

Receptor protein tyrosine phosphatase α is essential for hippocampal neuronal migration and long-term potentiation

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Despite clear indications of their importance in lower organisms, the contributions of protein tyrosine phosphatases (PTPs) to development or function of the mammalian nervous system have been poorly explored. *In vitro* studies have indicated that receptor protein tyrosine phosphatase α (RPTP α) regulates SRC family kinases, potassium channels and NMDA receptors. Here, we report that absence of RPTP α compromises correct positioning of pyramidal neurons during development of mouse hippocampus. Thus, RPTP α is a novel member of the functional class of genes that control radial neuronal migration. The migratory abnormality likely results from a radial glial dysfunction rather than from a neuron-autonomous defect. In spite of this aberrant development, basic synaptic transmission from the Schaffer collateral pathway to CA1 pyramidal neurons remains intact in *Ptpra*^{-/-} mice. However, these synapses are unable to undergo long-term potentiation. Mice lacking RPTP α also underperform in the radial-arm water-maze test. These studies identify RPTP α as a key mediator of neuronal migration and synaptic plasticity.

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Introduction

Protein tyrosine phosphatases (PTPs) constitute a broad gene family, dating back to the earliest stages of animal evolution. They are essential to the reversibility of tyrosine phosphorylation as a regulatory mechanism, and are thus particularly relevant in processes where speed or dynamic turnover are of essence. Phosphorylation/dephosphorylation on tyrosine residues in proteins is continuous and highly dynamic, as indicated by the fact that PTP inhibition, even without kinase stimulation, quickly leads to excessive accumulation of phosphotyrosine and

activation of signaling pathways. Thus, it is the balance between kinase and PTP activities that determines the phosphorylation level of a protein or site. Tyrosine dephosphorylation is, in turn, regulated in complex and dynamic ways. The size and complexity of the PTP family are of the same order as the tyrosine kinases (Andersen *et al.*, 2001), and PTPs are subject to numerous regulatory mechanisms (Ostman and Bohmer, 2001).

Knowledge of the cellular and developmental functions of individual PTPs lags far behind that for tyrosine kinases. Much progress thus far is based on cellular models or genetic approaches in non-mammalian systems. A recurrent finding in cellular studies has been their intimate involvement in cell–substrate or cell–cell interactions; alteration of a cell's PTP profile affects cellular adhesion or migration (Petrone and Sap, 2000). The close link between PTPs and cell–cell or cell–matrix communication is underscored by the existence of multiple receptor PTPs (RPTPs), whose ectodomains can interact with matrix or cell adhesion molecules. Studies of such RPTPs in *Drosophila* and chicken reveal roles in axonal outgrowth and guidance, but the substrates and molecular mechanisms mostly remain to be elucidated (Stoker, 2001).

Current understanding of the role of RPTPs in the mammalian central nervous system (CNS) is limited, and the phenotypes of mammalian RPTP loss-of-function mutants are sometimes subtle. Mice lacking PTP σ display aberrant pituitary development, and a number of architectural abnormalities, many of which are resolved with age, suggesting a developmental delay (Elchebly *et al.*, 1999; Meathrel *et al.*, 2002). Mice deficient for PTP δ lack anatomical abnormalities, but combine strengthened hippocampal long-term potentiation (LTP) with impaired learning (Uetani *et al.*, 2000). The paucity of knowledge of the contributions of RPTPs to development and function of the mammalian CNS is surprising given some of their interaction partners and substrates. PTP β/ζ is a ligand for multiple cell adhesion molecules, and interacts with sodium channels; PTP β/ζ -null mice display no obvious developmental defects (Harroch *et al.*, 2000), but show impaired recovery from experimental demyelination (Harroch *et al.*, 2002). In the peripheral nervous system, PTP ϵ plays a role in myelination by Schwann cells, controlling potassium channel phosphorylation and activity (Peretz *et al.*, 2000).

Several considerations led us to address the function of RPTP α (encoded by the *Ptpra* locus) in the nervous system. First, the protein is abundantly and dynamically expressed during CNS development (den Hertog *et al.*, 1996; Ledig *et al.*, 1999), and remains high in the adult. Secondly, RPTP α associates with potassium channels, controlling their phosphorylation and activity in response to neurotransmitters (Tsai *et al.*, 1999; Imbrici *et al.*,

2000), and with the neural adhesion molecule contactin (Zeng *et al.*, 1999). Thirdly, RPTP α regulates kinases of the Src family (SFKs) (Ponniah *et al.*, 1999; Su *et al.*, 1999; Zheng *et al.*, 2000), by counteracting phosphorylation of their inhibitory C-terminal phosphorylation sites by the tyrosine kinase Csk. As a consequence, these sites are hyperphosphorylated in cells lacking RPTP α , the kinase activity of Src and Fyn in brain lysates and fibroblasts from *Ptpra*^{-/-} mice is reduced (Ponniah *et al.*, 1999), and integrin signaling in *Ptpra*^{-/-} fibroblasts is impaired (Su *et al.*, 1999; von Wichert *et al.*, 2003). Csk and its targets Src and Fyn have important roles in multiple aspects of CNS development and function. Inactivation of Csk disrupts early neural tube development (Imamoto and Soriano, 1993). Mice lacking Fyn display anatomical abnormalities in hippocampus and olfactory bulb, and defective LTP (Grant *et al.*, 1992). NMDA-receptor subunits are *in vivo* substrates for Fyn (Nakazawa *et al.*, 2001), and Src positively modulates NMDA-receptor function (Yu and Salter, 1999). While, individually, the CNS phenotypes of SFK ablation are relatively mild, their functions are highly redundant (Stein *et al.*, 1994).

We wished to address the significance of the numerous observations that relate RPTP α to molecules important for CNS function, and assessed the effect of ablating the *Ptpra* gene on CNS development and hippocampal synaptic plasticity. Because of the functional relationship between RPTP α and SFKs, we expected that this could also contribute new insights into the roles of this family of kinases in the CNS.

Results

Macroscopic analysis of *Ptpra*^{-/-} mice

Our previously reported approach to generate RPTP α -deficient mice led to complete loss of detectable RPTP α protein (Su *et al.*, 1999). *Ptpra*^{-/-} animals were born at close to Mendelian ratios, were viable, and had a normal lifespan. Closer scrutiny revealed impaired fertility, and modest reductions in weight and size, but no overt neurological or behavioral phenotypes. Macroscopic observation showed altered morphology of *Ptpra*^{-/-} forebrain, with the mutant brain displaying a less elongated, more stubby appearance compared with wild-type (WT) animals (Figure 1A). The cerebellum also appeared smaller in size.

Histological characterization of adult *Ptpra*^{-/-} brain

Histological analysis revealed a striking pattern of disorganization in *Ptpra*^{-/-} hippocampus. Cell bodies in the pyramidal cell layer (*stratum pyramidale*; SP) in the CA1 region were much less compacted in mutant than WT animals, in extreme cases even showing layer discontinuity (Figure 1B). At the same time, excess cell bodies were found in the white matter of *Ptpra*^{-/-} *stratum oriens* (SO), with the mutant SO tending to be thicker than in WT controls. This phenotype was statistically highly significant (Figure 1B). The total number of cell bodies in adult CA1 (i.e. SO + SP + SR) did not differ between WT and mutant ($P = 0.20$; $n = 7$). However, whereas in WT 11% of total cell bodies were found in SO, this number was increased to 28% in the mutant ($P < 0.001$; $n = 7$ /genotype). Furthermore, cell bodies in WT SO were

almost always located close to the SP proper, while most cell bodies in mutant SO were located at significant distances from the SP, often closer to the *Alveus* than to the SP. This phenotype was observed at all adult stages, and was independent of genetic background (it manifested itself equally on a mixed 129SVJ \times 129SvEv background, or after five backcrosses to C57/Bl6). No such hippocampal malformation was seen in *Ptpra*^{+/-} heterozygotes (see Supplementary figure 1 available at *The EMBO Journal* Online).

