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Hippocampal Spatial Memory Impairments Caused by the Familial Alzheimer's Disease-Linked Presenilin 1 M146V Mutation

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Key Words
Knock-in mouse model · Brain · Water maze task · γ-Secretase · Spatial memory · Hippocampus · Presenilin

Abstract
Mutations in presenilins (PS) 1 and 2 are the major cause of familial Alzheimer's disease. Conditional inactivation of *PS1* in the mouse postnatal forebrain leads to mild deficits in spatial learning and memory, whereas inactivation of both *PS1* and *PS2* results in severe memory and synaptic plasticity impairments, followed by progressive and substantial neurodegeneration. Here we investigate the effect of a familial Alzheimer's disease-linked PS1 missense mutation using knock-in (KI) mice, in which the wild-type *PS1* allele is replaced with the M146V mutant allele. In the Morris water maze task, *PS1* KI mice at 3 months of age exhibit reduced quadrant occupancy and platform crossing in the probe trial after 6 days of training, though their performance was normal in the probe trial after 12 days of training. By the age of 9 months, even after 12 days of training, *PS1* homozygous KI mice still exhibit reduced platform crossing in the post-training probe trial. ELISA analysis revealed a selective increase in cortical levels of β-amyloid 42 in *PS1* KI mice, whereas production of β-amyloid 40 was normal. Histological and quantitative real-time RT-PCR analyses showed normal gross hippocampal morphology and unaltered expression of three genes involved in inflammatory responses in *PS1* KI mice. These results show hippocampal spatial memory impairments caused by the PS1 M146V mutation and age-related deterioration of the memory impairment, suggesting that *PS1* KI mice are a valuable model system for the study of memory loss in AD.

Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly. At the early stages of the disease, memory loss is the cardinal clinical symptom, which at later stages is followed by deficits in multiple cognitive domains, and eventually dementia. Mutations in presenilin 1 (PS1) are a major cause of familial AD (FAD) and lead to an aggressive form of dementia with a very early onset [1]. PS1 is a transmembrane protein required for the γ-secretase activity responsible for the proteolytic cleavage of the amyloid precursor protein (APP) and Notch [2–4]. Using *PS1*-null and neural progenitor cell-restricted *PS1* conditional knockout (cKO) mice, we have previously shown that PS1 plays essential roles in the maintenance of neural progenitor population, neuronal

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differentiation and migration during embryonic development [5–7]. Our recent analysis of postnatal forebrain-restricted *PS1* single cKO and *PS1/2* conditional double KO (cDKO) mice revealed that PS play central roles in synaptic plasticity, learning and memory, and neuronal survival in the adult cerebral cortex [8, 9], and that PS inactivation also leads to age-dependent, progressive inflammatory responses [10].

Based on the central role of PS1 in FAD and in the control of neuronal processes that are relevant to AD pathogenesis, here we investigate the in vivo effect of a FAD-linked PS1 missense mutation using knock-in (KI) mice, in which the exon 5 of the wild-type *PS1* gene is replaced with a mutant form containing the M146V mutation. Hippocampal neurons of *PS1* KI mice display increased vulnerability to excitotoxic necrosis and to β-amyloid (Aβ) toxicity [11, 12]. Aβ-induced cell death in these neurons has been associated with increased superoxide production and caspase activation, suggesting that increased oxidative stress may be involved in the pathogenesis mediated by FAD-linked PS1 mutations [12]. Oligodendrocytes from these mice exhibit enhanced damage induced by glutamate, Aβ42, homocysteine, or the demyelinating agent cuprizone [13, 14]. In addition, inositol triphosphate-evoked Ca^{2+} responses are increased in brain slices from mice of the same line [15]. It has been reported that after cuprizone treatment, spatial learning and memory is impaired in *PS1* KI mice, as compared to wild-type (+/+) mice [13]. In another study, mice with one *PS1* KI and one *PS1*-null allele displayed deficits in associative learning and in adult neurogenesis, compared to *PS1*+/- mice, whereas associative learning in *PS1* KI homozygous (KI/KI) mice was grossly normal [16]. Although these studies have provided important insights into cellular and molecular processes that are affected by the introduced mutation, learning and memory in *PS1* KI mice without additional mutations, and a possible age effect have not been studied, and it is also unclear whether these mice exhibit increases in Aβ production or inflammatory responses, both of which are important features of AD pathology.

In this study, we examined hippocampal spatial learning and memory in *PS1* KI mice using the Morris water maze task and found age-related deterioration of memory impairments. We further examined hippocampal morphology, Aβ generation and the expression of three genes involved in inflammatory reponses in these mice. We found that the M146V mutation results in selectively increased Aβ42 generation, with no apparent effect on inflammation or hippocampal neuronal survival.

