Neurobiology of Disease

Conditional Inactivation of Presenilin 1 Prevents Amyloid Accumulation and Temporarily Rescues Contextual and Spatial Working Memory Impairments in Amyloid Precursor Protein Transgenic Mice

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Accumulation of β-amyloid (Aβ) peptides in the cerebral cortex is considered a key event in the pathogenesis of Alzheimer's disease (AD). Presenilin 1 (PS1) plays an essential role in the γ-secretase cleavage of the amyloid precursor protein (APP) and the generation of Aβ peptides. Reduction of Aβ generation via the inhibition of γ-secretase activity, therefore, has been proposed as a therapeutic approach for AD. In this study, we examined whether genetic inactivation of PS1 in postnatal forebrain-restricted conditional knock-out (*PS1* cKO) mice can prevent the accumulation of Aβ peptides and ameliorate cognitive deficits exhibited by an amyloid mouse model that overexpresses human mutant APP. We found that conditional inactivation of PS1 in *APP* transgenic mice (*PS1* cKO;*APP* Tg) effectively prevented the accumulation of Aβ peptides and formation of amyloid plaques and inflammatory responses, although it also caused an age-related accumulation of C-terminal fragments of APP. Short-term PS1 inactivation in young *PS1* cKO;*APP* Tg mice rescued deficits in contextual fear conditioning and serial spatial reversal learning in a water maze, which were associated with *APP* Tg mice. Longer-term PS1 inactivation in older *PS1* cKO;*APP* Tg mice, however, failed to rescue the contextual memory and hippocampal synaptic deficits and had a decreasing ameliorative effect on the spatial memory impairment. These results reveal that *in vivo* reduction of Aβ via the inactivation of PS1 effectively prevents amyloid-associated neuropathological changes and can, but only temporarily, improve cognitive impairments in *APP* transgenic mice.

Key words: **Alzheimer's disease; β-amyloid; γ-secretase; mouse; behavior; synaptic plasticity**

Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder in which progressive memory loss is accompanied by cognitive decline. Neuropathologically, AD is characterized by a progressive loss of synapses and neurons as well as the presence of amyloid plaques and neurofibrillary tangles. Mutations in the genes encoding β-amyloid precursor protein (APP) and presenilins are linked to familial AD (FAD) (Hutton and Hardy, 1997). According to the "amyloid cascade" hypothesis, accumulation of β-amyloid (Aβ) peptides, which are the main constituents of amyloid plaques, is the key event initiating AD pathogenesis (Hardy and Selkoe, 2002).

Transgenic (Tg) mice overexpressing mutant forms of APP reproduce several key neuropathological features of AD, such as amyloid plaques and inflammatory responses (Games et al., 1995; Borchelt et al., 1996; Hsiao et al., 1996; Masliah et al., 1996; Sturchler-Pierrat et al., 1997; Mucke et al., 2000). In addition, *APP* transgenic mice can exhibit age-related impairments of hippocampal synaptic plasticity and learning and memory (Hsiao et al., 1996; Chapman et al., 1999; G. Chen et al., 2000; Morgan et al., 2000; Koistinaho et al., 2001; Corcoran et al., 2002). The synaptic and cognitive deficits often appear before amyloid plaque deposition and synaptic loss (Hsia et al., 1999; Larson et al., 1999; Koistinaho et al., 2001).

If Aβ accumulation is causal with respect to progression of the disease, limiting its accumulation should be beneficial therapeutically. An experimental test of this idea would be to limit the accumulation of Aβ at the point of synthesis or degradation. Aβ peptides are generated by two sequential proteolytic cleavages of APP mediated by β- and γ-secretases. β-Secretase [β-site APP-

Received March 31, 2005; revised June 1, 2005; accepted June 1, 2005.

This work was supported by National Institute of Neurological Disorders and Stroke Grant R01NS041783 (J.S.), the Alzheimer's Association (C.A.S., J.S.), the Medical Research Council, and the Alzheimer's Research Trust (R.G.M.M). We thank L. Mucke for the *APP* transgenic mice, D. Selkoe for the C7 and Aβ antibodies, M. Shoji for the Saeko antiserum, and W. Xia and J. Zheng for ELISA. We are grateful to V. Beglopoulos, W. Cheng, M. Goldberg, and C. Lemere for assistance.

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DOI:10.1523/JNEUROSCI.1247-05.2005

 0270-6474/05/256755-10$15.00/0

cleaving enzyme (BACE)] is an aspartyl protease that cleaves APP to generate the β-C-terminal fragment (β-C-TF) of APP (Vassar et al., 1999). Thus one route to limit the accumulation of Aβ would be to block or inhibit BACE (Ohno et al., 2004). An alternative route is via γ-secretase, or presenilin, an essential component of the γ-secretase complex, which further cleaves β-C-TF to generate Aβ. This has led to the hypothesis that targeting presenilin function by γ-secretase inhibitors also may be a suitable therapeutic strategy for AD (Hardy and Selkoe, 2002).

A major problem with this strategy is that presenilin 1 (PS1) plays essential roles *in vivo* (Wong et al., 1997; Xia et al., 2001; Pan et al., 2004), including neuronal differentiation and migration during embryonic development (Shen et al., 1997; Handler et al., 2000; Wines-Samuelson et al., 2005). However, *PS1* conditional knock-out (*PS1* cKO) mice, in which PS1 inactivation is restricted to the postnatal forebrain, show reduced Aβ generation and only subtle spatial memory impairment (Yu et al., 2001). Nevertheless, complete inactivation of both presenilins in the adult cerebral cortex results in hippocampal memory and synaptic plasticity impairments, followed by progressive neurodegeneration (Saura et al., 2004). Based on these results, we hypothesized that partial, but not complete, γ-secretase inactivation may prevent amyloid-related neuropathological changes and ameliorate the hippocampal-dependent learning deficits exhibited by *APP* transgenic mice. To test this hypothesis, we used here a multidisciplinary approach to examine the effects of partial inhibition of γ-secretase activity on amyloid-related neuropathology and cognitive impairments associated with *APP* transgenic mice via PS1 inactivation.

Materials and Methods

Mice. Generation of *PS1* cKO and *APP* transgenic mice (*APP* Tg; J20 line) has been described previously (Mucke et al., 2000; Yu et al., 2001). To generate *PS1* cKO;*APP* Tg mice, we first crossed *APP* transgenic mice, which were maintained in the hybrid background of C57BL/6 and DBA, with homozygous floxed *PS1* (*fPS1/fPS1*) mice, which expressed normal levels of PS1 and were maintained in the hybrid background of C57BL/6 and 129, to obtain the *fPS1/+*;*APP* Tg mice, which then were bred to *fPS1/fPS1* to obtain *fPS1/fPS1*;*APP* Tg mice. *fPS1/fPS1*;*APP* Tg mice then were bred to *fPS1/fPS1*;*CaM-Cre* (*PS1* cKO) to obtain *fPS1/fPS1*;*CaM-Cre*;*APP* (*PS1* cKO;*APP* Tg) mice. The mice used in the study, *PS1* cKO; *APP* Tg, *PS1* cKO, *APP* Tg, and control mice, were littermates obtained from the cross between *PS1* cKO;*APP* Tg and control (*fPS1/fPS1*) mice. The genetic background of all mice used in the study was in the same hybrid background of C57BL/6, 129, and DBA. Experimenters of the behavioral tests and the electrophysiological analysis were blind to the genotypes of the mice.

Western blotting and ELISA analyses. For biochemical analysis, the mice cortices were dissected and homogenized in cold lysis buffer [consisting of (in mM) 50 Tris-HCl, pH 7.4, 150 NaCl, 1 EDTA plus 1% NP-40, and 0.5% Triton X-100] containing protease and phosphatase inhibitors (Sigma, St. Louis, MO). Lysates were cleared by centrifugation (12,000 rpm for 15 min), and the same amount of total protein was resolved on SDS-PAGE. Polyvinylidene difluoride membranes were incubated with antibodies against PS1 ($PS1_{NT}$, 1:12,000; Calbiochem, La Jolla, CA), APP (Saeko antiserum $APP_{665-695}$; 1:7500), phosphorylated tau (Ser^{202}; CP13, 1:250), and β-actin (1:20,000; Abcam, Cambridge, UK) (Yu et al., 2001; Saura et al., 2004). For ELISAs, the total (soluble and insoluble) human $A\beta_{40}$ and $A\beta_{42}$ peptides from the cortex of *APP* Tg and *PS1* cKO;*APP* Tg mice ($n = 3–8$) were measured by the 2G3/3D6 and 21F12/3D6 sandwich ELISA, respectively (Johnson-Wood et al., 1997).

