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Increased expression of receptor phosphotyrosine phosphatase-β/ζ is associated with molecular, cellular, behavioral and cognitive schizophrenia phenotypes

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Schizophrenia is a serious and chronic mental disorder, in which both genetic and environmental factors have a role in the development of the disease. Neuregulin-1 (NRG1) is one of the most established genetic risk factors for schizophrenia, and disruption of NRG1 signaling has been reported in this disorder. We reported previously that NRG1/ErbB4 signaling is inhibited by receptor phosphotyrosine phosphatase-β/ζ (RPTP β/ζ) and that the gene encoding RPTPβ/ζ (*PTPRZ1)* is genetically associated with schizophrenia. In this study, we examined the expression of RPTPβ/ζ in the brains of patients with schizophrenia and observed increased expression of this gene. We developed mice overexpressing RPTPβ/ζ (*PTPRZ1*-transgenic mice), which showed reduced NRG1 signaling, and molecular and cellular changes implicated in the pathogenesis of schizophrenia, including altered glutamatergic, GABAergic and dopaminergic activity, as well as delayed oligodendrocyte development. Behavioral analyses also demonstrated schizophrenia-like changes in the *PTPRZ1*-transgenic mice, including reduced sensory motor gating, hyperactivity and working memory deficits. Our results indicate that enhanced RPTPβ/ζ signaling can contribute to schizophrenia phenotypes, and support both construct and face validity for *PTPRZ1*-transgenic mice as a model for multiple schizophrenia phenotypes. Furthermore, our results implicate RPTPβ/ζ as a therapeutic target in schizophrenia.
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Introduction

Schizophrenia (MIM 181500) is a serious and chronic mental disorder with a 1% lifetime prevalence, characterized by positive symptoms (that is, delusions and hallucinations), negative symptoms (that is, social withdrawal, anhedonia, and blunted affect) and cognitive dysfunction (that is, deficits in attention, working memory and executive function).[1–3] Population, family and twin studies indicate that schizophrenia is highly heritable, with additional non-genetic factors involved in the disease.[4,5] On the basis of pharmacological and postmortem brain studies, it has been argued that altered neuronal signaling and circuitry, including the glutamatergic,[6–8] dopaminergic[9–11] and GABAergic pathways,[12,13] as well as white matter abnormalities,[14,15] have important roles in the development of the disease, however, detailed molecular and cellular mechanism underlying schizophrenia pathogenesis are still unknown.

Several lines of evidence indicate that abnormalities in neuregulin-1 (NRG1) signaling, mediated via the ErbB receptor family, are involved in schizophrenia. Genetic association of the gene encoding NRG1 (*NRG1*) with schizophrenia is one of the most well-replicated findings in genetic dissection of schizophrenia.[16–25] NRG1 is a multifunctional protein that has important roles in development of central nervous system,[26] and it has been shown that *NRG1*+/− mice demonstrate several molecular changes resembling abnormalities observed in schizophrenia, such as altered glutamatergic,[27,28] dopaminergic[29] and GABAergic signaling,[30–34] as well as altered oligodendrocyte and myelin[35,36] development.[29,37–39] These mice have also shown abnormal behaviors relevant to schizophrenia.[19,33] Altered expression of *NRG1* mRNA in the postmortem brain of schizophrenia has been reported[40–42] and recent finding using cultured lymphocytes suggest that NRG1 signaling is suppressed in patients with schizophrenia.[43,44] There is also emerging data implicating ERBB4 in schizophrenia. Genetic association of *ERBB4*[20,35] and gene–gene interaction between *ERBB4* and *NRG1* in schizophrenia[20,36] and relevant cognitive dysfunction have been reported.[45–47] In addition, altered expression of ERBB4 in brain tissue from patients with schizophrenia has been observed.[48] Finally, ErbB4+/− and transgenic mice harboring dominant negative form of ErbB4 showed molecular and behavioral changes relevant to schizophrenia.[19,29]

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We previously reported that a central nervous system-specific phosphatase, receptor phosphotyrosine phosphatase-β/ζ (RPTPβ/ζ) is complexed with ErbB4 via MAGI scaffolding proteins, such that NRG1/ErbB4 signaling is negatively regulated by RPTPβ/ζ *in vitro*. We also found that the *PTPRZ1* gene, which encodes RPTPβ/ζ, is genetically associated with schizophrenia.[49] Recent copy number variation analyses have identified deletions in genes coding for ErbB4 and MAGI2[50] in schizophrenia.

On the basis of these results, we hypothesized that increased expression of RPTPβ/ζ would inhibit NRG1 signaling *in vivo*, thus, leading to schizophrenia phenotypes. To directly test this idea, we examined the expression levels of RPTPβ/ζ in the brains of patients with schizophrenia, and developed and characterized a mouse overexpressing RPTPβ/ζ. To generate the mouse we used a bacterial artificial chromosome (BAC) transgenic approach that has robust and stable expression, with expression regulated under the control of human *cis* elements, and hence closest to the native state.

Materials and methods

Postmortem human brain specimens. Total RNA from dorsolateral prefrontal cortex of 105 samples was provided by the Stanley Foundation (35 each for schizophrenia, bipolar disorder and healthy controls). The three diagnostic groups were matched by age, sex, race, postmortem interval (PMI), pH, side of brain, mRNA quality, smoking status and lifetime antipsychotics dose. Detailed demographic information is available (http://www.stanleyresearch.org/dnn/Portals/0/Stanley/Array%20Collection%20Demographic%20Details%20Chart-Final.pdf). cDNA was synthesized using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA, USA). Primers for short and long form of RPTPβ and four endogenous controls (*ACTB*, *GAPD*, *GUSB* and *PPIA*) were designed using the software provided for the Universal Probe Library system (Roche Applied Science; https://www.roche-applied-science.com/sis/rtpcr/upl/acenter.jsp). Analysis of variance (ANOVA) with Tukey's *post hoc* tests was used for group comparison.