Materials and Methods

Mice

The strategy for the generation of *PS1* KI mice and the characterization that they express mutant PS1 at normal levels have been described previously [11, 12]. The loxP-flanked neomycin resistance gene that was integrated into the intron 4 of the *PS1* gene has been deleted by Cre-loxP mediated recombination [16, and pers. commun. therein]. All three genotypic groups used in this study [KI/KI, *PS1* KI heterozygous (KI/+), and +/+] were littermates derived from breedings between *PS1* KI/+ mice. The genetic background of the mice used was C57BL6/129 hybrid.

Morris Water Maze

The water maze is a circular pool 160 cm in diameter. Each mouse was given 4 trials daily for 12 days, with maximum trial duration 90 s. When mice did not find the hidden platform, they were guided to the platform and allowed to stay on it for 15 s. The movement of the mice was monitored using an automated tracking system (HVS Image). Following training days 2, 6, and 12, mice were subjected to a 90-second probe trial in which the platform was removed and the mice were allowed to search for it. In the visible platform version of the task, which was carried out for 5 days with 4 trials per day, extramaze distal cues were removed and the platform was raised above the water level and marked with a red flag. The different genotypic groups were subjected to the task together and the experimenter was unaware of the genotypes. Both male and female mice were used. Significance was tested using two-way ANOVA analysis for group X quadrant interaction, and one-way ANOVA analysis with Scheffe's S test as post hoc for individual comparisons.

Aβ Extraction from Brain Tissue

Cortical tissue was dissected, weighed and homogenized in TBS (50 m*M* Tris, 150 m*M* NaCl, pH 7.4). The homogenates were centrifuged at 100,000 *g* for 20 min. The resulting pellets were resuspended in 5 *M* guanidine, 50 m*M* Tris, pH 8.0 and incubated at room temperature for 10–30 min. The suspension was then diluted 1:10 with loading buffer (10% Block ACE) and centrifuged at 11,000 *g* for 10 min. The supernatant was subjected to Aβ detection.

Sandwich Aβ Enzyme-Linked Immunosorbent Assay

The sandwich Aβ enzyme-linked immunosorbent assay (ELISA) was developed as described [17]. In brief, NUNC Maxisorb immunoassay plates (96 well) were coated with the antibodies 2G3 (anti-Aβ40) or 21F12 (anti-Aβ42) at 0.3 μg/well in PBS overnight at 4°C. Plates were subsequently blocked with Block ACE (Snow Brand Milk Products, 1:4 dilution of original solution) for 2 h at room temperature and washed with PBS-T briefly. The samples were loaded in the wells and incubated overnight at 4°C. After washing with PBS-T, the plates were incubated in a solution of biotinylated 4G8 for 2 h at 4°C. The plates were washed with PBS-T twice, followed by alkaline phosphatase treatment (Streptavidin-conjugated alkaline phosphatase, Amersham, 1:5,000 dilution) for 1.5 h at 4°C. The signal was amplified by adding 100 μl AttoPhos and measured with a Fluoroskan (Perkin Elmer). The detection limits are 3.125 pg/ml for both Aβ40 and Aβ42. The final values were normalized to the loading amount of wet tissue and analyzed for significance using the Student's t test.

Histology

Nissl staining was performed using 12-μm paraffin-embedded sagittal brain sections. Neuronal counts were carried out using the optical dissector technique as described [18], and significance was tested using the Student's t test.

Quantitative Real-Time RT-PCR

Quantitative real-time RT-PCR was performed as described [9]. Briefly, total cortical RNA was treated with DNase I and reverse transcribed in the presence of random hexamers. PCR reactions were performed using SYBR Green PCR mastermix in an ABI PRISM 7700 Sequence Detector (Applied Biosystems). Reactions were performed in duplicates and threshold cycle (Ct) values were normalized to 18S RNA. All procedures were carried out together for *PS1* KI and +/+ in gender-matched pairs. The PCR products were analyzed by electrophoresis to confirm their correct size. Significance was tested using one-way ANOVA analysis with Scheffe's post-hoc S test.