More subtle abnormalities were observed in other brain regions. Grossly, neocortical lamination appeared normal, with distinguishable layers IV and V in somato-sensory areas; however, transitions between neocortical layers appeared more diffuse. There was a modest but consistent tendency for the boundary between layers I and II to be less regular, and for layer I to be thinner (Figure 1C). The cerebellum displayed a slight reduction in Purkinje cell numbers and disturbed packing of Purkinje cell bodies (data not shown).

Ectopic cells in SO have a CA1 pyramidal phenotype

The ectopic cell population in SO displayed characteristics of pyramidal hippocampal neurons. Besides expressing the neuronal marker neuN (data not shown), they expressed SCIP (Figure 2A), a specific marker for pyramidal cells in CA1 absent from inhibitory interneurons (Grove and Tole, 1999). They were negative for the interneuronal marker parvalbumin (data not shown). Furthermore, MAP2 staining revealed dendrites emanating from the ectopic cell bodies in SO that extended through the pyramidal cell layer into *stratum radiatum* (SR; Figure 2C, arrowheads). This cytoarchitecture is similar to that of bona fide pyramidal cells, whose dendrites in SR receive synaptic input from the Schaffer collateral pathway emanating from CA3 pyramidal cells. This staining also revealed that, while the overall direction of dendritic targeting of pyramidal cells in *Ptpra*^{-/-} animals was normal, their trajectory was more disorganized and meandering than in WT animals (Figure 2C). Lastly, ectopic cells in adult *Ptpra*^{-/-} SO stained positive for *lacZ*, indicating that the *Ptpra* promoter is transcriptionally active in the ectopic cells, as it is in pyramidal cells within SP (Figure 2B). Strong *LacZ* staining also occurred in pyramidal cells of the phenotypically normal heterozygotes (data not shown).

Abnormal radial neuronal migration during development of *Ptpra*^{-/-} hippocampus

A recurrent feature in CNS development is that the eventual positions of neurons are distinct from the areas where their terminal mitosis has occurred. A major pathway of cell movement in forebrain development involves a radial course from periventricular proliferative zones towards the pial surface (Nadarajah and Parnavelas, 2002). Therefore, the accumulation of pyramidal cells in *Ptpra*^{-/-} SO, and their relative deficiency in SP, may reflect an underlying migratory abnormality, since SO is the zone through which these cells migrate in order to take up their eventual position in SP. Indeed, the *Ptpra*^{-/-} phenotype bears similarity to that of other mouse mutants where radial neuronal migration is disturbed, such as hetero-

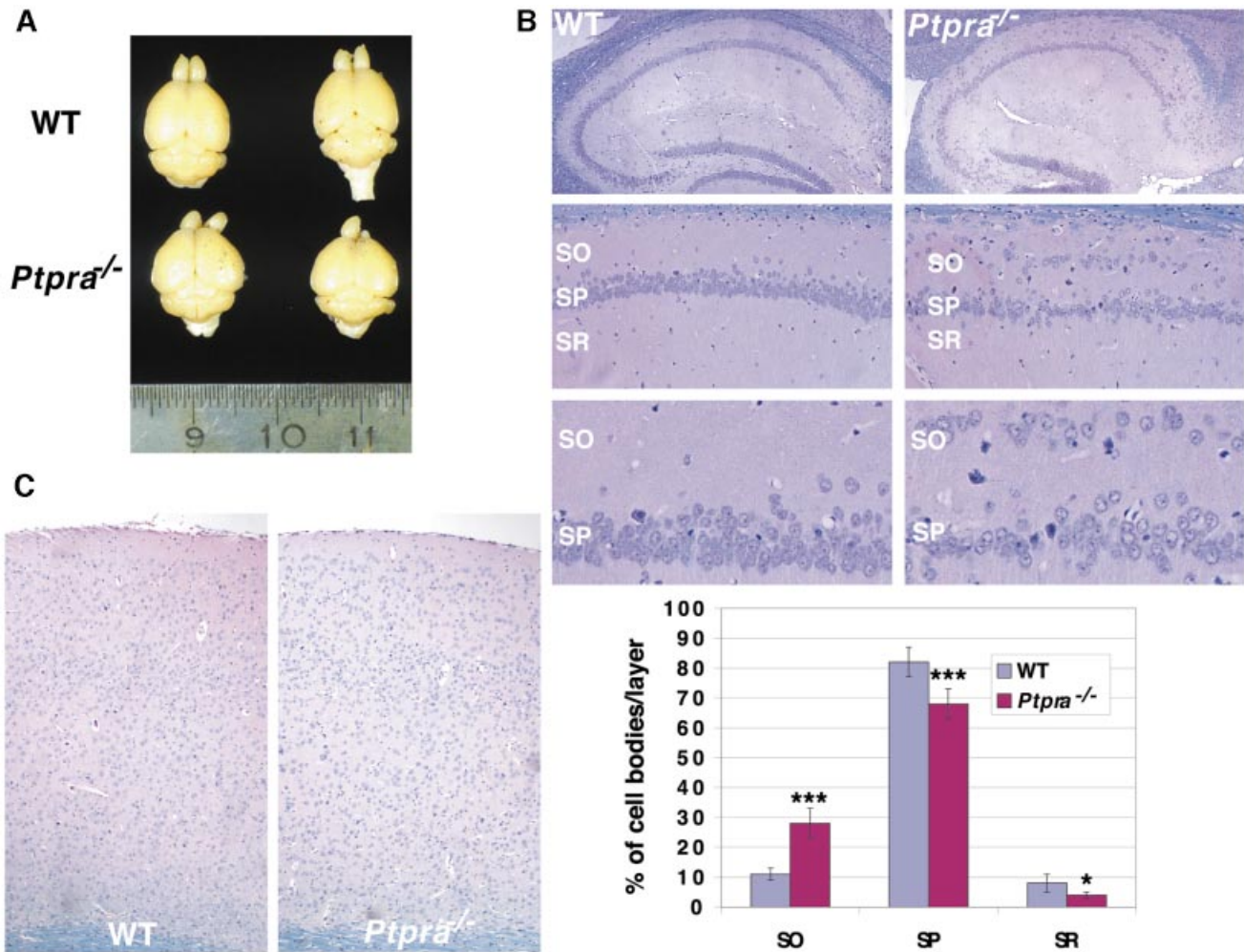


Fig. 1. Abnormal brain structure in adult *Ptptra*^{-/-} mice. (A) Overall brain morphology. Dorsal view of two WT (top) and two mutant (*Ptptra*^{-/-}, bottom) brains. Scale is in centimeters. (B) Hematoxylin–eosin/luxol blue staining of parasagittal sections of hippocampus from WT (left) and mutant (right). The lower panels show progressively higher magnifications of SP. The graph shows the relative distribution of cell bodies in SO, SP and SR for WT and mutant (*Ptptra*^{-/-}) CA1 (error bars indicate standard deviation; $n = 7$; *** $P < 0.001$; * $P < 0.05$; cells in SO + SP + SR = 100%). Cells were classified as belonging to SO when separated from the SP by at least one cell diameter; hence, this graph underestimates the mutant SP's disorganized texture and ragged boundary. (C) Hematoxylin–eosin/luxol blue staining of somatosensory cortex (left, WT; right, *Ptptra*^{-/-}). Note the recognizable layers IV and V (pyramidal) in both genotypes, and the less distinct nature of the layer I/II boundary in the mutant.

zygosity for *Lis1* (the gene for type I human lissencephaly), inactivation of the genes for VLDR and ApoER2, or hypomorphic alleles of *Dab1* (Trommsdorff *et al.*, 1999; Magdaleno and Curran, 2001; Herrick and Cooper, 2002; Nadarajah and Parnavelas, 2002).

