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Role of presenilin-1 in cortical lamination and survival of Cajal-Retzius neurons

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Abstract

Presenilin-1 (*PS1*), the major causative gene of familial Alzheimer disease, regulates neuronal differentiation and Notch signaling during early neural development. To investigate the role of PS1 in neuronal migration and cortical lamination of the postnatal brain, we circumvented the perinatal lethality of *PS1*-null mice by generating a conditional knockout (cKO) mouse in which PS1 inactivation is restricted to neural progenitor cells (NPCs) and NPC-derived neurons and glia. BrdU birthdating analysis revealed that many late-born neurons fail to migrate beyond the early-born neurons to arrive at their appropriate positions in the superficial layer, while the migration of the early-born neurons is largely normal. The migration defect of late-born neurons coincides with the progressive reduction of radial glia in *PS1* cKO mice. In contrast to the premature loss of Cajal-Retzius (CR) neurons in *PS1*-null mice, generation and survival of CR neurons are unaffected in *PS1* cKO mice. Furthermore, the number of proliferating meningeal cells, which have been shown to be important for the survival of CR neurons, is increased in *PS1*-null mice but not in *PS1* cKO mice. These findings show a cell-autonomous role for PS1 in cortical lamination and radial glial development, and a non-cell-autonomous role for PS1 in CR neuron survival.

Keywords: Neuronal migration; Radial glia; Notch; Meningeal fibroblasts; BrdU birthdating; Neural development

Introduction

Large numbers of mutations in *presenilin-1* (*PS1*) are the primary cause of familial Alzheimer disease. In addition to its involvement in neurodegeneration of the aged cerebral cortex, PS1 is required for maintaining the appropriate size of the neural progenitor population during development (Shen et al., 1997). In the absence of PS1, the ventricular zone and the developing cerebral cortex are thinner, indicating reductions in neural progenitor cells (NPCs) and neurons. The depletion of the neural progenitor population is caused by premature differentiation of the progenitor cells into postmitotic neurons, rather than alterations in proliferation or apoptotic cell death (Handler et al., 2000). The premature neurogenesis is associated with downregulation of the Notch signaling pathway, as evidenced by reduced expression of the Notch target genes in the *PS1*−/− embryonic brain (Handler et al., 2000) and reduced intramembrane cleavage of Notch1 in *PS1*−/− cells (De Strooper et al., 1999; Song et al., 1999).

In addition to regulation of the timing of neuronal differentiation, PS1 appears to be required for the survival of Cajal-Retzius (CR) neurons (Hartmann et al., 1999). In *PS1*−/− embryos, the cell density in the marginal zone appears unaffected at embryonic day 13 (E13), but the immunoreactivity specific for CR neurons is reduced in the marginal zone at E18 (Hartmann et al., 1999). CR neurons are transient pioneer neurons residing in layer I of the developing cerebral cortex. They secrete the extracellular glycoprotein Reelin, which provides a guidance signal for migrating cortical neurons towards the pial surface (Rice and Curran, 2001; Tissir and Goffinet, 2003). In the absence of Reelin, later born cortical neurons fail to migrate past the

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earlier born neurons, resulting in inverted cortical layers (Ogawa et al., 1995). The cause of the premature loss of CR neurons in *PS1*−/− mice is less clear. It was proposed that thickening of the meningeal layer, which is primarily composed of fibroblasts and has been shown to be important for CR neuron survival (Super et al., 1997a,b), may cause the degeneration of CR neurons in *PS1*−/− mice (Hartmann et al., 1999).

The reduction in CR neuron-specific immunoreactivity in *PS1*−/− mice raised the possibility that lack of PS1 may affect neuronal migration and cortical lamination. Consistent with this notion, BrdU birthdating analysis of *PS1*-null mice revealed that cortical neurons labeled at E10.5 fail to arrive at their appropriate position and form a well-defined cortical plate at E14.5, suggesting a neuronal migration defect (Handler et al., 2000). However, the severe cerebral hemorrhage associated with later embryonic stages of *PS1*−/− mice makes it difficult to extend such analysis to later developmental stages. The perinatal lethality of *PS1*−/− mice precluded further investigation into the postnatal stages. We therefore generated a *PS1* conditional knockout (cKO) mouse in which PS1 inactivation is restricted to the central nervous system (CNS) to address whether PS1 is required for normal cortical lamination and neuronal migration. Furthermore, since PS1 is inactivated only in CNS-derived cells, such as CR neurons, but not in meningeal cells in *PS1* cKO mice, we were able to determine whether PS1 is required for CR neuron survival in a cell-autonomous or non-cell-autonomous manner.

Materials and methods

Mice

PS1−/− embryos were obtained by timed mating between *PS1* heterozygous knockout mice with the day of the vaginal plug designated as E0.5. To generate *PS1* cKO mice, homozygous floxed *PS1* (*fPS1/fPS1*) mice, in which *PS1* exons 2 and 3 are *f*lanked by two l*ox* P sites (Yu et al., 2000, 2001), were crossed with *Nestin-Cre* transgenic mice (Tronche et al., 1999) to obtain mice heterozygous for the *fPS1* allele and hemizygous for the *Nestin-Cre* allele, which were then crossed with *fPS1/fPS1* mice to obtain *PS1* cKO (*fPS1/fPS1;Nestin-Cre* or *fPS1/fPS1*$^{\Delta}$*;Nestin-Cre*) mice. The *fPS1*$^{\Delta}$ allele represents a *PS1*-null allele, resulting from Cre-mediated excision of floxed *PS1* exons 2 and 3 in a small percentage of germ cells. The desired genotype was obtained at a ratio (approximately 1:36) much lower than the expected Mendelian ratio (1:4), indicating that the integration site of the *Nestin-Cre* transgene was on the same chromosome as *PS1* and that the crossover between the *PS1* locus and the integration site of the *Nestin-Cre* transgene occurs at 2.8% frequency (2.8 centi-Morgans). Because *PS1* cKO mice die at 2–3 months of age, they were not ideally suited to be used as breeding mice. We therefore crossed *fPS1/fPS1*$^{\Delta}$*;Nestin-Cre* with *fPS1/+* to obtain *+/fPS1*$^{\Delta}$*; Nestin-Cre* mice, which were then crossed with *fPS1/fPS1* female mice to obtain *fPS1/fPS1*$^{\Delta}$*;Nestin-Cre* (*PS1* cKO) and *fPS1/+* (control) mice at a ratio of 1:1, due to the linkage between the *fPS1*$^{\Delta}$ and *Nestin-Cre* alleles.

In situ hybridization and Western blotting

For in situ hybridization detection of *PS1* transcripts, sense and antisense probes were synthesized using PCR-generated DNA fragments containing *PS1* exons 2 and 3 as template and T7 and SP6 RNA polymerase, respectively. For quantitative in situ hybridization analysis using radioactive probes, tissue pretreatment and hybridization were performed as previously described with minor modifications (Frantz et al., 1994). Briefly, brain sections were pretreated with 1.5 μg/ml proteinase K (Roche Molecular Biochemicals, Indianapolis, IN) for 30 min at room temperature, followed by two washes in 2× SSC. Hybridization was carried out overnight at 60°C in 62.5% formamide, 12.5% dextran sulfate, 0.375 M NaCl, 1.25% Denhardt's solution, 12.5 mM Tris (pH 8), 1.25 mM EDTA, and 5×10^6 cpm/ml of *PS1* probe. After hybridization, brain sections were treated with 50 μg/ml RNase A (Roche Molecular Biochemicals) and washed in 0.1× SSC at 60°C. For higher resolution in situ hybridization using digoxigenin-labeled *PS1* probes, sections were pretreated with acetic anhydride (0.25% v/v) and triethanolamine (1.2% v/v) for 10 min at room temperature, then blocked with hybridization buffer [50% formamide, 5× SSC, 0.02% SDS, 0.1% *N*-lauroylsarcosine, and 2% blocking reagent (Roche Molecular Biochemicals)] in a humidified chamber for 6 h at room temperature. Probe was then added to hybridization buffer at 0.4 μg/ml and incubated overnight at 65°C. The following day, sections were RNase-treated (10 μg/ml) for 30 min at room temperature and washed in 0.1× SSC at 65°C. Sections were then blocked with 1% blocking reagent in maleic acid buffer (Roche Molecular Biochemicals) and incubated with anti-digoxigenin Fab fragments conjugated to alkaline phosphatase (1:5000; Roche Molecular Biochemicals) overnight at room temperature. Color development was performed in the dark at room temperature with BM purple AP substrate (Roche Molecular Biochemicals).

