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Deficient Neurogenesis in Forebrain-Specific *Presenilin-1* Knockout Mice Is Associated with Reduced Clearance of Hippocampal Memory Traces

Ruiben Feng,[1,5,7] Claire Rampon,[1,7] Ya-Ping Tang,[1] David Shrom,[1] Janice Jin,[1] Maureen Kyin,[1] Bryce Sopher,[2] George M. Martin,[2] Seong-Hun Kim,[4] Ronald B. Langdon,[3] Sangram S. Sisodia,[4] and Joe Z. Tsien[1,6]

[1]Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

[2]Department of Laboratory Medicine and
Department of Molecular Medicine
University of Washington
Seattle, Washington 98995

[3]New Jersey Neuroscience Institute
Seton Hall University
Edison, New Jersey 08818

[4]Department of Neurobiology, Pharmacology, and Physiology
University of Chicago
Chicago, Illinois 60637

[5]Shanghai Institute of Brain Functional Genomics
East China Normal University
Shanghai
China

Summary

To examine the in vivo function of *presenilin-1* (*PS1*), we selectively deleted the *PS1* gene in excitatory neurons of the adult mouse forebrain. These conditional knockout mice were viable and grew normally, but they exhibited a pronounced deficiency in enrichment-induced neurogenesis in the dentate gyrus. This reduction in neurogenesis did not result in appreciable learning deficits, indicating that addition of new neurons is not required for memory formation. However, our postlearning enrichment experiments lead us to postulate that adult dentate neurogenesis may play a role in the periodic clearance of outdated hippocampal memory traces after cortical memory consolidation, thereby ensuring that the hippocampus is continuously available to process new memories. A chronic, abnormal clearance process in the hippocampus may conceivably lead to memory disorders in the mammalian brain.

Introduction

Alzheimer's Disease (AD) is a degenerative disease of the central nervous system. Although the exact etiology has not been fully determined, the onset of AD has been linked to decreased neurotransmitters in neurons, genetic mutations, and other factors (Hardy, 1997). The disease is characterized by progressive dementia, histological signs of senile plaques, and neurofibrillary tangles. These abnormal deposits accumulate in the brain and lead to decreased neuronal connections and neuronal atrophy, largely in parts of the temporal lobe system such as the hippocampus and neocortex (Lendon et al., 1997).

Mutations in the *PS1* gene are associated with the early onset of AD (St. George-Hyslop, 2000; Haass, 1997; Price and Sisodia, 1998). The *PS1* gene encodes a 45 kDa polytopic membrane protein that is associated with γ-secretase. γ-secretase activity is necessary for processing amyloid precursor protein (APP), the precursor of the 40–43 amino acid Aβ peptides that accumulate in senile plaques (Kim and Tanzi, 1997; Guenette and Tanzi, 1999). PS1 also plays a critical role in facilitating intramembranous processing of Notch, a signaling receptor that is essential for neuronal fate specification and differentiation. PS1-dependent processing of Notch results in the release of the intracellular domain that translocates into the nucleus and transcriptionally regulates the expression of downstream genes (De Strooper, 1999; Selkoe, 2000). Presenilins are expressed during neuronal development and are present in neuronal cells in the hippocampus and the cortex, brain regions associated with learning and memory (Lee et al., 1996). However, the function of PS1 in postmitotic neurons is not known.

Both gene knockout and transgenic techniques are powerful means to study *PS1* and AD-related genes in vivo (Price and Sisodia, 1998). For example, *PS1* deficiency in mice is associated with severe developmental abnormalities and neonatal embryonic lethality (Wong et al., 1997; Shen et al., 1997; Handler et al., 2000), suggesting an essential role of *PS1* in development. However, the lethality of the *PS1* knockouts precludes functional analysis of *PS1* in adulthood. Because AD occurs as an age-associated malady, it is useful to develop an animal model that permits us to examine the long-term effects of *PS1* on brain function and cognitive behaviors.

Therefore, we make use of a previously developed brain subregion-specific gene knockout technique that is based on the *Cre/loxP* system (Tsien et al., 1996a, 1996b). Through a series of detailed analyses, we demonstrate that the *Cre/loxP* system works efficiently in the brain and allows us to manipulate genes in forebrain regions (cortex, hippocampus, striatum, etc.) or subregions (hippocampal CA1 region). Just as importantly, these transgenic *Cre* lines allow the gene knockout to occur from the fourth postnatal week to adulthood (Tsien et al., 1996a, 1996b; Rampon et al., 2000a), thereby eliminating major complications derived from gene deletion during early development. This type of region- and temporal-specific knockout technique is suitable for the study of the functions of genes linked to age-dependent phenomena in the adult brain. Here, we show that forebrain-specific *PS1* deletion results in reduced enrichment-induced neurogenesis in the dentate gyrus. We also uncover an effect of such deletion, not on the ability to form new memories under conventional conditions of study, but rather on the ability to clear old memory traces from the hippocampus after cortical memory consolidation.

[6]Correspondence: jtsien@princeton.edu
[7]These authors contributed equally to this work.

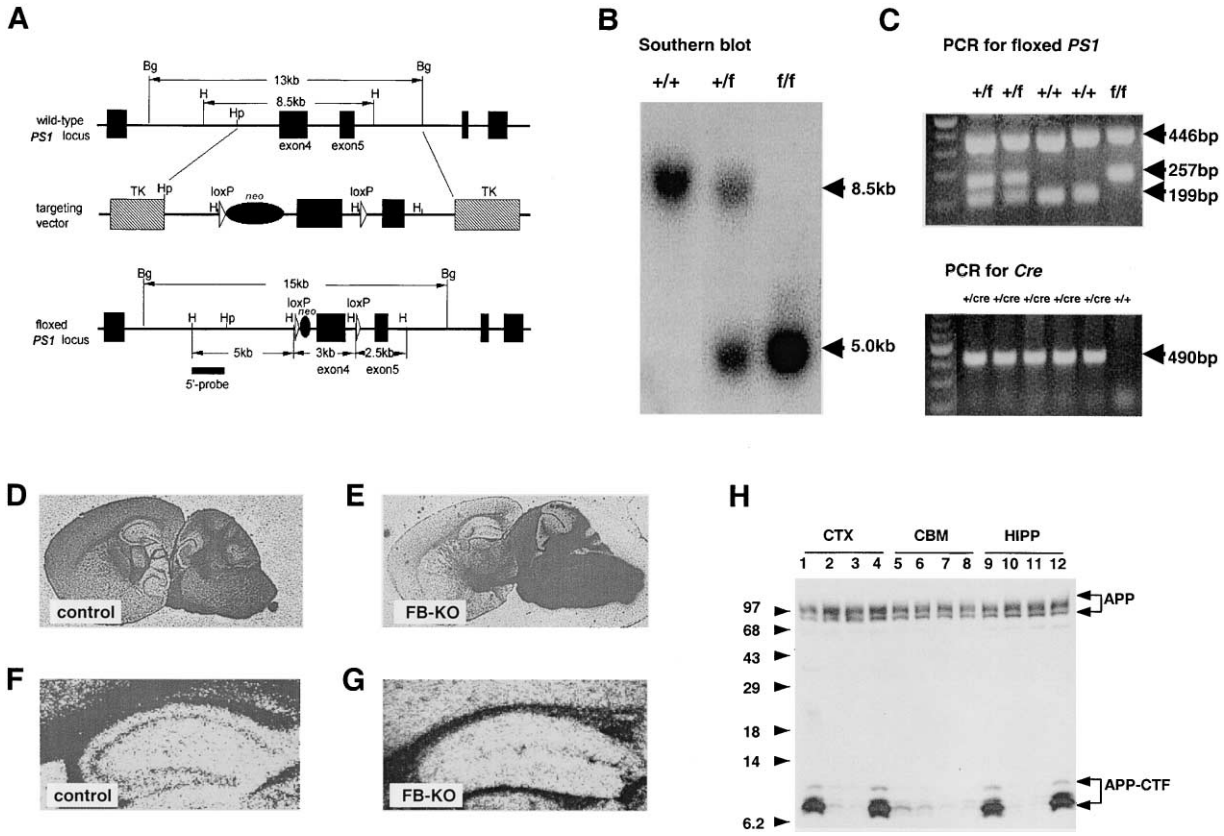

Figure 1. Generation of *PS1* FB-KO Mice

(A) Schematic description of the genomic targeting vector. Two *loxP* sequences were inserted to flank exon 4 of the *PS1* gene.

(B) Southern blot of tail DNA shows wild-type (+/+), heterozygous (+/f), and homozygous floxed (f/f) *PS1* gene.

(C) PCR detection of floxed *PS1* alleles (top panel) and *Cre* transgene (bottom panel). The sizes of the bands are marked.

(D) Expression of PS1 mRNA in control brain (10-months-old).

(E) Forebrain-specific knockout of *PS1* gene in FB-KO mice.

(F) Expression of *PS1* mRNA in the control hippocampus.

(G) Lack of *PS1* mRNA in the excitatory neurons of CA1, CA3, and dentate gyrus of FB-KO mice.

(H) Western blot shows the accumulation of APP-CTF in the cortex (CTX; lanes 1 and 4) and hippocampus (HIPP; lanes 9 and 12) of FB-KO mice (10-months-old), whereas the APP-CTF level in cerebellum (CBM; lanes 5 and 8) of the same FB-KO mice is unaltered. Full length APP and its CTF are indicated. Molecular weight markers are in kDa. Lanes 2, 3, 6, 7, 10, and 11 were prepared from control littermates (10-months-old).

Results

Production and Molecular Characterization of Forebrain-Specific *PS1* Knockout Mice

The overall strategy for making forebrain-specific *PS1* knockout mice is the same as previously published (Tsien et al., 1996a, 1996b), namely, crossing the forebrain-specific *Cre* transgenic mouse line with the floxed *PS1* mice in which the *PS1* gene is flanked by two *loxP* sequences. We constructed a genomic targeting vector in which two *loxP* sequences flanked exon 4 of the *PS1* gene (Figure 1A). The linearized vector was transfected into embryonic stem (ES) cells. Targeted ES cells with correct homologous recombination were isolated and then injected for the production of chimeric mice, which gave successful germline transmission to the offspring. The heterozygous or homozygous offspring were then backcrossed systematically with a forebrain-specific *Cre* transgenic mouse line to generate conditional knockout mice that carry both the *Cre* transgene and homozygous floxed *PS1* genes (Figures 1B and 1C). Those forebrain-specific *PS1* knockout mice (*fPS1/fPS1*; *Cre/+*) were simply termed as FB-KO mice, and their sibling nontransgenic littermates (*fPS1/+* or *fPS1/fPS1*) served as controls.