Immunohistochemistry and immunogold electron microscopy. Paraffin-embedded brain sections (10 μm) were deparaffinized, alcohol-dehydrated, and immunostained with an Aβ antiserum (R1282; 1:1000) or monoclonal glial fibrillary acidic protein (GFAP; 1:500; Sigma) or CD45 (1:5000; Serotec, Raleigh, NC) antibodies as described previously (Stoltzner et al., 2000; Saura et al., 2004). Brain sections were incubated with biotinylated secondary antibodies and developed by using the peroxidase avidin–biotin reagent and the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). For double immunostaining, the sections were immunostained with C7 antiserum ($APP_{676-695}$; 1:1000) and monoclonal synaptophysin (1:200; Sigma), NMDA receptor 1 (NMDAR1; 1:200; Chemicon, Temecula, CA), microtubule-activated protein 2 (MAP2; 1:250; Sigma), or R1282 and phosphorylated tau (Ser^{202}/Thr^{205}) (AT-8, 1:50; Innogenetics, Gent, Belgium) antibodies and were incubated with Alexa Fluor 488 or 594 secondary antibodies (Molecular Probes, Eugene, OR) (Saura et al., 2004). Images were analyzed with a Zeiss (Oberkochen, Germany) 510 confocal laser-scanning microscope.

Procedures for immunogold electron microscopic analysis have been described previously (Takahashi et al., 2002). For immunogold labeling, the free-floating sections were incubated with 369 antibody ($APP_{649-695}$) by the immunogold-silver procedure and goat anti-rabbit IgG conjugated to 1 nm gold particles (Amersham Biosciences, Arlington, IL) in 0.01% gelatin and 0.08% bovine serum albumin in PBS. Transmission electron microscopy was performed on a Philips CM10 electron microscope at the Division of Neurobiology of Weill–Cornell Medical College (New York, NY).

Contextual fear conditioning. For contextual fear conditioning, the mice were placed within the conditioning chamber for 3 min before the onset of the unconditioned stimulus (US; footshock; 1 s/1 mA) to allow them to develop a representation of the context via exploration. After the shock, they then were left in the chamber for 2 min (immediate freezing) and returned to the home cages. Conditioning was tested 24 h after training for 4 min in the same conditioning chamber. Freezing response was scored by using the FreezeFrame automated system (Actimetrics, Wilmette, IL).

Water maze. Mice used for all behavioral tests were littermates and were 3 and 15–17 months of age. The serial spatial reversal task was performed in a circular pool (2 m in diameter) with a hidden platform (20 cm in diameter) (G. Chen et al., 2000). For the visible cue task, the pool was surrounded with white curtains to hide the extramaze cues, and the platform was marked with a dark cylinder. Each mouse was given four trials daily for 5 d, with a maximum trial duration of 90 s and an intertrial interval of 10 min. For the serial spatial learning to a hidden platform, each mouse was given a maximum of eight trials daily before reaching the criterion of three trials in a row with an average escape latency of <20 s. After the criterion was reached, the platform was moved to a new position the next day, and the training started again. An initial series of five different hidden platform locations was used, and the mice were allowed to take as many days as they needed to learn each of the platform locations. With the use of additional platform locations, the maximum number that the animals could learn in 10 d was used as a measure of learning capacity. The movement of the mice was monitored by an automated tracking system (Watermaze; Actimetrics).

Electrophysiology. Hippocampal slice preparation was performed as described previously (Yu et al., 2001). Stimulation (200 ms) pulses were delivered with a bipolar concentric metal electrode. Synaptic strength was quantified as the initial slope of field potentials recorded with artificial CSF-filled microelectrodes (1–2 MΩ). Baseline responses were collected at 0.07 Hz with a stimulation intensity that yielded a half-maximal response. Long-term potentiation (LTP) was obtained by five episodes of theta burst stimulation (TBS) delivered at 0.1 Hz. Each episode contained 10 stimulus trains delivered at 5 Hz, and each train consisted of four pulses at 100 Hz. Average responses (mean ± SEM) were expressed as a percentage of pre-TBS baseline response (at least 10 min of stable responses). A repeated-measures ANOVA was used to assess statistical significance.

Results

To evaluate the consequence of partial γ-secretase inactivation on the neuropathological changes and memory impairments in *APP* transgenic mice, we crossed *PS1* cKO mice (Yu et al., 2001) with *APP* Tg mice, which overexpress a human APP containing

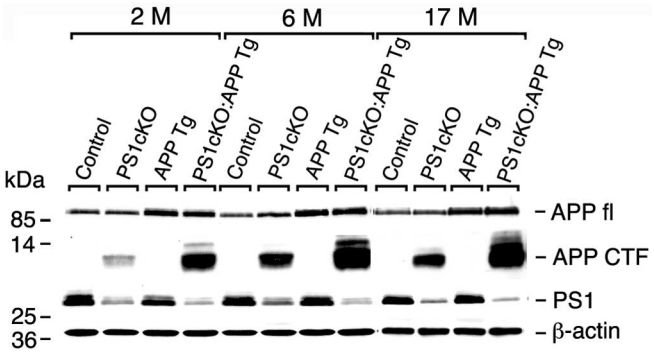

Figure 1. Age-dependent accumulation of APP C-TFs in *PS1* cKO;*APP* Tg mice. Levels of APP, APP C-TFs, and PS1 in cortical lysates of control, *PS1* cKO, *APP* Tg, and *PS1* cKO;*APP* Tg mice at 2, 6, and 17 months of age were analyzed by Western blotting with the use of antibodies specific for the C terminal of APP (Saeko) or PS1. An antibody specific for β-actin was used as a loading control. Levels of APP C-TFs in *PS1* cKO;*APP* Tg mice were increased at 6 and 17 months of age compared with 2 months of age.

the Swedish (K670N/M671L) and Indiana (V717F) mutations (Swe/Ind) (Mucke et al., 2000), to generate double-mutant *PS1* cKO;*APP* Tg mice. The *APP* transgenic mice have been shown previously to develop amyloid plaques and other AD-like features in an age-dependent manner (Mucke et al., 2000).

Accumulation of APP C-TFs in synaptic terminals

Using Western blot analysis, we first confirmed the marked reduction of PS1 in the cerebral cortex of *PS1* cKO;*APP* Tg mice at 2, 6, and 17 months of age (Fig. 1). The residual level of PS1 in the cortex of *PS1* cKO;*APP* Tg mice likely is attributable to the normal PS1 expression in glia and interneurons, where PS1 expression is not targeted to eliminate in *PS1* cKO mice (Yu et al., 2001). We next examined the consequence of PS1 inactivation on the proteolytic processing of APP. Western blot analysis showed an increase (approximately twofold) of full-length APP in *APP* Tg and *PS1* cKO;*APP* Tg mice compared with nontransgenic controls at 2, 6, and 17 months of age (Fig. 1). The level of APP C-TFs was increased markedly in the cortex of *PS1* cKO;*APP* Tg mice relative to *APP* Tg mice at 2, 6, and 17 months of age (Fig. 1). Similarly, the level of APP C-TFs was increased in *PS1* cKO mice, compared with the control, at all ages. Furthermore, the increase in APP C-TFs was higher at 6 and 17 months of age relative to that at 2 months of age ($n = 3$–4). These results indicate that there is an age-dependent increase in APP C-TFs in the cortex of *PS1* cKO;*APP* Tg mice.