For Western analysis, brains were dissected, and meninges were removed in ice-cold PBS. Lysates were prepared as previously described (Yu et al., 2001). For detection of PS1 and BLBP, lysates were loaded onto either 4–20% (PS1) or 12% (BLBP) Tris–glycine SDS–PAGE gels and transferred to PVDF membranes (BioRad, Hercules, CA). For detection of Nestin, lysates were loaded onto 3–8% Tris–acetate SDS–PAGE gels and transferred to nitrocellulose membranes (Protran BA83; Schleicher and Schuell, Keene, NH). Membranes were then blocked in 5% milk, 0.1% BSA in PBS (for the immunodetection of PS1, BLBP, and tubulin), or 5% milk, 0.1% Tween-20 in PBS (for the immunodetection of Nestin and α-spectrin),

and incubated overnight with a PS1 N-terminus-specific antiserum (1:8000) (Thinakaran et al., 1998), BLBP antibody (1:10,000) (Feng et al., 1994), Nestin antibody (1:1000; BD Biosciences Pharmingen, San Diego, CA), α-spectrin antibody (1:5000; Chemicon, Temecula, CA), or a mouse anti-tubulin antibody (1:100,000; Sigma, St. Louis, MO). Blots were then incubated with an HRP-conjugated secondary antibody (BioRad), and the signals were detected by chemiluminescence (SuperSignal West Pico kit, Pierce Biotechnology, Rockford, IL).

For quantification of Nestin and BLBP protein levels, autoradiographs were scanned, converted to digital image files in Adobe Photoshop, and assayed by densitometric analysis with NIH Image 1.62. Equal loading and transfer of protein were confirmed by probing the blots with antibodies for α-spectrin (for Nestin) or α-tubulin (for BLBP). Relative protein levels of control and mutant sample pairs were compared and evaluated for statistical significance by Student *t* test. For the E12.5 dataset, four pairs of control and mutant littermates were analyzed; for the E17.5 dataset, five pairs of controls and mutant littermates were analyzed. Error bars denote standard deviation.

Beta-galactosidase staining of brain sections

For detection of β-galactosidase activity in *Nestin-Cre;Rosa26* (Soriano, 1999) double transgenic mice, embryos at E17.5 were removed from the uterus, and brains were dissected in ice-cold PBS and lightly fixed by immersion in 4% paraformaldehyde at 4°C for 1 h. Brains were cryoprotected in 30% sucrose/PBS overnight at 4°C, embedded in OCT medium, and sectioned at 12 μm. Sections were washed with 100 mM $MgCl_2$, 0.1% deoxycholic acid, and 0.5% NP-40 in PBS and subsequently incubated with 1 mg/ml X-gal + 5 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ diluted in the same buffer at 37°C. Following staining, sections were washed with PBS, fixed with 4% paraformaldehyde, and mounted with Crystal Mount medium (Biomeda, Foster City, CA).

Tissue preparation and immunohistochemical analysis

For immunostaining, whole heads of embryos at E11.5–15.5 or dissected brains at E17.5 were immersion fixed in 4% paraformaldehyde for 1.5–3 h at 4°C, then transferred to 30% sucrose/PBS for cryoprotection. Heads or brains were embedded in OCT and stored at −80°C. Cryostat sections were cut at 12 μm, thaw-mounted on Colorfrost slides (Fisher), and stored at −20°C. Brain sections were blocked with 3% normal goat serum, 1% BSA, and 0.1% Triton-X 100 for 1 h at RT and then incubated with monoclonal RC2 antibody (1:5; Developmental Studies Hybridoma Bank, Iowa City, IA), anti-BLBP polyclonal antibody (1:3000) (Feng et al., 1994), anti-phosphohistone H3 (Ser10) polyclonal antibody (1:200; Upstate, Lake Placid, NY) (Mahadevan et al., 1991), or anti-MAP2 monoclonal antibody AP-20 (1:500; Sigma) overnight at 4°C. Slides were then incubated with Alexa Fluor secondary antibodies (1:300; Molecular Probes, Eugene, OR) for 1–2 h at RT, washed in PBS, and mounted with Vectashield aqueous mountant (Vector Laboratories, Burlingame, CA). For detection of Reelin, slides were processed using the Histomouse SP kit (Zymed Laboratories, San Francisco, CA) following the manufacturer's suggestions, except that the brain sections were incubated with anti-Reelin monoclonal antibodies CR-50 (1:400) (Ogawa et al., 1995) or G10 (1:1000) (de Bergeyck et al., 1997) for 4 h at room temperature and that streptavidin-FITC (1:1000; BD Biosciences, San Diego, CA) was used instead to increase the signal intensity.

BrdU labeling and detection

For BrdU pulse labeling, timed pregnant female mice were injected intraperitoneally with BrdU (100 mg/kg) and sacrificed 30 min postinjection. For birthdating experiments, timed pregnant female mice were injected with BrdU (50 mg/kg) five times with 3-h intervals as previously described (Chae et al., 1997), the pups at postnatal day 21 (P21) were sacrificed and perfused intracardially with 4% paraformaldehyde, and the brains were then dissected and postfixed for 2 h at 4°C. Fixed brains were transferred to PBS and processed for paraffin embedding. Coronal sections were cut (14 μm) on a Leica microtome, deparaffinized in xylenes for immunostaining, and counterstained with hematoxylin. For BrdU immunostaining, the brain sections were denatured in 2 N HCl for 45 min at 37°C, neutralized in borate buffer (pH 8.4) for 10 min at RT, rinsed twice in PBS, blocked in 3% NGS, 0.1% Triton-X 100 in PBS for 1 h at RT, and then incubated with rat anti-BrdU monoclonal antibody (1:20; Harlan Bioproducts, Madison, WI). The signal was detected using the Vectastain Elite ABC peroxidase staining and DAB substrate kits (Vector Laboratories).

Quantification of BrdU-positive neurons

BrdU-positive (BrdU+) neurons were scored in brains from mice at P21 by capturing digital images on an Olympus BX40 light microscope equipped with an Olympus DP11 camera (Olympus, Melville, NY), assembling composite images spanning the width of the cerebral cortex in Adobe Photoshop, applying a grid overlay of defined dimensions to each composite sample image, and counting labeled neurons in each bin. The grid overlay width was set at 800 μm, and the length was divided into five equal bins, with specific bin length proportional to cortical thickness of the sample; the range was 225–275 μm per bin. Numerical order of the bins corresponds to positions of the cortical layers, with Bin 1 defined as the most superficial layer and Bin 5 as the deepest layer. For neurons labeled at E12, a stage when many progenitors are undergoing cell division, only neurons with higher levels of BrdU incorporation were scored; for neurons labeled at E16, all neurons with BrdU-

positive nuclei were counted. For each brain, three comparable coronal sections from control and mutant littermates were analyzed. Statistical significance was evaluated by Student *t* test.

Quantification of CR neurons and meningeal cells

The number of CR neurons at E13.5 was quantified by counting Reelin-positive (Reelin+) neurons in layer I of the telencephalon from the rostral interhemispheric fissure to the caudal tip using transverse sections. The number of CR neurons at E17.5 was quantified by counting Reelin- or calretinin-positive neurons in layer I of the telencephalon from the dorsal interhemispheric fissure to the level of the ventral tip of the fimbria using coronal sections. Only those with clearly stained cell bodies visible in the plane of the section were counted; morphology of each CR neuron was verified by visual inspection. The number of BrdU-positive meningeal cells at E17.5 was quantified in the telencephalon from the dorsal interhemispheric fissure to the level of the ventral tip of the fimbria using coronal sections. BrdU-positive nuclei were counted only if they contained strong immunoreactivity and were localized in the meningeal layers directly above the telencephalon. Phosphohistone H3-positive meningeal cells were scored in both hemispheres of four consecutive sections from the dorsal interhemispheric fissure to the ventralmost extent of the telencephalon. At least three sections per brain were used for counting BrdU+ and Reelin+ cells, and a minimal number of three brains per genotype were used for each marker scored. Quantification of Reelin+ cells at E17.5 by an investigator who was blind to genotype confirmed the original data. Student *t* test was used to assess statistical significance.

Result

Generation of neural progenitor cell-specific PS1 conditional knockout mice

To overcome the perinatal lethality of *PS1*−/− mice, which is caused by respiratory failure due to malformation of the ribcage (Shen et al., 1997), we employed the Cre/loxP recombination system to restrict *PS1* inactivation to the CNS. We first generated a floxed *PS1* (*fPS1*) mouse by introducing two *loxP* sites into introns 1 and 3, which does not affect transcription, splicing, or translation of *PS1* (Yu et al., 2001). In the presence of Cre recombinase, *PS1* exons 2 and 3 are excised by Cre-mediated site-specific recombination, resulting in a *PS1*-null allele (Yu et al., 2001). We then crossed *fPS1* mice with *Nestin-Cre* (*Nes-Cre*) transgenic mice, in which Cre is expressed in neural progenitor cells under the control of the *Nestin* promoter (Tronche et al., 1999), to generate a neural progenitor cell-specific *PS1* conditional knockout (*PS1* cKO) mouse. Expression of *Cre* transcripts in *Nes-Cre* transgenic mice begins at E9.0 in the spinal cord and the forebrain–midbrain junction, and then extends to the entire CNS by E10.5 (Yang et al., 2004). By crossing the same *Nes-Cre* mouse with a *Rosa26* reporter mouse, another report demonstrated that *Cre*-mediated site-specific recombination occurs in neural progenitor cells localized in the ventricular zone (Graus-Porta et al., 2001).