Results

Mild Memory Impairments in PS1 *KI Mice at 3 Months of Age*

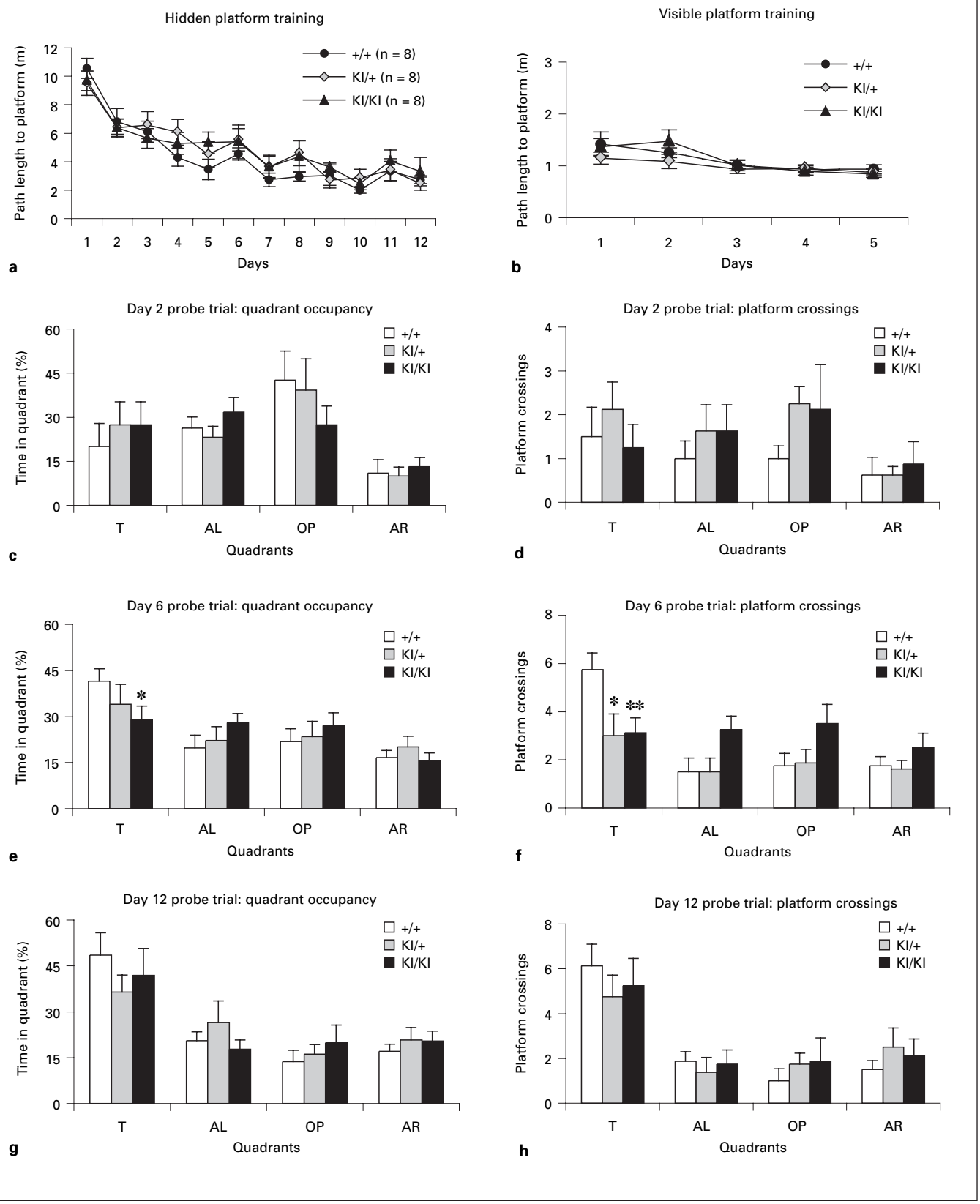
To evaluate spatial learning and memory in *PS1* KI mice, we used the Morris water maze task, a widely used behavioral paradigm for assessing hippocampus-dependent learning and memory. During the acquisition phase of the task, mice were given 4 trials a day for 12 days to learn the position of a hidden escape platform in a circular pool using distal spatial cues. We analyzed three genotypic groups of mice at the age of 3 months, *PS1* KI/KI mice, *PS1* KI/+ littermates, and +/+ littermates. The performance was assessed by the distance traveled to the platform (path length). During the course of training, all three groups improved significantly their performance (day 1 versus day 12, $p < 0.0001$) (fig. 1a).

After training days 2, 6 and 12, we performed probe trials, in which the platform was removed from the pool and the mice were allowed to swim freely for 90 s. The percentage of time they spent in each of the four quadrants of the pool (quadrant occupancy) and the number of times they swam above the former platform position (platform crossings) were used to evaluate the retention of the memory. After 2 days of training, all three genotypic groups did not show a significant preference for the target quadrant (fig. 1c). No significant difference was observed between +/+ mice and each of the mutant groups in the time they spent in the target quadrant (+/+ versus KI/+, $p = 0.49$; +/+ versus KI/KI, $p = 0.49$) (fig. 1c). Crossings of the original platform position were also not significantly different between groups (+/+ versus KI/+, $p = 0.48$; +/+ versus KI/KI, $p = 0.76$) (fig. 1d).