To determine the subcellular localization of the accumulated APP C-TFs in *PS1* cKO;*APP* Tg mice, we performed immunohistological analysis by using the C7 antibody on *PS1* cKO;*APP* Tg mice at 6 months of age and littermate *APP* Tg mice as controls. The C7 antibody was raised against the C-terminal region of APP, which can recognize both full-length APP and C-TFs of APP. We found that the C7 immunoreactivity was localized mainly in the cell body of hippocampal neurons in *APP* Tg mice, whereas in *PS1* cKO;*APP* Tg mice, the C7 immunoreactivity was mostly in neuronal processes, suggesting an ectopic localization of the accumulated APP C-TFs in the absence of PS1 (Fig. 2*A–C*). To address this issue further, we performed confocal microscopy analysis by using the C7 antibody and antibodies specific for MAP2 (somatodendritic), NMDAR1 (a postsynaptic marker), or synaptophysin (a presynaptic marker). We found that the C7 (green) and MAP2 (red) immunoreactivity were not colocalized in *PS1* cKO;*APP* Tg mice (Fig. 2*A*). The C7 immunoreactivity, however, did overlap partially (yellow) with the NMDAR1 immunoreactivity in *PS1* cKO;*APP* Tg mice, although there was some C7 immunoreactivity (green) that did not overlap with the NR1 immunoreactivity (red) (Fig. 2*B*). In contrast, the C7 immunoreactivity overlapped almost entirely with synaptophysin, suggesting that APP C-TFs were localized primarily to the presynaptic terminal (Fig. 2*C*).

To confirm the synaptic localization of APP C-TFs, we also performed electron microscopy analysis by using the 369 antibody, which is also specific for the C-terminal region of APP. We found that the 369 immunoreactivity was localized predominantly in the presynaptic terminal of cortical and hippocampal neurons in *PS1* cKO;*APP* Tg brains, whereas no immunoreactivity was detected in nontransgenic control or *APP* Tg mice (Fig. 2*D*) (data not shown). These results indicate that partial inhibition of γ-secretase activity by PS1 inactivation causes an accumulation of APP C-TFs in the presynaptic terminal.

Prevention of amyloid-related neuropathological changes

To determine whether increased levels of APP C-TFs were accompanied by a decreased production of Aβ peptides, we measured the amount of total (soluble and insoluble) human Aβ peptides in the cerebral cortex of *PS1* cKO;*APP* Tg and *APP* Tg mice at 2–3, 6, and 17 months of age. Our previous study has shown a reduction of endogenous mouse Aβ peptides in the cortices of *PS1* cKO mice (Yu et al., 2001). Sandwich ELISA revealed that Aβ peptides accumulated in an age-dependent manner in the cerebral cortex of *APP* Tg mice, and the increase in $A\beta_{42}$ was more marked than $A\beta_{40}$ at 6 and 17 months of age (Fig. 3). In the cortex of *PS1* cKO;*APP* Tg mice, the cortical $A\beta_{40}$ levels were reduced significantly at 2–3 months of age (~58%; $p < 0.006$; $n = 8$), 6 months of age (~78%; $p < 0.0001$; $n = 7$), and 17 months of age (~96%; $p < 0.0001$; $n = 3$). Similarly, levels of $A\beta_{42}$ were reduced at 2–3 months of age (~55%; $p < 0.002$; $n = 8$), 6 months of age (90%; $p < 0.0001$; $n = 7$), and 17 months of age (~99%; $p < 0.0001$; $n = 3$). These results indicate that inactivation of PS1 resulted in reductions in the levels of Aβ peptides and prevented age-dependent accumulation of Aβ peptides.

Histological analysis with an antibody (R1282) specific for Aβ confirmed the age-dependent deposition of amyloid plaques in the hippocampus and neocortex of *APP* transgenic mice starting at ~5 months of age (Fig. 4*A*), as reported previously (Mucke et al., 2000). In the cortex of *PS1* cKO;*APP* Tg mice, the R1282 immunoreactivity (Fig. 4*A*) and thioflavine S staining (data not shown), which recognizes fibrillar Aβ, were absent, indicating the lack of amyloid plaque formation in these mice. Similar results were obtained from an analysis of *PS1* cKO;*APP* Tg mice at 12 months of age (data not shown). These results demonstrate that targeting PS1 function in the adult brain effectively prevents amyloid plaque formation in *APP* transgenic mice.

In addition to amyloid deposition, inflammatory responses such as astrogliosis, microgliosis, and dystrophic neurites are prominent neuropathological features in *APP* transgenic mice (Masliah et al., 1996; Frautschy et al., 1998; Matsuoka et al., 2001). Reactive astrocytes and activated microglial cells labeled by GFAP and CD45 immunoreactivity, respectively, were present in the hippocampus of *APP* Tg mice but absent in that of *PS1* cKO;*APP* Tg mice at 6 months of age (Fig. 4*B*). Similarly, dystrophic neurites labeled with antibodies against phosphorylated tau (AT-8) were present in the cortices of *APP* Tg mice but absent in *PS1* cKO;*APP* Tg mice (Fig. 4*C*). Western blot analysis also indicated a modest but statistically insignificant decrease in the level of phosphorylated tau (Ser^{202}) in cortical lysates of *PS1* cKO;*APP*

Tg mice compared with that in *APP* Tg mice at 6 months of age (*APP* Tg, 100 ± 28%; *PS1* cKO;*APP* Tg, 70.3 ± 2.7%; $p > 0.05$; $n = 3$). This likely is attributable to the fact that dystrophic neurites immunoreactive for phosphorylated tau were restricted to areas in or surrounding compact amyloid plaques in *APP* Tg mice and that the number of compact amyloid plaques was low and varied among individual mice at this age. Overall, these results demonstrate that inactivation of *PS1* in *APP* transgenic mice prevented amyloid-associated inflammatory responses and neuritic degeneration.

Age-dependent rescue of contextual memory deficits

To determine whether reduced levels of Aβ peptides and the absence of amyloid plaques in *PS1* cKO;*APP* Tg mice were associated with the rescue of cognitive deficits, we examined littermate *PS1* cKO;*APP* Tg, *APP* Tg, *PS1* cKO, and control mice by using two behavioral tasks assessing their learning and memory. Although levels of Aβ peptides are similar in *PS1* cKO;*APP* Tg mice between 2–3 and 6 months of age, levels of APP C-TFs accumulate with age. This accumulation may limit the extent of cognitive rescue. We therefore tested these mice at different ages (3 and 6 months).

We first tested all four genotypic groups in contextual fear conditioning, in which memory can be acquired in a single training session. In contextual fear conditioning, the mice learn to associate a conditioned stimulus (CS; test chamber) with a US (footshock) (Phillips and LeDoux, 1992). After the pairing of CS and US, a robust associative memory of the CS–US is formed such that the CS alone can elicit a fear response (e.g., freezing). At 3 months of age, *PS1* cKO;*APP* Tg, *APP* Tg, *PS1* cKO, and control mice displayed similar levels of freezing immediately after training (Fig. 5*A*). However, *APP* Tg mice showed significantly reduced levels of freezing to the context 24 h after training compared with the other three genotypic groups ($F_{(3,31)} = 3.39$; $p < 0.03$). *Post hoc* analysis revealed a significant reduction in freezing responses in *APP* Tg mice (29.3 ± 7.4%; $n = 7$) compared with control (50.1 ± 3.0%; $n = 10$), *PS1* cKO (49.0 ± 5.2%; $n = 9$), and *PS1* cKO;*APP* Tg (48.1 ± 6.2%; $n = 9$) mice ($p < 0.032$). The fact that *PS1* cKO;*APP* Tg mice performed as well as control mice ($p = 0.42$) suggests that inactivation of PS1 had prevented the impairment of contextual fear memory normally displayed by *APP* transgenic mice at this age.