To determine the spatial and temporal pattern of *PS1* inactivation, we performed quantitative in situ hybridization analysis on *PS1* cKO (*fPS1/fPS1*$^{\Delta}$*;Nestin-Cre*) and littermate control (*fPS1/+*) mice between the ages of E9.5 and E15.5 using a radioactive probe specific for exons 2 and 3. We began to detect a slight reduction in *PS1* transcripts in cKO brains at E10.5 (Fig. 1A). By E12.5, *PS1* transcripts become undetectable in cKO brains (Fig. 1A). Western analysis confirmed the absence of PS1 protein in cKO brains at E12.5 (Fig. 1B). These results show that inactivation of PS1 expression begins at E10.5 and is complete by E12.5 in *PS1* cKO brains. The delay of PS1 inactivation relative to the time of Cre expression is likely caused by the slow turnover of the existing *PS1* transcripts and protein (Bazan and Lukiw, 2002; Counts et al., 2001).

To confirm the absence of *PS1* transcripts in layer I neurons at E17.5, we performed in situ hybridization on *PS1* cKO and littermate control brains using a digoxigenin-labeled probe. In Fig. 1C, high-magnification images show that, in control brains, *PS1* transcripts are more abundant in the cortical layers than in the meningeal layer. In *PS1* cKO brains, intact *PS1* transcripts are eliminated in the cortical layers, whereas *PS1* transcripts remain in the meningeal layer where Cre recombinase is not expressed. To confirm the occurrence of Cre-mediated recombination in CR neurons but not in meningeal cells, we crossed *Nes-Cre* transgenic mice to *Rosa26* reporter mice. *Lac*Z staining of double transgenic *Rosa26;Nes-Cre* brains at E17.5 revealed *Lac*Z-positive cells in the cortex, including the large horizontal CR neurons in layer I. The meninges directly superficial to layer I are negative for β-galactosidase activity, indicating the lack of Cre recombinase activity, consistent with previous findings in these mice (Graus-Porta et al., 2001). These results confirm that Cre-mediated recombination occurs in CR neurons but not in meningeal cells.

PS1 cKO mice live up to 2–3 months of age, allowing investigation of the role of PS1 in postnatal cortical development. *PS1* cKO mice are often visually distinguishable from their littermate controls by their smaller body and head size. With increasing age, they exhibit excessive grooming behavior, become withdrawn from social interactions, and eventually lose much of their body weight. Their early mortality may be a consequence of decreased food intake, widespread intracranial hemorrhage, and hydrocephalus, which could cause brain herniation. Due to the expected developmental defects in these mice, they are not well suited for the study of presenilin function in the adult brain. Our analysis of postnatal forebrain-restricted

PS1 single and *PS1/PS2* double conditional knockout mice revealed that presenilins are essential for learning and memory, synaptic plasticity, and neuronal survival in the adult brain (Saura et al., 2004; Yu et al., 2001).

Reduction of neural progenitors in PS1 cKO mice

We previously reported that, in the absence of PS1, neural progenitor cells prematurely differentiate into post-mitotic neurons (Handler et al., 2000). In *PS1*−/− mice, more MAP2-immunoreactive neurons are present in the diencephalon and telencephalon, followed by a marked reduction of the number of BrdU-positive neural progenitor cells (Handler et al., 2000). Similar to *PS1*−/− brains, more MAP2-immunoreactive neurons are present in the telencephalon and the diencephalon of *PS1* cKO mice ($n = 3$) at E12.5 compared to controls ($n = 3$) (Figs. 2A–D). At E13.5, fewer BrdU-positive neural progenitors were labeled in *PS1* cKO brains ($n = 3$) in the lateral ganglionic eminence of the telencephalon (Figs. 2E, F) and the diencephalon (Figs. 2I, J). By E17.5, much fewer BrdU-positive progenitors remain in the ventricular zone of *PS1* cKO brains ($n = 3$) (Figs. 2K–N) relative to control brains ($n = 3$). These results are consistent with our previous findings in *PS1*−/− mice, showing premature neuronal differentiation and reduced neural progenitor population in the *PS1* cKO brain.

Disrupted cortical lamination in PS1 cKO mice

To determine whether lack of PS1 affects cortical lamination and neuronal migration, we performed birthdating analysis by labeling early- and late-born neurons with BrdU at E12 and E16, respectively, and then examining their final destination in the neocortex at postnatal day 21 (P21). Most neurons generated at E12 are properly positioned in the deeper cortical layers of *PS1* cKO mice, although a few BrdU-labeled cells are found in the more superficial layers (Figs. 3A, B). To quantify the final cortical position of labeled neurons, we divided the neocortex into five equal segments or bins, and counted the number of BrdU-positive neurons in each bin. Quantitative analysis of neurons born at E12 containing moderate to high levels of BrdU revealed no significant difference between control ($n = 3$) and *PS1* cKO ($n = 2$) mice in each of the bins ($P > 0.05$) (Fig. 3G).

In contrast to the largely normal positioning of the early-born neurons, many of the later born neurons labeled by BrdU at E16 fail to arrive at their appropriate positions in

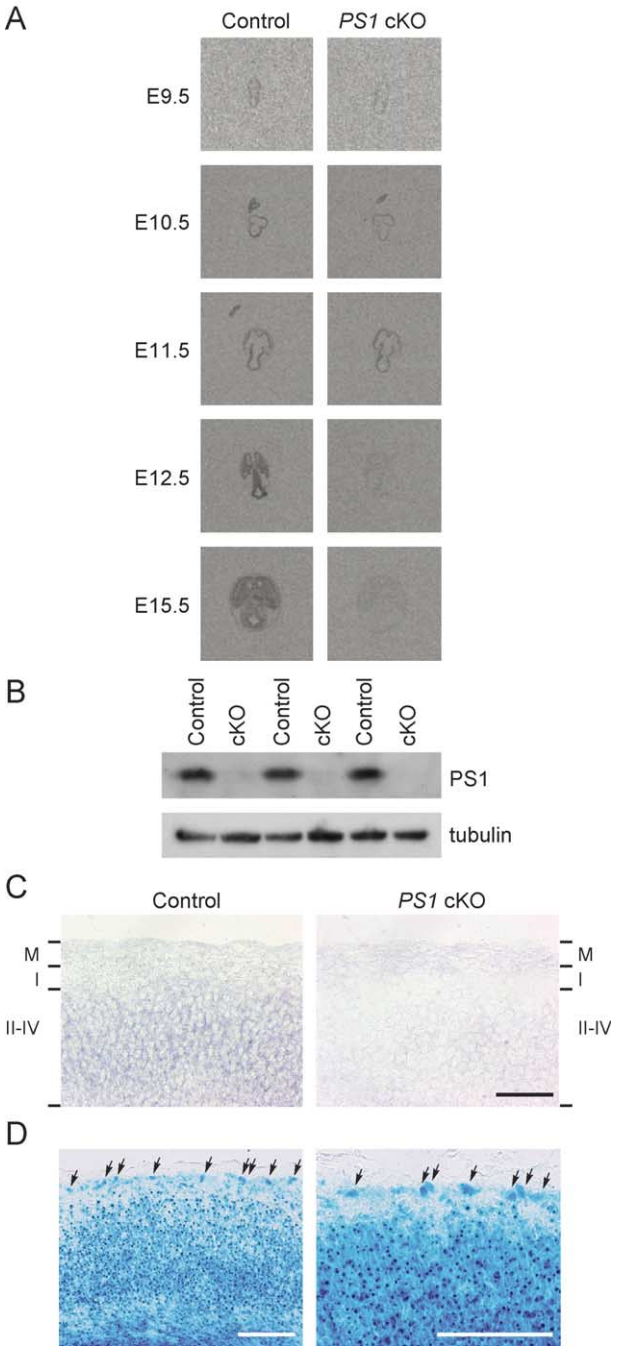
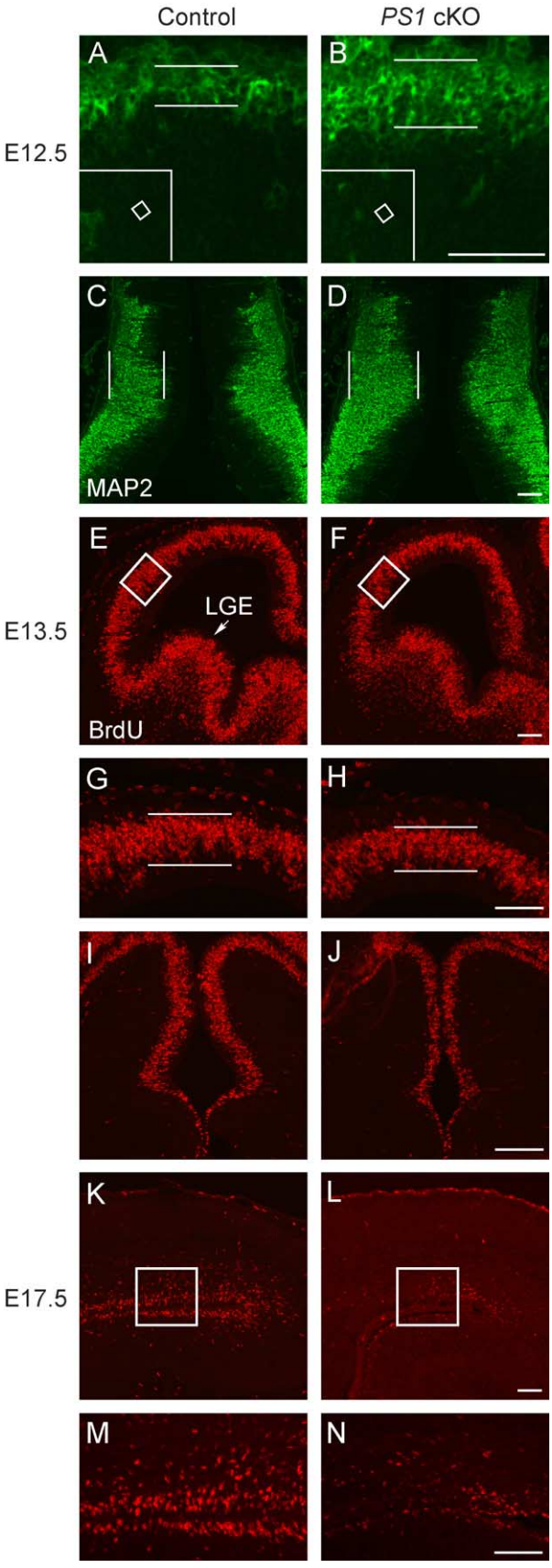