To test the hypothesis of defective radial migration during morphogenesis of *Ptptra*^{-/-} hippocampus, we used BrdU birthdating. Pregnant heterozygote females, mated with heterozygote males, were injected with a single pulse of BrdU on day E15.5, the expected peak of radial migration in hippocampus. Because of the short half-life of BrdU, this intervention marks cells undergoing mitosis during a narrow time-window. Since BrdU is diluted out from nuclei of cells that undergo subsequent rounds of mitosis, only cells that underwent their terminal mitosis at the time of injection remain strongly labeled, and their eventual location in the adult can be tracked.

This analysis revealed that, as expected, the majority of cells (91%) labeled by BrdU at E15.5 in WT embryos had migrated to the hippocampal SP in the adult, with only 9% taking up residence in the SO (Figure 3A and B). In

contrast, in *Ptptra*^{-/-} littermates, 45% of BrdU-labeled cells did not reach SP but were found in ectopic locations in SO (Figure 3A and B) ($P < 0.001$; $n = 8$ /genotype). This experiment also allowed us to analyze the fate of BrdU-labeled cells in neocortex. It is well known that radial migration follows an 'inside-out' pattern, with cells born later progressively migrating over longer distances and taking up more apical positions (Nadarajah and Parnavelas, 2002). While this phenomenon holds in both archi- and neocortex, it is most easily observed in the latter. We found that the distribution of labeled cells in *Ptptra*^{-/-} neocortex largely tracked that of WT littermates (Figure 3C), with early BrdU pulses (E13.5) predominantly labeling deeper layers (data not shown). Thus, the inside-out migratory pattern is conserved in the mutant, and no inversion has occurred. However, in *Ptptra*^{-/-} animals, there was a consistent tendency for E15.5-birthdated neocortical neurons to assume adult positions that were scattered more broadly over the various neocortical layers (Figure 3C).

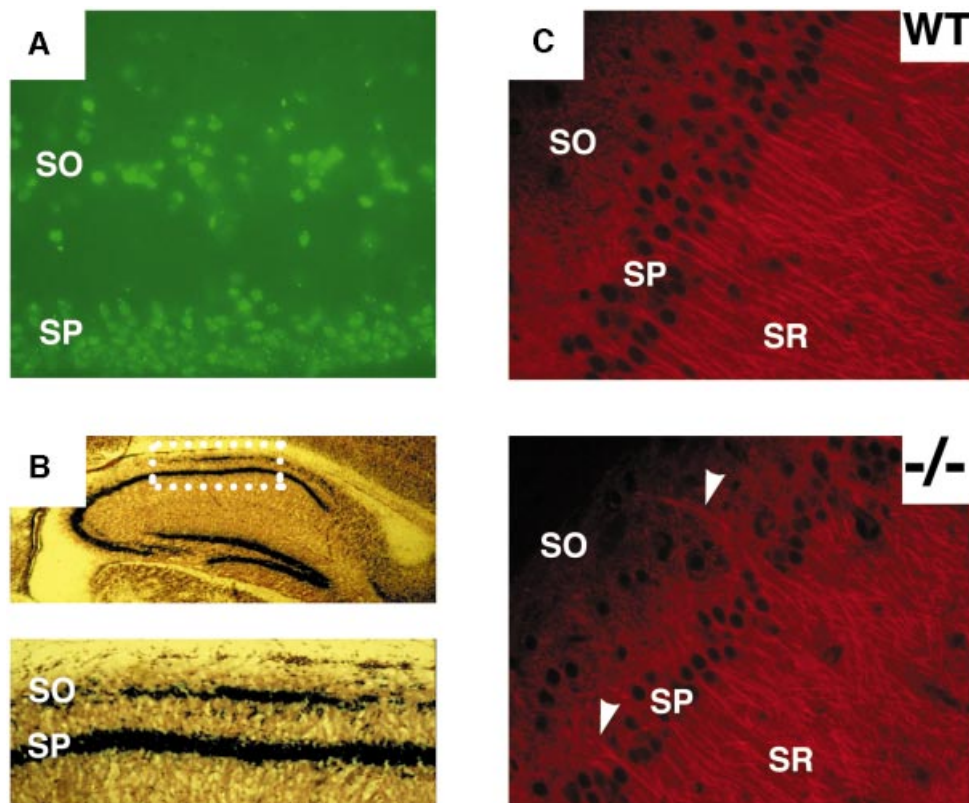


Fig. 2. Ectopic cells in hippocampal CA1 express RPTP α and pyramidal cell markers. (A) Staining of *Ptptra*^{-/-} CA1 for the CA1 pyramidal cell marker SCIP. Neurons in SP, as well as ectopic cells in SO, stain positive. (B) *LacZ* staining of *Ptptra*^{-/-} hippocampus. The CA1 region in the top panel (dotted box) is enlarged in the bottom panel. *LacZ* is expressed from the *Ptptra* promoter as a consequence of the homologous recombination strategy used (Su *et al.*, 1999). Expression is seen both in bona fide pyramidal cells in SP, and in the ectopic cells in SO. (C) MAP2 immunostaining of WT (top) and mutant (bottom) hippocampus. Arrowheads indicate the presence of radially oriented dendrites in mutant SO.

RPTP α expression and radial glial morphology during forebrain development

During forebrain development, post-mitotic neurons are born from radial glial precursors, the latter having elaborate radial processes extending from their cell bodies in the periventricular zone to the pial surface, which provide the substrate for neuronal ‘gliophilic’ migration (Noctor *et al.*, 2002). To determine the reason for the abnormal migration in *Ptptra*^{-/-} mice, we analyzed the normal pattern of RPTP α expression and radial glial morphology during development of *Ptptra*^{-/-} embryos.

Staining of E15.5 *Ptptra*^{+/-} heterozygote embryos for the *lacZ* reporter (introduced downstream of the *Ptptra* promoter; Su *et al.*, 1999) showed RPTP α expression to be highest in deeper forebrain layers, and widespread among cells in intermediate to ventricular zones. Strikingly, hardly any RPTP α expression was detected among neurons located in the densely packed cortical plate, the destination layer into which neurons are actively migrating and assuming their final positions at this developmental stage; this pattern applied to prospective hippocampus as well as neocortex (Figure 4A and B).

Using anti-BLBP (brain lipid-binding protein) immunostaining (Feng and Heintz, 1995), we found that, while the overall structure of the radial glial processes was retained during embryonic development, *Ptptra*^{-/-} radial glia manifested a more wavy and irregular appearance, with a more extensive subpial arborization than in WT.

This phenotype was particularly pronounced at E16.5 (Figure 4C). Taken together with the low RPTP α levels in cortical plate neurons, this suggests that the migratory defect in *Prpra*^{-/-} embryos may occur in the early steps of the migratory path, perhaps with a primary origin in the radial glial cells themselves.

Normal basal synaptic transmission but defective LTP in adult *Ptptra*^{-/-} CA1

We wished to determine whether the aberrant development of hippocampus in *Ptptra*^{-/-} mice correlated with deficiencies in synaptic function, plasticity and higher-order information processing. This question appeared relevant, since the *Ptptra*^{-/-} mutation, as opposed to other more catastrophic migratory defects, left the major outlines of hippocampal organization recognizable, and did not lead to an overt neurological phenotype.