Mice. All animal procedures were approved by the Institutional Animal Care and Use Committee of Mount Sinai School of Medicine and the James J Peters Veterans Affairs Medical Center. Human BAC RP11-367M11, containing the entire *PTPRZ1* gene was introduced to C57BL/6xC3H F1 hybrid mice and the allele was maintained on a C57Bl6 background. The BAC is 180 kb in length and with flanking genomic sequences of 110 and 42 kB around the *PTPRZ1* gene. The human gene codes for a protein that shares 85% identity and 82% similarity with the mouse gene product.

***In situ* hybridization.** *In situ* hybridization was performed using digoxygenin-labeled cRNA probes, with sequence corresponding to 5228–5746 bp of mouse cDNA for RPTPβ/ζ (NM_001081306). Sense and antisense probes were synthesized and digoxygenin-labeled by *in vitro* transcription using Sp6 and T7 RNA polymerase (Roche, Basel, Switzerland). *In situ* hybridization was performed using IsHyb *in situ* hybridization kit (BioChain, Hayward, CA, USA) on 20-μm thick sagittal cryosections, according to the manufacturer's protocol.

NRG1 stimulation in hippocampal slices. A NRG1 stimulation assay was performed as previously described.[51] Hippocampi were dissected and 200-μm thick slices were prepared using a tissue chopper (Vibratome, Bannockburn, IL, USA). Slices were incubated with 200 ng ml^{-1} of recombinant human neuregulin (NRG1-beta EGF domain, R&D systems, Minneapolis, MN, USA) or vehicle in ice-cold Krebs-Ringer solution containing 118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose supplemented with complete protease EDTA-free inhibitor tablets (Roche Applied Science, Penzberg, Germany) and protein phosphatase inhibitor set (Millipore, Temecula, CA, USA) (pH 7.4) with aeration. After 20 min of stimulation, ice-cold EGTA was added to terminate the reaction. Tissue was then homogenized in lysis buffer (150 mM NaCl, 20 mM Tris-HCl, 10% glycerol, 5 mM EDTA and 1% NP-40) supplemented with protease and phosphatase inhibitors, and separated on 10% SDS-PAGE gels. Phosphorylation of extracellular signal-regulated kinase (ERK) was normalized to ERK levels. ANOVA with Tukey's *post hoc* tests was used for group comparisons.

Quantitative PCR assays. Total RNA was isolated and cDNA was prepared using the high-capacity cDNA archive kit (Applied Biosystems). The mRNA levels of the target genes were measured by qPCR using the Roche Universal Probe Library system (Roche Applied Science, Indianapolis, IN, USA). Four endogenous control genes (*Ppia*, *Actb*, *Gusb* and *B2m*) were also analyzed for reference. The ABI Prism 7900 sequence detection system (Applied Biosystems) was used for the PCR reaction. Relative expression levels for control sample were calculated with qBase software (http://medgen.ugent.be/qbase/).[52] Unpaired *t*-tests were used for group comparisons.

Western blotting. Western blotting was performed according to standard protocols. The antibodies and their concentration used were as follows: RPTPβ (mouse monoclonal; 1:250; Molecular Probes, Eugene, OR, USA), phosophotyrosine-ERK (SC-7383; mouse monoclonal, 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), ERK (SC-1647; mouse monoclonal, 1:1000, Santa Cruz Biotechnology), NR2B (rabbit polyclonal; 1:1000; R&D Systems), phospho-NR2B (rabbit polyclonal; 1:1000; R&D Systems), GSK3β (mouse monoclonal, 1:2000, Transduction Laboratories, Franklin Lakes, NJ, USA), GSK3β phosphorylated at Ser9 (rabbit polyclonal, 1:1000, Cell Signaling, Danvers, MA, USA).

Electrophysiology. Sections were prepared as described above and electrophysiology was carried out as we have recently described.[53,54] In brief, individual slices were placed in a submerged recording chamber and were perfused with recording solution at a rate of 1–2 ml min^{-1}. Schaffer collateral–commissural fibers were stimulated every 20 s. Stimulus intensity was adjusted to evoke 30–40% maximal stimulation, and the initial slopes of field excitatory

postsynaptic responses were measured for field potential recordings. Long-term potentiation was induced by either tetanic or theta-burst stimulation. The responses were divided by the initial slope of the first field potential to normalize the data. Tetanic stimulation consisted of five trains of 100 Hz stimulation, lasting 200 ms at an intertrain interval of 10 s. Synaptic fatigue was studied by measuring the synaptic responses during the first high-frequency train. Theta-burst stimulation consisted of 10 trains of four pulses delivered at a frequency of 100 Hz separated by 200 ms.

Immunohistochemistry. Mice were deeply anesthetized and perfused intracardially with 4% (parvalbumin (PV) staining) or 2% (oligodendrocyte staining) paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were removed, postfixed overnight in the same solution at 4 °C and cut into 25-μm thick sagittal sections. Sections were then incubated in blocking buffer PGBA (0.1 M phosphate buffer, 0.1% gelatin, 1% bovine serum albumin, 0.002% sodium azide) containing 10% normal goat serum for 30 min and then incubated overnight at room temperature with primary antibodies. Primary antibodies and their concentration used were as follows: PV (mouse monoclonal; 1:1000, Millipore), NG2 (rabbit monoclonal; 1:200; Millipore) and CC1/APC (mouse monoclonal; 1:50; EMD Bioscience, San Diego, CA, USA). After rinsing in phosphate-buffered saline, sections were incubated with the relevant secondary antibodies (goat anti-mouse IgG-HRP, 1:250, Santa Cruz Biotechnology for PV, Alexa Fluor 488 goat anti-mouse for CC1/APC, Alexa Fluor 555 donkey anti-rabbit for NG2, both 1:10 000, Invitrogen, Carlsbad, CA, USA). 4',6-Diamidino-2-phenylindole (1:1000; Molecular Probes) was used as a nuclear counterstain. Parvalbumin-positive cells were counted using Zeiss Axiovision2 (Zeiss, Oberkochen, Germany) and Stereo investigator software (MBF Bioscience, Williston, VT, USA). A minimum of four sections per mouse and three mice were evaluated for the quantification. Student's *t*-tests were used for comparisons. Confocal images of NG2 and CC1/APC-positive cells were obtained using a Zeiss LSM 510 Meta confocal laser-scanning microscope (Zeiss). Optical sections of confocal epifluorescence images were sequentially acquired with LSM5 Image Browser software (Zeiss). Immunoreactive cells for NG2 and CC1/APC were counted with the ImageJ cell counting plug-in (http://rsbweb.nih.gov/ij/). A minimum of three sections per mouse and three mice were evaluated for the quantification. χ^2-tests were used for group comparisons.