These FB-KO mice were indistinguishable from their control littermates. They mated, grew normally, and exhibited normal open field behavior. We confirmed the forebrain-specific deletion of *PS1* by in situ hybridization using a probe that recognizes the *PS1* mRNA specifically encoded by exon 4. As expected, *PS1* mRNA was absent in the cerebral cortex, striatum, and hippocampus (CA1, CA3, and dentate gyrus) of FB-KO mice (10-months-old), whereas *PS1* mRNA in the other regions, such as the olfactory bulb, thalamus, brain stem, and cerebellum, was unchanged (Figures 1D–1G).

Accumulation of C-Terminal Fragments of APP and APP-like Protein

It is well established that α- and β-secretases process APP to generate membrane-tethered APP-CTFs (C-terminal

fragments) and that PS1 is required for further cleavage of these APP-CTFs at the γ-secretase site to produce Aβ peptides. *PS1* deficiency is associated with increased accumulation of APP-CTFs and diminished Aβ production. We determined whether APP-CTF accumulates in the forebrain regions of FB-KO mice by Western blots. In comparison to APP-CTF levels in control mice, we observed that the level of APP-CTF was dramatically elevated in the cortex and the hippocampus, but not in the cerebellum, in FB-KO mice (Figure 1H).

APP is a member of the family of proteins that includes amyloid precursor-like proteins 1 and 2 (APLP1 and APLP2) that are expressed in the nervous system. Although APLPs are highly homologous to APP in the N- and C-terminal domains, they lack the Aβ domain. Immunohistochemical studies in the brain reveal considerable overlap in the distributions of APP and APLP1 (Bayer et al., 1997). Thus, we decided to examine the level of CTF derived from the APP homolog APLP1-CTF. Indeed, we observed a similarly marked accumulation of APLP1-CTF in the cortex and the hippocampus, but not in the cerebellum (data not shown). Therefore, our data not only demonstrate that PS1 is essential for the processing of APP and APLP1 in the adult brain, but also provide further in vivo functional verification for the occurrence of the region-specific PS1 knockout in the FB-KO mice.

Brain Histology of FB-KO Mice

We used Nissl staining to analyze the brain anatomy under a light microscope and observed no differences between the FB-KO mice and the controls (Figures 2A and 2B). Furthermore, we investigated whether the postnatal knockout of *PS1* altered the number of neurons in the FB-KO brain. We focused on the hippocampal dentate gyrus and conducted quantitative cell counting. We found that the total cell densities were the same between FB-KO and control mice (Figure 2C).

Because the dentate gyrus of the hippocampus has the highest levels of neurogenesis in the mammalian brain (Altman and Das, 1965; Kaplan and Hinds, 1977; Kornack and Rakic, 1999), we decided to measure the number of newborn cells in the dentate gyrus of the adult mouse brain. To label those cells, we injected BrdU (100 mg/kg of body weight) intraperitoneally twice daily (2 hr apart) into five FB-KO and five wild-type littermates (10- to 12-months-old) for 4 consecutive days. The animals were sacrificed and perfused 12 hr after the last BrdU injection. We found that BrdU-labeled cells were noticeable in the dentate gyrus but at low numbers (Figure 3A). Our quantitative analysis revealed no significant differences in the densities of BrdU-labeled cells in the dentate gyrus (Figure 2D). Moreover, our analysis within subregions (the hilus, the granular cell layer, and the molecular layer) of the dentate gyrus also showed no differences between BrdU-positive cells in FB-KO and control mice (data not shown).

We further determined the role of *PS1* in the differentiation of nascent cells into glial cells and neurons in the dentate gyrus. We employed a double-labeling technique (Liu et al., 1998) to distinguish newborn glial cells from neurons using antibodies against an astroglial marker, glial fibrillary acidic protein (GFAP), and a neuronal marker, NeuN (Figure 2E). Our quantitative analysis revealed no statistically significant difference in either newborn neurons or newborn glial cells between FB-KO and control mice (Figures 2F and 2G).

Deficiency of Enrichment-Induced Neurogenesis in FB-KO Mice

An enriched experience is known to promote structural growth (Rosenzweig, 1966; Nilsson et al., 1999; Greenough et al., 1990; Rampon et al., 2000a) and neurogenesis (Kornack and Rakic 1999; Kempermann et al., 1997) in adult animals, hence we sought to examine the role of PS1 in enrichment-induced cell proliferation in the adult mouse brain. Using a similar enrichment protocol as we previously described (Rampon et al., 2000a; Tang et al., 2001), we placed mice (10- to 12-month-old males) in an enriched environment for 3 hr a day for 2 weeks; these animals are termed "enriched mice." We changed various toys, spin wheels, small tunnels, and houses every day to stimulate exploration. During the last 4 days of enrichment, the animals were injected with BrdU twice daily at a 2 hr interval, and then the animals were sacrificed and perfused 12 hr after the last BrdU injection. "Naive mice" (10- to 12-month-old males) were not subjected to enriched environments and were kept in laboratory cages. Indeed, we found that our enrichment protocol resulted in robust increases in BrdU-positive cells in the dentate gyrus of control mice (6-fold over basal levels) and FB-KO mice (4.2-fold) (Figures 3A–3C); however, FB-KO mice showed a significantly lower level of BrdU-positive cells compared with control mice. The average density of BrdU-labeled cells in the enriched FB-KO mice was 37% less than that of the enriched control counterparts ($p < 0.015$; Figure 3C).

We performed double labeling to further classify the newborn cells into glial cells and neurons. Our analysis revealed that the number of newborn neurons in FB-KO dentate gyrus was 34% less than that of the control mice ($p < 0.05$; Figure 3D), suggesting that PS1 function is important for enrichment-induced neurogenesis in the adult dentate gyrus. We saw no difference between FB-KO and controls in newborn glial cells (Figure 3E).

We then examined the spatial distribution of nascent neurons within the dentate gyrus. The majority of newborn neurons were located in the subgranular zone (SGZ), the proliferative zone in the dentate gyrus. There were 39% less newborn neurons in SGZ of FB-KO mice than in the control mice ($p = 0.028$; Figure 3F). We also found a significant difference in the molecular layer, where there were 48% less newborn neurons in FB-KO mice ($p < 0.05$; Figure 3H). Therefore, our results show that loss of PS1 leads to a significant deficiency in enrichment-induced neurogenesis in FB-KO mice.

Electrophysiological Measurements of FB-KO Mice

Observations of adult neurogenesis in the dentate gyrus and other regions of the brain have led to a number of interesting functional hypotheses that for the most part remain untested. We decided to use our FB-KO model to examine whether deficient neurogenesis would have any appreciable effect on synaptic plasticity and learning behaviors. We measured synaptic responses and

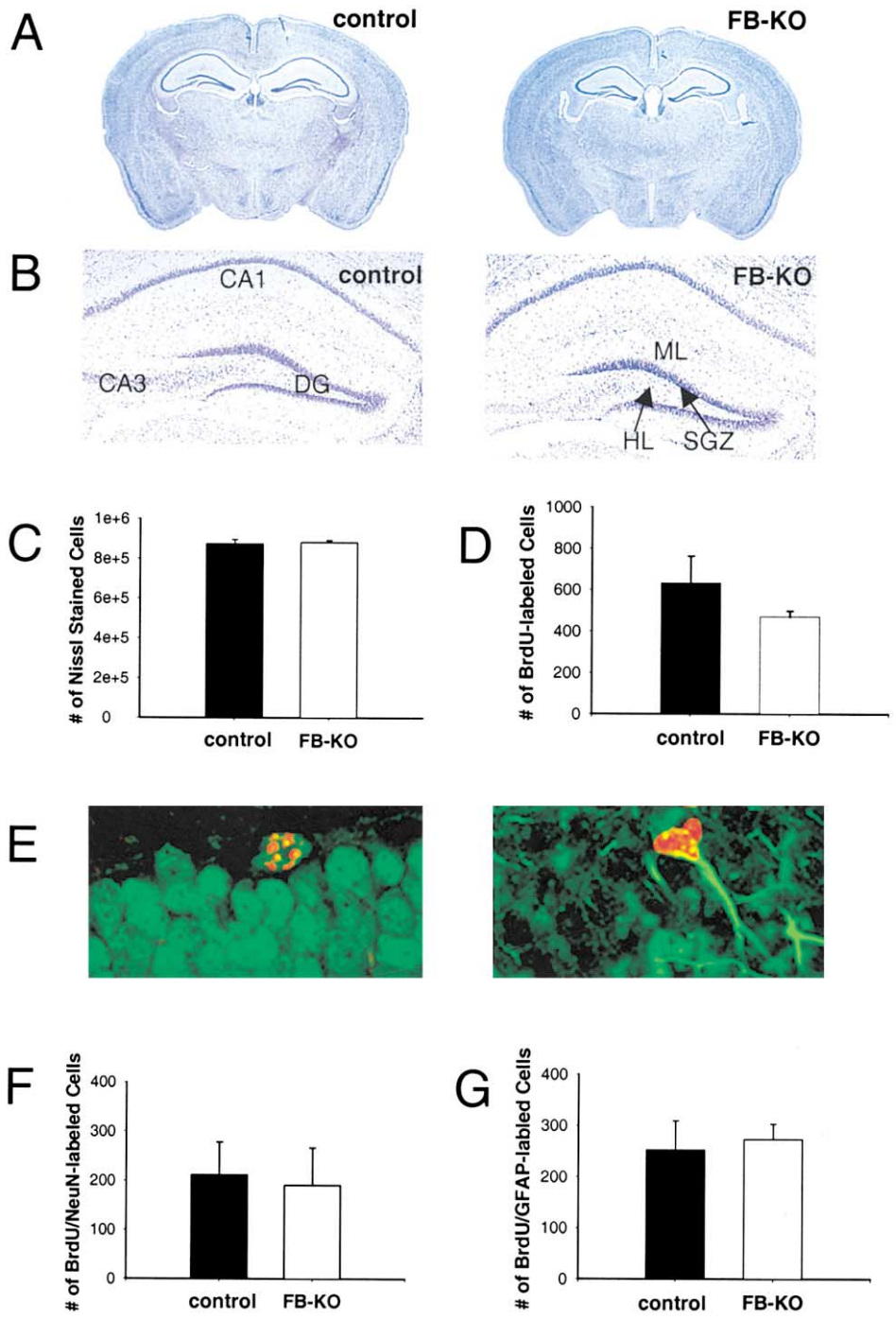

Figure 2. Brain Histology of Naive FB-KO Mice

(A) Normal appearance of brain anatomy by Nissl staining from a control (left panel) and FB-KO (right panel) mouse.