To determine whether the absence of RPTP α affects synaptic transmission, we measured input–output relationships between the Schaffer collateral pathway and CA1 neurons in WT and *Ptptra*^{-/-} hippocampal slices. The stimulus–response curves of field excitatory post-synaptic potential (fEPSP) slope versus presynaptic fiber volley amplitude did not differ between WT and mutant slices across a range of stimulus intensities (*t*-test *P* = 0.27; Figure 5A). We conclude that, in spite of the anatomical abnormality, basal synaptic transmission remained normal in *Ptptra*^{-/-} CA1.

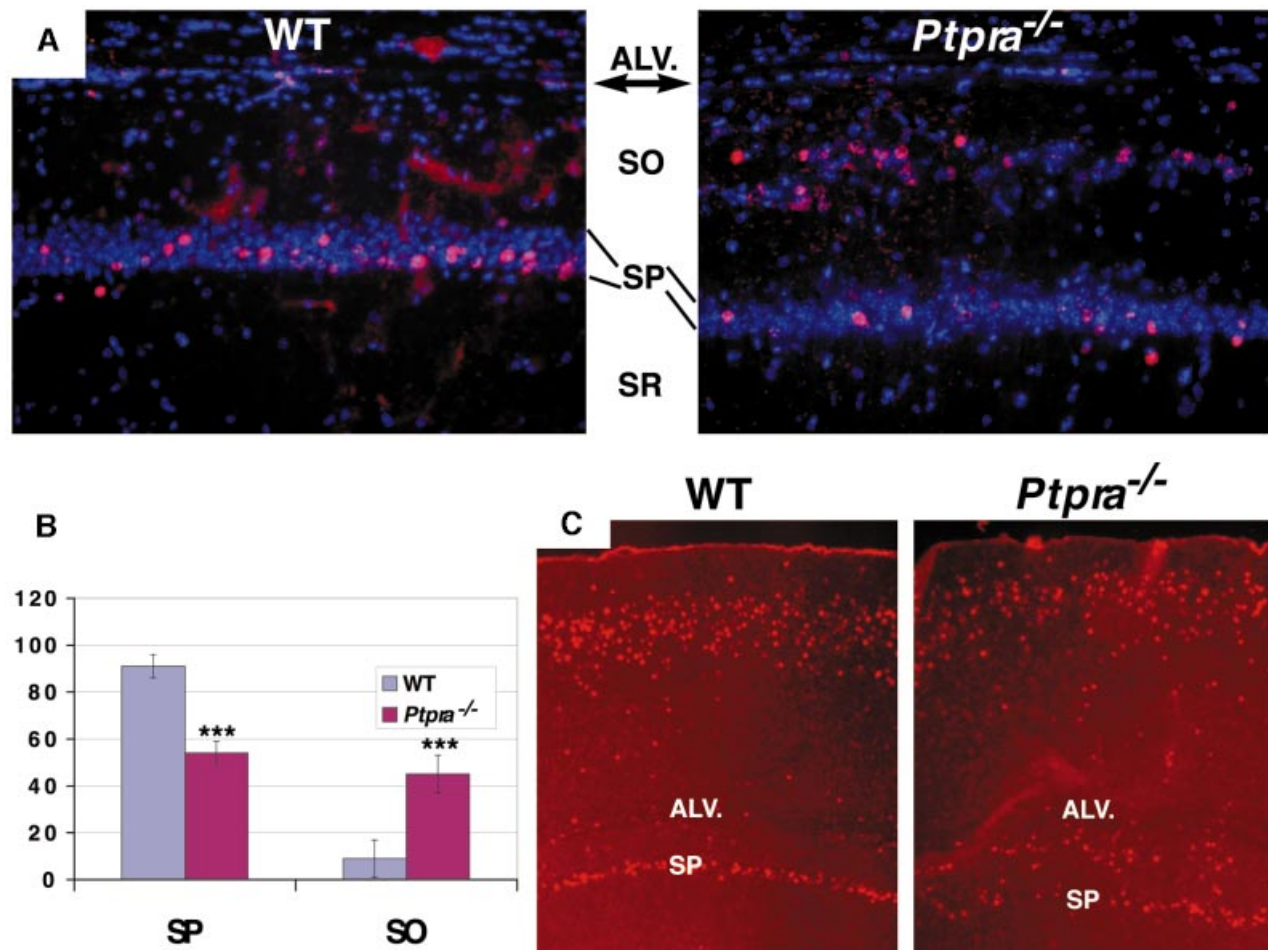


Fig. 3. BrdU birthdating analysis. (A) Anti-BrdU immunostaining on coronally sectioned hippocampi of WT and *Ptptra*^{-/-} littermates derived from a litter whose mother was injected with a single pulse of BrdU on day 15.5 of gestation. Sections were obtained at the adult stage and stained with anti-BrdU (red) and DAPI (blue) (ALV, *Alveus*). (B) Quantitation of data obtained in (A). Mean percentage of total number of anti-BrdU-stained cells in SP and SO (cells in SO + cells in SP = 100%) for each genotype that had reached either of these layers. Error bars denote standard deviations ($n = 8/\text{genotype}$; *** $P < 0.001$). (C) Similar experiment as in (A), but at lower magnification, also showing neocortex.

Another synaptic parameter examined was paired-pulse facilitation (PPF), the enhanced response of the second of two closely spaced stimuli. It is thought to reflect build-up of residual Ca^{2+} due to the action potential from the first depolarization of the terminal, leading to enhanced transmitter release at the arrival of the second stimulus. As the time between stimuli increases, facilitation approaches zero, since they are seen as independent. Therefore, PPF is considered a measure of presynaptic function. As shown in Figure 5B, PPF was observed for stimuli applied at 10–200 ms intervals in both WT and mutant mice, with no significant difference between both genotypes ($P = 0.19$). Thus, lack of RPTP α does not compromise the presynaptic mechanism of PPF in CA1.

Synapses from the same animal were next tested for their ability to undergo plastic changes. The ability to support LTP, a lasting enhancement of synaptic efficacy, is widely utilized as a model of learning and memory. In CA1 of hippocampal slices, θ -burst-induced LTP (three series of 10 burst trains) was severely affected in *Ptptra*^{-/-} mice compared with WT littermates (Figure 5C and D). At 60 min after LTP induction, when WT mice showed a potentiated response in the fEPSP slope of $240.9 \pm 22.1\%$

of the baseline pre- θ -burst stimulation ($n = 10$), potentiation was only $144.7 \pm 28.2\%$ of baseline value in *Ptptra*^{-/-} mice ($n = 12$). This impairment in potentiation in the mutant animals was of high statistical significance ($P = 0.0045$ by ANOVA). LTP was also impaired in *Ptptra*^{-/-} mice when it was elicited with a weak tetanus (one series of 10 burst trains); at 60 min after LTP induction using this protocol, *Ptptra*^{-/-} mice showed $140.4 \pm 8.6\%$ potentiation ($n = 6$) compared with $181.4 \pm 9.5\%$ potentiation in WT ($n = 6$; $P = 0.0057$; data not shown). Elimination of RPTP α expression thus severely compromises the capability of synaptic plastic change in the CA1 region.

Although to a lesser extent, LTP was also impaired in heterozygous *Ptptra*^{+/-} mice. At 60 min after LTP induction (three series of 10 burst trains), *Ptptra*^{+/-} mice showed $179.9 \pm 19.8\%$ potentiation ($n = 6$) compared with $229.3 \pm 17.9\%$ in WT littermates ($n = 6$; $P = 0.046$; Figure 5E).