High-performance liquid chromatography. Dopamine, homovanillic acid and 3,4-dihydroxyphenylacetic acid levels were measured as previously described,[55] using an isocratic high-performance liquid chromatography system with electrochemical detection, consisting of a pump (ESA model 582, ESA, Chelmsford, MA, USA), automatic injector (ESA model 542 autosampler) and a Coulochem III detector (ESA) in conjunction with a guard cell (ESA model 5020) and an analytical cell (ESA model 5014B). Cell potentials were set at 350, 150 and 220 mV for the guard, E1 and E2 cells, respectively. A volume of 25 μl per sample was injected with a flow rate of 0.6 ml min^{-1}, and passed through a 150-mm column (ESA model MD-150X3.2) with a particle size of 3 μm and pore size of 12 nm. The mobile phase consisted of 10% acetonitrile, 90 mM NaH_2PO_4, 1.7 mM 1-octane sulfonic acid, 50 mM citric acid and 50 μM EDTA, pH 3. Data were collected and analyzed using the EZStart software (Agilent Technologies, Santa Clara, CA, USA). Standards were run in parallel. Unpaired *t*-tests were used for group comparisons.

Behavioral analyses. Three cohorts of transgenic mice and wild-type littermate controls were studied. Each cohort contained 20 transgenic mice and 20 wild-type animals (10 males and 10 females for each genotype). Behavioral testing was performed in the Mouse and Rat Phenotyping Shared Research Facility of Mount Sinai School of Medicine located at the James J Peters VA Medical Center, Bronx, NY, USA. Animals were bred at the Mount Sinai School of Medicine and transferred to the Veterinary Medical Unit at the James J Peters VA Medical Center for behavioral testing at 10 weeks of age. After transfer, animals were singly housed and allowed an acclimation period before initiation of testing, which begun at 14–16 weeks of age. All mice were housed in standard clear plastic cages at a constant temperature of 21–22 °C, with rooms kept under 12-h light/dark cycles with lights switched on at 0700 h. Access to food and water was *ad libitum*. Animals were housed on racks in random order to prevent rack position effects. Testing was performed by a single investigator blinded to the genotype of the animals.

Open-field test. Open-field testing was performed as previously described.[56] Mice were brought into the experimental room and were allowed to acclimate for 1 h before testing. Each mouse was placed for 30 min in a square open-field box under low lighting conditions. An automated infrared beam array system measured locomotion in the center and periphery of the test arena. Experimentally naive mice were used for all experiments and activity data were collected in 5-min intervals over the 120-min open-field session. Where indicated, MK-801 (0.3 mg kg^{-1}) was injected intraperitoneally at 25 min before behavioral testing. Similarly, amphetamine (2 mg kg^{-1}) was injected intraperitoneally at 25 min before behavioral testing for five consecutive days. Total distance traveled was analyzed with two-way ANOVA (genotype and treatment) for the study with MK-801. Total distance traveled during the first 40 min was compared by using a three-way ANOVA (genotype, day (repeated measure) and treatment) for the study with amphetamine. Sphericity was assessed using the Mauchly test.

Prepulse inhibition test. Prepulse inhibition was assessed as previously described.[56] Mice were brought into the experimental room and were allowed to acclimate for a minimum of 1 h before testing. Mice were individually placed in a startle enclosure in the startle chamber with a background white noise of 70 dB and were left undisturbed for 10 min. Then a session was started that consisted of 10 trials of each condition (no discrete stimulus, prepulse only, prepulse-pulse and pulse only) presented in a pseudorandomized order. Prepulse–pulse trial started with a 50-ms null period, followed by a 20-ms prepulse white noise of 72 dB. After a

100-ms delay, the startle stimulus was presented (a 40-ms 120 dB white noise), followed by a 290-ms recording time. The total duration of each trial was 500 ms. The formula 100−((startle response on acoustic prepulse + pulse stimulus trials/pulse stimulus response alone trials) × 100) was used to calculate percent prepulse inhibition. Unpaired *t*-tests were used for group comparison.

Light/dark transition test. Light/dark transition testing was performed as previously described.[56] Mice were placed in the dark side of a two-chamber light/dark apparatus and were allowed to move freely between the two chambers with the door kept open for 10 min. The total number of transitions and time spent in each side were recorded. Unpaired *t*-tests were used for group comparisons.

Social interaction. Social interaction was tested using a three-chamber social interaction and recognition test.[57] The subject was offered a choice between investigating an unfamiliar mouse and a novel object. Interaction time was collected in 5-min segments for each trial. Unpaired *t*-tests were used for group comparisons.

Eight-arm radial maze test. Working memory was measured by win–shift task using an eight-arm radial maze.[58] Mice were food-restricted to 80–85% of their free-feeding weights. Mice were first habituated to an eight-arm radial maze for 10 days by freely foraging for food rewards located at the end of the maze arms. Training was given during daily trials for 10 days that consisted of two phases. During the first phase, mice were put in the center of the maze and four random arms were opened. The animals then had 5 min to collect all of the food rewards located at the end of the arms. The mice were then removed into a holding chamber for 1 min, and the center and arms of the maze were cleaned with 70% ethanol. During the second phase, the mice were returned to the center of the maze and all eight arms of the maze were opened. However, only the four previously unopened arms contained food reward for which the animals had 5 min to retrieve. During testing, the delay between phases was increased from 1 min to 5, 30 and 60 min on consecutive days. Errors were classified as within-phase (re-entries into target arms) or across-phase (entries into arms baited in the first phase). Data were analyzed for each error type by using two-way repeated measures ANOVA.