(B) Normal appearance of the hippocampus of control mice (left panel) and an FB-KO mouse (right panel) revealed by Nissl staining. CA1, CA3, dentate gyrus (DG), the subgranular zone (SGZ), the hilus (HL), and the molecular layer (ML) of the dentate gyrus are marked.

(C) Quantitative measurement of Nissl staining cells in the dentate gyrus of control littermate ($n = 10$) and FB-KO ($n = 9$) mice.

(D) Quantitative measurement of newborn cells labeled with BrdU in the dentate gyrus of naive control ($n = 6$) and naive FB-KO ($n = 5$) mice. No statistical difference was found between genotypes.

(E) Confocal microscopic image of a newborn neuron labeled by BrdU/NeuN double staining (left panel; BrdU is in bright orange, whereas NeuN is stained in green). A newborn glial cell is shown by BrdU/GFAP double labeling (right panel; BrdU is in bright orange, whereas GFAP is stained in green).

(F) Quantification of newborn neurons in the dentate gyrus of naive control ($n = 6$) and naive FB-KO ($n = 5$) mice. No statistical difference was found between genotypes.

(G) Quantification of newborn glial cells in the dentate gyrus of naive control ($n = 6$) and naive FB-KO ($n = 5$) mice. No statistical difference

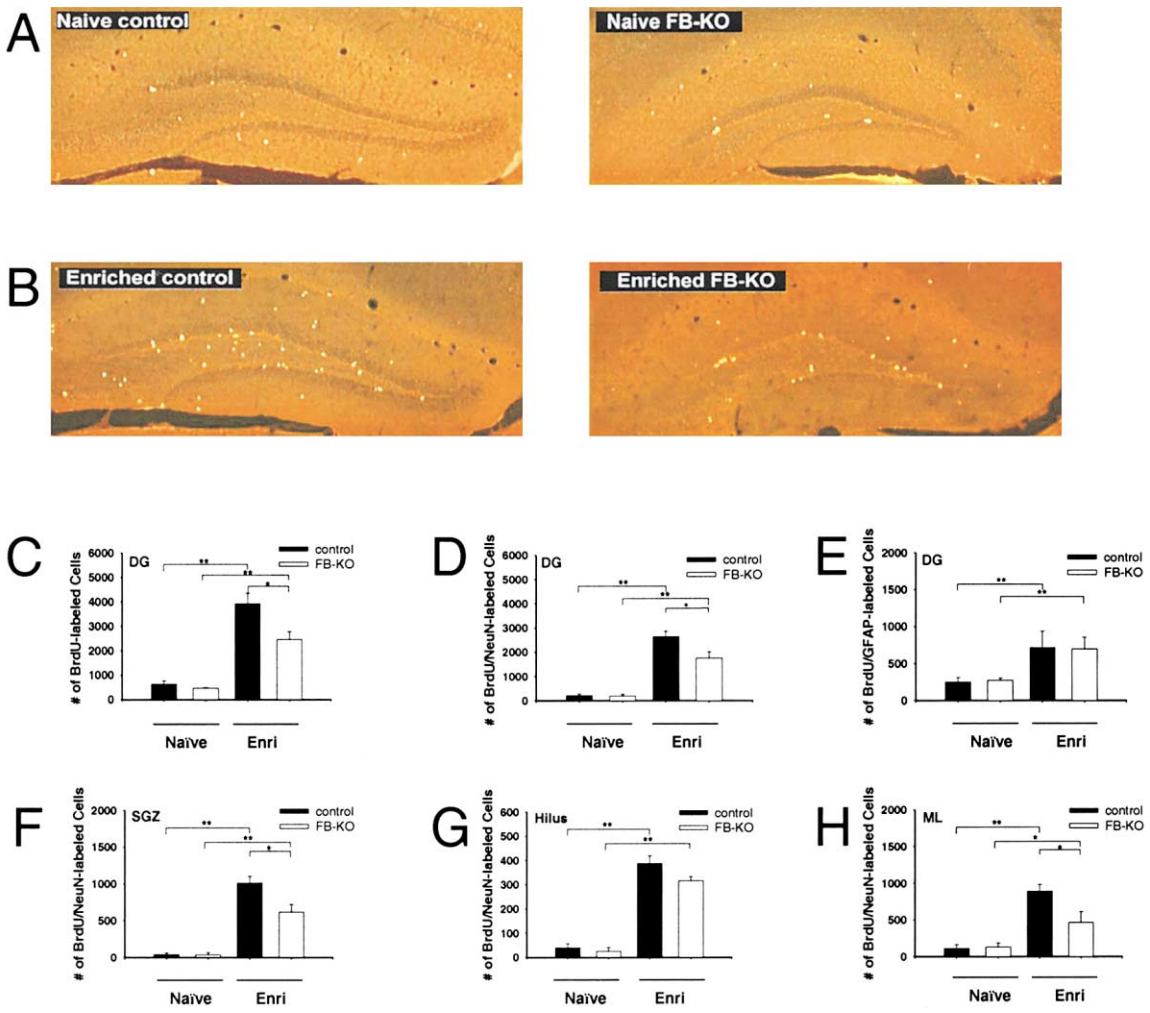

Figure 3. Deficiency of Enrichment-Induced Neurogenesis in FB-KO Mice

(A) Light microscopic image of BrdU labeling in the dentate gyrus of a naive control (left panel) and a naive FB-KO (right panel) mouse.

(B) Light microscopic image of BrdU labeling in the dentate gyrus of an enriched control (left panel) and an enriched FB-KO (right panel) mouse.

(C) Enrichment-induced increase in the number of newborn cells in the dentate gyrus. Enrichment-induced BrdU-positive cells in enriched FB-KO mice is 37% less than that in control mice.

(D) Enrichment-induced new neurons in the dentate gyrus from both control and FB-KO mice. FB-KO mice exhibited 34% less nascent neurons than control mice.

(E) Enrichment-induced new glial cells in the dentate gyrus from both control and FB-KO mice. No difference was found between enriched genotypes.

(F) Deficiency of enrichment-induced neuronal proliferation in the SGZ of the dentate gyrus of enriched FB-KO mice. They exhibited 39% less nascent neurons than enriched control littermates.

(G) Enrichment-induced increase in the newborn cells in the hilus of the dentate gyrus in enriched control and FB-KO mice. No statistical difference was found.

(H) Deficiency of enrichment-induced increase in nascent neurons in molecular cell layer (ML) of the dentate gyrus of enriched FB-KO mice. They exhibited 48% less nascent neurons than enriched control mice. (* indicates a significant difference, $p < 0.05$; ** indicates a significant difference, $p < 0.01$). Naive control mice ($n = 6$); naive FB-KO mice ($n = 5$); enriched control mice ($n = 4$); and enriched FB-KO mice ($n = 4$). Data were expressed as mean ± SEM.

their plasticity elicited in the hippocampal dentate gyrus by input via the medial perforant path (MPP) in brain slices prepared from four groups of mice (9- to 13-months-old): naive control, naive FB-KO, enriched control, and enriched FB-KO (Figures 4A and 4B). As a control, we concurrently recorded synaptic responses elicited in the same postsynaptic population by input from the lateral perforant pathway (LPP). Recording and

was found between genotypes.

(H) Quantification of newborn glial cells in the dentate gyrus of naïve control ($n = 6$) and naive FB-KO ($n = 5$) mice. No statistical difference was found between genotypes. Data were calculated as mean ± SEM.

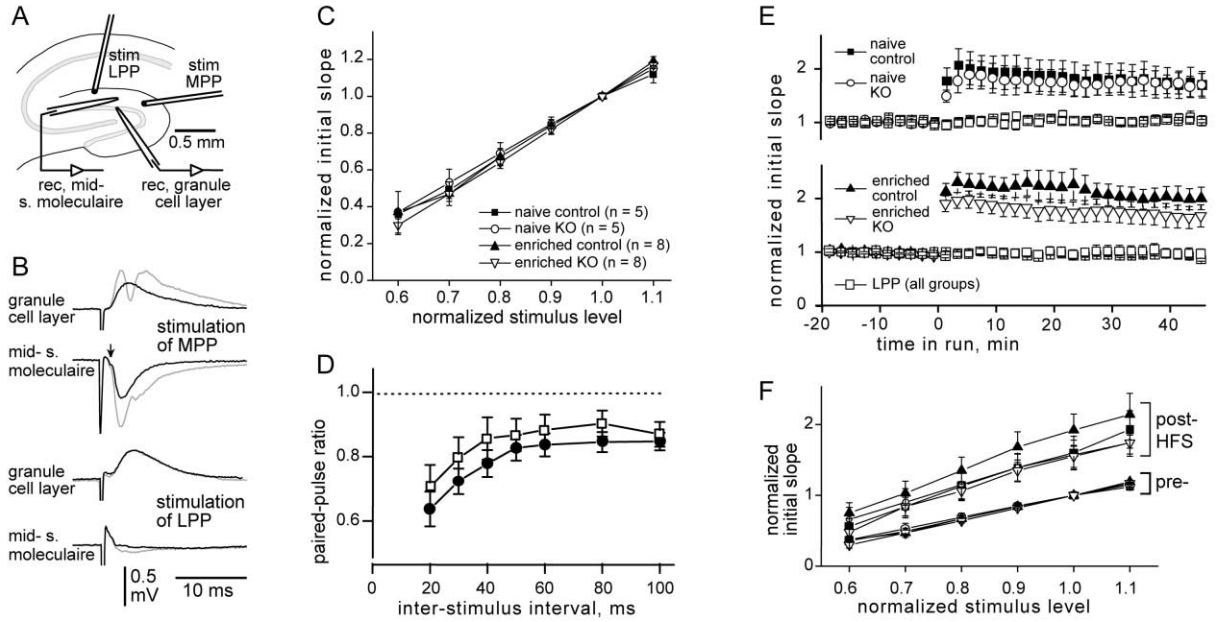

Figure 4. Synaptic Physiology and Plasticity in the Dentate Gyrus in Control and FB-KO Mice

(A) The positions of the LPP and MPP stimulating (stim) electrodes and the mid-s. moleculaire and granule cell layer recording (rec) electrodes in the dorsal blade of the dentate gyrus.