Fig. 1. Expression of *PS1* transcripts and proteins is inactivated by E12.5 in the CNS-restricted *PS1* cKO mouse. (A) In situ hybridization analysis of *PS1* cKO and control mice between the ages of E9.5 and E15.5 using a radioactive probe specific for *PS1* exons 2 and 3. At each age, comparable brain sections of *PS1* cKO and control mice are shown. The level of *PS1* transcripts is slightly reduced at E10.5 and E11.5, and decreases below detection at E12.5 and E15.5. (B) Western analysis of *PS1* cKO and control brains at E12.5. *PS1* protein is present in the control brain but absent in the cKO brain. Meninges were removed from the embryonic brain to prevent the detection of *PS1* expressed in meningeal fibroblasts. (C) In situ hybridization analysis of *PS1* cKO and control mice at E17.5 using a digoxigenin-labeled probe specific for *PS1* exons 2 and 3. Comparable brain sections are shown. Similar intensity of *PS1* transcripts is present in the meningeal layer of both *PS1* cKO and control brains. *PS1* transcripts are expressed highly in the cortical layers of control brains but not in the *PS1* cKO brain. M indicates meningeal layer; I, cortical layer I; II–IV, cortical layers II–IV. Scale bar: 50 μm. (D) *LacZ* staining of *Nestin-Cre; Rosa26* transgenic mice at E17.5. Cre-mediated recombination activated expression of β-galactosidase is found in cortical neurons including Cajal-Retzius neurons, which exhibit their characteristic large, horizontal shape (indicated by arrows). In contrast, cells in the meningeal layer located directly above layer I are negative for *LacZ* staining. Scale bars: 100 μm.



layer II/III and are scattered throughout the cortical layers, including layers V and VI (Figs. 3C, D). To quantify the migration defect, we counted late-born neurons labeled with BrdU at E16 in each bin. The number of labeled cells is significantly reduced (22.1%; $P = 0.016$) in Bin 2 and significantly increased in Bins 3 (8.6%; $P = 0.002$) and 5 (15%; $P = 0.007$) of *PS1* cKO mice ($n = 3$) compared to controls ($n = 3$) (Fig. 3H). Consistent with these data, gross histological analysis of *PS1* cKO brains also showed less clear demarcations of the cortical layers (Figs. 3E, F). These results indicate that, in the absence of PS1, the late-born neurons exhibit a more pronounced migration defect and fail to migrate past the early-born neurons to arrive at their appropriate destination in the superficial cortical layers.

Reduced radial glial generation in PS1 cKO mice

Previous studies have shown that PS1 is required for normal Notch signaling during neural development (De Strooper et al., 1999; Handler et al., 2000; Song et al., 1999). Furthermore, Notch signaling has been shown to be involved in radial glial development (Gaiano et al., 2000; Patten et al., 2003; Schmid et al., 2003). In addition to serving as neural progenitor cells, radial glia are known to provide scaffolding for migrating cortical neurons towards the pial surface (Goldman, 2003; Rakic, 2003). We therefore examined *PS1* cKO brains at E11.5–17.5 to determine whether radial glial generation is affected in the absence of PS1, which may explain the migration defect of cortical neurons. RC2 immunoreactivity, a commonly used marker for radial glia (Misson et al., 1988), is similar in the telencephalon of *PS1* cKO and control mice at E11.5 and E12.5, suggesting normal radial glial generation at these early stages (Figs. 4A–D). Western analysis of other radial glial markers, Nestin (Frederiksen and McKay, 1988) and

Fig. 2. Premature differentiation of neurons and depletion of neural progenitor cells in *PS1* cKO brains. (A–D) Differentiated neurons are labeled by MAP2 immunoreactivity in the telencephalon (A, B) and the diencephalon (C, D) at E12.5. Comparable sections of the *PS1* cKO and control brains are shown. An increase in MAP2 immunoreactivity is found in the superficial layer of the lateral telencephalon of the *PS1* cKO brain (indicated by pairs of horizontal white lines), indicating the presence of a greater number of neurons. Boxes within inset panels in A and B identify the position of images A and B within the telencephalon. In C and D, a noticeable increase in MAP2-positive neurons is present also within the diencephalon of *PS1* cKO brains (delimited by pairs of vertical lines). (E–N) Proliferating neural progenitors are identified by BrdU incorporation at E13.5 and E17.5. A brief pulse of BrdU (30 min) followed by BrdU immunostaining reveals a noticeable reduction in neural progenitors in the ventral telencephalon (e.g., lateral ganglionic eminence, LGE) at E13.5 (E, F). G and H are higher power views of the boxed areas in E and F, indicating a slight reduction in proliferating progenitors in the dorsal telencephalon of *PS1* cKO brains as compared to the littermate control brain. In the diencephalon (I, J), the neural progenitor population is markedly decreased in *PS1* cKO mice. By E17.5, few proliferating neural progenitor cells remain in the ventricular zone of the *PS1* cKO telencephalon relative to control brain (K, L). M and N are higher power views of the boxed areas in K and L. Scale bar: 100 μm.