Conditional fear conditioning test. For contextual fear conditioning, the conditioning chamber (Coulbourne FreezeFrame system, White Hall, PA, USA) consisted of a metal and plexiglass chamber ($7 \times 7 \times 12''$) equipped with a grid floor for delivery of the conditioned stimulus and an overhead camera to monitor movement and freezing. The conditioning chamber was placed inside a soundproof isolation cubicle. Training occurred in the dark with background noise generated by a small fan. Each mouse was placed inside the conditioning chamber for 2 min before delivery of a 2 s foot shock (0.6 mA) accompanied by a white house-light. Each mouse remained in the chamber for an additional 60 s after which another foot shock was given. Each mouse was returned to its home cage after another 30 s. Freezing was defined as a lack of movement (except for respiration). Baseline freezing was measured during 0–2 mins of the training day. Contextual fear memory was determined at 24 h after training by measuring freezing during a 3-min test in the conditioning chamber. Unpaired *t*-tests were used for group comparisons.

Statistical analysis. Statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA). The results were expressed as mean values ± s.e.m. Statistical significance was set at level of 0.05.

Results

Increased expression of RPTPβ/ζ in schizophrenia. We had previously observed a genetic association of *PTPRZ1* with schizophrenia and were interested in assessing whether expression of the gene product RPTPβ/ζ might be altered in schizophrenia as further evidence that it may contribute to pathogenesis. Analysis by qPCR showed increased expression of RPTPβ/ζ in the dorsolateral prefrontal cortex of schizophrenia (Figure 1) (ANOVA with Tukey's *post hoc* tests, $P < 0.05$). Although the samples were matched on these measures, we also carried out multiple regression analysis which showed that possible confounding factors including PMI, brain pH, brain side, smoking status and lifetime antipsychotic dose did not affect expression of RPTPβ/ζ (data not shown).

Generation of *PTPRZ1*-transgenics. On the basis of the postmortem brain data above, we generated mice overexpressing human RPTPβ/ζ by introducing a BAC,[59] which included the entire human *PTPRZ1* gene (RP11-367M1). The *PTPRZ1*-transgenics were viable and showed

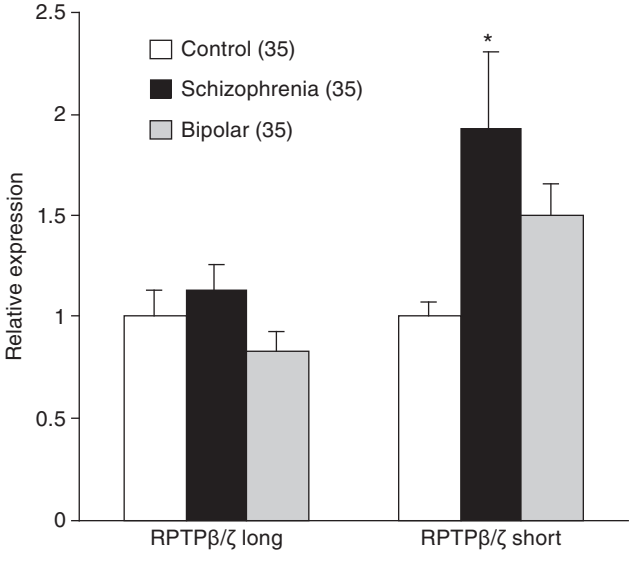

Figure 1 Elevated RPTPβ/ζ expression in schizophrenia. Analysis by qPCR showed increased expression of the RPTPβ/ζ-short form in the dorsolateral prefrontal cortex of patients with schizophrenia compared with controls. $n = 35$ for each diagnostic group. Data represent mean ± s.e.m. $^*P < 0.05$. Error bars represent s.e.m.

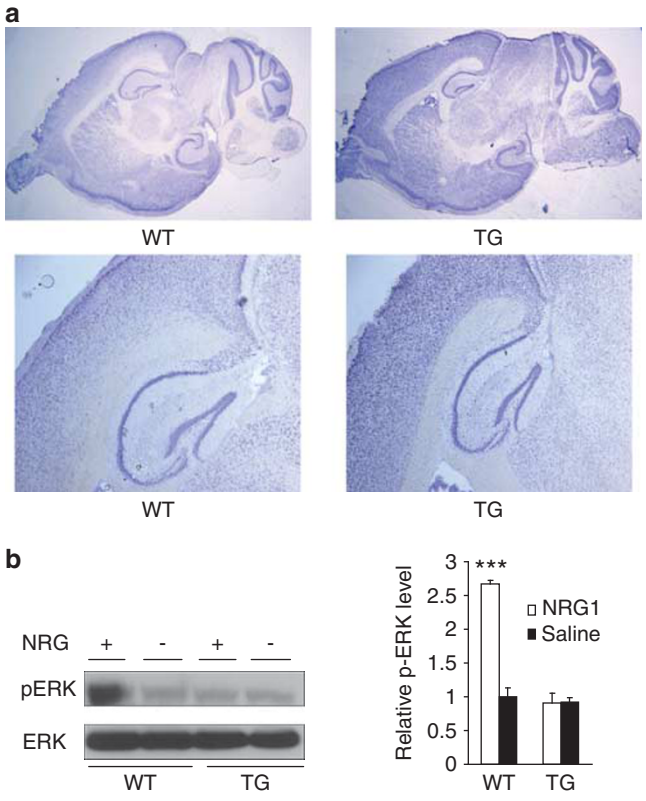

Figure 2 Generation and validation of *PTPRZ1*-transgenics. (**a**) Increased RPTPβ/ζ expression in *PTPRZ1*-transgenics. *In situ* hybridization was carried out on wild-type and transgenic littermates. Lower magnification (top) and higher magnification (bottom) images demonstrate increased expression in the transgenics, with similar expression patterns of *PTPRZ1* in both. (**b**) Reduced NRG1 signaling in *PTPRZ1*-transgenics. Hippocampal slices of wild-type mice and *PTPRZ1*-transgenics were dissected and incubated with recombinant human neuregulin (NRG1) or vehicle in ice-cold Krebs-Ringer solution. Phosphorylation levels of ERK, a downstream signaling molecule of Nrg1, was measured by western blotting. Left, representative experiment; right, quantification (mean ± s.e.m.) of three experiments. WT, wild-type mice; TG, transgenic mice. ***$P<0.001$.

no obvious alterations in gross brain structure or hippocampal cytoarchitecture. *In situ* hybridization using a probe that recognizes both human and mouse transcripts of *PTPRZ1* demonstrated that expression of *PTPRZ1* was increased in the transgenics but the regional distribution pattern was similar between the transgenics and wild-type littermates (Figure 2a). We also confirmed that BAC transgenesis resulted in ~50% increase in expression of both RPTPβ/ζ mRNA and protein (Supplementary Figures S1a–c).