(B) Typical response waveforms. Each is an average of eight consecutive trials recorded with the stimulating and recording electrodes as indicated. Response waveforms did not differ appreciably between the animal groups; this particular example is from an enriched FB-KO mouse.

(C) Comparison of baseline i/o relationships between the four experimental groups. No differences in mean initial slope were significant at any stimulus level examined (all, $p > 0.05$; n represents the number of animals).

(D) MPP paired-pulse depression in slices from control (open squares, $n = 12$) and FB-KO (filled circles, $n = 12$) mice (naive and enriched groups pooled).

(E) A comparison of the time course of long-term potentiation in the four animal groups. There were no statistically significant differences between the different groups (all, $p > 0.05$; both one-way ANOVA test and two-tailed t test).

(F) Comparison of i/o relationships determined at 45 min after HFS between four groups. The symbols used have the same meaning as in (C). No differences in mean were statistically significant at any stimulus level tested (all, $p > 0.05$; both one-way ANOVA test and two-tailed t test).

analysis of the physiological data were done blind to genotype.

Baseline synaptic responses were evaluated by three parameters that characterized input/output (i/o) relationships and two that related to response time course. More specifically, we measured (1) stimulus thresholds for elicitation of granule cell (GC) population spikes, (2) the amplitudes of field EPSPs elicited in the stratum moleculaire (s. moleculaire) by stimulation at this level, (3) the level of stimulation that elicited a half-maximal field EPSPs (field EPSPs that were half the amplitude of those elicited by stimulation at the GC population spike threshold), (4) the latency of field EPSPs at 90% of peak (latency-to-peak), and (5) the durations of these potentials at half amplitude (field EPSP half-width). We observed no significant differences in the means for any of these parameters between genotypes (Table 1; all, $p > 0.05$). In a further analysis, we calculated and compared mean i/o relationships for each experimental group and found these to be very similar, with no significant differences in mean at any level of stimulation ($p > 0.05$, Figure 4C). This suggests that *PS1* deletion did not produce any gross alterations in membrane excitability, the baseline efficacy of glutamatergic neurotransmission, or the kinetic properties of ligand- and voltage-gated channels that determine rates of impulse conduction and the duration of postsynaptic currents.

We then examined the short-term plasticity elicited by paired-pulse stimulation. It has been shown that dentate granule cell response to MPP input characteristically exhibits paired-pulse depression (McNaughton, 1980; Colino and Malenka, 1993). Our responses to stimulation applied to the mid-s. moleculaire displayed paired-pulse depression, consistent with our interpretation that such stimulation specifically activated MPP input. This depression of field EPSPs diminished from ~35% to 20% as interstimulus intervals (ISI) were increased from 20 ms to 60 ms, and it persisted at the 20% level as the ISI was further increased to 80 and 100 ms. Control and FB-KO mice exhibited statistically indistinguishable patterns of this short-term plasticity (Figure 4D).

Finally, we examined LTP in the four groups of mice. Application of high-frequency stimulation (HFS) elicited LTP comparable to that which has been observed by others in this pathway (Colino and Malenka, 1993; Chapman et al., 1999). Comparing the time course of changes in the initial slope of half-maximal field EPSPs (determined from the initial 0.8–1.5 ms of response waveforms), we observed no statistically significant differences among any of the four experimental groups (Figure 4E;

Table 1. Parameters of Baseline Synaptic Transmission in Naive and Enriched FB-KO and Control Mice

Animal group	Stimulus threshold for GC spike response, (μA)	Stimulus threshold for half-maximal field EPSP response, (μA)	Field EPSP amplitude at the GC spike threshold, (mV)	Field EPSP latency-to-peak (90% rise) (ms)	Field EPSP half-width (ms)	n
Naive control	17.5 ± 1.2	12.0 ± 0.9	0.91 ± 0.16	2.55 ± 0.08	2.99 ± 0.15	5
Naive FB-KO	19.7 ± 2.1	13.3 ± 0.9	1.11 ± 0.16	2.79 ± 0.15	3.11 ± 0.08	5
Enriched control	15.4 ± 2.2	11.2 ± 1.5	0.79 ± 0.10	2.53 ± 0.09	3.01 ± 0.17	8
Enriched FB-KO	16.4 ± 1.7	12.2 ± 1.6	0.99 ± 0.10	2.62 ± 0.05	3.01 ± 0.05	8

All ranges given are SEM. The stimulus thresholds for half-maximal field EPSP responses (as defined in the text) were determined graphically from i/o plots. Among the groups, there were no statistically significant differences in mean. n, number of animals.

one way ANOVA test and two-tailed t test; all, $p > 0.05$). In a parallel analysis, the same conclusion was reached based on examination of peak amplitudes (data not shown). As a further test, we also compared the i/o relationships between the four experimental groups 45 min post-HFS and again found that no differences in mean were statistically significant (Figure 4F; one-way ANOVA test and two-tailed t test; all, $p > 0.05$).

Therefore, the results of our electrophysiological experiments suggest that basal synaptic properties and the ability to induce and express LTP in the dentate gyrus are not affected by deletion of the *PS1* gene and are not correlated with levels of adult neurogenesis.

Basic Learning and Memory Function in Naive FB-KO Mice

To examine the effect of forebrain-specific *PS1* deletion on learning and memory, we conducted a series of behavioral tests in adult FB-KO mice and their littermate controls. In all behavioral experiments, the experimenters were blind to the genotype of the animals throughout the experiments. We used three hippocampal-dependent tasks and one hippocampal-independent behavioral task to assess general functions of learning and memory in naive FB-KO mice and their control littermates.

We first tested the naive mice in a novel object recognition test (Tang et al., 1999; Rampon et al., 2000a). This task requires both hippocampal and cortical function. During the 15 min of training, FB-KO and control mice spent the same amount of time exploring two novel objects (Figure 5A), indicating that they had comparable levels of motivation, interest, and curiosity. Retention tests were conducted 1, 3, and 5 days after the training. During the retention tests, one of the two familiar objects was replaced with a novel one. The animals were allowed to explore the two objects for 5 min and the exploratory preference was measured. Moreover, as shown in Figure 5B, both genotypes exhibited significant exploratory preferences for the novel object in the day 1 and the day 3 tests, but not in the day 5 test. This indicates that FB-KO mice have normal object recognition memory.

Next we measured the behavioral performance of the mice in the hidden-platform water maze. We found that FB-KO mice exhibited escape latency indistinguishable from that of control mice (Figure 5C). The normal spatial learning of FB-KO mice was further confirmed in the transfer test conducted on day 7, as indicated by strong preference toward the target quandrant (Figure 5D), suggesting normal water maze learning in FB-KO mice.

Finally, we conducted fear conditioning tests in naive FB-KO and control mice. In the hippocampal-dependent contextual fear conditioning test, the mice learned to fear the environment associated with an aversive stimulus, such as a mild foot shock (Phillips and Ledoux, 1992; Kim et al., 1992; Davis et al., 1987; Tang et al., 1999). We measured the contextual fear memories in the two groups of mice 24 hr after training and found a similar level of freezing responses in both groups (Figure 5E).

Because the knockout of the *PS1* gene is in the forebrain (including the amygdala and the neocortex), we also examined the performance of these mice in an amygdala-dependent memory task, namely, cued fear conditioning. In this task, mice learned to fear a neutral stimulus, such as a tone, by pairing it with an electrical foot shock (Davis et al., 1987; Phillips and LeDoux, 1992). Similar to the results in the contextual conditioning, we found no significant difference in the freezing response measured 24 hr after training between these two naive groups (Figure 5F). The above experiments on naive mice suggest that the loss of *PS1* in the forebrain regions had no significant effect on the performance of the learning and memory tasks tested here.

Effects of Enrichment on Learning and Memory in FB-KO Mice

It has been speculated that adult neurogenesis in the dentate gyrus may play a crucial role in the formation of hippocampal-dependent new memories (Shors et al., 2001; Gould et al., 1999). We decided to genetically test the validity of this hypothesis. The enrichment protocol that we have used is also known to promote behavioral improvement in several learning and memory tests (Rampon et al., 2000a; Tang et al., 2001). We conducted the same set of behavioral tests on another group of FB-KO and control mice that had been subjected to 2 weeks of daily enrichment (3 hours/day) prior to behavioral training and tests (pre-learning-enriched group).