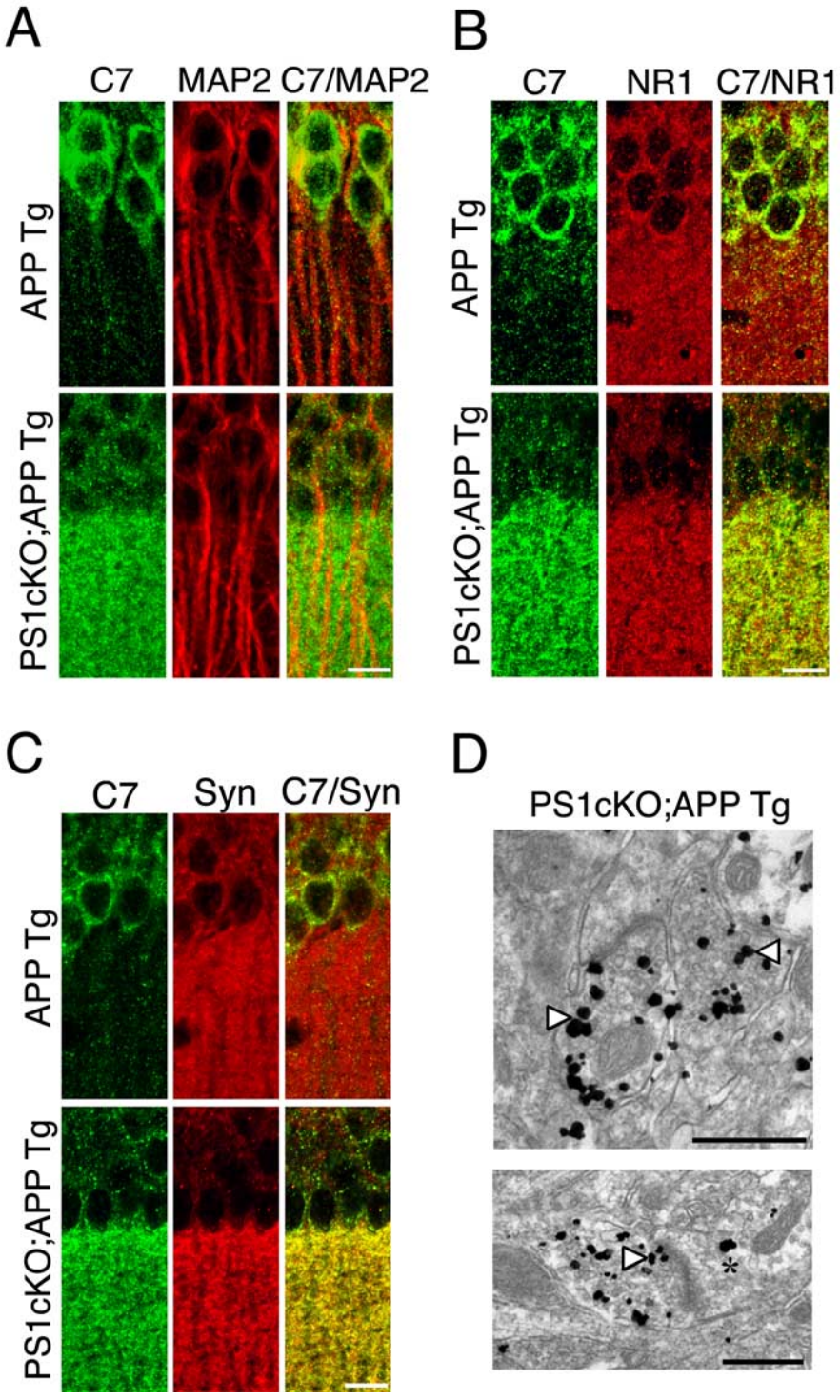

Figure 2. Accumulation of APP C-TFs in presynaptic terminals of *PS1* cKO;*APP* Tg mice. ***A***, Confocal microscopic images showing a marked increase in APP C-TFs recognized by C7 immunoreactivity (green) and a lack of colocalization (yellow) between the C7 immunoreactivity and the somatodendritic marker MAP2 immunoreactivity (red) in CA1 pyramidal neurons of 6-month-old *PS1* cKO;*APP* Tg mice. ***B***, Confocal microscopic images showing an increase in APP C-TFs recognized by C7 immunoreactivity (green) and its partial colocalization (yellow) with the postsynaptic marker NMDAR1 immunoreactivity (NR1; red) in CA1 pyramidal neurons of *PS1* cKO;*APP* Tg mice. ***C***, Confocal microscopic images showing a marked increase in APP C-TFs recognized by C7 immunoreactivity (green) and its abundant colocalization (yellow) with the presynaptic marker synaptophysin immunoreactivity (Syn; red) in CA1 pyramidal neurons of *PS1* cKO;*APP* Tg mice. ***D***, Electron microscopic images showing higher levels of accumulation of APP C-TFs in presynaptic compartments (arrowheads) compared with postsynaptic compartments (asterisk) in CA1 (top) and cortical (bottom) neurons from *PS1* cKO;*APP* Tg mice at 6 months of age. Magnification: top, 19,000×; bottom, 13,500×. Scale bars: ***A–C***, 25 μm; ***D***, 500 nm.

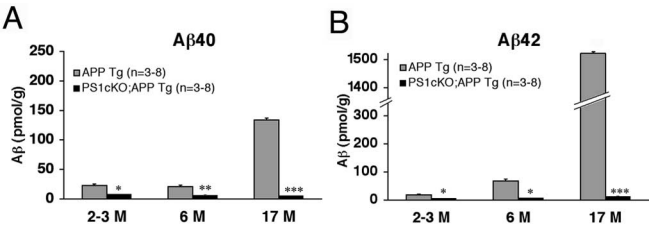

Figure 3. Reduced accumulation of Aβ peptides in *PS1* cKO;*APP* Tg mice. Levels of $A\beta_{40}$ (***A***) and $A\beta_{42}$ (***B***) in cortical lysates of *APP* Tg and *PS1* cKO;*APP* Tg mice were quantified by ELISA. In *APP* Tg mice, the levels of $A\beta_{40}$ and $A\beta_{42}$ were increased from 2 to 17 months of age; levels of $A\beta_{42}$ were higher than those of $A\beta_{40}$ at 6 and 17 months of age. In the *PS1* cKO;*APP* Tg mice, $A\beta_{40}$ and $A\beta_{42}$ were reduced significantly at all ages. Data represent the mean ± SEM; pmol/g signifies picomoles of Aβ per gram of cortex. $*p < 0.01$; $**p < 0.001$; $***p < 0.0001$.

A different pattern was observed at 6 months of age. All four genotypic groups again showed similar levels of freezing responses immediately after the footshock (Fig. 5*B*). However, in the memory retention test conducted 24 h after the initial training, there was no longer any rescue associated with the conditional *PS1* inactivation. The overall analysis showed a significant main effect of genotype in freezing responses 24 h after the initial training session ($F_{(3,43)} = 9.0$; $p < 0.0001$). *Post hoc* analysis revealed that the freezing responses shown by *APP* Tg (28.1 ± 3.4%; $n = 12$) and *PS1* cKO;*APP* Tg (22.8 ± 4.4%; $n = 13$) mice were reduced significantly compared with control (50.0 ± 5.04%; $n = 16$) and *PS1* cKO (48.8 ± 5.1%; $n = 6$) mice ($p < 0.02$) (Fig. 5*B*). These results indicate that the ability of *PS1* cKO;*APP* Tg mice to display a rescue of the memory deficit shown by *APP* transgenic mice deteriorated with age.

Age-dependent rescue of serial spatial learning

Previously, platelet-derived growth factor promoter APP (PDAPP) mice have been shown to exhibit both age-independent and age-related and amyloid plaque-related deficits in serial spatial learning in the water maze (G. Chen et al., 2000). The age-related component of these changes in learning ability occurred gradually over the range of 8–16 months in the PDAPP mice. To determine whether the *APP* transgenic mice used in our study exhibit similar age-related changes in learning and whether these could be rescued by PS1 inactivation, we used the identical training protocol. The only change was to start testing at a younger age (3 months) at which the *APP* transgenic mice used in this study previously exhibited an impairment in contextual fear conditioning. The full battery of tests (see Materials and Methods) consisted of cued navigation (5 d), followed by training-to-criterion in each of five different successively learned spatial locations and then continued training for 10 d to obtain a measure of learning capacity.

In the cue task, all genotypic groups at 3 and 15–17 months of age showed excellent learning to locate a visible platform, with rapidly declining swim latencies across days (Greenhouse–Geisser correction for multiple comparisons; $F = 99.42$; $p < 0.001$) (Fig. 6*A*,*B*). Thus there were no gross sensorimotor abnormalities that might have limited their capacity to learn the more complex hidden platform task that was trained next. In the serial spatial learning task, the escape latency is measured on each swim trial, but the primary measures for analysis are trials-to-criterion and learning capacity. Trials-to-criterion is the number of trials taken to learn each of the five successive spatial locations to the criterion of three trials averaging <20 s. Learning capacity is the total number of spatial locations learned in 10 d. Although distinct, these measures are related and thus not strictly independent.