Inhibition of NRG1 signaling in *PTPRZ1*-transgenics. To determine whether increased expression of RPTPβ/ζ led to decreased NRG1 signaling, hippocampal slices were prepared from the *PTPRZ1*-transgenics and littermate controls and stimulated with NRG1. As we predicted based on our prior *in vitro* studies,[49] stimulation increased levels of phosphorylated ERK in wild-type mice, but not in transgenic littermates (Figure 2b) (ANOVA with Tukey's *post hoc* tests, $P<0.001$).

Reduced GABA signaling in *PTPRZ1*-transgenics. One of the most established findings from postmortem brain studies of schizophrenia is the reduced expression of GAD67 in the prefrontal cortex of patients, and this change has been attributable to a reduced number of PV-positive cells in this region.[60,61] It has also been demonstrated that NRG1/Erbb4 signaling controls the development of inhibitory circuits in the brain, and that reduced signaling suppresses expression of GAD67 in interneurons,[32] providing a mechanistic link between altered NRG1 signaling and reduced GABA signaling in schizophrenia.[21] Interestingly, we found reduced expression of GAD67 in the medial prefrontal cortex of *PTPRZ1*-transgenic mice, when examined by qPCR (Figure 3a) (unpaired *t*-test, $P<0.05$). In addition, we observed that the numbers of PV-positive neurons were significantly reduced in this region of the *PTPRZ1*-transgenics, as compared with wild-type littermates (Figure 3b) (unpaired *t*-test, $P<0.05$).

Increased dopamine signaling in *PTPRZ1*-transgenics. We next evaluated dopaminergic activity in *PTPRZ1*-transgenic mice. Dopamine abnormalities are considered core features of schizophrenia, given the molecular targets of antipsychotics and that elevated dopamine signaling precedes the onset of schizophrenia and can lead to cognitive dysfunction.[62] We found ~40% increase in the levels of dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid in the striatum in the transgenics compared with wild-type littermates (Figure 3c) (unpaired *t*-test, $P<0.05$). Analysis of qPCR showed overexpression of the dopamine D2 receptor in this region (Figure 3d) (unpaired *t*-test, $P<0.001$). These findings are of interest in light of a recent study showing that disruption of NRG1/Erb signaling in oligodendrocytes induces hyperdopaminergia in the striatum.[29]

Levels of phosphorylated GSK3β, a key mediator of D2 receptor signaling, were significantly reduced, which could be reversed by clozapine (Supplementary Figures S2a, b). Amphetamine treatment induces synaptic dopamine release, and can cause psychotic symptoms in healthy controls while exaggerating symptoms in patients with schizophrenia.[63] The *PTPRZ1*-transgenics showed an increased response to amphetamine, consistent with enhanced dopaminergic activity in these mice (Figure 3e) (three-way ANOVA (genotype, day (repeated measure) and treatment), $P<0.05$).

Altered NMDA signaling in *PTPRZ1*-transgenics. NRG1 also has an important role in glutamatergic neurotransmission,[21] previously implicated in psychosis and schizophrenia in part because non-competitive agonists of the *N*-methyl-D-aspartate receptors induce psychotic features resembling schizophrenia.[6] One effect of NRG1 signaling is the enhanced phosphorylation of the NR2B subunit, and this phosphorylation is attenuated in heterozygous *Nrg1*- and *Erbb4*-deficient mice (*Nrg1*+/− and *Erbb4*+/−)[28] and reduced phosphorylation may contribute to *N*-methyl-D-aspartate receptor dysfunction in schizophrenia. Phosphorylation of NR2B was significantly reduced in the hippocampus of the *PTPRZ1*-transgenics (Figure 4a) (unpaired *t*-test, $P<0.001$), without