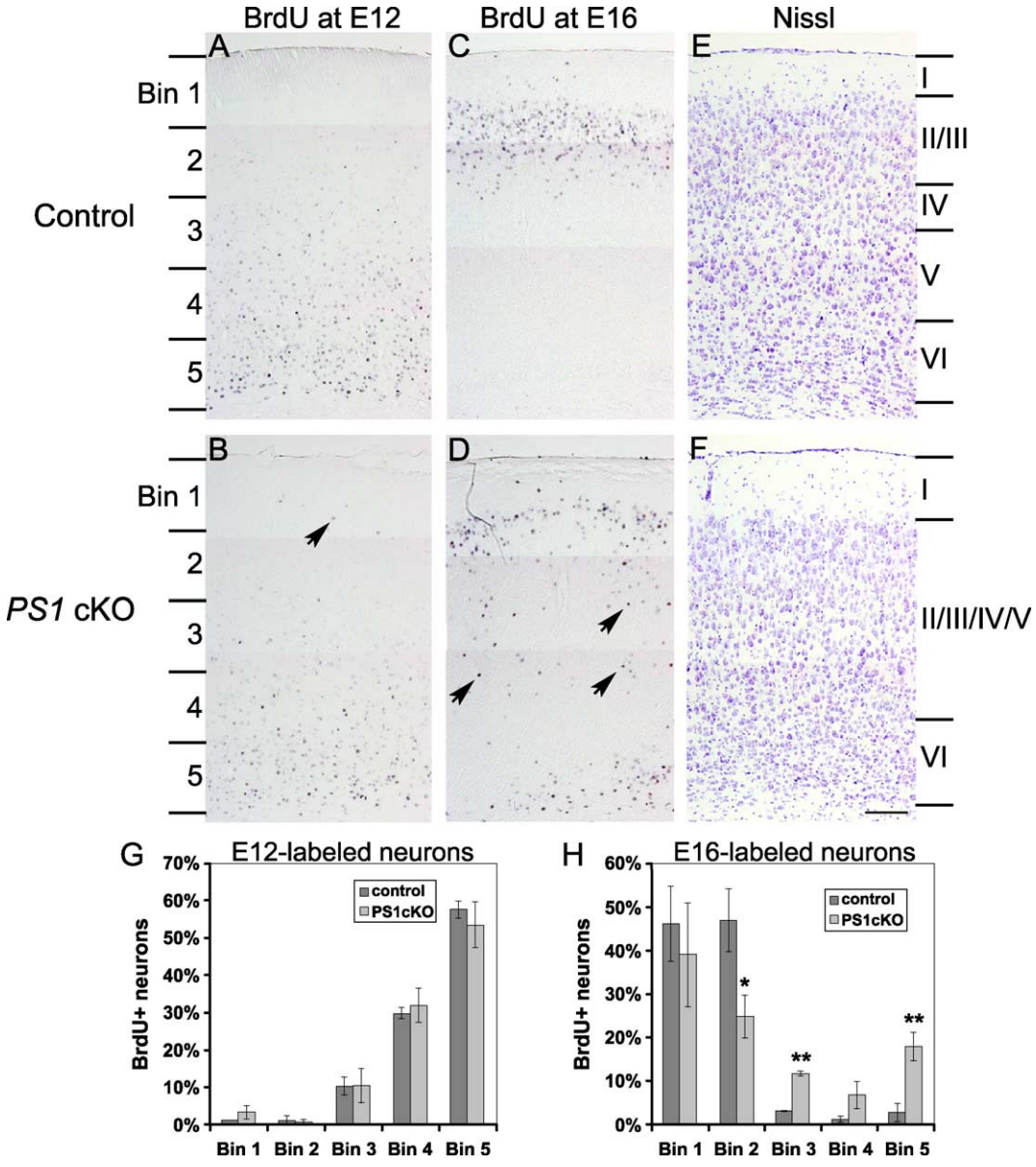


Fig. 3. BrdU birthdating analysis reveals perturbed neuronal migration and cortical lamination in the postnatal brain of *PS1* cKO mice. (A, B) The final destination of the early-born neurons labeled by BrdU at E12 is shown in the postnatal brain of *PS1* cKO and control mice at P21. Comparable areas in the medial cortex of *PS1* cKO and control brains are shown. The darkly stained neurons are those that exit the cell cycle immediately after the injection of BrdU at E12, while the less intensely stained neurons are those that exit the cell cycle after one or multiple cell divisions. In the control cortex, the labeled neurons are localized in the deeper cortical layers (layers V and VI). In the *PS1* cKO cortex, most of the labeled neurons are appropriately localized in the deeper cortical layers, while a few labeled neurons, indicated by the arrow, are scattered in the superficial layer (layer II/III). (C, D) The final destination of the late-born neurons labeled by BrdU at E16 is shown in the postnatal brain of *PS1* cKO and control mice at P21. Comparable areas in the medial cortex of *PS1* cKO and control brains are shown. In the control cortex, the BrdU-labeled late-born neurons are all appropriately localized in the superficial cortical layer (layer II/III). In the *PS1* cKO cortex, the late-born neurons labeled by BrdU are not restricted to layer II/III but scattered throughout the cortical layers. The arrows indicate neurons displaying aberrant migration. (E, F) Nissl-stained coronal brain sections of *PS1* cKO and control mice at P21. The area shown is comparable to those shown in A–D. At this age, even in the control mice, the demarcations between the adjacent cortical layers are not as clear as those in older brains. In *PS1* cKO brains, the demarcations among the cortical layers appear even less clear than those in the control. The thickness of the cortical layers in the medial cortex at the level of the posterior parietal association area is slightly thinner in *PS1* cKO mice compared to the control. The thinning of the cortical layers is more severe in the lateral area of the cortex in *PS1* cKO mice (not shown). (G, H) Quantification of E12 and E16 BrdU-labeled neurons present in specific cortical layers at P21. The bar graph depicts the percentage of labeled neurons in each position, or bin, relative to the total number scored per brain. The majority of neurons born at E12 in both controls and *PS1* cKO mutants are localized to the deep layers (Bins 4 and 5); in contrast, a large percentage of neurons born at E16 in *PS1* cKO brains fails to reach their appropriate cortical position and remains in the deeper layers. Error bars were drawn using the standard deviation. One asterisk represents a P value of <0.05; two asterisks represent $P < 0.01$. Scale bar: 150 μm.

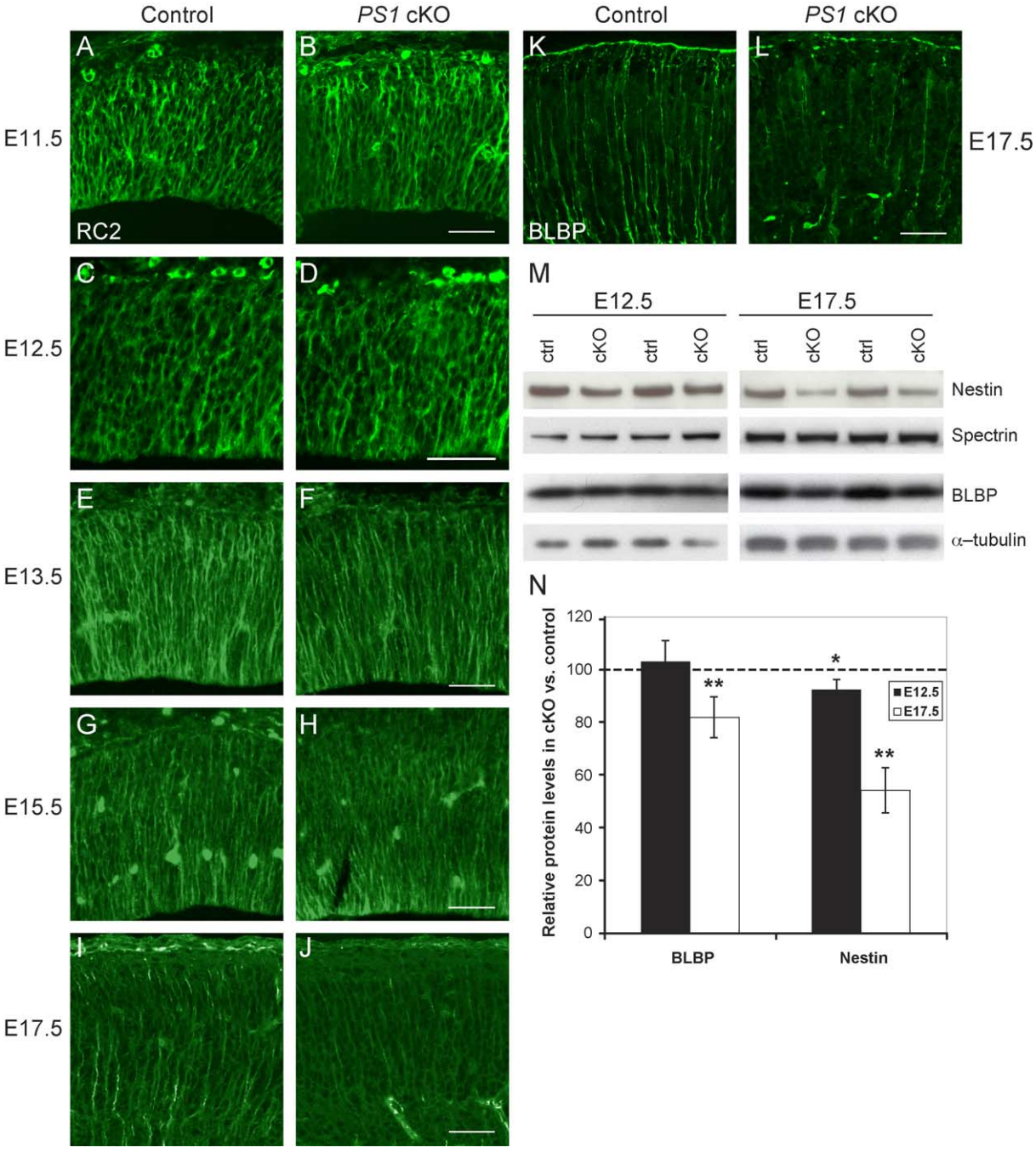


Fig. 4. Expression of glial markers, RC2, BLBP, and Nestin, is normal at E12.5 and progressively diminishes in *PS1* cKO mice. (A–L) Confocal images captured from the rostral medial telencephalon of embryonic brains immunostained for RC2 (A–J) or BLBP (K, L) are shown. (A–D) At E11.5 and E12.5, immunohistochemical analysis shows similar RC2 immunoreactivity in the comparable area of the telencephalon in *PS1* cKO and control mice, indicating unaffected radial glia at this age. (E, F) At E13.5, RC2 immunoreactivity is reduced in *PS1* cKO brains, indicating reduction in radial glia. Comparable areas of the *PS1* cKO and control telencephalon are shown. (G, H) At E15.5, RC2 levels appear further diminished in the *PS1* cKO telencephalon, with shorter glial processes extending toward the pial membrane and more intense RC2 staining near the lateral ventricle. (I–L) At E17.5, RC2 immunoreactivity is markedly reduced in the telencephalon of *PS1* cKO mice. The decrease in radial glia is confirmed using another marker for radial glia, BLBP, the expression of which is upregulated upon contact with migrating neurons. Comparable areas of the *PS1* cKO and control telencephalon are shown. (M, N) BLBP protein levels are relatively normal in *PS1* cKO brain at E12.5, but decrease to 82% of control levels by E17.5 ($P = 0.007$). Nestin levels are slightly reduced in mutants versus controls at E12.5 (92%; $P = 0.019$) and diminish to 54% of control levels at E17.5 ($P = 0.001$). Alpha-spectrin, a 240-kDa structural protein, was used as a control for Nestin Western blots, since its molecular weight is similar to Nestin (approximately 200 kDa). Error bars represent standard deviation. The *P* values are indicated as follows: $P < 0.05$ (one asterisk); $P < 0.01$ (two asterisks) as evaluated by Student *t* test. Scale bar: 100 μm.

BLBP (Feng and Heintz, 1995; Feng et al., 1994), showed similar levels of BLBP (102%; $P = 0.695$) and Nestin (92%; $P = 0.019$) in *PS1* cKO ($n = 5$) relative to control ($n = 5$) brains at E12.5 (Figs. 4M, N).

