response to PCP (significant dose by genotype interaction; Ps < 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. 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
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 $\text{STEP}_61$, $\text{STEP}_61$ substrates and dopamine cAMP-regulated phosphoprotein-32. (Figure 4a, b) Primary cortical cultures were treated with the DA D1R agonist SKF-82958 (25 or 50 $\mu$M) or D2R antagonist sulpiride (25 or 50 $\mu$M). Both drugs at either concentration significantly increased phosphorylation of $\text{STEP}_61$ at Ser$^{221}$ ($\text{pSTEP}$), 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) $\text{STEP}_61$, (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; *$P < 0.05$). In all panels, phosphoproteins were first normalized to the total protein and then to GAPDH. 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, 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. Moreover, positron emission tomography scans suggest that hippocampal NMDAR binding is significantly reduced in untreated, but not antipsychotic-treated, SZ patients. Genetic association studies have identified several candidate genes implicated in NMDAR stability or trafficking, including neuregulin 1, disrupted-in-schizophrenia 1, and calcineurin. Given that NMDARs have an integral role in cognitive function and synaptic plasticity, 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 and aberrant NMDAR signaling in SZ. STEP levels are increased in two independent patient populations and two different brain regions (DLPCF and anterior cingulate cortex). It has been suggested that medication status might affect gene expression in SZ. Given that STEP levels are elevated in SZ patient populations both on...
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 STEP61 in human postmortem tissue.

We propose that increased STEP61 levels promote the dephosphorylation of STEP61 substrates, including GluN2B, leading to internalization and hypofunction of NMDARs. In support of this, acute treatment with the psychotomimetics MK-801 and PCP increase STEP61 levels and activity, decrease tyrosine phosphorylation of STEP61 substrates and reduce the surface expression of GluN2B-containing NMDARs in cultured neurons and in vivo. While we did not find significant changes in STEP61 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. Further studies will be needed to determine whether acute disruption of STEP61 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 Ca2+-dependent mechanisms, and degradation of glutamate receptor-interacting protein 1 is NMDAR-dependent. Moreover, Ca2+ influx through NMDARs and L-type voltage-gated Ca2+ channels activates CaMKII, a necessary component for proteasomal trafficking into dendritic spines. We found that MK-801 treatment impairs the UPS and led to an increase in STEP61. Previous studies establish that disruption of the UPS by β-amyloid promotes an elevation of STEP61. 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 STEP61 accumulation is an important contributor to the observed findings. Although we provide evidence for UPS involvement in the psychotomimetic-induced increase in STEP61 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 STEP61 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. Unlike other psychotomimetic drugs, PCP elicits positive and cognitive deficits, and cognitive deficits in humans. Our data are consistent with STEP KO being less sensitive than WT to PCP-induced hyperactivity and cognitive deficits. As STEP KO exhibited increased cortical NMDAR surface expression and ERK1/2 phosphorylation, it is possible that the enhanced tyrosine phosphorylation of STEP61 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 KO mice are less sensitive behaviorally to PCP than WT.

Another important finding was that neuroleptic treatments regulate STEP61 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. 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. 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 STEP61.

Administration of D2 antagonists to cortical cultures led to the phosphorylation of STEP61, a modification that prevents STEP61 from interacting with its substrates. Subchronic haloperidol treatment in mice led to a similar increase in STEP61 phosphorylation with no change in total STEP61 levels. Subchronic risperidone and clozapine treatment significantly increased the phosphorylation of STEP61. However, both of these neuroleptics also increased STEP61 levels in synaptosomal (P2) fractions. While the mechanism by which these two drugs increase STEP61 expression remains to be determined, it is possible that subchronic neuroleptic treatment promotes trafficking of STEP61 to the synapse as a homeostatic mechanism. As STEP61 phosphorylation is increased by antipsychotics to a greater magnitude than its expression level, it appears that increased STEP61 in the P2 fraction is insufficient to reverse the decreased STEP61 activity and enhanced NMDAR surface expression elicited by neuroleptic treatment.

