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

N Takahashi1,2, T Sakurai1,2*, O Bozdagi-Gunal1,2, NP Dorr1, J Moy1, L Krug1, M Gama-Sosa1,3, GA Elder1,3,4, RJ Koch3, RH Walker3,5, PR Hof6, KL Davis1 and JD Buxbaum1,2,6,7

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.

Translational Psychiatry (2011) 1, e8; doi:10.1038/tp.2011.8; published online 10 May 2011

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 glutameric,6–8 dopaminergic9–11 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 glutamatergic27,28 dopaminergic29 and GABAergic signaling,30–34 as well as altered oligodendrocyte and myelin35,36 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 reported40–42 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 ERBB430,35 and gene–gene interaction between ERBB4 and NRG130,36 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

1Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA; 2Friedman Brain Institute, Mount Sinai School of Medicine, New York, NY, USA; 3Neurology Service, James J Peters Veterans Affairs Medical Center, Mount Sinai School of Medicine, New York, NY, USA; 4Research and Development Service, James J Peters Veterans Affairs Medical Center, Mount Sinai School of Medicine, New York, NY, USA; 5Department of Neurology, Mount Sinai School of Medicine, New York, NY, USA; 6Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA and 7Department of Genetics, Mount Sinai School of Medicine, New York, NY, USA.

*Present address: Kyoto University School of Medicine, Medical Innovation Center, Laboratory for CNS Drugs (TK project).
Correspondence: Professor JD Buxbaum, Department of Psychiatry, Mount Sinai School of Medicine, One Gustave L Levy Place, Box 1668, New York, NY 10029, USA.
E-mail: joseph.buxbaum@mssm.edu

Keywords: animal model; dopamine; GABA; glutamate; neuregulin; schizophrenia

Received 28 March 2011; accepted 9 April 2011
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. Recent copy number variation analyses have identified deletions in genes coding for ErbB4 and MAGI2 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 (PPIA 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 comparisons.

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/6JxC3H 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 digoxigenin-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 digoxigenin-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. 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 CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃, 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/). 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), phosphotyrosine-ERK (SC-7383; mouse monoclonal, 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), ERK (SC-1647; mouse monoclonal, 1:1000, Santa Cruz Biotechnology), NRG1 (rabbit polyclonal; 1:1000; R&D Systems), phospho-NRG1 (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. 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), CC1/APC, Alexa Fluor 555 donkey anti-rabbit for NG2, both (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 1:10000, Invitrogen, Carlsbad, 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 1:10000, Invitrogen, Carlsbad, CA, USA). 4′,6-Diamidino-2-phenylindole in 0.1 M phosphate buffer, pH 7.2. The brains were cut into 25-μm-thick sagittal sections. Sections were then incubated overnight in the same solution at 4 °C 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 1:10000, 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 Axiovision 2 (Zeiss, Oberkochen, Germany) and Stereo investigator software (MBF Biotechnology, 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. χ²-tests were used for group comparisons.

**High-performance liquid chromatography.** Dopamine, homovanillic acid and 3,4-dihydroxyphenylacetic acid levels were measured as previously described, 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 NaH₂PO₄, 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 began 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. 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. 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 $100 - (\text{startle response on acoustic prepulse} + \text{pulse stimulus trials/pulse stimulus response alone trials}) \times 100$ 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 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 BMI, brain pH, brain size, 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
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.\textsuperscript{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,\textsuperscript{32} providing a mechanistic link between altered NRG1 signaling and reduced GABA signaling in schizophrenia.\textsuperscript{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.\textsuperscript{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.\textsuperscript{29}

Levels of phosphorylated GSK3\(\beta\), 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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{6} One effect of NRG1 signaling is the enhanced phosphorylation of the NRG2 subunit, and this phosphorylation is attenuated in heterozygous Nrg1- and Erbb4-deficient mice (Nrg1\(^{+/-}\) and Erbb4\(^{+/-}\))\textsuperscript{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...
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 and NRG1 has been implicated in myelination.29 RPTPβζ is expressed in oligodendrocytes, including adult oligo-
dendrocyte progenitors, and RPTP$\beta/\gamma$ activity inhibits the differentiation of these cells.\textsuperscript{65} PTPRZ1-trangenic 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) ($\chi^2$-test, $P<0.001$).