At E13.5 and E15.5, RC2 immunoreactivity appears reduced in the *PS1* cKO telencephalon (Figs. 4E, F). By E17.5, RC2 and BLBP immunoreactivity is markedly reduced in *PS1* cKO (Figs. 4I–L), indicating reduced radial glia. The reduction was confirmed by Western blot analysis, showing reduced BLBP (approximately 80%; $P = 0.007$) and Nestin (approximately 50%; $P = 0.001$) levels in *PS1* cKO brains ($n = 5$) compared to control brains ($n = 5$) at E17.5 (Figs. 4M, N). These data indicate that PS1 inactivation is accompanied by progressive reductions in radial glia, which could explain the migration defect of the late-born neurons in *PS1* cKO mice. The reduction in radial glia is consistent with reduced neural progenitors in *PS1*-null (Handler et al., 2000) and *PS1* cKO (Fig. 2) brains, as radial glia have been shown to be neural progenitors in the developing brain (Doetsch, 2003; Goldman, 2003).

Normal numbers of CR neurons in PS1 cKO mice but reduced numbers of CR neurons in PS1 −/− mice

Although the progressive reduction in radial glia provides an explanation for the disrupted cortical lamination observed in *PS1* cKO mice, loss of CR neurons could also contribute to this neuronal migration defect. Furthermore, CR neurons are important for the maintenance of the radial glial scaffold (Super et al., 2000). We therefore examined *PS1* cKO ($n = 3$) and control ($n = 3$) mice at E13.5 and E17.5 to determine whether inactivation of PS1 affects generation and survival of CR neurons. We used both Reelin and calretinin as markers for CR neurons (de Bergeyck et al., 1997; Glezer et al., 1992; Ogawa et al., 1995; Winsky et al., 1989). Similar levels of Reelin (Figs. 5A–D) and calretinin (data not shown) immunoreactivity were observed in the marginal zone of the telencephalon in *PS1* cKO and control mice at E13.5 and E17.5. At E13.5, CR neurons were counted in the marginal zone around the entire telencephalon in transverse sections; at E17.5, CR neurons were scored within the marginal zone in the dorsal aspect of coronal sections (from the interhemispheric fissure to the level of the distal tip of the fimbria). Quantification of Reelin-positive CR neurons in the marginal zone showed similar numbers of CR neurons in *PS1* cKO (E13.5: 161 ± 3; E17.5: 148 ± 7) and control (E13.5: 156 ± 3; E17.5: 157 ± 9) telencephalon at E13.5 ($P = 0.11$) and E17.5 ($P = 0.25$) (Figs. 5E, F). These results indicate unaffected generation and survival of CR neurons in *PS1* cKO mice.

It has been reported that immunoreactivity for calretinin and Reelin is reduced in the marginal zone of *PS1 −/−* brains at E18, while cell number in the marginal zone appears unaffected at E13.5, suggesting a premature loss of CR neurons (Hartmann et al., 1999). To determine quantitatively whether there is indeed a premature degeneration of CR neurons, we counted the number of Reelin-immunoreactive CR neurons in the marginal zone of *PS1 −/−* and control brains at E13.5 ($n = 3$) and E17.5 ($n = 3$). Similar numbers of CR neurons are present in *PS1 −/−* (193 ± 5) and littermate control (180 ± 12) mice at E13.5 ($P = 0.18$), while significantly fewer CR neurons remain in *PS1 −/−* mice (127 ± 15) compared to the control (177 ± 8) by E17.5 ($P < 0.02$) (Figs. 5G–L). Similar results were obtained using calretinin as marker for CR neurons (data not shown). These results confirmed the previous observation (Hartmann et al., 1999) and demonstrated that normal numbers of CR neurons are generated, but fewer of them survive by E17.5 in *PS1 −/−* mice. Together, the results obtained from *PS1* cKO and *PS1 −/−* mice show that PS1 is required for CR neuron survival in a non-cell-autonomous manner.

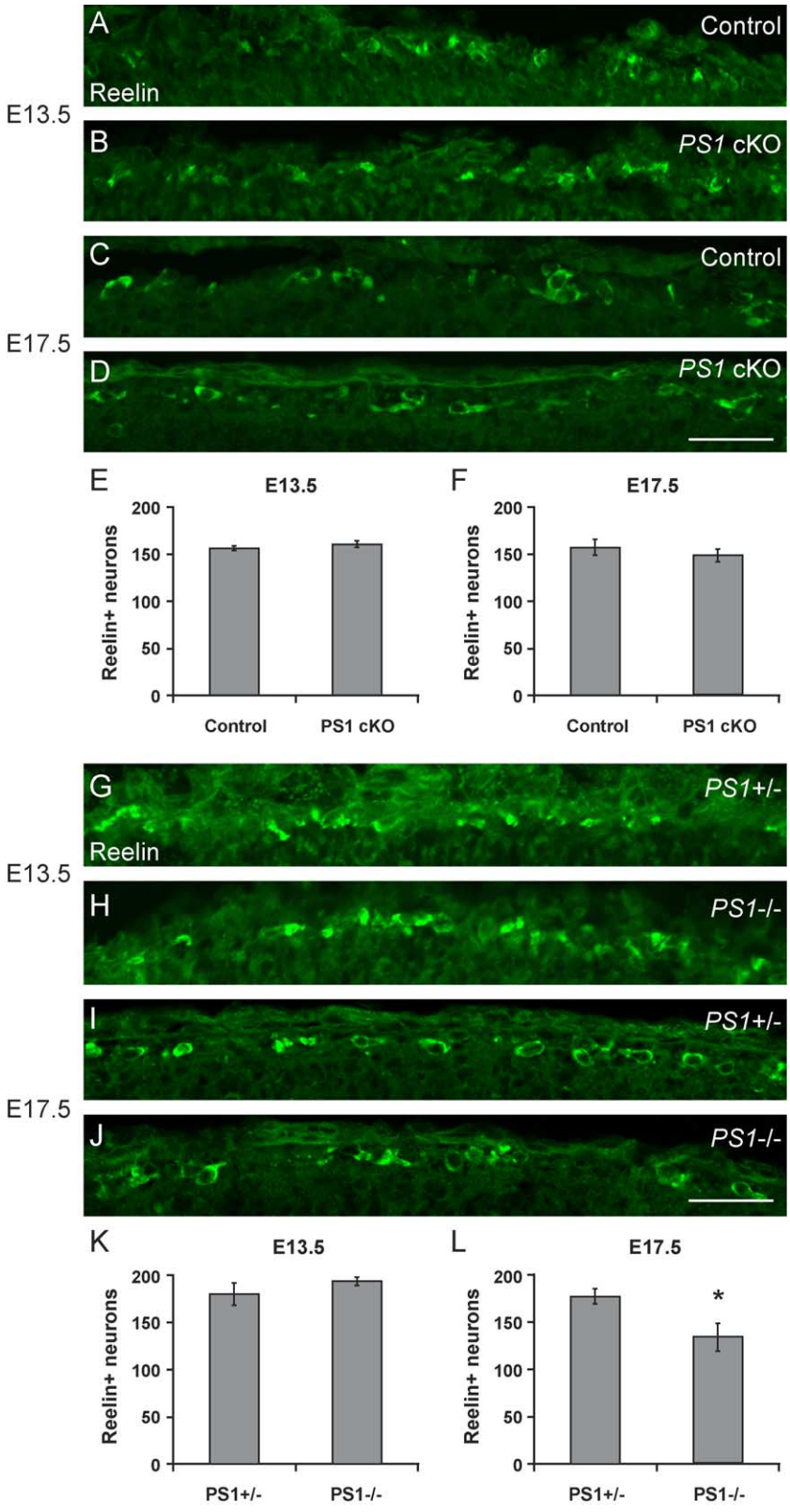
Overproliferation of meningeal cells in PS1 −/− but not PS1 cKO brains

With the premature degeneration of CR neurons in *PS1 −/−* mice but not in the NPC-restricted *PS1* cKO mice, we hypothesized that meningeal cells are the source of the difference in CR neuron survival between these mice. The meninges localized immediately above the CR neurons have been shown to be required for CR neuron survival, perhaps through secretion of trophic factors (Super et al., 1997a,b). In addition, previous studies have reported that PS1 affects proliferation of fibroblasts and keratinocytes (Kang et al., 2002; Xia et al., 2001). We therefore investigated whether loss of PS1 function in meningeal cells of the *PS1 −/−* brain similarly increases their proliferation. *PS1 −/−* ($n = 3$) and control ($n = 3$) embryos at E17.5 were pulse-labeled with BrdU, and the number of BrdU-labeled cells in the pia mater of the meningeal layers was counted. Significantly higher

Fig. 5. Cajal-Retzius neurons prematurely degenerate in *PS1 −/−* mice but not in *PS1* cKO mice. (A–D) Immunohistochemical analysis reveals similar numbers of Reelin-positive cells in the marginal zone of the telencephalon of *PS1* cKO and control mice at E13.5 and E17.5, although *PS1* inactivation is complete in neural progenitor cells and neurons by E12.5. Comparable areas are shown in *PS1* cKO and control brains. (E, F) Quantification of the number of Reelin-positive CR neurons confirms normal generation of CR neurons and the absence of premature loss of CR neurons in *PS1* cKO mice, demonstrating that loss of *PS1* in CR neurons does not compromise their survival. (G–J) At E13.5, the number of Reelin-positive cells is similar in the marginal zone of the telencephalon of *PS1 −/−* and control mice. By E17.5, fewer Reelin-positive cells appear to be present in *PS1 −/−* mice relative to the control. Comparable areas are shown in *PS1* cKO and control brains. (K, L) Quantification of the number of Reelin-positive CR neurons confirms normal generation of CR neurons at E13.5 and the premature degeneration of CR neurons at E17.5 in *PS1 −/−* mice, indicating a non-cell-autonomous effect of PS1 in the promotion of CR neuron survival. Scale bar: 50 μm. Asterisk represents a P value of 0.05.

numbers of BrdU-labeled meningeal cells were found in *PS1*−/− mice (201 ± 17) compared to the control (152 ± 15) ($P < 0.02$) (Figs. 6A, B), suggesting overproliferation of these cells. These results are in agreement with previous findings showing increased proliferation of fibroblasts in vitro (Xia et al., 2001).