An analysis of the LTP results revealed that post-tetanic potentiation (PTP) of the fEPSP was smaller in *Ptptra*^{-/-} mice (Figure 5C and D). PTP is believed to be an indication of presynaptic function. It reflects a period of

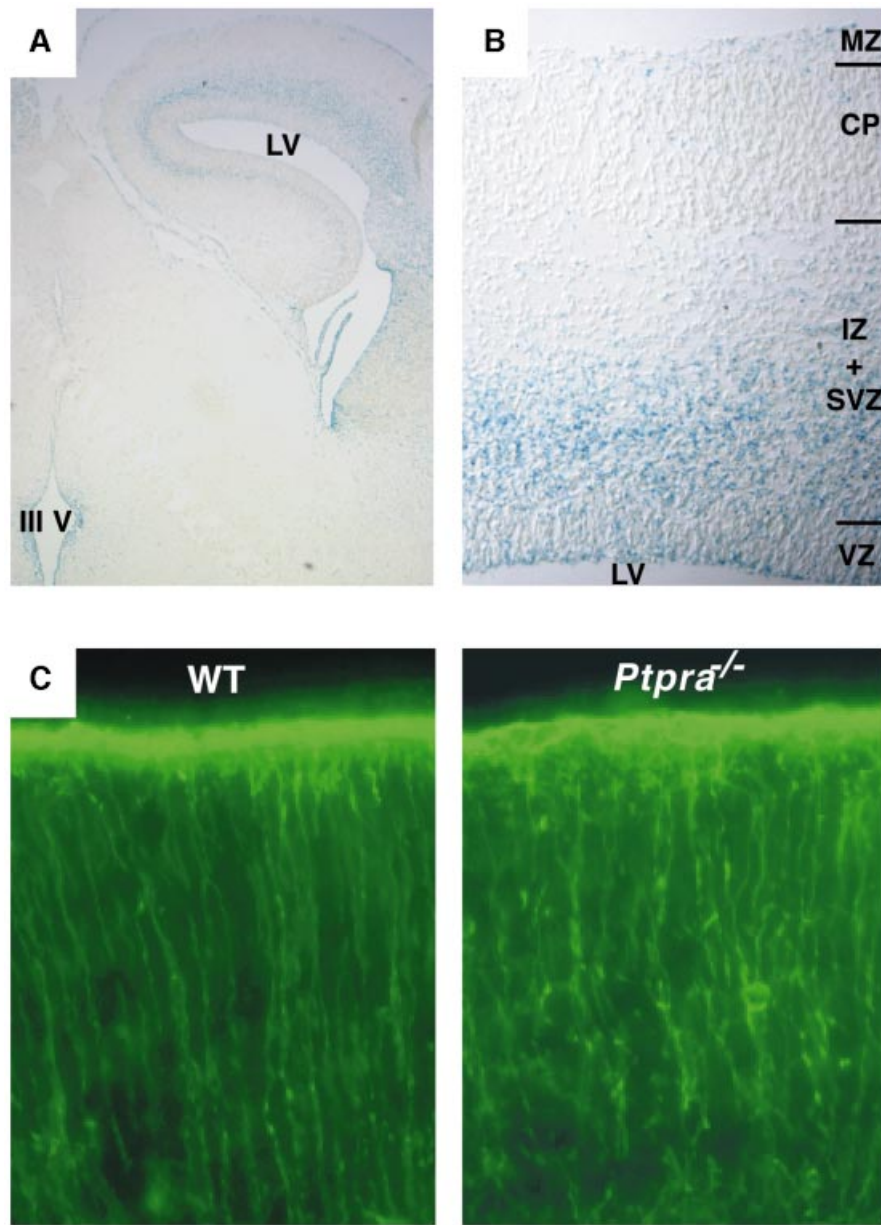


Fig. 4. Altered morphology of radial glia in developing *Ptptra*^{-/-} forebrain. (A) LacZ staining of coronally sectioned E15.5 *Ptptra*^{+/-} forebrain, indicating cells where the *Ptptra* promoter is active (LV, lateral ventricle; III V, third ventricle). (B) Higher magnification of lacZ-stained E15.5 *Ptptra*^{+/-} coronal section, showing forebrain cerebral wall only; the transmitted light-image was overlaid with that in differential interference contrast mode, so as to visualize the different layers (MZ, marginal zone; CP, cortical plate; IZ + SVZ, intermediate and subventricular zones; VZ, ventricular zone). (C) Coronal sections of E16.5 WT and mutant embryos stained for BLBP (Feng and Heintz, 1995). In the mutant, note the more wavy appearance of the radial glial fibers in the cortical plate, and their more abundant arborization below the meningeal basement membrane.

enhanced transmitter release due to loading of the presynaptic terminal with Ca²⁺ during the tetanus. To further test whether PTP impairment in *Ptptra*^{-/-} mice was caused by a reduction in transmitter release during the tetanus, we induced PTP in the presence of the NMDA antagonist D-APV to block LTP-inductive mechanisms. We did not see any difference in the degree of PTP between WT and *Ptptra*^{-/-} mice (Figure 5F), suggesting that impaired transmitter release during the tetanus was not responsible for the LTP reduction.

Another possible explanation for the LTP impairment in *Ptptra*^{-/-} mice is that synaptic fatigue instead of potentiation *per se* is responsible for the abnormal plasticity. To address this issue, a train of 40 pulses at

the same frequency as the θ -burst stimulation (100 Hz) was applied to a group of slices from *Ptptra*^{-/-} mice and WT controls. No difference in the change of the fEPSP slope during the tetanus was observed between the two genotypes: the percentage of the 40th fEPSP slope over the first during the tetanus was $60 \pm 5\%$ in *Ptptra*^{-/-} versus $65 \pm 4\%$ in WT ($n = 7$ for both groups; data not shown), suggesting that synaptic fatigue does not account for the LTP impairment.

Impaired memory in *Ptptra*^{-/-} animals

We used the radial-arm water-maze task, considered capable of assessing working (short-term) memory, to examine learning in *Ptptra*^{-/-} mice. In this test, which is