After the 6th day of training, +/+ and KI/+ mice exhibited significantly higher occupancies of the target quadrant relative to the other quadrants (+/+, $p < 0.0001$; KI/+, $p < 0.05$), whereas KI/KI mice failed to show such a preference ($p = 0.22$) (fig. 1e). Comparison of the target quadrant occupancies between the groups showed significantly lower values displayed by KI/KI, as compared to +/+ mice ($p < 0.05$), and no significant difference between KI/+ and +/+ mice ($p = 0.31$) (fig. 1e). Crossings of the original platform position were significantly lower in both mutant groups, as compared to the +/+ group (+/+ versus KI/+, $p < 0.05$; +/+ versus KI/KI, $p < 0.01$) (fig. 1f). These results indicate that KI/KI and, to a lesser extend, KI/+ mice display impairments in spatial memory.

The deficits observed in KI/KI and KI/+ mice appeared to be reversed with further training. After training day 12, all three groups searched for the platform preferentially in the target quadrant (+/+, $p < 0.0001$; KI/+, $p < 0.05$; KI/KI, $p < 0.001$) (fig. 1g). No significant difference was observed between +/+ and KI/+ or KI/KI mice in target quadrant occupancy (+/+ versus KI/+, $p = 0.18$; +/+ versus KI/KI, $p = 0.55$) (fig. 1g) or platform crossings

Fig. 1. Impaired spatial learning and memory in *PS1* KI mice at 3 months of age. *PS1* KI/KI, KI/+, and +/+ mice were subjected to the Morris water maze task. **a** The three groups showed similar path lengths to the hidden platform over 12 days of training (4 trials per day). **b** No significant difference was observed between groups in path length to platform in the visible platform version of the task over 5 days of training (4 trials per day). **c** All three groups did not show a preference for the target quadrant in the training day 2 probe trial. **d** Platform crossings in the training day 2 probe trial were not significantly different across genotypic groups. **e** +/+ and KI/+ mice spent significantly more time in the target quadrant relative to the other quadrants in the training day 6 probe trial [target quadrant (T) versus adjacent left quadrant (AL), opposite quadrant (OP), adjacent right quadrant (AR): +/+, $p < 0.0001$; KI/+, $p < 0.05$], whereas KI/KI mice spent similar amounts of time in all quadrants ($p = 0.22$). Group X quadrant interaction: +/+ versus KI/+, $F(3, 56) = 0.76$, $p = 0.06$; +/+ versus KI/KI, $F(3, 56) = 3.60$, $p = 0.02$. **f** Platform crossings in the training day 6 probe trial were significantly lower in the KI/+ and KI/KI groups, as compared to the +/+ group. Group X quadrant interaction: +/+ versus KI/+, $F(3, 56) = 3.10$, $p = 0.03$; +/+ versus KI/KI, $F(3, 56) = 6.66$, $p = 0.006$. **g** All three genotypic groups swam preferentially in the target quadrant in the training day 12 probe trial (T versus AL, OP, AR: +/+, $p < 0.0001$; KI/+, $p < 0.05$; KI/KI, $p < 0.001$). Group X quadrant interaction: +/+ versus KI/+, $F(3, 56) = 1.60$, $p = 0.20$; +/+ versus KI/KI, $F(3, 56) = 0.72$, $p = 0.54$. **h** No significant difference in platform crossings was found among the three groups in the training day 12 probe trial. Group X quadrant interaction: +/+ versus KI/+, $F(3, 56) = 1.43$, $p = 0.24$; +/+ versus KI/KI, $F(3, 56) = 0.56$, $p = 0.64$. * $p < 0.05$, ** $p < 0.01$ (comparison with +/+).



(+/+ versus KI/+, p = 0.30; +/+ versus KI/KI, p = 0.56) (fig. 1h).

To address the possibility that the poor performance of KI/KI and KI/+ mice in the training day 6 probe trial could be due to poor vision, motivation, and/or sensorimotor abilities, all mice were tested in the visible platform version of the task. We found no significant difference in path length between groups (fig. 1b), indicating that the differences in target quadrant occupancy and platform crossings between the +/+ group and the mutant groups in the training day 6 probe trial reflect specific learning and memory deficits.