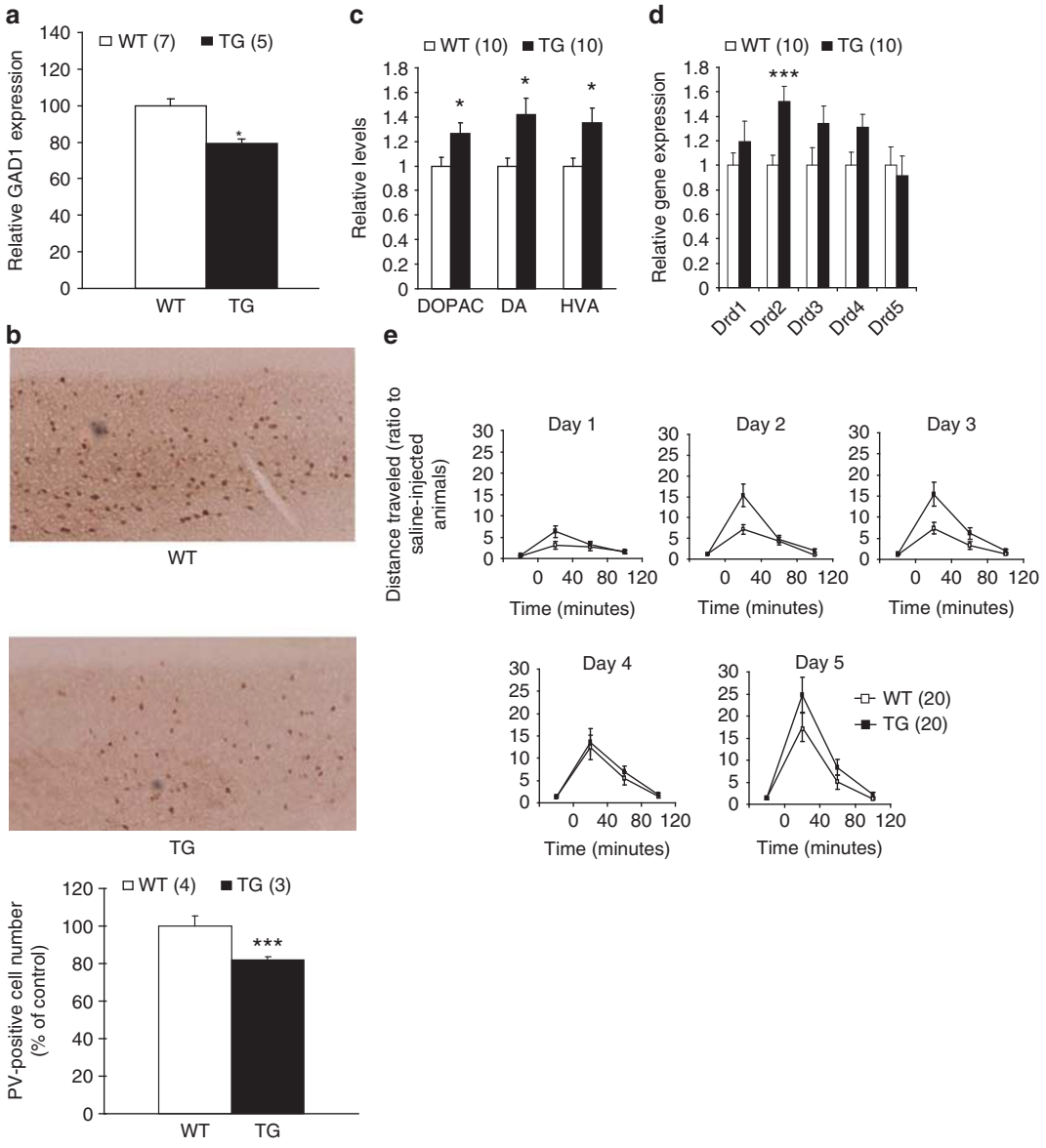

Figure 3 Reduced GABA and increased dopamine signaling in *PTPRZ1*-transgenics. (**a**) Reduced expression of GAD1 in the medical prefrontal cortex of *PTPRZ1*-trangenics. Data represent mean ± s.e.m. for expression of GAD1 determined by qPCR. (**b**) Decreased numbers of parvalbumin (PV)-positive cells in the medical prefrontal cortex of *PTPRZ1*-trangenics. Upper, representative images of the staining pattern; lower, quantification of the number of cells stained (mean ± s.e.m.). WT, wild-type mice; TG, transgenic mice. (**c**) Elevated levels of dopamine and metabolites in *PTPRZ1*-trangenics. Levels of dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid were assessed by high-performance liquid chromatography. Data represent mean ± s.e.m. (**d**) Increased expression of *Drd2* in the striatum of *PTPRZ1*-transgenics. Gene expression was determined by qPCR and error bar represents s.e.m. (**e**) Enhanced amphetamine induced locomotion in *PTPRZ1*-trangenics ($P<0.001$). Amphetamine (2 mg kg^{-1}) was injected intraperitoneally at 25 min before open-field testing for five consecutive days. Data represent mean ± s.e.m. WT, wild-type mice; TG, transgenic mice. $^{*}P<0.05$; $^{***}P<0.001$.

corresponding changes in the expression of NR2B or other NMDA or AMPA receptors (Supplementary Figures S3a, b). *PTPRZ1*-transgenics showed increased locomotor activity when challenged with the *N*-methyl-D-aspartate receptor antagonist MK801 (Figure 4b) (unpaired *t*-test, $P<0.05$), consistent with altered glutamate signaling in these mice.

As intact glutamatergic transmission is necessary for long-term potentiation, we next measured long-term potentiation in these mice and found that long-term potentiation induced by either tetanus or theta-burst stimulation was significantly reduced in the *PTPRZ1*-transgenic mice (Figure 4c) (unpaired *t*-test, $P<0.05$), similar to what has been reported in *Nrg1*+/− mice.[28]

Delayed oligodendrocyte development in *PTPRZ1*-transgenics. White matter and oligodendrocyte abnormalities have been implicated in schizophrenia[14,64] and NRG1 has been implicated in myelination.[29] RPTPβ/ζ is expressed in oligodendrocytes, including adult oligo-