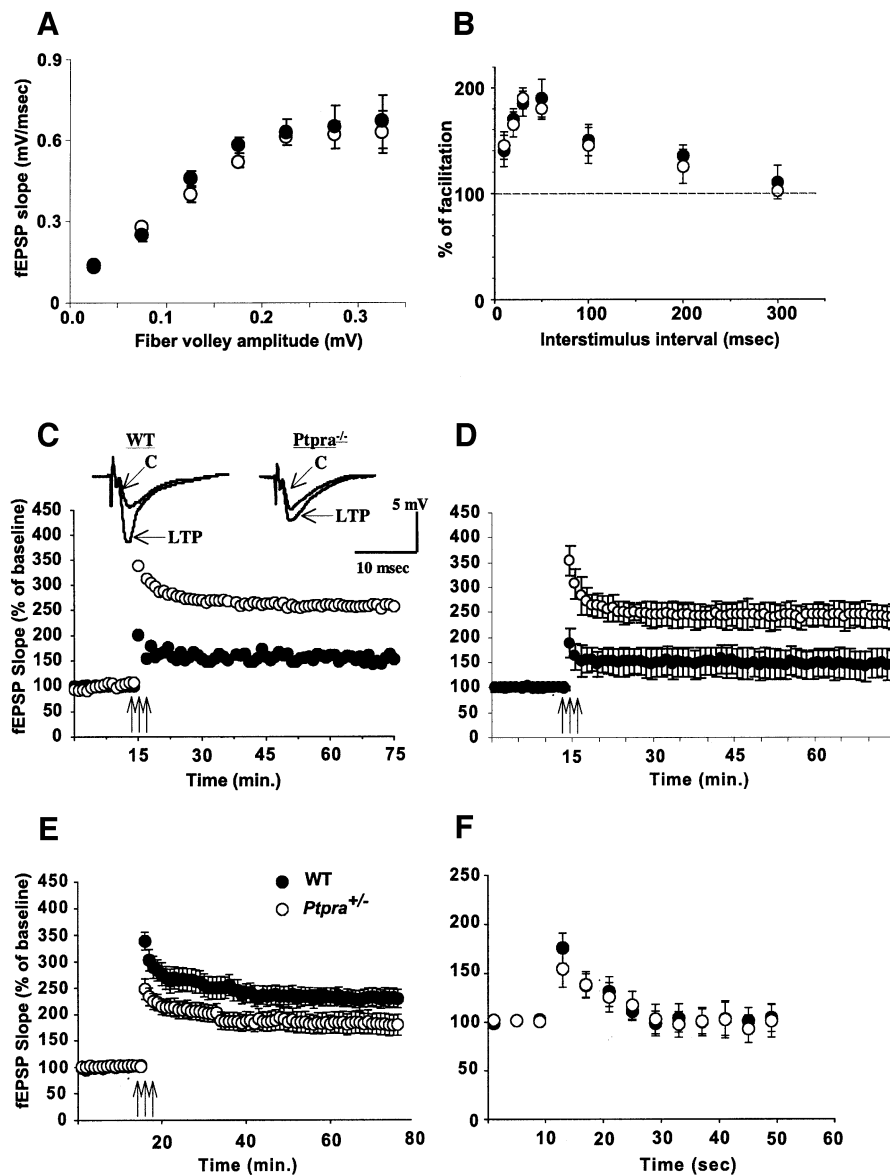


Fig. 5. Lack of RPTP α impairs LTP in hippocampal slices. (A) Summary graph of field input–output relationships for WT (open circles) and *Ptpra*^{-/-} (closed circles). Both genotypes showed similar relationships ($P = 0.27$). (B) Summary graph of PPF in WT (open circles) and *Ptpra*^{-/-} (closed) mice. Facilitation was similar at all interstimulus intervals tested ($P = 0.19$). (C) Representative values of fEPSP slopes from single experiments taken from WT (open circles) and *Ptpra*^{-/-} (closed) littermates that underwent potentiation. Insets show traces taken 1 min before (C) and 60 min after potentiation (LTP). (D) Summary graphs of LTP in WT (open circles) and *Ptpra*^{-/-} (closed) mice. The average fEPSP slope is normalized to the baseline value (ANOVA $P = 0.0045$). (E) Summary graph of LTP in WT (closed circles) and heterozygote *Ptpra*^{+/-} (open) mice (ANOVA $P = 0.046$). (F) Summary graph of PTP (in the presence of 25 μ M D-APV) in WT (open circles) and *Ptpra*^{-/-} (closed).

more efficient in sample size requirements than other memory tasks typically used for rodents, mice are required to learn and memorize the location of a hidden platform in one of the arms of a maze with respect to spatial clues. The test has been used in the analysis of other types of transgenic mice, and depends upon hippocampal function (Diamond *et al.*, 1999; Arendash *et al.*, 2001).

WT mice showed strong learning and memory capacity (Figure 6A, open circles); they averaged about eight errors on the first trial as they sought out the new platform location for that day, but averaged less than one error by acquisition trial four. They then retained memory of the platform location during the 30 min delay until the retention (5th) trial. In contrast, *Ptpra*^{-/-} mice showed

impaired learning (Figure 6A, closed circles), still scoring about five errors on the 4th and retention trials (ANOVA $P < 0.0001$; $n = 10$ /group).

Possible explanations for poor performance in the radial-arm water maze might be an impairment of vision, motor coordination, or motivation as a consequence of disabling the *Ptpra* gene. To rule out these possibilities, we also tested the same mice in the visible platform task. In this version of the task, *Ptpra*^{-/-} mice showed no difference compared with WT littermates in swimming speed (Figure 6B; $P > 0.05$), or in time to find a visible platform (Figure 6C; $P > 0.05$). Thus, impaired working memory is the most obvious explanation for the under-performance of *Ptpra*^{-/-} mice.

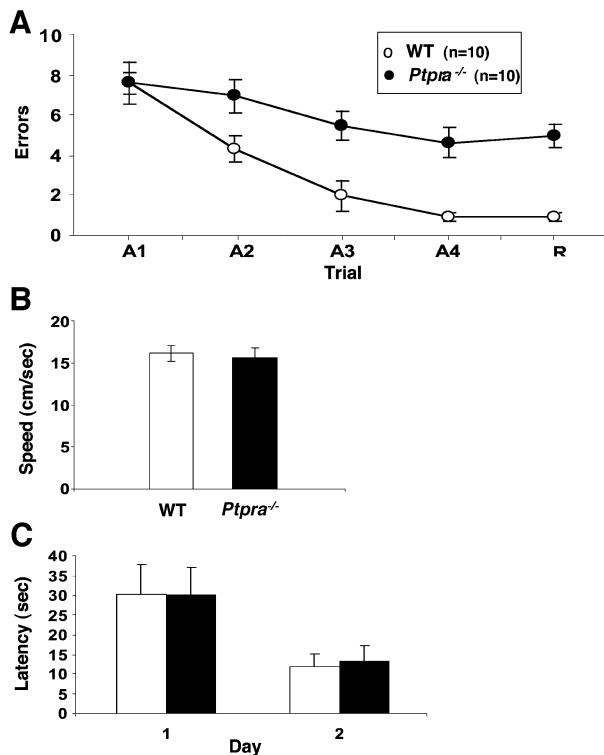


Fig. 6. Radial-arm water-maze performance of *Ptptra*^{-/-} mice is impaired. (A) Radial-arm water-maze test for working memory. A1–A4 denote the acquisition trials, whereas R denotes the retention trial after a 30 min delay. The WT mice (open circles) learned (trials A2–A4) and remembered (trial R) the platform location. *Ptptra*^{-/-} mice (closed circles) showed impaired learning and memory for platform location at the acquisition trials A2–A4 and at the retention trial R compared with WT mice (ANOVA $P < 0.0001$). (B and C) WT and *Ptptra*^{-/-} mice performed similarly in visible platform trials with regard to speed (B) ($P > 0.05$) and time to reach the platform (C) ($P > 0.05$).

Discussion

Developmental and functional abnormalities in *Ptptra*^{-/-} hippocampus

Our data demonstrate essential roles for RPTP α in hippocampal development and plasticity. The functional relevance of these findings is indicated by the impaired performance of animals lacking RPTP α in the radial-arm water-maze test.

Inactivation of the *Ptptra* gene causes accumulation of ectopic cells in CA1 SO, accompanied by a reduction of cell numbers in SP. The CA1 pyramidal identity of the ectopic cells indicates the nature of the developmental origin of the defect. First, ‘duplication’ of the pyramidal cell layer is characteristic of mutations affecting radial neuronal migration. Secondly, BrdU birthdating directly demonstrated aberrant radial migration during development of *Ptptra*^{-/-} embryos: cells ‘born’ simultaneously to the bona fide pyramidal cells that will populate the SP in the WT are, in the mutant, scattered ectopically along the path of radial migration, i.e. in SO (Figure 3A and B). An alternative interpretation, that pyramidal cells also accumulate in aberrant positions during WT development but that absence of RPTP α exempts such cells from a culling process that normally occurs in the WT, seems less likely; it requires more underlying assumptions, and we are not aware of evidence for such a process in the WT. We also

detected more subtle neocortical abnormalities (Figure 3C). We conclude that RPTP α constitutes a novel member of the class of proteins important for radial migration, which is particularly important for hippocampal pyramidal cells.