More Severe Memory Impairments in PS1 *KI Mice at 9–11 Months of Age*

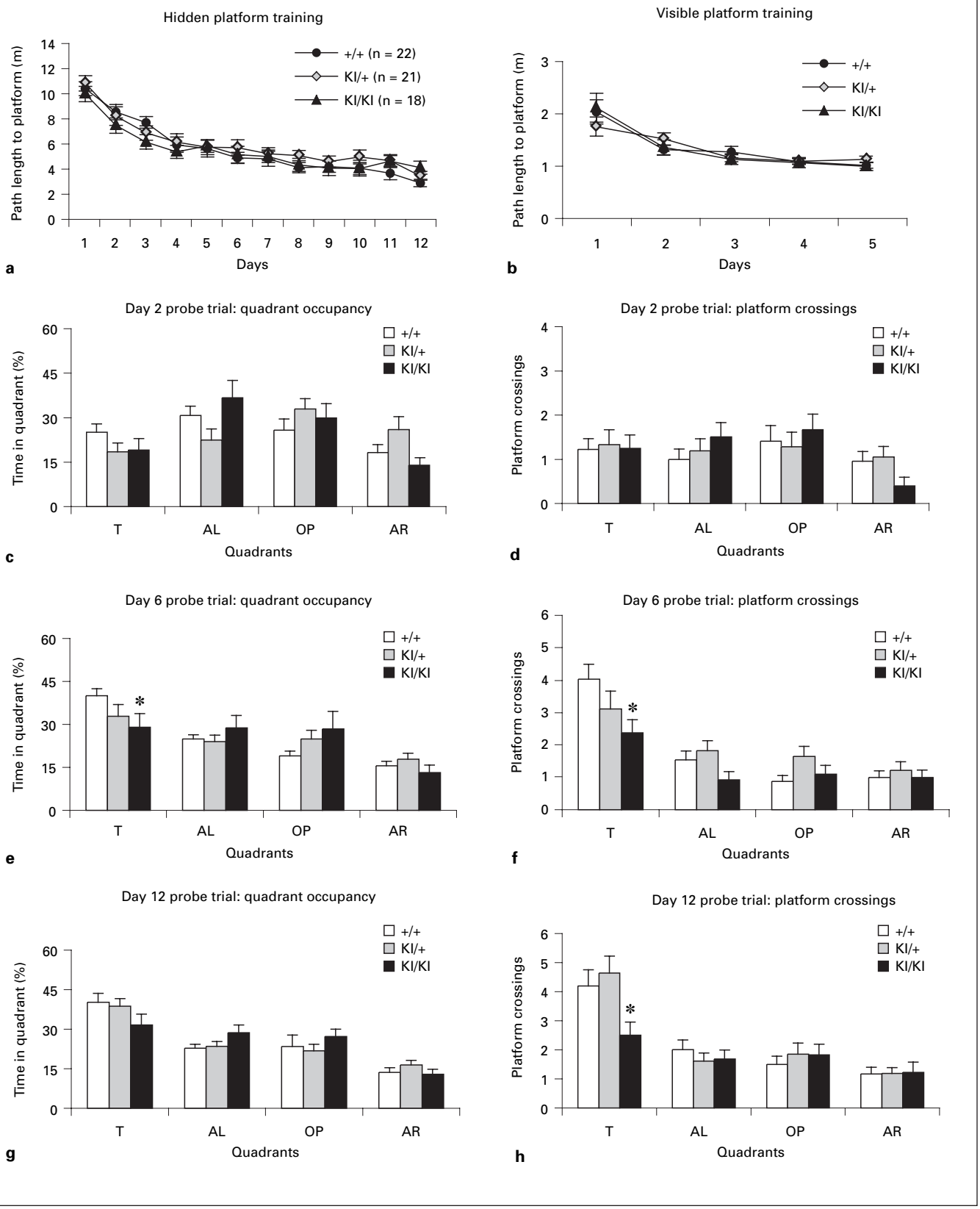
The progressive nature of memory loss in AD prompted us to investigate whether *PS1* KI mice exhibit worse memory as they age. Using the same Morris water maze protocol, we examined spatial learning and memory in all three genotypic groups of naïve mice at 9–11 months of age. We found that all three genotypic groups improved significantly their performance during the acquisition phase of the task, as demonstrated by their decreasing path lengths (day 1 versus day 12, $p < 0.0001$) (fig. 2a).