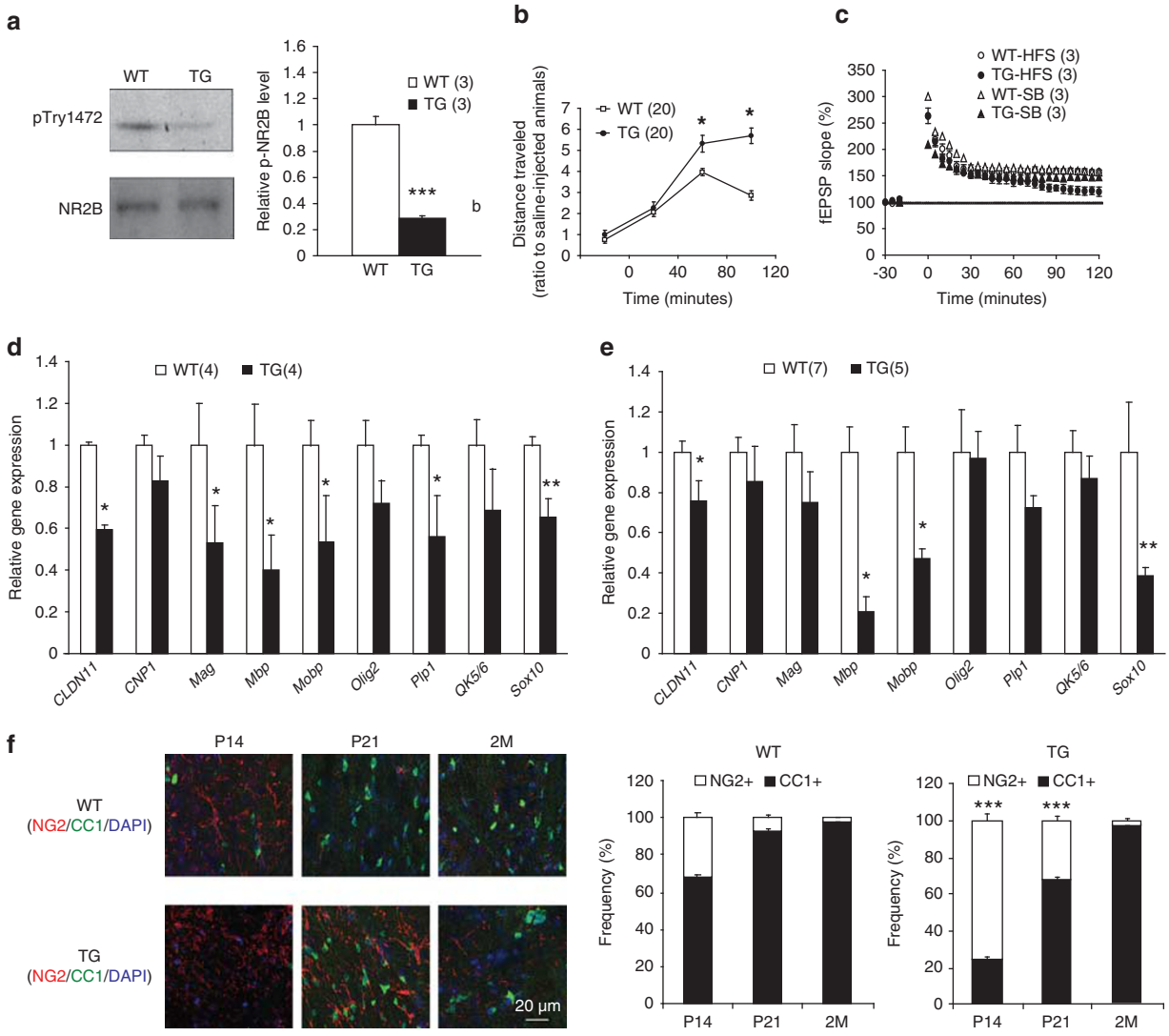

Figure 4 Altered *N*-methyl-D-aspartate signaling and delayed oligodendrocyte development in *PTPRZ1*-transgenics. (**a**) Reduced phosphorylation of NR2B in *PTPRZ1*-transgenics. Left, representative experiment; right, quantification (mean ± s.e.m.) of three experiments. (**b**) Enhanced MK-801 induced locomotion in *PTPRZ1*-trangenics. MK-801 (0.3 mg kg^{-1}) was injected intraperitoneally at 25 min before behavioral testing. Data represent mean ± s.e.m. (**c**) Impaired long-term potentiation in *PTPRZ1*-trangenics. Long-term potentiation was induced either by a high-frequency stimulus or theta-burst stimulus. In both cases there was reduced long-term potentiation in the transgenics ($P<0.05$). WT, wild-type mice; TG, transgenic mice. Error bar represents s.e.m. (**d**, **e**) Reduced expression of oligodendrocyte- and myelin-related genes in 14- (**d**) and 21- (**e**) day-old *PTPRZ1*-transgenics. Data represent mean ± s.e.m. for gene expression determined by qPCR. (**f**) Delayed maturation of oligodendrocytes in *PTPRZ1*-transgenics. The corpus collosum was stained with markers for immature (NG2) and mature oligodendrocytes (CC1) in 14-day- (P14), 21-day- (P21) or 2-month (2M)-old mice. Left, representative confocal images; right, quantification (mean ± s.e.m.) of three animals. Scale bar = 20 μm. WT, wild-type mice; TG, transgenic mice. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

dendrocyte progenitors, and RPTPβ/ζ activity inhibits the differentiation of these cells.[65] *PTPRZ1*-trangenics showed a transient reduction in expression of oligodendrocyte- and myelin-related genes up to 3 weeks of age (Figures 4d and e) (unpaired *t*-test, $P<0.05$ for CLDN11, CNP1, Mag, Mbp, Mobp and Plp1, $P<0.01$ for Sox10 at 2 weeks of age; $P<0.05$ for CLDN11, Mbp, and Mobp, $P<0.01$ for Sox10 at 3 weeks of age). Immunohistochemical analyses demonstrated a delay in the development of oligodendrocyte lineage cells in the transgenic animals (Figure 4f) (χ^2-test, $P<0.001$).

Schizophrenia-related behavioral alterations in *PTPRZ1*-transgenics. *PTPRZ1*-transgenics showed reduced prepulse inhibition of startle (Figure 5a) (unpaired *t*-test, $P<0.05$), reflecting sensory gating abnormalities that are also observed in schizophrenia.[66] Furthermore, prepulse-elicited reactivity, considered to be more specific to psychotic symptoms of schizophrenia,[66] was significantly reduced in the transgenics (Figure 5b) (unpaired *t*-test, $P<0.05$). Importantly, the *PTPRZ1*-transgenics showed impaired working memory—considered to be a core cognitive dysfunction in schizophrenia—as shown by increased