Functionally, *Ptptra*^{-/-} mice suffer from reduced synaptic plasticity in hippocampal CA1, and underperform in the radial-arm water maze, a test thought to measure hippocampus-dependent working memory (Diamond *et al.*, 1999; Arendash *et al.*, 2001). In a more narrow sense, the underperformance of *Ptptra*^{-/-} mice in this test may reflect a defect in spatial representation or memory resulting from the anatomical abnormality of the hippocampus of *Ptptra*^{-/-} mice (e.g. mislocalization of place cells). Alternatively, synaptic dysfunctions in the *Ptptra*^{-/-} adult may be affecting memory function in a more fundamental and independent way. Behavioral analysis of conditionally targeted loss-of-function mice, including the use of tests for non-spatial memory, will ultimately be needed to address this issue. In the meantime, several considerations argue for a specific and direct contribution of RPTP α to synaptic function and learning that may go beyond its developmental role. First, in spite of the anatomical defect, presynaptic function and synaptic coupling *per se*, as measured by PPF, PTP, synaptic fatigue and EPSP stimulus–response relationships, are unaffected by lack of RPTP α . Secondly, other mutations that alter hippocampal morphology due to abnormal migration have been shown to be associated with different specific alterations, rather than a generalized impairment, of synaptic activity. For example, heterozygosity at the *Lis1* locus in mice altered extracellular input–output curves, but did not impair LTP in CA1 (Fleck *et al.*, 2000). These electrophysiological results are opposite to what we observed in *Ptptra*^{-/-} mice, even though *Lis1*^{+/-} mice showed incomplete neuronal migration and multiple layers of pyramidal cells in CA1, similar to *Ptptra*^{-/-} hippocampus. Thirdly, heterozygote animals have a partial impairment of LTP in the absence of an ectopic cell phenotype in hippocampus (Supplementary figure 1). Lastly, recent antibody perturbation experiments indicated a role for RPTP α in NMDA receptor-mediated synaptic responses; intracellular application of anti-RPTP α antibody into CA1 neurons (where RPTP α is highly expressed; Figure 2B) reduced the amplitude of the long-lasting excitatory postsynaptic current after tetanic stimulation, while RPTP α intracellular domain enhanced it (Lei *et al.*, 2002). In this respect, we also find that phosphorylation of Y1472 in the NR2B subunit of NMDA receptor, a major Fyn target site (Nakazawa *et al.*, 2001), is reduced in *Ptptra*^{-/-} mice (Supplementary figure 2). All these observations are consistent with an independent involvement of RPTP α in plasticity and memory function of the adult brain.

Neuron-autonomous versus radial glial roles for RPTP α in neuronal migration

Forebrain stratification is a remarkable process of histogenesis, involving development of a ventricular-to-pial axis (corresponding, in hippocampus, to the direction SO–SP–SR–hippocampal fissure). Following birth in ventricular and subventricular zones, neurons migrate outward along a scaffold provided by their radial glial precursors, and assume their eventual positions according

to birth order and extracellular cues, resulting in an inside-out gradient. While such glia-guided locomotion is the predominant process used by pyramidal cells, early-generated neurons also rely on somal translocation (Nadarajah and Parnavelas, 2002).

Aberrant glia-guided locomotion may therefore result from intrinsic deficiencies in migrating neurons, from abnormalities in radial glia, or both. The majority of studies on genes that control migration have focused on cell-autonomous defects in neuronal signaling pathways or cytoskeletal function. For instance, aberrant migration can result from dysfunction in the complex signaling pathways activated in neuronal cells by reelin. Secreted by Cajal–Retzius cells, reelin provides positional cues by signaling through at least two classes of neuronal receptors: the lipoprotein-receptor-like VLDLR and ApoER2, and $\alpha_3\beta_1$ integrins (Nadarajah and Parnavelas, 2002). Transduction of the reelin signal inside neurons involves downregulation and tyrosine phosphorylation of its effector, Disabled-1 (Dab1) (Howell *et al.*, 2000). Besides mediating reelin-induced detachment from radial glia (Dulabon *et al.*, 2000), β_1 -integrin may also function extrinsically to neurons, e.g. in development of radial glial endfeet and remodeling of the meningeal basement membrane (Graus-Porta *et al.*, 2001; Magdaleno and Curran, 2001). The connection between *Lis1* and genes governing nucleokinesis highlights the additional key contribution of the neuronal microtubule cytoskeleton in the migratory process (Nadarajah and Parnavelas, 2002). At the cellular level, one functional consequence of reelin signaling may be to antagonize migration, by inducing detachment of neurons from radial glia, or by acting as a repellent. This ‘stop-signal’ model is supported by the finding that hypomorphic alleles of *Dab1* cause invasion of late-born cortical neurons into the marginal zone (Herrick and Cooper, 2002). However, this model clearly does not do justice to the full repertoire of reelin’s functions (Magdaleno *et al.*, 2002).

The ectopic neurons found in *Ptpra*^{-/-} SO have remained abnormally close to the former inner edge of the structure where they originated. Assuming RPTP α functions cell-autonomously in migrating neurons, and reasoning within the stop-signal model for reelin action, such premature arrest might reflect hypersensitivity to the reelin ‘stop’ signal. Accordingly, RPTP α would act as a negative regulator of the neuronal ‘stop’ response to reelin. However, evidence supporting this hypothesis has so far failed to materialize. Combining the *Ptpra*^{-/-} genotype with heterozygosity for the *reln* (reelin) mutation yielded no evidence of antagonistic or epistatic interactions between both genes (data not shown). One consequence of reelin signaling is a decrease in the level of its effector Dab1 (Howell *et al.*, 2000). Fyn (and to a lesser extent Src) is involved in tyrosine phosphorylation of Dab1 (Arnaud *et al.*, 2003), and these kinases are known targets for RPTP α . Immunoblotting of E16.5 forebrain lysates revealed identical levels of Dab1 protein in WT and *Ptpra*^{-/-} embryos (data not shown), indicating no major dysfunction in reelin signaling in the mutant. Lastly, *lacZ* staining reveals little or no RPTP α expression within cortical plate neurons that are in the vicinity of the source of the reelin signal and express high levels of Dab1 (Figure 4A and B). Therefore, we presently do not favor

deregulation of reelin signaling as a mechanism explaining the *Ptpra*^{-/-} migratory defects.

The above hypothesis assumed that the effect of *Ptpra* gene ablation is neuron-autonomous, i.e. intrinsic to the migrating neurons themselves. By contrast, the aberrant radial glial morphology in developing forebrain may indicate a primary origin in this cell type for the *Ptpra*^{-/-} migratory phenotype. Clearly, definitive determination of the relative contributions of aberrant neuronal versus radial glial cells to the *Ptpra*^{-/-} phenotype will require lineage-specific *Ptpra* gene inactivation and *in vitro* heterologous reconstitution approaches. In the meantime, independent evidence is consistent with a primary radial glial origin for the *Ptpra*^{-/-} phenotype. In a comprehensive analysis in the avian retinotectal system, RPTP α stood out from other RPTPases in that its expression was mostly restricted to Muller glia and radial glia of the tectum, prompting the authors to suggest a function for RPTP α in neuronal migration (Ledig *et al.*, 1999). RPTP α is also expressed in chicken cerebellar Bergmann glia (Fang *et al.*, 1996). We have been unable to unambiguously demonstrate the presence of RPTP α in mouse radial glial processes by immunostaining. However, *lacZ* staining of *Ptpra*^{+/-} E15.5 embryos reveals that RPTP α is predominantly expressed in cell bodies within deeper layers of the developing forebrain, including the ventricular and subventricular zones. In contrast, no expression is found in cell bodies that have reached the cortical plate, where post-mitotic neurons are responding to reelin secreted by marginal zone Cajal–Retzius cells (Figure 4A and B). This expression pattern is compatible with a radial glial function, or at least with a role for RPTP α in the early steps of migration.