Analyses of trials-to-criterion performed separately for young and old mice revealed overall group differences in the young mice, but only a trend in the old mice (for young, $F = 5.24$, df 3/43, $p < 0.005$; for old, $F = 2.82$, df 3/40, $0.10 > p > 0.05$). The pattern in the young mice revealed no significant difference among *PS1* cKO;*APP* Tg, *PS1* cKO, and control groups ($p > 0.10$), with all three groups taking fewer trials to learn the five platform locations than the *APP* Tg group ($F = 18.00$; df 1/43; $p < 0.001$) (Fig. 6*C*). Thus, as in contextual fear conditioning, *PS1* cKO;*APP* Tg mice at 3 months of age showed a rescue of the impaired phenotype displayed by *APP* Tg mice. However, as shown in Figure 6*D*, this rescue effect was weaker in the older mice. In these animals, the performance of the *APP* Tg, *PS1* cKO; *APP* Tg, and *PS1* cKO mice did not differ from each other ($p > 0.10$), but the average performance of these groups was poorer than that shown by the control group ($F = 5.25$; df 1/40; $p < 0.05$).

Next we analyzed the learning capacity of mice at 3 and 15–17 months of age (i.e., the number of serial spatial locations they could learn in 10 d). These analyses showed overall differences across groups in both the young and old mice (for young, $F = 4.99$, df 3/43, $p < 0.005$; for old, $F = 4.19$, df 3/40, $p < 0.05$). In the young mice, orthogonal comparisons showed a rescue effect in the *PS1* cKO;*APP* Tg mice, which performed as well as control and *PS1* cKO mice and better than the *APP* Tg group ($p < 0.001$) (Fig. 6*E*). In the older mice, the *APP* Tg, *PS1* cKO;*APP* Tg, and *PS1* cKO groups did not differ significantly, but the average performance of these three groups was significantly poorer than for controls ($F = 9.23$; df 1/40; $p < 0.01$) (Fig. 6*F*). We conclude that, in the serial spatial learning task, which displays an age-related decline in learning by control and *APP* Tg mice, inactivation of PS1 ameliorates the learning deficit in young *APP* Tg mice, but this apparent rescue of learning ability deteriorates with age.

Failure to rescue synaptic dysfunction

It has been reported that transgenic mice overexpressing human mutant APP exhibit impaired synaptic transmission and/or plasticity (Chapman et al., 1999; Fitzjohn et al., 2001). To determine whether inactivation of PS1 in *PS1* cKO;*APP* Tg mice can rescue the hippocampal synaptic deficits associated with *APP* Tg mice, we examined synaptic transmission and plasticity in the Schaffer collateral pathway in control, *APP* Tg, and *PS1* cKO;*APP* Tg mice at 6 months of age [*PS1* cKO mice were reported previously by Yu et al. (2001)], because at this age both *APP* Tg and *PS1* cKO;*APP* Tg mice exhibited contextual memory impairments (Fig. 5*B*). To measure basal synaptic transmission, we first determined input/output (I/O) curves in acute hippocampal slices by plotting the amplitude of the fiber volley (FV), which is a measure of the number of recruited axons, versus the initial slope of the evoked field EPSP (fEPSP). ANOVA of the average of the FV–fEPSP slope showed a significant main effect of genotype ($F_{(2,84)} = 6.19$; $p < 0.003$). *Post hoc* analysis revealed reduced basal synaptic transmission in *APP* Tg and *PS1* cKO;*APP* Tg mice compared with controls ($p < 0.02$) (Fig. 7*A*). Paired pulse facilitation (PPF), a presynaptic form of short-term plasticity that correlates inversely with the probability of transmitter release, was similar in all genotypic groups ($F_{(2,12)} = 1.99$; $p = 0.14$) (Fig. 7*B*). These results are consistent with previous reports showing reduced synaptic transmission and unchanged PPF in similar *APP* Tg mice (Hsia et al., 1999; Jolas et al., 2002). Our previous study has shown that induction of LTP was normal in *PS1* cKO mice (Yu et al., 2001); we therefore examined synaptic plasticity in hip-

pocampal slices from *APP* Tg and *PS1* cKO;*APP* Tg mice and littermate controls by inducing LTP with TBS. The initial induction of LTP was reduced in *APP* Tg (155.3 ± 11.1) and *PS1* cKO;*APP* Tg (154.3 ± 8.7) mice compared with control mice (180.4 ± 8.1) (Fig. 7*C*). Repeated-measures ANOVA of the magnitude of LTP showed a significant main effect of genotype ($F_{(2,212)} = 4.65$; $p < 0.05$), indicating that LTP was impaired in *APP* Tg and *PS1* cKO;*APP* Tg mice.

To determine whether basal synaptic transmission and LTP changes in *APP* Tg and *PS1* cKO;*APP* Tg mice were age dependent, we also performed electrophysiological recordings in younger mice at 3 months of age. ANOVA of the FV–fEPSP slope indicated similar responses in younger control (7.74 ± 0.54; $n = 5$), *APP* Tg (8.13 ± 0.65; $n = 5$), and *PS1* cKO;*APP* Tg (8.27 ± 0.58; $n = 5$) mice ($F_{(2,64)} = 0.21$; $p = 0.81$), suggesting normal basal synaptic transmission in mutant mice at this age (Fig. 7*D*). Similar to the older age, *APP* Tg and *PS1* cKO;*APP* Tg mice at 3 months of age showed PPF at each interstimulus interval that was similar to that seen for control mice ($F_{(2,12)} = 2.1$; $p = 0.13$), indicating intact presynaptic short-term plasticity in these mutant mice (Fig. 7*E*). Induction of LTP in CA1 hippocampal synapses of younger *APP* Tg and *PS1* cKO;*APP* Tg mice elicited larger responses than in control mice (Fig. 7*F*). Repeated-measures ANOVA of the magnitude of LTP in the last 10 min showed a significant main effect of genotype ($F_{(2,253)} = 47.9$; $p < 0.0001$), indicating an enhancement of LTP induction in younger *APP* Tg and *PS1* cKO;*APP* Tg mice at 3 months of age. This enhancement of LTP that occurs before amyloid deposition has been observed previously in the CRND8 transgenic mouse, which also overexpresses the mutant $APP_{Swe/Ind}$ and develops hippocampal-dependent cognitive deficits (Janus et al., 2000; Jolas et al., 2002). Although the exact mechanism or mechanisms underlying the age-dependent synaptic plasticity alterations in these mutants are unclear, varying levels of Aβ and APP C-TFs in *APP* Tg and *PS1* cKO; *APP* Tg mice at these two ages may account for such a phenotype. Previous *in vitro* studies have shown both reduction and enhancement of LTP after acute perfusion of Aβ in hippocampal slices (Wu et al., 1995; Q. S. Chen et al., 2000). Secreted forms of APP also have been found to increase LTP (Ishida et al., 1997).