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

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Altered N-methyl-D-aspartate signaling and delayed oligodendrocyte development in PTPRZ1-trangenic. (a) Reduced phosphorylation of NR2B in PTPRZ1-trangenic. Left, representative experiment; right, quantification (mean ± s.e.m.) of three experiments. (b) Enhanced MK-801 induced locomotion in PTPRZ1-trangenic. 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-trangenic. 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) Reduced expression of oligodendrocyte- and myelin-related genes in 14- (d) and 21- (e) day-old PTPRZ1-trangenic. Data represent mean ± s.e.m. for gene expression determined by qPCR. (f) Delayed maturation of oligodendrocytes in PTPRZ1-trangenic. 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. *$P<0.05$; **$P<0.01$; ***$P<0.001$.}
\end{figure}
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 transgensics 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 RPTPζζζζ expression is upregulated in schizophrenia. One caveat with our study is that we measured expression of RPTPζζζζ only in dorsolateral prefrontal cortex because of the limited availability of other tissue, therefore, we cannot conclude that RPTPζζζζ 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 RPTPζζζζ 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 RPTPζζζζ 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 brain35,36 and that GABAergic dysfunction induced elevated dopaminergic activity,60,69 one could speculate that increased expression of RPTPζζζζ 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 RPTPζζζζ with synaptic proteins such as PSD9571 and MAGIs72 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 glutamatergic, 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 face satisfactory validity for multiple facets of schizophrenia. The genetic association of PTPRZ1 with schizophrenia that we previously reported, and the elevated expression of RPTPz/z 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 RPTPz/z in the brain in schizophrenia indicates that the development of specific inhibitors of RPTPz/z activity and/or function represents a novel thera-
peutic approach for schizophrenia.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. We acknowledge Dr Kevin A Kelly and The Mouse Genetics Shared Research Facility (SRF) at Mount Sinai for their help in generation of transgenic mice. We thank Dr Michelle Ehrlich for a critical reading of this manuscript and for helpful comments in experimental design. We also thank Dr Patricia Casacchia-Bonelli for her help and direction and William Janssen for providing support in the use of microscope. This study was supported by the National Institutes of Health Grant P50 MH063832 (JBD, KLD, PRH), the Mitsubishi Pharma Research Foundation (NT) and a Stanley Medical Foundation Research Grant (GER-1427, TS).

1. Jaaro-Peled H, Hayashi-Takagi A, Seshadri S, Kamiya A, Brandon NJ, Sawa A. Neurons contain glutamatergic NMDA receptors that are upregulated in the frontal cortex of schizophrenia patients. Arch Neurol 2004; 61: 872–877.

2. Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. Arch Gen Psychiatry 2004; 61: 969–704.

3. Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. Trends Neurosci 2006; 29: 362–368.

4. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: basic re-
conceptualization. Biol Psychiatry 2003; 54: 593–601.

5. Silberberg G, Darvasi A, Pinkas-Kramarski R, Navon R. The involvement of ErbB4 with NRG1 and ERBB4 genes with schizophrenia in a Japanese population. Proc Natl Acad Sci USA 2007; 104: 939–944.

6. Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia. J Clin Invest 2003; 11: 161–168.

7. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Trends Neurosci 2000; 23: 197–202.

8. Coyle JT, Tsai G. The NMDA receptor glycine modulatory site: a therapeutic target for schizophrenia? Trends Pharmacol Sci 2009; 30: 121–128.

9. Shiota S, Tochigi M, Shimada H, Ohashi J, Kasai K, Kato N et al. Neuregulin 1-stimulated phosphorylation of AKT in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. Proc Natl Acad Sci USA 2007; 104: 5219–5224.

10. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and mechanism for neuropsychiatric disorders. Translational Psychiatry 2009; 9: 606–609.
Increased expression of PTPRZ1 and schizophrenia

N Takahashi et al

44. Kei S, Benicky S, Kelterman O. Suppression of the P50 evoked response and neuregulin 1-induced AKT phosphorylation in first-episode schizophrenia. Am J Psychiatry 2010; 167: 444–450.

45. Nicodemus KK, Law AJ, Radulescu E, Luna A, Kolachana B, Vakkalanka R et al. Biological validation of increased schizophrenia risk with NRG1, ERBB4, and AKT1 epistasis via functional neuroimaging in healthy controls. Arch Gen Psychiatry 2010; 67: 891–1001.

46. Nicodemus KK, Luna A, Vakkalanka R, Goldberg T, Egan M, Straub RE et al. Further evidence for association between ErbB4 and schizophrenia and influence on cognitive intermediate phenotypes in healthy controls. Mol Psychiatry 2008; 11: 1063–1065.

47. Zuliani R, Moorhead TW, Bastin ME, Johnstone EC, Lawrie SM, Brambilla P et al. Genetic variants in the ErbB4 gene are associated with white matter integrity. J Neurosci 2008; 30: 539–543.

48. Law AJ, Kleinman JE, Weinberger DR, Weickert CS. Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. Hum Mol Genet 2007; 16: 129–141.

49. Buxbaum JD, Georgieva L, Young JJ, Plescia C, Kajiwara Y, Jiang Y. Increased expression of neuregulin 1-induced AKT phosphorylation in first-episode schizophrenia. Proc Natl Acad Sci USA 2010; 107: 915–921.

50. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 2008; 320: 136–140.

51. Hahn CG, Wang HY, Cho DS, Talbot K, Gur RE, Berrettini WH et al. Altered neuregulin 1–erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. Nat Med 2006; 12: 824–828.