In the probe trial given after training day 2, all three genotypic groups did not show a significant preference for the target quadrant, indicating that they have not learned the task after 2 days of training (fig. 2c). Target quadrant occupancies were not significantly different between +/+ and KI/+ or KI/KI mice (+/+ versus KI/+, p = 0.14; +/+ versus KI/KI, p = 0.25) (fig. 2c), as well as platform crossings (+/+ versus KI/+, p = 0.80; +/+ versus KI/KI, p = 0.99) (fig. 2d). After 6 days of training, as in the case of mice at 3 months of age, +/+ and KI/+ mice spent significantly more time in the target quadrant relative to the other quadrants (+/+, $p < 0.0001$; KI/+, $p < 0.01$), whereas KI/KI mice failed to show such a preference (p = 0.30) (fig. 2e). Comparison between groups showed significantly lower target quadrant occupancies (fig. 2e) and platform crossings (fig. 2f) in the KI/KI group, compared to the +/+ group ($p < 0.05$, for both parameters). No significant difference was found between the KI/+ and +/+ groups in target quadrant occupancy (p = 0.13) (fig. 2e) or platform crossings (p = 0.29) (fig. 2f). In contrast to mice at the age of 3 months, after training day 12, KI/KI mice still performed worse than +/+ mice. Although all three groups searched for the platform preferentially in the target quadrant (+/+, $p < 0.0001$; KI/+, $p < 0.0001$; KI/KI, $p < 0.05$), KI/KI mice crossed the original position of the platform significantly fewer times than +/+ mice ($p < 0.05$) (fig. 2h). Target quadrant occupancies were also lower in the KI/KI group than in the +/+ group, but not statistically significant (p = 0.10) (fig. 2g). No significant difference was observed between KI/+ and +/+ mice in target quadrant occupancy (p = 0.73) (fig. 2g) or platform crossings (p = 0.59) (fig. 2h). These results indicate that the impairments in spatial learning and memory in KI/KI mice become more severe with age, and at the age of 9–11 months they cannot be compensated for by extended training. In the visible platform version of the task, no significant difference in path length was observed across the genotypic groups (fig. 2b), confirming the learning and memory specificity of the probe trial phenotype.

Selectively Increased Aβ42 in PS1 *KI Mice*

To evaluate whether the introduced mutation in *PS1* KI mice affects Aβ generation, which could be associated with the observed memory deficits, we measured the cortical levels of Aβ40 and Aβ42 in KI/KI, KI/+, and +/+ mice by sandwich ELISA. At both 3 and 9–11 months of age, levels of Aβ40 were similar between the three genotypes (fig. 3). In contrast, Aβ42 was significantly increased in the mutant groups, as compared to +/+ mice (+/+ versus KI/+, $p < 0.05$; +/+ versus KI/KI, $p < 0.01$, at both

Fig. 2. Impaired spatial learning and memory in *PS1* KI mice at 9–11 months of age. **a** Path lengths to the hidden platform in the acquisition phase of the Morris water maze task indicate similar ability across the KI/KI, KI/+ and +/+ groups to learn the position of the platform. **b** Path lengths to platform in the visible platform version of the task show no significant difference between groups. **c** Mice from all genotypic groups did not show a preference for the target quadrant in the training day 2 probe trial. **d** Platform crossings in the training day 2 probe trial were not significantly different between groups. **e** +/+ and KI/+ mice displayed significantly higher occupancies of the target quadrant relative to the other quadrants in the training day 6 probe trial (T versus AL, OP, AR; +/+, $p < 0.0001$; KI/+, $p < 0.01$), whereas KI/KI mice failed to show such a preference (p = 0.30). Group X quadrant interaction: +/+ versus KI/+, $F(3, 164) = 2.48$, p = 0.06; +/+ versus KI/KI, $F(3, 152) = 3.79$, p = 0.01. **f** Platform crossings in the training day 6 probe trial were significantly lower in the KI/KI, as compared to the +/+ group. Group X quadrant interaction: +/+ versus KI/+, $F(3, 164) = 1.89$, p = 0.13; +/+ versus KI/KI, $F(3, 152) = 2.77$, p = 0.04. **g** The three groups of mice searched for the platform preferentially in the target quadrant in the training day 12 probe trial (T versus AL, OP, AR: +/+, $p < 0.0001$; KI/+, $p < 0.0001$; KI/KI, $p < 0.05$). Group X quadrant interaction: +/+ versus KI/+, $F(3, 164) = 0.33$, p = 0.80; +/+ versus KI/KI, $F(3, 152) = 2.32$, p = 0.08. **h** KI/KI mice exhibited significantly lower platform crossings than +/+ mice in the training day 12 probe trial. Group X quadrant interaction: +/+ versus KI/+, $F(3, 164) = 0.50$, p = 0.68; +/+ versus KI/KI, $F(3, 152) = 2.95$, p = 0.03. * $p < 0.05$ (comparison with +/+).