Our previous studies showed that enrichment enhances memory function in the novel object recognition test in young adult mice (Rampon et al., 2000a; Tang et al., 2001). However, we did not observe enrichment-induced improvement in both the 1 day and the 3 day

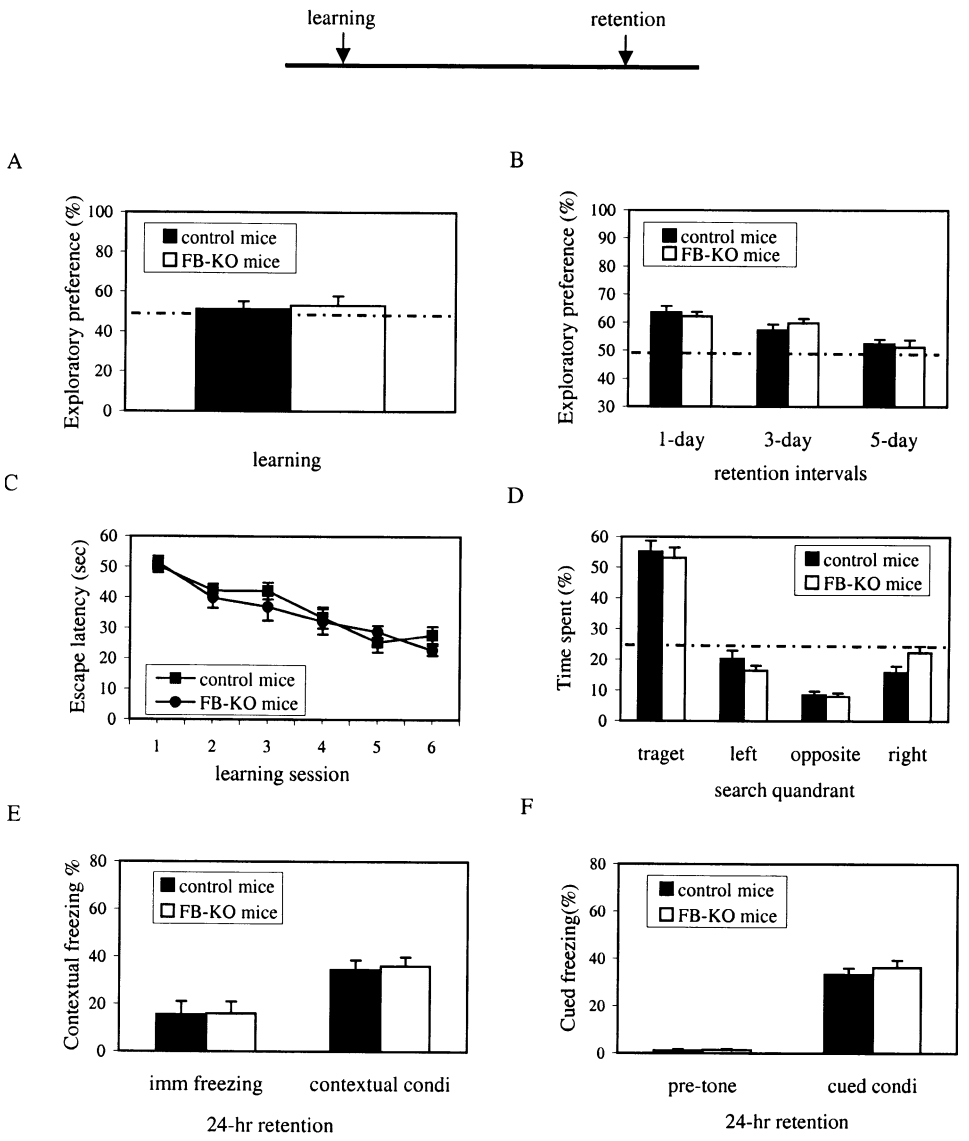

Figure 5. Comparable Learning and Memory Function in Naive Control Littermates and Naive FB-KO Mice as Assessed by Four Behavioral Tests

(A) Equal amount of time spent in exploring the two novel objects during training sessions of the novel object recognition tests.

(B) Novel object recognition retention test reveals no difference in the performances of FB-KO and control mice. One day retention: control mice, n = 8; FB-KO, n = 9. Three day retention: control mice, n = 10; FB-KO, n = 9. Five day retention: control mice, n = 10; FB-KO, n = 11. Recognition memory is expressed in terms of exploratory preference. 50% is about random chance.

(C) Normal learning curve in FB-KO mice as measured by decreased escape latency in the hidden-platform water maze during 6 days of training (four trials per session, one session per day). Control mice, n = 11; FB-KO mice, n = 13.

(D) Normal retention of spatial memory (platform location) as measured by the transfer test in the water maze. Both mutant and control mice exhibited a strong preference towards the target quandrant where the hidden platform was previously located during training.

(E) Similar contextual fear conditioning in control and FB-KO mice. No difference was observed in freezing responses measured immediately after training (imm freezing). Also, there is no difference in the amount of freezing in 24 hr retention tests. Control mice, n = 10; FB-KO, n =1 1.

(F) Similar retention of 24 hr cued fear memories in control and FB-KO mice. During retention tests, the amounts of freezing were measured before onset of tone (pre-tone) as well as during the presentation of 30 s of tone. There was no difference between the groups. Control mice, n = 10; FB-KO, n = 11. The data are expressed as mean ± SEM.

retention tests in either FB-KO or control mice (Figures 6A and 6B). We excluded the possibility of irreproducibility as the reason since we have reproducibly observed the enrichment effect in young adult (3- to 5-months-old) in a more recent study (Tang et al., 2001). Because our animals were much older (11- to 16-months-old), our observation is consistent with the many reports that enrichment-induced improvement of learning behaviors is often age dependent.

On the other hand, in both contextual and cued fear conditioning tests, enriched FB-KO and control mice showed significant enhancement over naive mice ($p < 0.01$, Student's t test) (Figures 6C and 6D), suggesting the effectiveness of prelearning enrichment in promot-

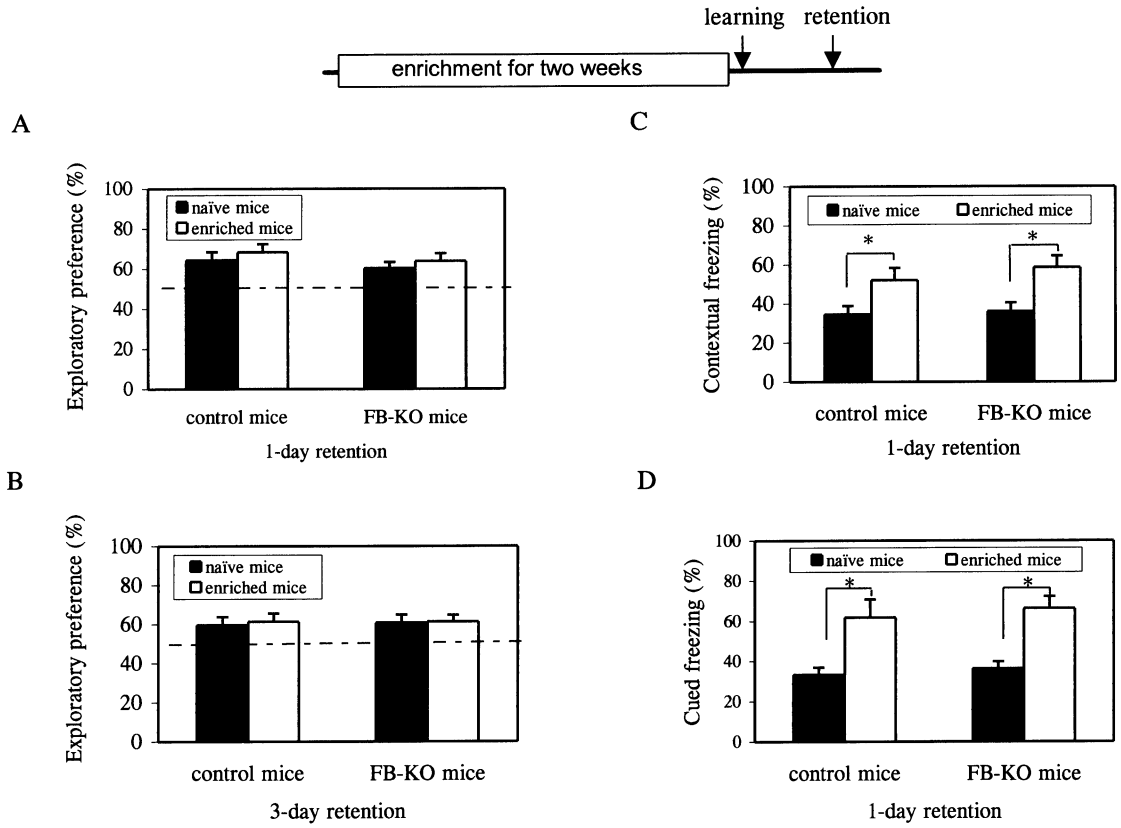

Figure 6. Comparable Learning and Memory Function in Prelearning-Enriched Control and FB-KO Mice as Assessed by the Enrichment-Learning-Retention Test Paradigm

(A) One day retention of novel object recognition memory in FB-KO and control mice after 2 weeks of prelearning enrichment. No enhancement was detected in either enriched group when compared to the naive groups. Naive control mice, n = 10; enriched control mice, n = 10; naive FB-KO mice, n = 9; enriched FB-KO mice, n = 11.

(B) Three day retention of novel object recognition memory in FB-KO and control mice after 2 weeks of enrichment. No enhancement was detected in either enriched group when compared to naive groups. Naive control mice, n = 10; enriched control mice, n = 10; naive FB-KO mice, n = 9; enriched FB-KO mice, n = 11.

(C) Enhanced contextual fear memory in both FB-KO mice and control mice after 2 weeks of prelearning enrichment. There is no difference in the 24 hr retention tests between enriched control and enriched FB-KO mice ($p > 0.05$, post hoc analysis). Naive control mice, n = 10; enriched control mice, n = 10; naive FB-KO mice, n = 11; enriched FB-KO mice, n = 11.

(D) Enhanced cued fear memory in FB-KO and control mice after 2 weeks of prelearning enrichment. There was no difference between the enriched groups ($p > 0.05$, post hoc analysis). Naive control mice, n = 10; enriched control mice, n = 10; naive FB-KO mice, n = 11; enriched FB-KO mice, n = 11. Data were calculated as mean ± SEM.

ing behavioral performance in fear conditioning tasks. Interestingly, a comparison between the genotypes within enriched groups did not reveal any differences, indicating that FB-KO mice preserved normal responsiveness to enrichment procedures despite the lack of *PS1* expression in forebrain regions.

Thus, our above experimental paradigms on both naive and enriched mice suggest that the large accumulations of APP-CTF and APLP1-CTF and a deficiency in enrichment-induced adult neurogenesis in the FB-KO brain did not lead to memory deficits in either hippocampal-dependent (contextual memory, hidden-platform water maze, and novel object recognition) or hippocampal-independent (cued fear conditioning) memory tests.

Differential Alterations of Contextual Memory by Postlearning Enrichment

It is well established that postlearning external events can influence or interfere with the processing and storage of newly acquired memory. These observations prompted us to examine whether FB-KO and control mice respond differently to postlearning external events, such as postlearning enrichment activities, and whether neurogenesis could alter previously formed memories.