52. Hellerman J, Mortier G, De Paepe A, Speelman F, Vanesloopenje J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 2007; 8: R19.

53. Bzdagi O, Wang XB, Nikkizcz JS, Anderson TR, Blais EB, Radice GL et al. Persistence of coordinated long-term potentiation and dendritic spine enlargement at mature hippocampal CA1 synapses requires N-cadherin. J Neurosci 2010; 30: 9884–9898.

54. Lilliehook C, Bzdagi O, Yoo J, Gomez-Ramirez M, Zaidi NF, Wasco W et al. Altered Abeta formation and long-term potentiation in a calsenilin knock-out. J Neurosci 2003; 23: 9097–9106.

55. Walker RH, Koch RJ, Moore C, Meshul CK. Subthalamic nucleus stimulation and lesioning have distinct state-dependent effects upon striatal dopamine metabolism. J Neurosci 2009; 30: 136–146.

56. Elder GA, Ragnauth A, Dorr N, Franciosi S, Schmeidler J, Haroutunian V et al. Altered neuregulin 1–erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. Nat Med 2006; 12: 824–828.

57. Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, Barbaro RP et al. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Schizophr Bull 2009; 35: 244–255.

58. Keri S, Beniczky S, Kelemen O. Suppression of the P50 evoked response and neuregulin and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35: 13–24.

59. Sm FJ, Lang JK, Walsdau B, Roy NS, Schwartz TE, Pichler WH et al. Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation. Ann Neurol 2006; 59: 763–779.

60. Li M, Shi J, Gao F, Niu L, Liao H, Zhang J et al. Increased neuregulin 1 expression is associated with white matter integrity. J Neurosci 2008; 28: 63–69.

61. Ting AK, Chen Y, Wen L, Yin DM, Shen C, Tao Y et al. Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons. J Neurosci 2011; 31: 15–25.

62. Simpson EH, Kellendonk C, Kandel E. A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. Neuron 2010; 65: 585–596.

63. Lanuelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phases. Biol Psychiatry 1999; 46: 56–72.

64. Takahashi N, Sakurai T, Davis KL, Buxbaum JD. Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35: 13–24.

65. Sim FJ, Lang JK, Walsdau B, Roy NS, Schwartz TE, Pichler WH et al. Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation. Ann Neurol 2006; 59: 763–779.

66. Cosmor PA, Yee BK, Feldon J, Theodoridou A, Studerus E, Vollenweider FX. Impaired prepulse inhibition and prepulse-elicited reactivity but intact reflex circuit excitability in unmedicated schizophrenia patients: a comparison with healthy subjects and medicated schizophrenia patients. Schizophr Bull 2009; 35: 244–255.

67. Piantonopoulos H, Woo TU, Lim MP, Lange N, Berretta S. Extracellular matrix-glia abnormalities in the amygdala and entorhinal cortex of subjects diagnosed with schizophrenia. Arch Gen Psychiatry 2010; 67: 155–166.

68. Balla A, Nattini ME, Sershen H, Latija A, Dunlop DS, Javitt DC. GABAB/NMDA receptor interaction in the regulation of extracellular dopamine levels in rodent prefrontal cortex and striatum. Neuropharmacology 2009; 58: 915–921.

69. Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S et al. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci 2009; 31: 234–242.

70. Ting AK, Chen Y, Wen L, Yin DM, Shen C, Tao Y et al. Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons. J Neurosci 2011; 31: 15–25.

71. Fujikawa A, Chow JP, Shimizu H, Fukada M, Suezki R, soda M. Tyrosine phosphorylation of ErbB4 is enhanced by PDE9 and repressed by protein tyrosine phosphatase receptor type Z. J Biochem 2007; 142: 343–350.

72. Adamsky K, Arnold K, Sabanay H, Peles E. Functional protein MAGI-3 interacts with receptor tyrosine phosphatase beta (RPTP beta) and tyrosine-phosphorylated proteins. J Cell Sci 2003; 116: Pt 7: 1279–1289.

73. Morkogdan M, Yamauchi T, Tatsuki K, Okuda H, Tokeda T, Kucli K et al. Demyelination in the juvenile period, but not in adulthood, leads to long-lasting cognitive impairment and deficient social interaction in mice. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33: 978–985.

74. Kato T, Kazai A, Mizuno M, Fengyi L, Shintani N, Maeda S et al. Phenotypic characterization of transgenic mice overexpressing neuregulin-1. PLoS One 2010; 5: e11485.

75. O’Tuathail VM, Kirby BP, Moran PM, Waddington JL. Mutant mouse models: genotype–phenotype relationships to negative symptoms in schizophrenia. Schizophr Bull 2010; 36: 271–285.

76. van den Buisse M. Modeling the cognitive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr Bull 2010; 36: 246–270.

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