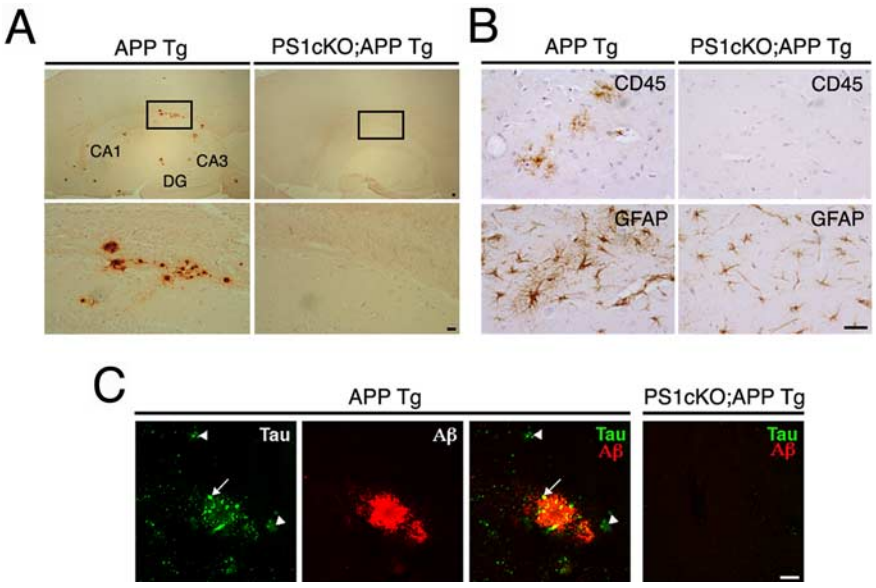

Figure 4. Absence of amyloid plaques and inflammation in *PS1* cKO;*APP* Tg mice. ***A***, Sagittal brain sections from *APP* Tg and *PS1* cKO;*APP* Tg mice were stained with an Aβ antibody (R1282) to reveal the presence of amyloid plaques in the hippocampus of *APP* Tg mice and their absence in *PS1* cKO;*APP* Tg mice at 6 months of age. Higher-power views of the boxed areas in the top panels are shown at the bottom. CA1 and CA3, CA1 and CA3 fields of the hippocampus, respectively; DG, dentate gyrus. Scale bar, 10 μm. ***B***, Brain sections of *APP* Tg and *PS1* cKO;*APP* Tg mice at 6 months of age were stained with antibodies specific for CD45 and GFAP. Activated microglial cells labeled by CD45 antibody and reactive astrocytes, which express high levels of GFAP and extend elaborate processes, are found only in the hippocampus of *APP* Tg mice, but not in *PS1* cKO;*APP* Tg mice. Scale bar, 10 μm. ***C***, Confocal microscopy analysis of *APP* Tg and *PS1* cKO;*APP* Tg brain sections double-labeled with antibodies for phosphorylated tau (green) and Aβ (red). Dystrophic axons immunoreactive for phosphorylated tau either extend into plaques (arrow) or remain in the surrounding area of the plaque (arrowheads) in the hippocampus of *APP* Tg mice, whereas the *PS1* cKO;*APP* Tg brain lacks such processes. Scale bar, 10 μm.

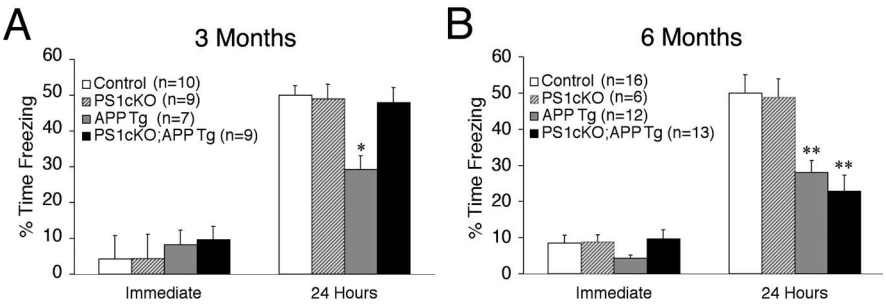

Figure 5. Age-dependent rescue of contextual memory in *PS1* cKO;*APP* Tg mice. ***A***, Control ($n = 10$), *PS1* cKO ($n = 9$), *APP* Tg ($n = 7$), and *PS1* cKO;*APP* Tg ($n = 9$) mice at 3 months of age were tested in the one-shock contextual fear conditioning task. All four groups showed similar levels of freezing immediately after the footshock. Control, *PS1* cKO, and *PS1* cKO;*APP* Tg mice showed similar levels of freezing (~50%) at 24 h, whereas *APP* Tg mice exhibited significantly reduced levels of freezing (~30%) ($F_{(3,31)} = 3.39$; $^*p < 0.03$). ***B***, Control ($n = 16$), *PS1* cKO ($n = 6$), *APP* Tg ($n = 12$), and *PS1* cKO;*APP* Tg ($n = 13$) mice at 6 months of age were tested in the one-shock contextual fear conditioning task. All four groups showed similar levels of freezing immediately after the footshock. However, *APP* Tg and *PS1* cKO;*APP* Tg mice showed reduced levels of freezing when compared with control and *PS1* cKO mice at 24 h ($F_{(3,43)} = 9.0$; $^{**}p < 0.0001$). Data represent the mean ± SEM.

Discussion

Genetic and pharmacological approaches designed to prevent or slow cognitive decline in AD should be evaluated in animal models by a variety of appropriate behavioral tests of learning and memory. The possible causal role of Aβ in AD pathogenesis raises the possibility of therapeutic strategies based on processes that regulate the level of Aβ peptides. Our previous finding that inactivation of PS1 in the postnatal forebrain results in a significant reduction of Aβ peptides without major deleterious consequences in mice supports the view that selective inhibition of PS1 or γ-secretase activity may represent a suitable therapeutic strategy for AD (Yu et al., 2001). Our recent investigations have revealed, however, that disruption of PS1 and PS2 in the mouse postnatal forebrain causes synaptic and memory deficits and age-dependent neurodegeneration (Saura et al., 2004). Because the pharmacological targeting of presenilin function or γ-secretase