We designed a learning-enrichment-retention test paradigm. We focused on the contextual and cued fear conditioning tests for three reasons: first, unlike the water maze, which requires repetitive training trials and involves rather complex external cues and multiple strategies/procedures, fear conditioning cues are simple and clearly defined (shock chamber, grid floor, and tone); second, unlike the novel object recognition test, fear learning occurs in seconds, and the memory is robust and long lasting; and third, lesion studies show that the hippocampus is a site for temporary storage and retrieval of contextual memory up to 2–3 weeks after initial learning (Kim and Fanselow, 1992). Moreover, our recent studies using an inducible gene knockout tech-

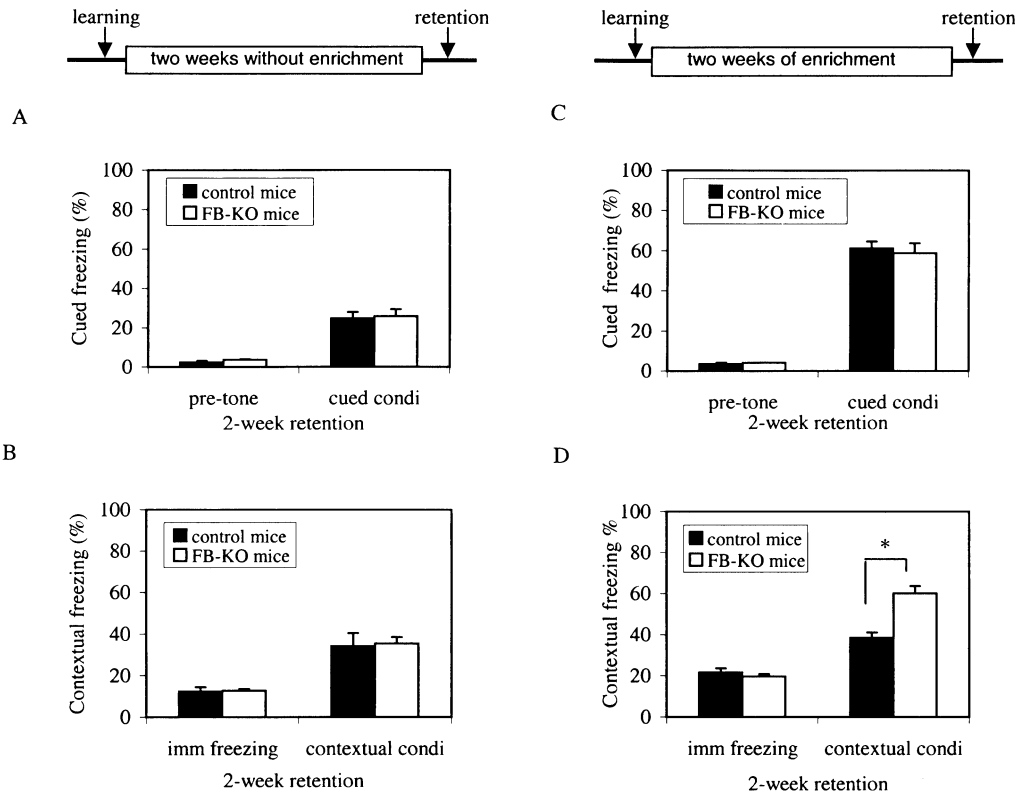

Figure 7. Effects of Postlearning Enrichment on the Retentions of 2-Week-Old Fear Memories by the Learning-Enrichment-Retention Test Paradigm

(A) Similar retention of 2-week-old cued fear memory in the naive FB-KO and control mice with no postlearning enrichment.

(B) Similar retention of 2-week-old contextual fear memory in the naive FB-KO and control mice with no postlearning enrichment.

(C) Similar cued fear memory in the same group of FB-KO and control mice subjected to postlearning enrichment.

(D) Differential effect of postlearning enrichment on contextual fear memory between the same set of control and FB-KO mice. There is a significant difference between control and FB-KO mice ($p < 0.05$, post hoc analysis). Naive control mice, n = 10; enriched control mice, n = 9; naive FB-KO mice, n = 10; enriched FB-KO mice, n = 8. Data were calculated as mean ± SEM.

nique revealed that there is a time-limited period (initial weeks) during which postlearning NMDA reactivation is required for the consolidation of long-term memory (Shimizu et al., 2000).

We trained a set of naive mice using fear conditioning training on day 1 and then kept one group of FB-KO and littermate controls in their home cages for 2 weeks (naive group). Another group of mice were subjected to 2 weeks of daily enrichment (3 hr/day) (postlearning-enriched group). The retention tests on contextual and cued fear memories in those mice were then conducted on day 15. Consistent with the results obtained for 1 day retention tests (Figures 5E and 5F), naive FB-KO and control mice both exhibited comparable freezing responses in both cued and contextual conditioning in the 2 week retention tests (Figures 7A and 7B). Moreover, postlearning-enriched FB-KO and control mice also exhibited the same level of freezing responses in the retention tests of cued fear memory (Figure 7C). However, in contextual retention tests, the same enriched FB-KO mice showed significantly more freezing responses than did the postlearning-enriched controls ($p < 0.05$; Figure 7D).

Discussion

Important Role of *PS1* in Regulating Adult Neurogenesis

In our current study, we used our previously established region-specific *Cre/loxP* system and generated a mouse model in which *PS1* is selectively deleted in the forebrain regions. In light of the lethality and severe developmental abnormalities of conventional *PS1* knockouts, we were somewhat surprised that the postnatal deletion of *PS1* in the forebrain regions did not lead to any overt impairment in synaptic electrophysiology or basic learning and memory, even at relatively advanced ages.

As was expected from in vitro experiments of *PS1*-deficient cells (Naruse et al., 1998; De Strooper et al., 1999; Xia et al., 1998), we confirmed that loss of PS1 resulted in large accumulations of C-terminal fragments derived from APP and the APP homolog, APLP1, selectively in the hippocampus and the cortex. A recent study by Yu et al. (2001) also showed that conditional *PS1* knockout mice have abnormal APP processing. Unlike the finding by Yu et al. (2001) in which subtle learning deficits were reported in the water maze (e.g., no spatial preference after 5 days of training for both mutant and

control mice), we found that FB-KO mice showed normal performance in the hidden-platform water maze, as measured by the escape latency during training; furthermore, FB-KO mice exhibited strong place preference during the probe test conducted after 6 days of training. The mice used in all our behavioral experiments were at more advanced ages (between 10- and 17-months-old) compared to the younger mice (5- or 8-months old) used in Yu et al. (2001); therefore, our mice should, in theory, be more sensitive for the detection of any potential cognitive impairments. We also did not think that age variations in our animal population could explain the difference between the two studies. The vast majority of animals described in our various behavioral tasks were in fact between 12- and 15-months-old, which is close to the ages of the mice used for BrdU-labeling studies. To confirm our impression that there was no difference in memory performance as a function of age in the mice we used, we conducted further "regression analysis" (see Experimental Procedures) and concluded that the age difference did not contribute to the performance difference. Furthermore, normal basic learning and memory function in our FB-KO mice was further supported by similar findings from our three additional learning tests: novel object recognition, contextual fear conditioning, and cued fear conditioning.

In our present study, we focused on the effect of *PS1* deletion on enrichment-induced adult neurogenesis in the dentate gyrus and on the effect of deficient adult neurogenesis on synaptic transmission, synaptic plasticity, and various aspects of memory processes.

Using BrdU-labeling techniques and quantitative measurements, we repeatedly found a consistent reduction of enrichment-induced neurogenesis in the dentate gyrus in FB-KO mice, indicating the essential role of PS1 activity in activity-dependent neurogenesis in the adult brain. Because of the potential implication of neurogenesis for therapeutic treatments of various neurodegenerative diseases, there is enormous interest in understanding the basic mechanisms underlying the regulation of adult neurogenesis in the brain. Our study now reports a crucial gene that regulates experience-dependent neurogenesis in the adult mammalian brain. Considering the emerging evidence for *PS1* in interacting with multiple intracellular pathways, including the APP and Notch signaling pathways (Handler et al., 2000; Berezovska et al., 2000), it is not clear at this stage how the altered signals from those pathways might play a role in the deficient enrichment-induced neurogenesis in the FB-KO animals. It is also not clear whether abnormal APP processing is directly related to deficient neurogenesis. Further experiments are required to explore these issues in detail.

Disassociation of Dentate Neurogenesis and Hippocampal Learning

In the mammalian brain, the dentate gyrus is the region associated with the highest levels of neurogenesis. However, largely due to the lack of a specific method for perturbing neurogenesis, the functional significance of adult neurogenesis remains speculative (Nowakowski and Hayes, 2000; Kornack and Rakic, 1999; Gould et al., 1999; Greenough et al., 1999). A recent pharmacological study shows that the DNA methylating agent, methylazoxymethanol acetate, which reduces adult dentate neurogenesis, impairs hippocampal learning (Shors et al., 2001). However, because this DNA methylating agent is quite toxic (Kisby et al. 1999; Mehl et al.,2000), it is not clear whether its detrimental effects on learning can be solely attributed to the reduction in adult neurogenesis. Our observation of deficiency in enrichment-dependent neurogenesis in the *PS1* conditional knockout mice now allows us to genetically test the correlation between adult neurogenesis and memory formation. Such "correlation analysis" approaches that do not necessarily give simple answers are powerful methods to associate or, particularly, to disassociate the relationships of complex phenomena in biological systems such as cellular changes and cognitive behaviors (for a review, see Tsien, 2000).

Because there is a significant difference in enrichment-induced neurogenesis, we reasoned that if adult neurogenesis is essential for the formation of new hippocampal memory, we may find that FB-KO mice do not do as well as their littermate controls if both are subjected to prelearning enrichment. Therefore, we designed an enrichment-learning-retrieval paradigm to investigate whether the difference in enrichment-induced neurogenesis between conditional mutants and controls would lead to any observable differences in memory function. Interestingly, in the three different learning tasks, we did not observe any differences in performance between prelearning-enriched FB-KO and control mice. Combining the normal learning and memory performance of naive mouse groups, our behavioral experiments on prelearning-enriched animals, while not exhaustive, appear to suggest a disassociation between adult neurogenesis and learning (in at least three types of learning tasks). In consideration of a widely held view that memory in the mammalian brain is encoded and distributed mainly among the preexisting neurons in the network, our finding may not be entirely surprising, since adult-generated newborn neurons constitute a tiny fraction of total neurons in the network (e.g., 0.0025%–0.03% of dentate granule cells estimated in our 12-month-old mice), thus the addition of new neurons through adult neurogenesis is not essential for learning. In fact, recent works show that memories can be processed and stored in the hippocampal CA1 region (Shimizu et al., 2000) or the amygdala (Repa et al., 2001), sites with no appreciable adult neurogenesis in mice (data not shown) or other mammals (Bayer, 1980; Kordower et al., 1992).