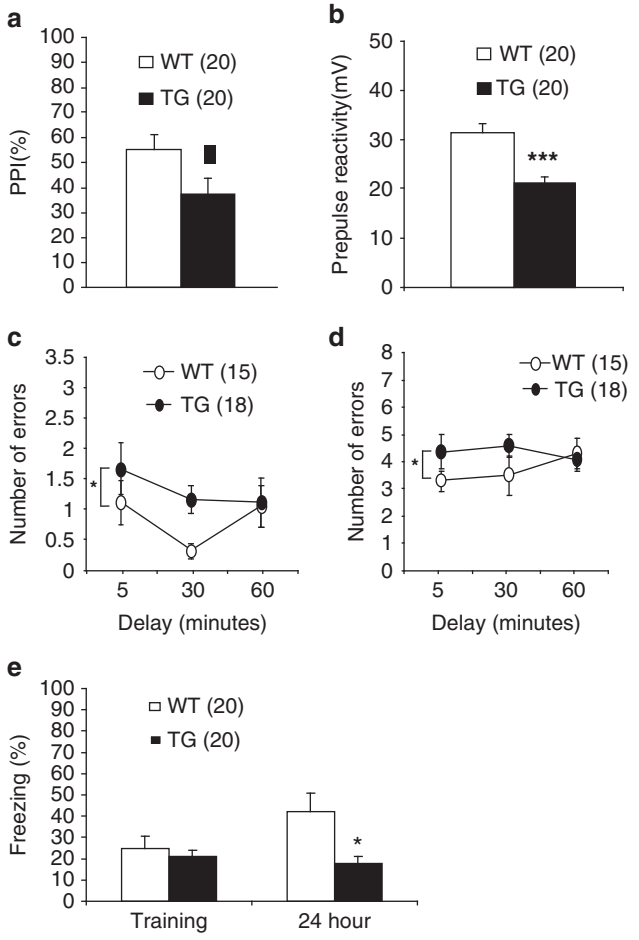

Figure 5 Behavioral and cognitive alterations in *PTPRZ1*-transgenics. (**a**, **b**) Blunted prepulse inhibition in *PTPRZ1*-trangenics. Prepulse inhibition was measured with a 78-dB prepulse stimulus. The test showed reduced prepulse inhibition (**a**) and prepulse-elicited reactivity (**b**) in the *PTPRZ1*-transgenics. Data represent mean ± s.e.m. (**c**, **d**) Deficits in working memory in *PTPRZ1*-trangenics. Eight-arm radial maze testing showed increased within phase (**c**) and across phase (**d**) error numbers in the *PTPRZ1*-transgenics. Data represent mean ± s.e.m. (**e**) Impaired associative learning and memory in conditional *PTPRZ1*-trangenics. Fear conditioning test showed reduced freezing time at 24 h after training. WT, wild-type mice; TG, transgenic mice; $^{*}P<0.05$; $^{***}P<0.001$.

errors in the win-shift task using the eight-arm radial maze test (Figures 5c and d) (two-way repeated measures ANOVA, $P<0.05$). In addition, the transgenics were impaired in a contextual fear conditioning test, suggesting problems in associative learning and memory (Figure 5e) (unpaired *t*-test, $P<0.05$). Other measures associated with the schizophrenia phenotype were also subtly altered, including increased locomotor activity, reduced social activity and increased anxiety (Supplementary Figures S4a–c).

Discussion

We observed that RPTPβ/ζ expression is upregulated in schizophrenia. One caveat with our study is that we measured expression of RPTPβ/ζ only in dorsolateral prefrontal cortex because of the limited availability of other tissue, therefore, we cannot conclude that RPTPβ/ζ is overexpressed more broadly; hence, our finding requires replication in an independent cohort and with additional brain regions. Interestingly, since we initiated this study, an additional study has reported increased expression of this gene in the amygdala in independent samples in schizophrenia.[67]

We observed increased expression of RPTPβ/ζ could inhibit NRG1 signaling in slices and led to several molecular features that capture aspects of schizophrenia, including reduced GABAergic signaling, enhanced dopamine signaling in the striatum and altered NMDA signaling, as well as behavioral and cognitive changes in mice. Furthermore, we observed delayed oligodendrocyte and myelin development in the *PTPRZ1*-transgenics, which reflects an additional deficit observed in schizophrenia. Taken together, our results indicate that increased expression of RPTPβ/ζ *in vivo* captures many key aspects of the molecular, cellular, behavioral and cognitive phenotypes of schizophrenia.

The mechanisms by which *PTPRZ1* overexpression leads to these multiple abnormalities are currently unknown but some relationships can be proposed. On the basis of recent studies showing that ErbB4 is predominantly expressed in interneurons in adult mouse brain[32,33] and that GABAergic dysfunction induced elevated dopaminergic activity,[68,69] one could speculate that increased expression of RPTPβ/ζ may inhibit ErbB4 function in interneurons leading to subsequent changes, including GABAergic dysfunction and elevated dopaminergic activity.

We also found additional molecular and behavioral changes relevant to glutamatergic signaling in the *PTPRZ1*-transgenics, however, effects of NRG1 on this pathway have not yet been completely clarified.[70] There is a possibility that these changes in glutamate signaling might be a consequence of direct interaction of RPTPβ/ζ with synaptic proteins such as PSD95[71] and MAGIs[72] rather than via the NRG1 pathway. It has been proposed that insults affecting myelination in early life could induce imbalances in the development of brain circuits relevant to schizophrenia pathogenesis,[73] hence, one can also speculate that delays in oligodendrocyte development in the transgenic animals will have impact on the development of the circuitry underlying glutamateric, GABAergic and/or dopaminergic signaling.

Some postmortem brain studies have suggested that NRG1 signaling is upregulated in schizophrenia.[42,51,60] Interestingly, a recent study using transgenic mice, which overexpress *NRG1*, demonstrated that even though these transgenics showed molecular changes in the opposite direction to the findings from postmortem brain studies of schizophrenia—including increased PV-positive cells, increased expression of oligodendrocyte markers and decreased expression of dopamine synthetic enzymes—they manifested almost the same behavioral phenotypes as Nrg +/− or ErbB4 +/− mice.[74] It is therefore possible that for certain critical pathways, over or under activity are equally disruptive and can produce overlapping end results.

Many schizophrenia-relevant phenotypes have been defined in mice ([75,76]; and see Supplementary Table S1) and extensive efforts have been made to develop mouse models with face, construct and predictive validity in schizophrenia. We have demonstrated that *PTPRZ1*-transgenic mice

demonstrate reduced NRG1/Erb signaling, which in turn is associated with many of these schizophrenia phenotypes (Supplementary Table S1), making it a potentially useful model for understanding the biology of schizophrenia. The diverse phenotypes affected in the *PTPRZ1*-transgenic mice indicate that they satisfy face validity for multiple facets of schizophrenia. The genetic association of *PTPRZ1* with schizophrenia that we previously reported, and the elevated expression of RPTPβ/ζ in the brain in schizophrenia shown here indicate that the model also has construct validity for schizophrenia. More studies will be needed to assess the degree to which the *PTPRZ1*-transgenic model has predictive validity, although we are encouraged to see that dopamine signaling abnormalities were reversed by clozapine. The elevated expression of RPTPβ/ζ in the brain in schizophrenia indicates that the development of specific inhibitors of RPTPβ/ζ activity and/or function represents a novel therapeutic approach for schizophrenia.

Conflict of interest

The authors declare no conflict of interest.

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