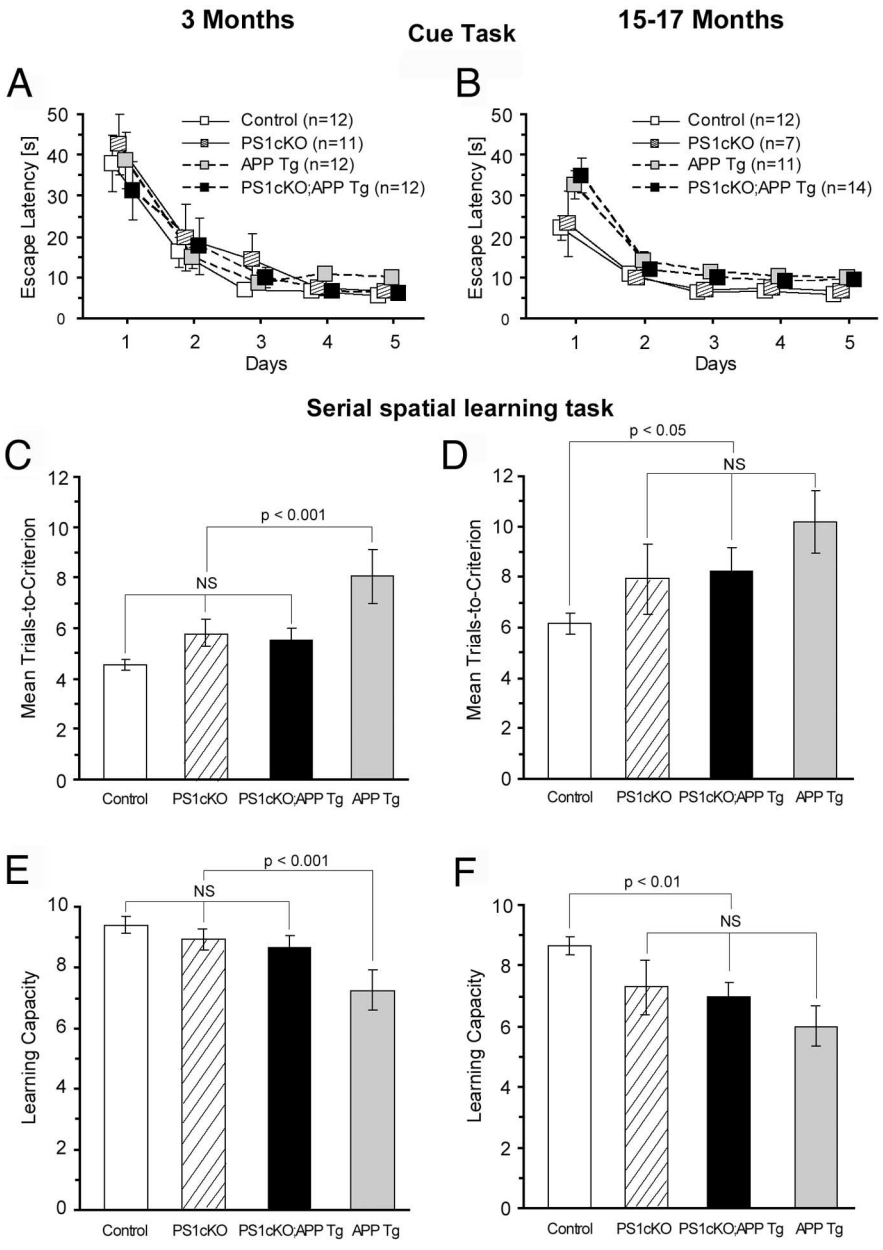

Figure 6. Age-dependent rescue of serial spatial learning in *PS1* cKO;*APP* Tg mice. ***A***, ***B***, Control, *PS1* cKO, *APP* Tg, and *PS1* cKO;*APP* Tg mice at 3 months of age (***A***) and 15–17 months of age (***B***) were trained in the visible platform version of the water maze for 5 d consecutively (4 trials/d). *APP* Tg and *PS1* cKO;*APP* Tg mice showed normal performance in the cue task. The number of mice used in each group is indicated in parentheses. ***C***, Mice were trained in the serial spatial reversal task in a water maze for 10 d. Analysis of trials to reach criterion revealed no difference among control, *PS1* cKO, and *PS1* cKO;*APP* Tg groups ($p > 0.10$), but these three groups took fewer trials to learn the five platform locations than the *APP* Tg group ($p < 0.001$). These data indicate that *PS1* cKO;*APP* Tg mice at 3 months of age exhibit a rescue of spatial working memory. ***D***, Analysis of trials-to-criterion of mice at 15–17 months of age indicated that performances of the *PS1* cKO, *APP* Tg, and *PS1* cKO;*APP* Tg mice did not differ from each other ($p > 0.10$), and their performances were poorer than that shown by the control group ($p < 0.05$). ***E***, Learning capacity was measured as the total number of spatial reversal locations learned in 10 d. Analysis of learning capacity showed similar learning capacity for the control, *PS1* cKO, and *PS1* cKO;*APP* Tg groups and reduced learning capacity in the *APP* Tg group at 3 months of age ($p < 0.001$). These data indicate a rescue of learning capacity in *PS1* cKO;*APP* Tg mice at 3 months of age. ***F***, The learning capacity of *APP* Tg, *PS1* cKO;*APP* Tg, and *PS1* cKO groups at 15–17 months of age did not differ significantly, but the average performance of these three groups was significantly lower than that of the control group ($p < 0.01$). Data represent the mean ± SEM.

activity is likely to lead to partial rather than complete inhibition of γ-secretase, a more relevant genetic mouse model of γ-secretase inhibition is a partial γ-secretase inactivation as investigated here, using postnatal forebrain-restricted *PS1* cKO mice.

Our findings show that partial inactivation of γ-secretase by conditional inactivation of PS1 ameliorated amyloid-related phenotypes displayed by *APP* transgenic mice. Specifically, our biochemical and histological analyses indicated that selective inactivation of PS1 in the cerebral cortex reduced the accumulation of Aβ peptides and prevented amyloid plaque formation and inflammatory responses associated with *APP* transgenic mice (Figs. 3, 4). These results are consistent with a previous report showing reduced Aβ peptides and amyloid deposition in another line of *APP* transgenic mice with the use of a neuronal-specific *PS1* cKO mouse (Dewachter et al., 2002). Furthermore, our behavioral analysis that used the contextual fear conditioning and serial spatial reversal learning revealed that inactivation of PS1 rescued the hippocampal-dependent learning and memory deficits of young *APP* transgenic mice (Figs. 5, 6). Our observation that Aβ-associated deficits normally observed in two separate hippocampal-dependent memory tasks were rescued by limiting the synthesis of human Aβ in *APP* transgenic mice via PS1 inactivation is consistent with a previous report stating that germline disruption of the *BACE1* gene in another line of *APP* transgenic mice rescued memory deficits in social recognition and spatial alternation tasks (Ohno et al., 2004).

The second key finding of our study is that the improvement of the cognitive impairment associated with *APP* Tg mice via PS1 inactivation is age-related and transient. For example, the impairment of contextual memory exhibited by *APP* Tg mice no longer was rescued by PS1 inactivation in *PS1* cKO;*APP* Tg mice at 6 months of age. Furthermore, alterations in basal synaptic transmission and/or LTP induction observed in *APP* Tg mice also failed to be rescued by PS1 inactivation in *PS1* cKO; *APP* Tg mice (Fig. 7). Our previous study of PDAPP mice in the serial spatial reversal task showed an age-related decline in performance that could be observed over a period of almost 1 year (G. Chen et al., 2000). Thus older *PS1* cKO;*APP* Tg mice at 15–17 months of age were tested in the serial spatial reversal task, and the amelioration of the learning deficits associated with the *APP* transgenic mice by PS1 inactivation was no longer statistically significant. This age-related rescue of hippocampal-dependent cognitive function in *PS1* cKO;*APP* Tg mice may explain the apparent discrepancies between our findings and a previous report showing that inactivation of PS1 in neurons of *APP*[V7171I] transgenic mice rescued LTP impairments but failed to rescue the impairment in

spontaneous object recognition at 3–6 months of age (Dewachter et al., 2002).

It may seem surprising that the learning and memory deficits exhibited by old *APP* transgenic mice are not ameliorated by the prevention of Aβ accumulation in *PS1* cKO;*APP* Tg mice. However, any beneficial effect on learning that results may be more than offset by other biochemical changes taking place in these older mice. Several observations of this study point to the possibility that age-dependent accumulation of APP C-TFs, as a result of γ-secretase inhibition, may underlie the memory deterioration in older *PS1* cKO; *APP* Tg mice. Confocal and electron microscopy analyses showed marked accumulation of APP C-TFs at synaptic terminals in *PS1* cKO;*APP* Tg mice (Fig. 2). The accumulation of APP C-TFs could be caused by deficient trafficking and/or processing of APP and APP C-TFs. Consistent with this interpretation, PS1 has been implicated previously in the trafficking of APP (Naruse et al., 1998; Leem et al., 2002; Cai et al., 2003). PS1 may be regulating the processing of APP and cleavage of APP C-TFs in or near synaptic sites where these precursors containing the entire Aβ domain accumulate (Schubert et al., 1991; Buxbaum et al., 1998). Indeed, we found recently that Aβ_{42} peptides accumulate in multivesicular bodies at neuronal processes and synaptic compartments of *APP* transgenic mice and AD brains (Takahashi et al., 2002, 2004). It has been suggested that the Aβ released from presynaptic sites is deposited in extracellular plaques (Buxbaum et al., 1998; Lazarov et al., 2002). Finally, a deleterious effect of abnormally high levels of APP C-TFs on synaptic function is supported by previous findings showing LTP and memory impairments in mice injected with or expressing APP C-TFs (Cullen et al., 1997; Nalbantoglu et al., 1997; Choi et al., 2001).

An alternative explanation of the age-dependent deterioration of contextual fear conditioning and serial spatial reversal in *PS1* cKO;*APP* Tg mice is that inactivation of PS1 itself causes cognitive impairments. However, we have demonstrated previously, by using an intensive training protocol, that *PS1* cKO mice perform similarly to littermate controls in contextual fear conditioning and spatial reference memory (Saura et al., 2004), although they do exhibit a spatial reference memory deficit under a more difficult training protocol (Yu et al., 2001). Furthermore, *PS1* cKO mice exhibit normal synaptic transmission and plasticity in the Schaffer collateral pathway (Yu et al., 2001). These ob-