On the other hand, lack of profound plasticity- and learning deficits in FB-KO mice can be argued as a potential failure for reaching the critical threshold or possible genetic compensation by the *presenilin-2* gene (*PS2*), the other member in the presenilin family. For example, although we did not see any statistical differences in LTP responses at any of the test stimulation levels between enriched control and enriched FB-KO mice, we did notice that LTP in the enriched controls appeared to be slightly larger. However, the difference never reached a statistically significant level even after we increased the number of mice to eight mice in each group. This possibility of genetic compensation may exist since the loss of PS2 expression also appears

to interfere with Notch signaling (Steiner et al. 1999). Moreover, when viable conventional *PS2* knockout mice were crossed with conventional *PS1* knockout mice, the double-knockout mice died much earlier (at embryonic day 9.5), showing phenotypes that closely resemble mice with a *Notch 1* deficiency (Donoviel et al., 1999). Therefore, it would be useful to generate conditional *PS1/PS2* double-knockout mice in the future, not only to address the issues of genetic compensation, but also to create more complete phenotypes that should further facilitate data interpretation.

Role of Dentate Neurogenesis in Hippocampal Memory Clearance?

Given the significant deficiency in enrichment-induced neurogenesis in the FB-KO model, we considered an alternative role for adult neurogenesis; adult neurogenesis in the dentate gyrus may represent a powerful mechanism for a clearance process of outdated memory traces in the hippocampus after the memory is transferred and consolidated in the cortex, thus allowing the hippocampal system to be continuously available to process new memories. Our notion is based on three considerations: (1) theoretical works predict that the addition of new neurons into the learned network can result in "interference" of stored information (McClelland et al., 1995); (2) adult-generated neurons in the dentate gyrus are known to rapidly make new synapses into the CA3 region, thus, they should be capable of altering the firing patterns of the neural network (Gaarskjaer, 1986; Hastings and Gould, 1999); (3) these adult-generated new neurons in the rodent dentate gyrus are short-lived, typically with a life span of 3 weeks after their generation (Cameron et al., 1993; Hastings and Gould, 1999), and the 3 week turnover rate of these newborn neurons correlates well with the stability of hippocampal memory before being transferred elsewhere (Kim and Fanselow, 1992; Shimizu et al., 2000).

To test the "neurogenesis-memory clearance" hypothesis, we designed a learning-enrichment-retrieval paradigm for fear conditioning. The paradigm that we used to assess the stability of memory traces in the hippocampus was the contextual fear conditioning test. This task is particularly suitable for the study of the stability of memory traces because learning occurs in a single trial (in seconds), and the hippocampus is known to be a temporary processing and storage site for contextual fear memory for up to 3 weeks after initial learning (Kim and Fanselow, 1992; Shimizu et al., 2000).

Using such a paradigm, we have indeed found that *PS1* FB-KO mice showed significantly more freezing responses (Figure 7D) than the controls did at the 15 day retention test, whereas, in the same groups of FB-KO mice, the tone-elicited fear memories were similar in comparison to control mice (Figure 7C). This observation is consistent with the notion that active neurogenesis in the dentate gyrus of the control mice degraded the memory traces selectively in the hippocampus, but not in the amygdala. Moreover, deficient dentate neurogenesis in FB-KO mice prevented the clearance process of contextual memory traces in the hippocampus, thereby resulting in better retrievals of 2-week-old contextual fear memories.

We considered several other scenarios that may potentially affect the above interpretation. For example, enrichment produces a variety of neurogenesis-independent changes that contribute to the observed phenotype. Specifically, there may be differential changes induced by enrichment in electrophysiological properties and synaptic plasticity in the hippocampal circuits of control and FB-KO mice. Our finding of indistinguishable dentate synaptic transmission and LTP between control and FB-KO mice either before or after enrichment seems to argue against this scenario.

Another potential scenario is that differential changes in the structures of the existing neurons between the control and FB-KO mice were the main reason for producing the difference in contextual memory retrievals under the training-enrichment-retrieval paradigm (Rampon and Tsien, 2000; Rampon et al., 2000b). However, there are two pieces of evidence that do not support this notion. First, if this is the case, we might expect to see some performance differences between prelearning-enriched FB-KO and control mice, which we did not. Second, there was no difference in tone-elicited fear freezing responses between genotypes in the postlearning-enriched animals (Figure 7C), because this type of cued fear memory is sensitive to the enhancement effect of enrichment and was also equally enhanced by prelearning enrichment in both FB-KO and control mice (Figure 6D).

Theoretical Considerations for Dentate Neurogenesis as a Memory Clearance Mechanism

Why should memory traces in the hippocampus be destabilized periodically by adult dentate neurogenesis? The hippocampus is crucial for converting short-term memories into long-term memories (Squire, 1987; Cohen and Eichenbaum, 1993) and can process and temporarily store new memories during this transition period before transferring those labile memories to the cortex for permanent storage. In rodents this transition period is often about 3 weeks, which coincides with the turnover rate (3 weeks) of adult generated neurons in the dentate gyrus. Because the hippocampus has limited storage capacity, such a closely correlated time course makes dentate neurogenesis an attractive mechanism to degrade those temporarily stored memory traces in the hippocampus once the consolidation of cortical memories has taken place, thus preventing the hippocampus from overload and making room for a new round of memory acquisition and processing.

This neurogenesis-memory clearance hypothesis has three major predictive features. First, the neurogenesis-based memory clearance should be a time-dependent process, because continuous production and periodic turnover of newborn neurons predict that neurogenesis-based destabilization of memory traces is a gradual and accumulative process. Second, such a clearance mechanism should be preserved in many mammalian species and should be available throughout the entire adult life. Recent findings that neurogenesis occurs in the dentate gyrus of monkeys and humans, even at old ages, appear to be consistent with our hypothesis (Gould, et al., 1999c; Kornack and Rakic, 1999; Eriksson,

et al. 1998). Third, this clearance process is also use dependent, and levels of neurogenesis should be positively correlated with the amount of experience or memory acquisition. As more memories are formed and processed in the hippocampus, more active neurogenesis is required to meet the demand for removing more old memory traces. Indeed, a series of experiments reports that hippocampal learning, enrichment, or even running exercise (which certainly produces episodic memories) increases neurogenesis in the dentate gyrus (Greenough, et al., 1999).

What, then, is the advantage of choosing dentate gyrus neurogenesis to perform memory clearance in the hippocampus? The dentate gyrus is known to be the first input station within the trisynaptic hippocampal circuits. Adult-generated neurons in the dentate gyrus are known to insert themselves into the granule layers and extend axons into CA3 even during migration, rapidly making new synapses long before they become fully mature (Gaarskjaer, 1986; Hastings and Gould, 1999). It has been estimated that one granule cell can contact a dozen CA3 pyramidal cells, and each CA3 cell then, in turn, contacts at least 40–60 other nearby CA3 pyramidal cells and 20–30 nearby inhibitory cells (Traub and Miles, 1991; Claiborne et al., 1986; Ishizuka et al., 1990). Therefore, such an upstream location for the addition of "transient new neurons" in the dentate gyrus makes it ideal for amplifying the destabilization effect within the entire hippocampus. This may perhaps explain why the dentate gyrus of the hippocampus has continuous, and the most robust, adult neurogenesis in the entire mammalian brain.

In conclusion, by using conditional knockout techniques, we generated forebrain-specific *PS-1* knockout mice. These conditional knockout mice exhibited significant impairments in both APP processing and enrichment-induced neurogenesis in the dentate gyrus. Our study leads us to conclude that *PS1* is crucial for normal APP processing and the regulation of experience-dependent neurogenesis in the adult brain. We also conclude that adult neurogenesis in the mammalian brain is not essential for memory formation and retrieval in several behavioral tasks. Furthermore, our postlearning enrichment experiments lead us to postulate that adult neurogenesis in the dentate gyrus may represent a cellular mechanism for the periodic clearance of outdated memory traces within the hippocampus after cortical memory consolidation, thereby ensuring that the hippocampal system is continuously available to process new memories. It is conceivable that a chronic, abnormal clearance process in the hippocampal system, caused by a *presenilin*-mediated neurogenesis deficiency, may lead to memory disorders in the mammalian brain. Similarly, it is also conceivable that the uncontrolled addition and differentiation of new neurons into existing circuits via transplantation of neuronal stem cells may potentially disrupt, rather than improve, CNS function in human patients.

Experimental Procedures

Production of Conditional PS1 Knockout Mice

We constructed a targeting vector in which two *loxP* sequences were inserted into the *PS1* gene. The first *loxP* sequence, along with *neo* cassette, was inserted in the BglII site positioned 678 bp upstream of the 5′ end of exon 4, while the second *loxP* sequence was inserted in a second BglII site positioned 75 bp downstream of the 3′ end of exon 4 (Figure 1A). Mice heterozygous for the *loxP-PS1* exon 4-*loxP* sequence (called floxed *PS1* or *fPS1*) were mated with B6/CBA hybrid (BCF) wild-type mice, a line that we have used extensively in various transgenic studies (Tang et al., 1999; Shimizu et al., 2000; Rampon and Tsien, 2000; Rampon et al., 2000a, 2000b). Heterozygous (*fPS1*/+) mice and homozygous *fPS1* mice (*fPS1*/*fPS1*) were viable and indistinguishable from wild-type mice (+/+). These *fPS1* mice were then crossed with *Cre* transgenic mice (on BCF hybrid background) to generate FB-KO mice (*fPS1*/*fPS1*; *Cre*/+) and control littermates (*fPS1*/+ or *fPS1*/*fPS1*). Since *fPS1*/+ and *fPS1*/*fPS1* mice exhibited no difference in any of the tests that we measured, data were pooled together. In the study described in this paper, sibling knockout and control mice (>9-months-old) were used for all our experiments.