To investigate further whether the normal survival of CR neurons in *PS1* cKO mice correlates with normal



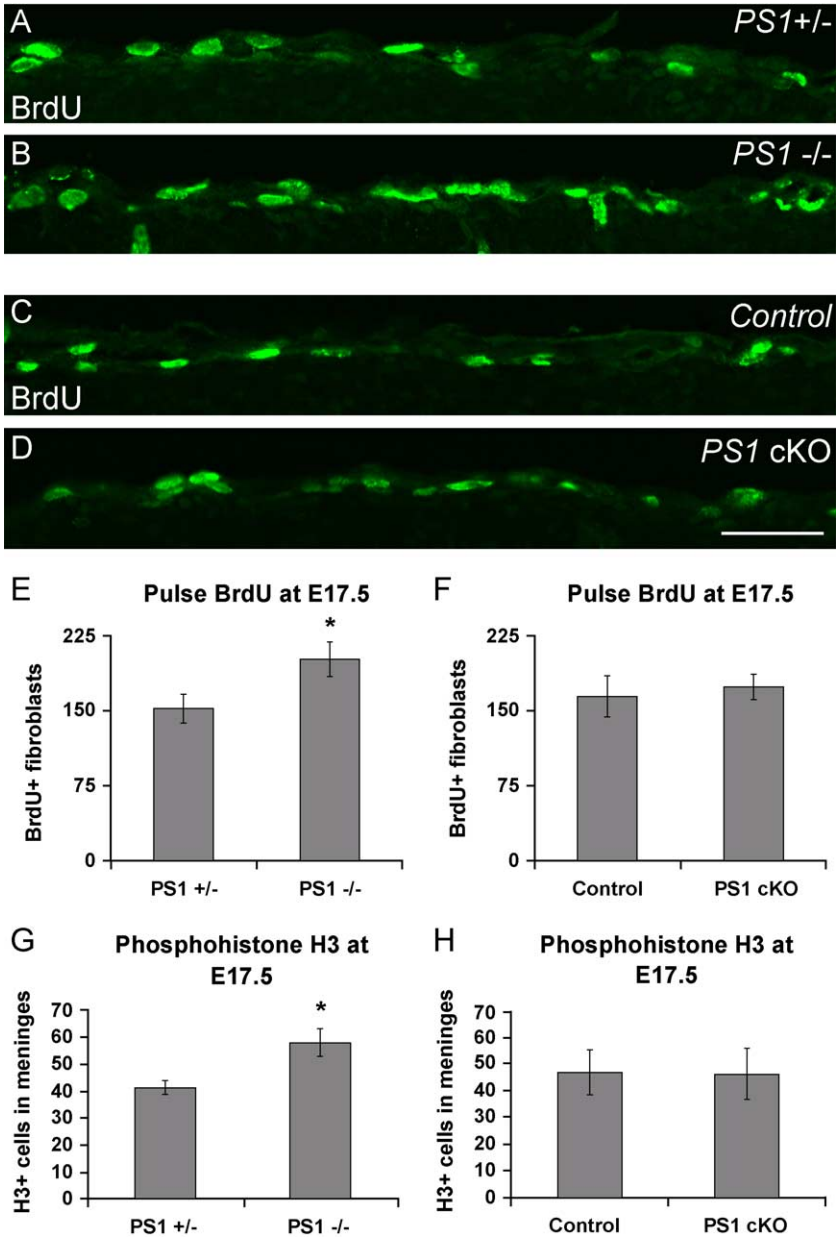


Fig. 6. Increased numbers of BrdU-labeled and phosphohistone H3-positive proliferating meningeal cells in *PS1*−/− mice but not in *PS1* cKO mice. (A–D) Proliferating meningeal cells in S phase are pulse-labeled for 30 min by BrdU at E17.5. Immunohistochemical analysis reveals increased numbers of BrdU-labeled cells in the meningeal layer of *PS1*−/− mice compared to the control, while similar numbers of BrdU-labeled cells appear to be present in the meningeal layer of *PS1* cKO and control mice. Comparable areas are shown in *PS1* cKO and control brains. (E, F) Quantification of the number of BrdU labeled cells in the meningeal layer confirms a significant increase in the dividing meningeal cells in *PS1*−/− but not in *PS1* cKO mice, compared to their respective controls. (G, H) Quantification of the number of phosphohistone H3-labeled cells in the meningeal layer further illustrates a significant increase in the number of dividing M phase cells in *PS1*−/− but not in *PS1* cKO mice, compared to controls. Scale bar: 50 μm. Asterisk represents a *P* value of 0.05.

proliferation of the meningeal cells, *PS1* cKO and control mice at E17.5 were pulse-labeled for 30 min with BrdU, and the number of BrdU-positive meningeal cells was determined. Similar numbers of proliferating cells were found in the pia mater of the meningeal layers of both *PS1* cKO (174 ± 13) and control (164 ± 20, $P = 0.50$) mice (Figs. 6C, D). We then used another marker for dividing cells. These results support the notion that the premature loss of CR neurons in *PS1*−/− mice may be caused by overproliferation of meningeal cells, which may lead to altered levels of trophic factors provided to CR neurons by these cells.

As an independent assessment of increased proliferation of *PS1*−/− meningeal cells, we immunostained brain sections with a marker of mitosis or M phase of the cell cycle, phosphohistone H3 (Ser10) (Mahadevan et al., 1991). Phosphohistone H3-positive meningeal cells were scored in coronal sections from control ($n = 3$) and *PS1*−/− ($n = 3$) littermates at E17.5 (Fig. 6G). A clear increase (40.3%) in the number of H3-positive cells was detected in *PS1*−/−

meninges (58 ± 5) compared to controls (41 ± 2.5, P = 0.015). Similar analysis performed with *PS1* cKO (n = 3) and control littermate (n = 3) brain sections revealed no discernable difference in phosphohistone H3-positive cells in the meningeal layer between *PS1* cKO (46 ± 9.6) and control (47 ± 8.4) mice (P = 0.93) (Fig. 6H). These results confirm a significant role of PS1 as a negative regulator of meningeal proliferation.

Discussion

PS1 is required for normal neuronal migration and cortical lamination

Previous studies of *PS1*−/− mice established an important role for PS1 in the maintenance of the neural progenitor population and the regulation of the Notch signaling pathway during early mammalian neural development (Handler et al., 2000; Shen et al., 1997). The perinatal lethality of *PS1*−/− mice and the severe cerebral hemorrhage associated with older *PS1*−/− embryos precluded elucidation of PS1 function in neuronal migration and cortical lamination of the postnatal brain. To overcome these problems, we generated a CNS-restricted *PS1* conditional knockout mouse in which PS1 is selectively inactivated in neural progenitor cells and neural progenitor cell-derived neurons and glia. This unique mouse model enabled us to extend the study of PS1 function to late embryonic and postnatal stages when cortical layers form and develop. Interestingly, by BrdU birthdating analysis, we found that a significant proportion (approximately 45%) of the late-born neurons fail to migrate to their appropriate positions in the superficial cortical layer II/III. In contrast, the vast majority of the early-born neurons are positioned properly in the deeper cortical layers.

The difference between the early- and late-born neurons in *PS1* cKO mice could be explained by progressive reduction of radial glia, which are known to provide scaffolding for migrating cortical neurons and serve as neural progenitor cells (Goldman, 2003; Rakic, 2003). At E12.5, radial glial generation is largely normal, as indicated by similar RC2 immunoreactivity and similar levels of Nestin and BLBP in *PS1* cKO and control brains (Fig. 4). Consistent with this result, the majority of early-born neurons labeled by BrdU at E12 are appropriately positioned in the deeper cortical layers (Fig. 3). By E17.5, levels of Nestin and BLBP as well as immunoreactivity of RC2 and BLBP are markedly decreased in *PS1* cKO mice (Fig. 4), indicating a progressive reduction in radial glia, which is consistent with decreased neural progenitor cells at this age. Accompanying this reduction in radial glia, a significant proportion of late-born neurons fail to migrate to their appropriate superficial cortical positions but rather remain in the deeper cortical layers (Fig. 3).