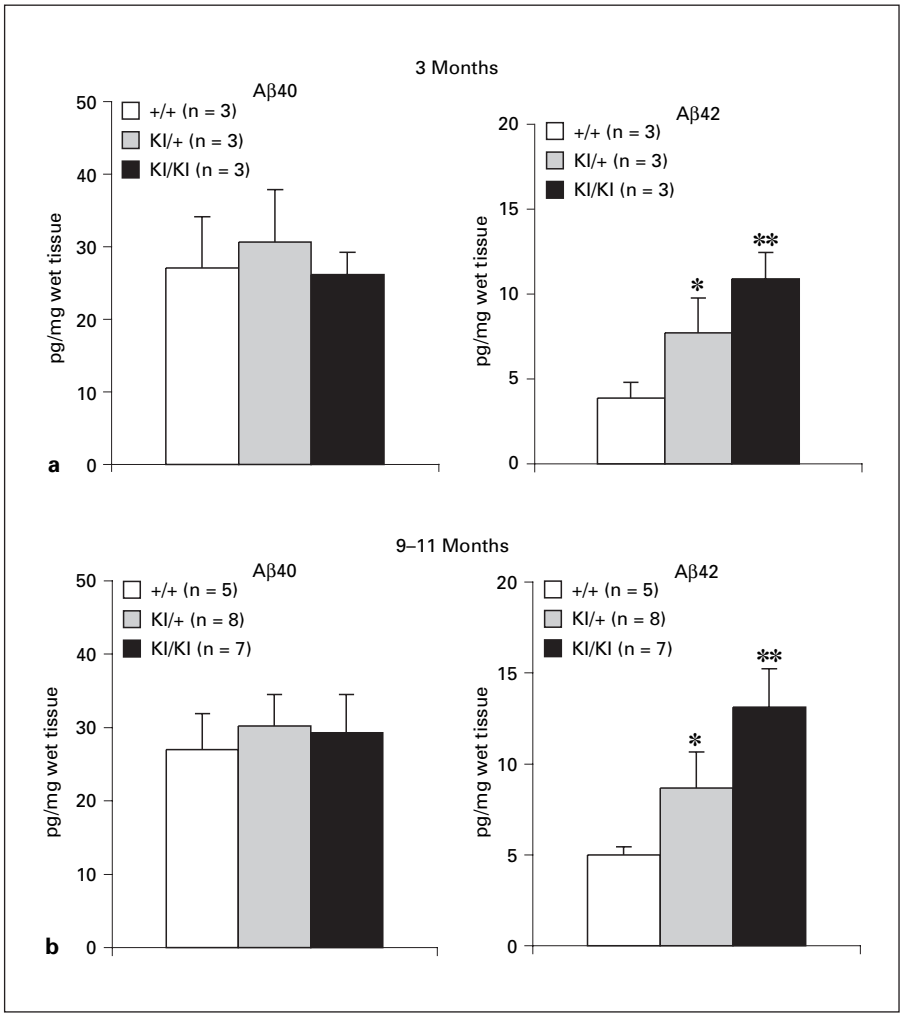


Fig. 3. Selectively increased Aβ42 in *PS1* KI mice. Cortical homogenates from *PS1* KI/KI, KI/+, and +/+ mice at the ages of 3 months (**a**) and 9–11 months (**b**) were analyzed by ELISA for levels of Aβ40 and Aβ42. No difference was observed in Aβ40 levels (left panels). However, Aβ42 was significantly increased in both KI/KI and KI/+ mice, as compared to +/+ mice, in a gene-dose dependent manner, with similar levels at the two ages tested (right panels). * $p < 0.05$, ** $p < 0.01$.

ages) (fig. 3). KI/KI mice exhibited the highest levels of Aβ42 among the three genotypes, whereas Aβ42 levels in KI/+ mice were intermediate comparing to +/+ and KI/KI mice (fig. 3), indicating a dosage effect.

Normal Hippocampal Morphology in PS1 *KI Mice*

We next performed histological studies to address potential morphological abnormalities in the brain of *PS1* KI mice. Because of the hippocampus-dependent nature of the Morris water maze task, we focused our analysis on the morphology of the hippocampus. Nissl-stained sections from +/+, KI/+, and KI/KI mice at the age of 9 months showed similar morphology between the three groups in the hippocampus, as well as in the other parts of the brain (fig. 4a, and data not shown). Stereological analysis of the hippocampal regions CA1 and CA2/CA3 revealed no significant differences in neuronal number or volume across the three genotypes (fig. 4b). These results indicate normal gross hippocampal morphology in *PS1* KI mice.