Molecular effectors for RPTP α

Thus far, tyrosine kinases of the Src family (SFKs), particularly Src and Fyn, are the best established targets for RPTP α . In fibroblasts, RPTP α activates these kinases by dephosphorylating their inhibitory C-terminal site of tyrosine phosphorylation (Ponniah *et al.*, 1999; Su *et al.*, 1999; Zheng *et al.*, 2000). The heterogeneity of brain tissue makes it difficult to easily assess to what extent RPTP α similarly regulates SFKs in brain, but Src activity is reduced in *Ptpra*^{-/-} brain homogenates (Ponniah *et al.*, 1999), and RPTP α can clearly activate Src in neuronal cells (den Hertog *et al.*, 1993; Yang *et al.*, 2002). A suggestive genetic insight into the possible involvement of SFKs in the *Ptpra*^{-/-} phenotype is provided by the phenotype of *Fyn* knockout mice, which also display aberrant hippocampal development, defective LTP and impaired spatial learning (Grant *et al.*, 1992). However, the developmental abnormality in *Fyn*^{-/-} mice affects granule cells in the dentate gyrus, and pyramidal cells in CA3. Thus, the *Fyn*^{-/-} and *Ptpra*^{-/-} phenotypes overlap only partially. This is not surprising if RPTP α acts on substrates other than Fyn (e.g. Src), or if RPTP α does not activate Fyn with respect to all substrates of the latter. No neuroanatomical or behavioral abnormalities have been described for other SFK knockouts. However, the phenotypic synergism in compound knockouts (Stein *et al.*, 1994) suggests that SFKs perform partially overlapping and redundant functions. Detection of a migratory phenotype similar to the one described here in

compound knockouts for SFKs, or epistatic interactions between RPTP α and SFKs, may provide genetic evidence for a function of RPTP α upstream of SFKs in neuronal migration.

Further insights into the molecular basis for the *Ptpra*^{-/-} phenotypes can come from considering known SFK roles and substrates. SFKs participate in integrin signaling, and fibroblast studies clearly implicate RPTP α in this process (Su *et al.*, 1999; von Wichert *et al.*, 2003). The multiple stages of the development of forebrain lamination in which integrins participate (Magdaleno and Curran, 2001) may provide clues for future analysis of the molecular and biophysical basis for the role of RPTP α in neuronal migration.

Tyrosine phosphorylation is a mechanism for regulating NMDA-receptor channel function, with Src and Fyn firmly implicated in this process (Yu and Salter, 1999; Nakazawa *et al.*, 2001). Recent findings demonstrate *in vivo* association between RPTP α , Src and NMDA receptors, and antibody interference indicates that RPTP α positively regulates NMDA receptor-mediated whole-cell currents and induction of synaptic LTP in hippocampal neurons (Lei *et al.*, 2002). Our finding of lack of synaptic plasticity in *Ptpra*^{-/-} brain slices is consistent with a critical role of RPTP α in CA1 LTP. This involvement of RPTP α may reflect a role in controlling (directly, or through an interposed SFK) tyrosine phosphorylation of NMDA-receptor subunits or associated proteins. Indeed, we find that Fyn-mediated tyrosine phosphorylation of the NR2B subunit is reduced in *Ptpra*^{-/-} hippocampus (Supplementary figure 2). Conceivably, NMDA receptor-unrelated mechanisms may further contribute to the lack of plasticity and the memory disorder in *Ptpra*^{-/-} mice. For instance, RPTP α participates in regulation of potassium channels by neurotransmitters (Tsai *et al.*, 1999; Imbrici *et al.*, 2000), and suppression of specific channels of this family has been shown to impair LTP in CA1 (e.g. Meiri *et al.*, 1998). In these issues, as in that of radial migration, identification of the upstream regulators and downstream effectors of RPTP α will provide a rewarding handle to identify novel players in brain development, synaptic plasticity and learning and memory, and their interconnections.

Materials and methods

Gene targeting

This was as described previously (Su *et al.*, 1999). Briefly, a IRES/ β -geo cassette was inserted under the control of the endogenous *Ptpra* promoter; this left no detectable RPTP α protein expressed (Su *et al.*, 1999). The mutation was propagated on a mixed 129SvEv \times 129SvJ background, and for electrophysiological and behavioral studies was also backcrossed five times into C57/Bl6.

Histology and immunohistochemistry

Animals were perfused with 4% PFA, postfixed, and embedded in OCT or paraffin. LacZ and immunostaining was performed following standard procedures. Anti-BrdU staining was preceded by 1 h denaturation (2 N HCl, 25°C), and neutralization by 100 mM tetraborate pH 8.6 for 15 min. The sources of the antibodies were: BrdU, Dako; BLBP, Dr N.Heintz (Rockefeller University); MAP-2, Boehringer; SCIP, Dr G.Lemke (Salk). Fluorescently labeled secondary antibodies were from Jackson Immunoresearch.

Electrophysiological studies

Hippocampal slices were prepared as described previously (Vitolo *et al.*, 2002). Both the stimulating and the recording electrodes were placed in CA1 SR. In determining input–output relationships, the input was the peak amplitude of the fiber volley, and the output was the slope of the 10–90% fEPSP. PPF, assessed using 10, 20, 30, 50, 100, 200 and 300 ms interstimulus intervals, was defined as the second fEPSP slope expressed as a percentage of the first. For LTP, baseline stimulation was delivered every min (0.01 ms pulse) at an intensity corresponding to 35% of that evoking maximum response. Baseline responses were recorded for 15 min prior to the LTP-inducing stimulation. LTP was induced using θ -burst stimulation (four pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including three 10 burst trains separated by 15 s). In some experiments, θ -burst stimulation consisted of one 10 burst train instead of three. For PTP measurement, three 10 burst trains were applied in the presence of 25 μ M D-APV. Synaptic fatigue was measured with a tetanus consisting of 40 pulses at 100 Hz. Statistical analysis was by factorial ANOVA with post hoc correction. Differences were considered significant when $P < 0.05$.

Behavioral studies

Experimenters were blind to the genotype. The radial-arm water maze consisted of a round tank (120 cm diameter) filled with water made opaque by white water-color #14 (Rich Art, NJ). Walls in the tank produced six swim paths radiating from a central area. Spatial cues were present on the walls and ceiling of the room. At the end of one of the arms was a 10 cm plexiglas platform, submerged 1.5 cm deep, which remained in the same location for every trial in one day, but was moved about randomly from day to day. On each trial, the mouse started the task from a different randomly chosen arm. The mouse could not use long-term memory of the location of the platform on previous days, but had to rely on the short-term memory of its location on the same day based on spatial cues. Each trial lasted 1 min and errors were counted each time the mouse entered the wrong arm with four paws, or needed more than 20 s to reach the platform. After each error, the mouse was gently pulled back to the start arm for that trial. After four consecutive trials, it was placed in its home cage for 30 min, and then administered a retention trial. Testing was considered complete when WT mice reached asymptotic performance (below one error on trials four and five; 10 training days). Scores for each mouse on the last 3 days of testing were averaged and used for statistical analysis.

Visible-platform tests to detect visual, motor or motivational deficits were performed in the same pool, but without arms and with the platform marked with a black flag, once the radial-arm water-maze studies were complete. Platform location was varied randomly to eliminate any contribution of external spatial cues. Four trials per day were administered over 2 days. Each animal was allowed to swim for 1 min from a random location. Once the mouse reached the platform (or was helped there if it did not reach it on its own), it was allowed to rest there for 30 s. Failures to reach the platform were scored as 60 s. Data were recorded using a ceiling-mounted camera and analyzed with an HVS-2020 video-tracking system.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

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