The progressive reduction in glia may explain the selective migration defect of the later born neurons in *PS1* cKO mice, while neurons born at E12 prior to radial glial reduction are spared. Alternatively, it is possible that the lamination defects in *PS1* cKO mice may not stem entirely from reduced levels of radial glia but may derive from both radial glial deficits and defects intrinsic to the migrating neurons themselves. However, inactivation of PS1 in all neural progenitor cells and NPC-derived neurons and glia using *Nestin-Cre* transgenic mice does not allow these two possibilities to be addressed experimentally. Further analysis using a postmitotic neuron-specific *PS1* cKO mouse would be necessary to shed light on this issue.

Unlike the gross disorganization and inversion of the cortical layers observed in *reeler*, *scrambler*, *mDab1*−/−, VLDL/*ApoER2*−/−, *Cdk5*−/−, and *p35*−/− mice (Chae et al., 1997; Gilmore et al., 1998; Gonzalez et al., 1997; Howell et al., 1997; Ogawa et al., 1995; Ohshima et al., 1996; Sweet et al., 1996; Trommsdorff et al., 1999), the defects of cortical lamination in *PS1* mutant mice are less dramatic and rather specific. In addition, in contrast to *reeler*, *scrambler*, *mDab1*−/−, and VLDL/*ApoER2*−/− mice (Gonzalez et al., 1997; Howell et al., 1997; Ogawa et al., 1995; Sweet et al., 1996; Trommsdorff et al., 1999), the preplate forms and splits properly into the marginal zone and the subplate in *PS1*−/− brains (M. Wines-Samuelson and J. Shen, unpublished results). Similar abnormalities in cortical lamination and radial glial development have been reported in other mutant mice (Caric et al., 1997; Gambello et al., 2003; Gotz et al., 1998; Wang et al., 2003). In *Lis1* hypomorphic mutant mice, neurons born at E15.5 are not localized in their appropriate superficial layer but scattered randomly throughout the cortical layers, although radial glia indicated by RC2 immunoreactivity appear normal (Gambello et al., 2003). Mice deficient for the estrogen receptor β display disorganized radial glia and fewer late-born neurons localized in the superficial layer II/III (Wang et al., 2003). Inactivation of Pax6 in naturally occurring *Small eye* mutant or *Pax6*−/− mice results in accumulation of late-born neurons in the ventricular and subventricular zones, and alterations in the generation and morphology of radial glia (Caric et al., 1997; Gotz et al., 1998).

Although our study has shown a requirement for PS1 in radial neuronal migration, it is unclear whether PS1 is involved in tangential migration. A very recent paper on the analysis of *PS1*−/− mice reported that lack of PS1 results in enhanced migration of *Dlx2*-positive interneurons and aberrant placement of migrating *Gad67*-positive interneurons in the *PS1*−/− embryonic brain (Louvi et al., 2004). However, the severe hemorrhaging and morphological changes associated with the *PS1*−/− brain at later developmental stages could confound interpretation of the migration results. It would be interesting to determine whether PS1 is required for tangential migration using *PS1* cKO mice, which provide a cleaner system than *PS1*−/−

mice to identify specific defects in tangential migration of interneurons.

PS1 is required for the survival of CR neurons in a non-cell-autonomous manner

It was previously reported that Reelin and calretinin immunoreactivity are reduced in the marginal zone of *PS1*−/− brains at E18, suggesting that loss of CR neurons occurs prematurely in the absence of *PS1* (Hartmann et al., 1999). In the present study, our quantification analysis showed normal numbers of Reelin-immunoreactive CR neurons in *PS1*−/− brains at E13.5 but significantly reduced (approximately 25%) numbers of CR neurons in *PS1*−/− brains at E17.5. These results indicate that the reduction of CR neurons at E17.5 is not due to reduced generation of CR neurons. Rather, the reduction results from premature degeneration of CR neurons, since CR neurons in the marginal zone are generated during early neural development and are maintained through the first postnatal week (Marin-Padilla, 1998). Our further quantification analysis of *PS1* cKO mice revealed normal numbers of CR neurons at both E13.5 and 17.5, indicating unaffected generation and survival of CR neurons in these *PS1* cKO mice. Therefore, premature loss of CR neurons in *PS1*−/− mice is due to the loss of *PS1* in nonneural tissues.

It remains theoretically possible that Cre-mediated inactivation of PS1 did not occur in all CR neurons in *PS1* cKO mice. By this hypothesis, small numbers of CR neurons which lack Cre expression and thus continue to express PS1 may contribute to the normal survival of CR neurons in *PS1* cKO embryos. However, this possibility is highly unlikely for the following reasons. First, while *PS1* is inactivated in all CR neurons in *PS1*−/− mice, 25% of CR neurons are lost by E17.5. If *PS1* is inactivated in 75% of CR neurons in *PS1* cKO, we would expect to see an approximately 19% (75% × 25%) reduction in CR neurons, which we did not see. Second, the half-life of *PS1* mRNA is 4–5 h (Bazan and Lukiw, 2002), and the half-life of PS1 protein is 22.5 h (Counts et al., 2001). Combined with the fact that the Nestin-Cre transgene is expressed in the CNS starting at E9 (Yang et al., 2004), it is very unlikely that a good percentage of CR neurons still retains *PS1* expression by E17.5.

Five classes of genes are known to control the specification, function, or survival of Cajal-Retzius neurons: the winged-helix transcriptional repressor *Foxg1*, genes in the Reelin signaling pathway, the *Extramacrochaete* (*Emc*)-related homeobox genes *Emx1* and *Emx2*, and the chemokine *stromal cell-derived factor-1* (*SDF-1*) and its receptor *CXCR4* (Gonzalez et al., 1997; Hanashima et al., 2004; Mallamaci et al., 2000; Stumm et al., 2003; Trommsdorff et al., 1999). In *Foxg1*−/− mice, increased numbers of Reelin-expressing CR neurons are dispersed through the cortical layers, suggesting a role for *Foxg1* in suppression of CR neuron specification in other cortical neurons (Hanashima et al., 2004). Inactivation of the Reelin receptors *VLDLR/ApoER2* or the Reelin downstream gene *mDab1* abolishes the function of Reelin in cortical development (Trommsdorff et al., 1999; Ware et al., 1997). Similar to *PS1*−/− mice, targeted inactivation of *Emx2* causes premature loss of CR neurons, although the phenotype exhibited by *Emx2*−/− mice is more severe with few Reelin-positive CR neurons remaining by E15.5 (Mallamaci et al., 2000). *SDF-1*- and *CXCR4*-null mutant mice display a similar reduction in the number of CR neurons as in *PS1*−/− mice (Stumm et al., 2003). SDF-1 is expressed in meningeal cells but not in CR neurons (Stumm et al., 2003). Similar to PS1, the promotion of CR neuronal survival by SDF-1 is mediated through the meninges. Whether Emx2 and CXCR4 regulate CR neuron survival via a cell-autonomous or non-cell-autonomous mechanism is unclear.

Although the detailed molecular mechanism by which PS1 promotes the survival of CR neurons is not yet known, our analysis of *PS1* cKO and *PS1*−/− mice indicated a non-cell-autonomous mechanism. Meningeal cells, which normally express PS1 (Hartmann et al., 1999), are critical for CR neuron survival, based on prior reports showing that chemical or mechanical ablation of the meninges results in rapid loss of CR neurons (Super et al., 1997a). Our quantification analysis revealed a significant increase in BrdU-labeled (33%) and phosphohistone H3-positive (40%) proliferating meningeal cells in *PS1*−/− brains, suggesting overproliferation of these cells. Our finding correlates well with several prior studies indicating that PS1 negatively regulates cell proliferation in various tissues. A 15% increase in BrdU incorporation and accumulation of cyclin D1 were reported in fibroblasts derived from *PS1*−/− embryos at E15.5 (Soriano et al., 2001). Furthermore, lack of functional PS1 in keratinocytes in vivo causes abnormal proliferation and tumorigenesis (Xia et al., 2001). A separate study showed increased levels of cyclin D1 and enlargement of the spinal cord in *PS1*−/− mice (Kang et al., 2002). Therefore, increased proliferation of the meningeal cells in *PS1*−/− mice may impair their normal ability to provide trophic support for the survival of the adjacent CR neurons.

In summary, through the generation and analysis of CNS-specific *PS1* cKO and *PS1*−/− mice, we have established a role for PS1 in neuronal migration and radial glial development. The observed abnormalities in neuronal migration and cortical lamination in *PS1* cKO mice may result from reduced Notch signaling and impaired radial glial generation. Although PS1 could regulate neuronal migration through promotion of CR neuron survival, the cortical lamination defects in the absence of CR neuron loss in *PS1* cKO mice indicate that loss of PS1 in neural progenitor cells alone is sufficient to cause neuronal migration defects. Furthermore, our combined studies of *PS1*−/− and *PS1* cKO mice demonstrate a non-cell-autonomous requirement for PS1

in CR neuron survival, providing further genetic evidence for a key role of the meningeal cells in promotion of CR neuron survival.

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