In summary, our results implicate STEP61 as one likely mediator of NMDAR hypofunction in SZ. Increased STEP61 levels are present both in postmortem SZ brains and mice exposed to psychotomimetics, and genetically eliminating STEP61 results in progeny less sensitive behaviorally to these drugs. One possible explanation for increased STEP61 levels is impaired UPS function in SZ. It is likely that other mechanisms affect STEP61 expression, such as mutations in specific UPS enzymes or disruption of microRNAs that regulate STEP61 expression. The present findings establish that neuroleptics used to treat SZ act, at least in part, via STEP61 inactivation. Dysregulation of STEP61 represents a previously unappreciated pathophysiological contributor to SZ and implicates STEP61 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 (P.J.L.). 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; RCC performed the majority of behavioral tests with assistance from PCR and CP; PK and JB assisted in tissue and cell culture preparation and biochemistry; JK 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, Moghadam B. Effects of NMDA receptor antagonists: implications for the pathophysiology of schizophrenia. Arch Gen Psychiatry 2002; 59: 683–694.
3. Jentsch JD, Roth RH. The neuropeharmacophory of phenoclycin: 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: 1868–1902.
5. Meador-Woodruff JH, Clinton SM, Beneyto M, McCullumsmith RE. Molecular abnormalities of the glutamate synapse in the thalami in schizophrenia. J Neurocy 2003; 1003: 75–93.
6. Stone JM, Erlanson K, Anand A, Squassante L, Teneggi V, Bressan RA et al. Relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy: a [11C](++)S-1261 SPET study. Psychopharmacology 2008; 197: 401–408.
7. Krystal JH, Jasper LP, Seibyl JP, Freeman GK, Delaney P, Brenner JD et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychomimetic, 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 phenoclycin administration in the nonhuman primate: implications for schizophrenia. Adv Pharmacol 1998; 42: 810–814.
9. Stoit G, Snyder LH. Effects of the NMDA antagonist ketamine on task-switching: 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 W, Bast T, Feldon J. Microinjection 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 prepulse inhibition: re-examination in two strains of rats. Pharmacol Biochem Behav 2006; 85: 20942–20956.
12. Nakazawa T, Komai S, Tezuka T, Haisatune C, Umemori H, Semba K et al. Characterization of Fyn-mediated tyrosine phosphorylation pathway on GluR2 epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor: J Biol Chem 2001; 276: 693–699.
13. Venkitaramani DV, Picciotto MR, Lombroso PJ. The dopamine/D1 receptor mediates the phosphorylation and inactivation of the protein tyrosine phosphatase STEP. J Biol Chem 2001; 276: 401–408.
14. Paul S, Nairn AC, Lombroso PJ. A protein tyrosine phosphatase that regulates the duration of ERK signaling. J Biol Chem 2003; 278: 24274–24279.
15. Venkitaramani DV, Moua PJ, Picciotto MR, Lombroso PJ. Striatal-enriched tyrosine phosphatase (STEP) knockout mice have enhanced hippocampal memory. J Neurosci 2011; 31: 2238–2246.
16. Xu J, Kurup P, Bartos JA, Patnaik A, Heil JW, Lombroso PJ. SR141716A inhibits striatal-enriched tyrosine phosphatase (STEP) knockout mice have enhanced hippocampal memory. J Neurosci 2011; 31: 2238–2246.
17. Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. Striatal-enriched protein phosphatase in postmortem frontal cortex of individuals with mood disorders. Mol Psychiatry 2011; 16: 7821–7831.
18. Boulanger LM, Lombroso PJ, Raghunathan A, Durin MJ, Wahle P, Naegle JR. Cellular and molecular characterization of a brain-enriched tyrosine phosphatase. J Neurosci 1995; 15: 1532–1544.
19. Jentsch JD, Elsworth JD, Redmond Jr DE, Roth RH. Dysregulation of NMDA receptors coupled preferentially to excitotoxicity via calpain-mediated cleavage of the NMDA receptor subunit protein SYT4. J Biol Chem 2002; 277: 24274–24279.
20. Jentsch JD, Elsworth JD, Miller JR, Redmond Jr DE, Roth RH. Dysregulation of mesoprefrontal dopamine neurons induced by acute and repeated phenoclycin administration in the nonhuman primate: implications for schizophrenia. Adv Pharmacol 1998; 42: 810–814.
21. Stoit G, Snyder LH. Effects of the NMDA antagonist ketamine on task-switching: evidence for specific impairments of executive control. Neuropsychopharmacology 2006; 31: 1675–1681.
22. 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.
23. Sharma E, Zhao F, Bressan RA et al. Relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy: a [11C](++)S-1261 SPET study. Psychopharmacology 2008; 197: 401–408.
24. Krystal JH, Jasper LP, Seibyl JP, Freeman GK, Delaney P, Brenner JD et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994; 51: 199–214.