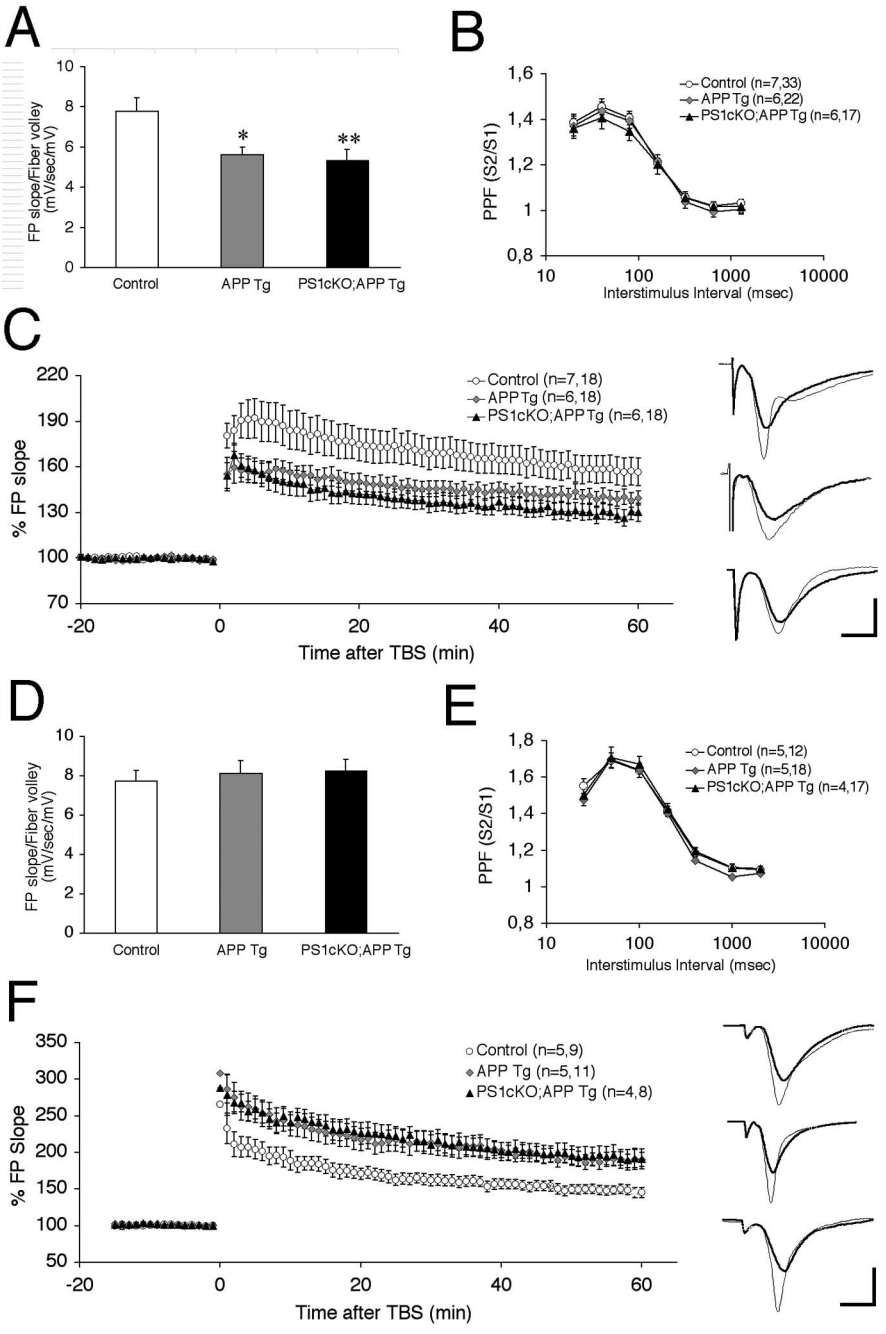

Figure 7. Similar hippocampal synaptic alterations in *APP* Tg and *PS1* cKO;*APP* Tg mice. ***A***, Basal synaptic transmission is reduced in *APP* Tg and *PS1* cKO;*APP* Tg mice at 6 months of age. The I/O slope was obtained by plotting the FV amplitude against the initial slope of the evoked fEPSP in acute hippocampal slices of control, *APP* Tg, and PS1 cKO;*APP* Tg mice. The graph represents the average slope of the I/O curves. $*p < 0.02$; $**p < 0.005$. ***B***, The graph depicts the paired pulse response ratio (second fEPSP/first fEPSP) obtained at different interstimulus intervals in 6-month-old mice. *APP* Tg and *PS1* cKO;*APP* Tg mice show normal PPF ($p = 0.14$). ***C***, Left, Time course of the effects of five episodes of TBS on the fEPSP initial slope in 6-month-old mice. Right, Examples of LTP induced in slices from control (top), *APP* Tg (middle), and *PS1* cKO;*APP* Tg (bottom) mice. Superimposed traces are averages of four consecutive responses recorded 1 min before (thin line) and 60 min after (thick line) TBS. *APP* Tg and *PS1* cKO;*APP* Tg mice show impaired LTP. ***D***, The graph represents the average slope of the I/O curves in 3-month-old mice. The average of FV–fEPSP slopes in *APP* Tg and *PS1* cKO;*APP* Tg mice is similar to that of control mice at 3 months of age ($p > 0.05$). ***E***, The graph depicts the paired pulse response ratio obtained at different interstimulus intervals in 3-month-old mice. *APP* Tg, *PS1* cKO;*APP* Tg, and control mice show similar PPF ($p = 0.13$). ***F***, Left, Time course of the effects of five episodes of TBS on the fEPSP initial slope in 3-month-old mice. Right, Examples of LTP induced in slices from control (top), *APP* Tg (middle), and *PS1* cKO;*APP* Tg (bottom) mice. Superimposed traces are averages of four consecutive responses recorded 1 min before (thin line) and 60 min after TBS (thick line). In ***B***, ***C***, ***E***, ***F***, the numbers of mice (left) and slices (right) are indicated in parentheses. Data represent the mean ± SEM. Calibration: (in ***C***, ***F***) 0.5 mV, 5 ms.

servations suggest that PS1 inactivation alone is unlikely to account for the observed phenotype. Moreover, although it may be argued that residual levels of Aβ in the cortex of *PS1* cKO;*APP* Tg mice contribute to the age-dependent cognitive deterioration, this is also unlikely, because similar residual levels of $A\beta_{40}$ and $A\beta_{42}$ were found in these mice at 2–3, 6, and 17 months of age. It is therefore reasonable to suggest that abnormal accumulation of APP C-TFs in neurons of the cerebral cortex, especially in the synaptic terminals, contributes to memory impairments in older *PS1* cKO;*APP* Tg mice. However, we cannot yet rule out the possibility that long-term PS1/γ-secretase inactivation could contribute to the described phenotype by the deficient processing of other known or still unidentified synaptic substrates (Kim et al., 2002; May et al., 2002; Marambaud et al., 2003; Schulz et al., 2003).

Are presenilins potential drug targets for AD therapy? The results presented here showing prevention of Aβ accumulation and amyloid deposition in *PS1* cKO;*APP* Tg mice provide support for the view that targeting PS1 function or γ-secretase activity could be an effective approach for anti-amyloidogenic treatment. In addition, our extensive behavioral analysis demonstrates that inactivation of PS1 can provide temporary benefits to improve cognitive function in *APP* transgenic mice. Nevertheless, presenilins have many important physiological functions ranging from synaptic plasticity to epidermal proliferation (Doerfler et al., 2001; Xia et al., 2001; Qyang et al., 2004; Saura et al., 2004; Tournoy et al., 2004), and complete loss of presenilin function in the cerebral cortex, which is the most relevant anatomical substrate for AD pathogenesis, leads to progressive memory loss and neurodegeneration (Saura et al., 2004). We recently proposed that loss of presenilin function together with increases in Aβ peptides collectively could lead to memory loss and neurodegeneration in FAD (Saura et al., 2004). The finding that PS1 mutations devoid of γ-secretase activity are associated with frontotemporal dementia (Raux et al., 2000; Amtul et al., 2002; Dermaut et al., 2004) also supports a possible link between loss of PS1 function and pathogenic mechanisms of dementia. The requirement of presenilins for synaptic plasticity, memory, and neuronal survival is mediated partly by the regulation of the cAMP response element-binding protein pathway, likely by γ-secretase-dependent Notch cleavage and signaling (Saura et al., 2004). Therefore, it is unlikely that γ-secretase inhibitors that affect Notch signaling will be useful as potential drugs for AD treatment. Alternatively, the development of specific γ-secretase inhibitors that inhibit Aβ production, and specifically $A\beta_{42}$, without disturbing other presenilin functions and specifically those involved in synaptic function, still may provide a rational therapeutic approach for the treatment of AD. In conclusion, we have demonstrated that conditional inactivation of PS1 in the postnatal forebrain efficiently prevents amyloid-associated neuropathological changes in *APP* transgenic mice. We provide evidence for the first time that reduction of Aβ as a result of γ-secretase inhibition *in vivo* may provide transient cognitive improvements in an amyloid mouse model. These findings raise the possibility that γ-secretase inhibitors that specifically inhibit Aβ generation without altering other essential presenilin functions may provide temporary therapeutic benefits.

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