Genotyping of the floxed *PS1* allele was conducted by both Southern blot and PCR methods. For the Southern blot, a probe outside of the target vector was used (see Figure 1A). For PCR, two primers (5′-CAGACATTAGCACTGTCTGTAAGGAGTC-3′ and 5′-GTTCCTAA ACCTCTAAACTTCCATGAGC-3′) were used to amplify genomic DNA sequences flanking exon 4. After an initial "hot start" at 94°C for 2 min, 35 cycles (94°C for 30 s, 62°C for 30 s, and 72°C for 1 min) were run. The expected PCR product is 645 bp for the wild-type allele and 703 bp for the targeted allele (due to the insertion of the 3′ *loxP* sequence). For easy detection, the PCR products were digested with BglII to produce 446/199 bp bands (wt) and 446/257 bp bands (floxed), respectively (Figure 1C). For genotyping the *Cre* transgene, we used the same PCR method described previously to detect a 490 bp band (Tsien et al., 1996a).

In Situ Hybridization

The overall procedures used for in situ hybridization were similar to the ones previously described (Rampon et al., 2000a). Briefly, an antisense 48-mer oligo probe (5′-CATTAGATATTGGCTCAGGGTTG TCAAGTCTCTGCCTGTCATGCTGCTGCTGCC-3′), which recognizes *PS1* exon 4, was end labeled with ^{35}S-dATP. After being hybridized with the probe (5×10^5 cpm/slide) at 48°C for 16 hr, brain sections (20 μm) were washed in 2× SSC at room temperature (RT), followed by two washes in 0.2× SSC at 60°C and one wash in 0.1× SSC at RT. All washing solutions contained 10 mM DTT.

Western Blot

Brain homogenates were prepared in TNE buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl, 5 mM EDTA) containing 2% SDS and protease inhibitor cocktail (a mixture of AEBSF, pepstatin A, E-64, bestain, leupeptin, and aprotinin; Sigma). Western blot membranes were prepared as previously described (Sisodia et al., 1993; von Koc et al., 1997) and probed with polyclonal antibodies CT15 and CT11 raised against peptides at the carboxyl termini of APP and APLP1, respectively. Bound antibodies were visualized using an enhanced chemiluminescence (ECL) detection system (New England Nuclear, Boston, MA).

Histology and Quantitative Analysis

Animals were anesthetized with tribromoethanol (avertin) and then perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer. The mouse brains were postfixed overnight, followed by transferral to 30% sucrose solution. The brains were mounted on a pedestal using Tissue-Tek O.C.T. compound and cut at 25 μm sections using a cryostat. The sections were stored at −80°C until use.

To assess for cell proliferation, mice were intraperitoneally injected with BrdU (100 mg/kg body weight; Sigma) twice daily (2 hr apart) for 4 consecutive days. The animals were then perfused within 12 hr of the last BrdU injection. BrdU was detected using a monoclonal mouse anti-BrdU antibody (Boerhinger Mannheim, 1:400) in combination with a biotinylated goat anti-mouse IgG and streptavidin as described by Nilsson et al. (1998). The procedures for double labeling are the same as previously described (Liu et al., 1998). Immunoreactive nuclei were counted on a computer monitor to improve visualization and in one focal plane to avoid oversampling.

For fluorescence immunolabeling, tissue was examined first with an Olympus fluorescence microscope (BX-60). Double-labeled cells were then verified using a confocal laser scanning microscope (Zeiss Axiovert 510 LSM).

All BrdU-labeled, BrdU/NeuN, or BrdU/GFAP double-labeled cells in the hilus, subgranular zone (SGZ), granule cell layer (GCL), and molecular layer (ML) were systematically counted in every eighth coronal section covering the complete dentate gyrus. The corresponding sample volumes, the total volume of dentate gyrus, and the absolute numbers of granule cells were determined in serial sections stained with Nissl and/or eosin (which labels nuclei). The measurements were conducted stereologically by a semiautomatic system on an Olympus microscope equipped with Image-Pro software and a video camera. The section thickness of 25 μm (cryostat setting) was used in the dissector. The resulting neuronal density was multiplied by the total volume to assess the total number of dentate granule cells. The SGZ was defined as a two-cell-body-wide zone along the border of the GCL and hilus. The numbers of labeled cells were estimated bilaterally and analyzed using one-way ANOVA followed by Tukey post hoc comparisons. Data was expressed as mean ± SEM.

Hippocampal Electrophysiology

For the study of synaptic physiology, transverse hippocampal slices were prepared from mice between 9 and 13 months of age and of either sex. Hippocampal slices were isolated and maintained as described (Holst et al., 1998). Since disinhibition is typically needed in order to elicit LTP in the dentate gyrus in vitro, we routinely added 0.1 mM picrotoxin to the ACSF during our recordings prior to collecting the data presented here. Addition of picrotoxin did not elicit any obvious changes in the amplitude and time course of the baseline responses, and, hence, the conclusions drawn here regarding baseline i/o relationships and response time course should be valid with respect to MPP neurotransmission either during disinhibition or with inhibition intact.

Electrical stimulation was applied via two pairs of formvar-insulated nichrome microwires (50 μm diameter): one lodged in the middle third of the s. moleculaire to stimulate the medial perforant path (MPP) and the other in the outer third to stimulate the lateral perforant path (LPP) (Figure 4A). Dendritic- and somatic level field potentials were recorded simultaneously using two electrodes: one lodged in the middle third of the s. moleculaire and the other in the granule cell (GC) layer. The data from the s. moleculaire were used to quantify synaptic responses elicited by MPP input, and the data from the GC layer were used in determining GC spike thresholds and the amplitude of responses to LPP input. Many response waveforms in the s. moleculaire began with a presynaptic fiber volley that either preceded or coincided with the beginning of the field EPSP (Figure 4B, arrow).

The standard test cycle consisted of a single stimulation applied to the LPP (the control pathway) followed by the application of a sequence of five stimuli to the MPP, each applied at a different level and 10 s after the former. The stimulus level changes were controlled through the programmed modification of voltage command steps sent to an analog stimulus isolation unit (Model 2200, A-M Systems). Each recording began with a preliminary i/o determination and the selection of a sequence of increasing stimulus levels that would start by eliciting a field EPSP that was between 0.2mV and 0.5mV in amplitude and ended by eliciting a granule cell (GC) population spike. This generally involved an ~2-fold increase in stimulus strength, starting from as low as 6 μA. The strongest stimulus applied in this study was 35 μA. All stimuli were 200 μs in duration. The recording electrodes were glass micropipettes filled with ACSF (2–5 MΩ). The electrode potentials were amplified 1000 times, low-pass filtered at 5 kHz, and then digitized at 10 kHz for online analysis and later review.

MPP LTP was induced by tetanic high-frequency stimulation (HFS), which consisted of three trains of 100 Hz stimulation for 1 s each, and was repeated three times at 20 s intervals. The stimulus level during such trains was the minimal suprathreshold for elicitation of GC spikes during single-shock stimulation. Among the four experimental groups studied with respect to LTP, the mean levels of stimulation applied during HFS differed insignificantly. For the naive control, naive FB-KO, enriched control, and enriched FB-KO groups, these means were 18.1 ± 1.4, 20.1 ± 2.0, 17.4 ± 2.5, and 18.4 ± 1.9 μA respectively. Differences between group means were evaluated using a two-tailed t test with the significance threshold $p < 0.05$. LTP was usually examined in only one or two slices per animal. In the latter case, the data from the two slices of the same animal were averaged prior to the analysis of group statistics. Thus, the sample sizes given in the figures are animal counts. Statistical differences between group means were evaluated using one-way ANOVA (with Ducan's multiple range test for post hoc comparison) and two-tailed t test.

Ages of Mice Used for Behavioral Experiments

The mice used in all behavioral experiments were between 10- and 17-months-old. However, overwhelming numbers of animals described in various behavioral tasks (a total of 64 control and 68 FB-KO mice) were in fact between 12- and 15-months-old, which is close to the ages of the mice used for BrdU-labeling studies. Only several of the mice used were at either end of the age scale (10-, 16-, or 17-month-old). Across all the ages, we did not see any statistical differences in memory performance as a function of age.

For example, in Figure 7, which highlights the memory clearance effect in enriched *PS1* knockout mice, the majority of both naive and enriched control mice as well as naive and enriched knockout mice were about 11- to 14-months-old, and we saw no difference in their performance as a function of age.

We further conducted regression analysis for each of the four groups in both contextual and cued fear memory as the function of age and found no difference within the age groups. The r^2 values are as follows: for 2 week contextual memory: naive control, 0.053; naive FB-KO, 1E-05; enriched control, 0.024; enriched FB-KO, 0.044; and for 2 week cued memory: naive control, 0.32; naive FB-KO, 0.027; enriched control, 0.0013; enriched FB-KO, 0.092. With our sample sizes, r^2 is required to be around 0.6 in order to reach statistical significance. Therefore, we conclude that the age difference did not contribute to the performance difference.

Enrichment Training

One group of adult littermates was kept in standard cages as the naive group, and the other group trained in an enriched environment for 3 hr daily for 2 weeks (enriched group). The enrichment environment was the same as described previously (Rampon et al., 2000a, 2000b; Tang et al., 2001).

Novel Object Recognition Task

Experiments were the same as described previously (Tang et al., 1999). Briefly, mice were handled for 1 week and then individually habituated to the test box for 5 days. During training sessions, two novel objects were placed into the box, and the animal was allowed to explore them for 15 min. During retention tests, one of the familiar objects used during training was replaced by a novel object, and the mouse was allowed to explore freely for 5 min. A preference index, a ratio of the amount of time spent exploring the familiar object or the novel one over the total time spent exploring both objects, was used to measure recognition memory. Data were calculated as mean ± SEM. ANOVA and post hoc Dunnett's test were used to determine genotype effects on the behavioral responses.

Water Maze Tests

Experiments and analysis were the same as described previously (Tang et al., 1999). All mice were accustomed to handling for 1 week before actual training begin (four sessions per day for 6 days). The transfer tests were conducted on day 7. Data were calculated as mean ± SEM. ANOVA and post hoc Dunnett's test were used to determine genotype effects on the behavioral responses.

Contextual and Cued Fear Conditioning Tests

Protocols were the same as those described previously (Tang et al., 1999; Rampon et al., 2000a). The mice were handled for 1 week and then were habituated to the training chamber for 15 min 1 day before the training began. The unconditional stimulus was a single foot shock (0.8 mA for 2 s) paired with an 86 dB sound at 2,800 Hz in the shock chamber (context). Data were calculated as mean ±

SEM. ANOVA and post hoc Dunnett's test were used to determine genotype effects on the behavioral responses.

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