25. Jentsch JD, Elsworth JD, Taylor JR, Redmond Jr DE, Roth RH. Dysregulation of mesoprefrontal dopamine neurons induced by acute and repeated phenoclycin administration in the nonhuman primate: implications for schizophrenia. Adv Pharmacol 1998; 42: 810–814.
49. Hu Y, Zhang Y, Venikaramani DV, Lombroso PJ. Translation of striatal-enriched protein tyrosine phosphatase (STEP) after beta-1-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, Hsi 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, Koetner L, Mastopalo J. The competitive NMDA antagonist CPP blocks MK-801-elicted popping behavior in mice. Neuropharmacology 1996; 35: 329–331.
54. Elsworth JD, Jentsch JD, Morrow BA, Redmond Jr DE, Roth RH. Clozapine normalizes cortical-dependent cognition in the rat. Life Sci 2005; 76: 251–257.
55. Thomas MJ, Zhou Z, Zhang L, Wang Y, Zhang YW, Zhong M et al. Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc Natl Acad Sci USA 2003; 100: 8987–8992.
56. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361: 31–39.
57. Patemi SH, Folsom TD, Reutnam 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.
58. Gao XM, Sakai K, Roberts RC, Conley RR, Dean B, Tamminga CA, et al. Ionotropic glutamate receptor modulation and signaling mechanisms. Neuropsychopharmacology 2001; 26: 253–257.
59. Yasmin E, Baldassano M, Barch E, Kwon H, et al. Tau protein is involved in drug resistant schizophrenia. J Neurol Neurosurg Psychiatry 2010; 81: 33–38.
60. Elsworth JD, Jentsch JD, Morrow BA, Redmond Jr DE, Roth RH. Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 1997; 277: 953–955.
61. 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.
62. Hashimoto K, Fuchita Y, Shizuka E, Iyo M. Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of dizapine, but not haloperidol. Eur J Pharmacol 2005; 519: 114–117.
63. Noda Y, Yamada K, Furukawa H, Nibeshima T. Enhancement of immunity in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. Br J Pharmacol 1995; 116: 2531–2537.
64. Nibeshima T, Himatsu 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 mice. Life Sci 1985; 36: 939–946.
65. Koosusva S, Owens SM, Elbein AD, Kanon CN. Differential and region-specific activation of mitogen-activated protein kinases following chronic administration of phencyclidine in rat brain. Neuropsychopharmacology 2001; 24: 267–277.
66. 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.
67. 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.
68. Heckers S, Konradi C. Hippocampal neurons in schizophrenia. J Neural Transm 2002; 109: 891–905.
69. 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.
70. Meador-Woodruff JH, Watson SJ. Postmortem studies in schizophrenic brain. J Psychiatr Res 1997; 31: 157–158.
71. Plochowsky LS. Probing targets for antipsychotic drug action with PET and SPET receptor imaging. Nucl Med Commun 2001; 22: 829–833.
72. Steffensen H, Sigurdsson E, Steinhodtröf T, Bjomsdottir S, Sigmundsson T, Ghosh S et al. Neuregulin 1 and susceptibility to schizophrenia. Arch Gen Psychiatry 2002; 59: 877–892.
73. 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.
74. Miyakawa T, Leiter LM, Gerber DJ, Gnanetdinov RR, Sotnikova TD, Zeng H et al. Conditional calichein knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc Natl Acad Sci USA 2003; 100: 8987–8992.
75. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361: 31–39.
76. Patemi SH, Folsom TD, Reutnam 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.
77. Girogetti ML, Nisenbaum LR, Bymaster F, Tewtillger R, Duman RS, Newton SS. Antipsychotic-induced gene regulation in multiple brain regions. J Neurochem 2010; 113: 175–187.
78. Bingle G, Wang CF, Amott D, Cheng D, Peng J, Sheng M. Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. Cell 2010; 140: 567–578.
79. 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.
80. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991; 148: 1301–1308.
81. Ellson GD, Keys AS. Persistent changes in brain glucose uptake following neurotrophic doses of phencyclidine which mirror the acute effects of the drug. Psychopharmacology 1996; 126: 271–274.
82. Molteni R, Cabalbrese F, Racagni G, Fumagalli F, Riva MA. Antipsychotic drug actions on gene modulation and signaling mechanisms. Pharmacol Ther 2009; 124: 74–85.
83. 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)