Normal Expression of Inflammatory Genes in PS1 *KI Mice*

Inflammatory responses are present in pathologically vulnerable regions of the AD brain and are considered to play an important role in the pathogenesis of AD [19]. To evaluate whether impaired learning and memory in *PS1* KI mice may be associated with inflammation pathology, we analyzed the expression levels of three of the most important genes that are involved in AD-related inflammatory responses. We used quantitative real-time RT-PCR to compare the cortical mRNA levels of glial fibrillary acidic protein, cathepsin S, and the α polypeptide of complement component C1q between +/+ and KI/KI mice at 9–10 months of age. Using the same approach, we have

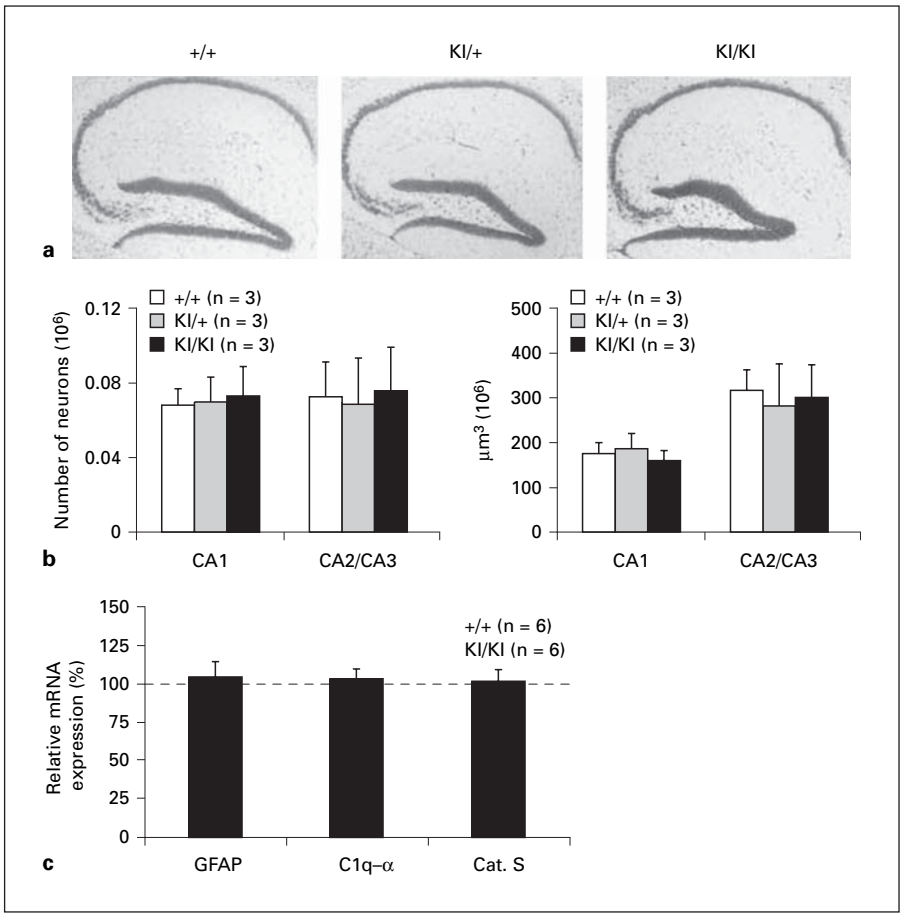


Fig. 4. Normal hippocampal morphology and inflammatory gene expression in *PS1* KI mice. **a** Nissl-stained brain sagittal sections show similar hippocampal morphology in *PS1* KI/KI, KI/+, and +/+ mice at the age of 9 months. **b** Stereological analysis of the hippocampal regions CA1 and CA2/CA3 revealed no significant difference in neuronal number (left panel) or volume (right panel) between the three genotypic groups. **c** Quantitative real-time RT-PCR analysis showed similar cortical mRNA levels between 9- to 10-month-old KI/KI and +/+ mice of glial fibrillary acidic protein (GFAP), cathepsin S, and the α polypeptide of complement component C1q, which are involved in inflammatory responses in AD. The dotted line represents control levels.

previously characterized the upregulation of these genes at the mRNA level in *PS* cDKO mice which develop a widespread inflammatory reaction [10]. In KI/KI mice, we found no significant difference in the expression levels of any of these genes, as compared to +/+ mice (fig. 4c), suggesting that the observed impairments in spatial learning and memory are not caused by increased inflammation.

Discussion

Among types of genetically modified mouse models that are engineered to express a given protein with a point mutation, KI models offer the advantage that the mutant protein is expressed under the control of the endogenous regulatory elements. This facilitates a genotype-phenotype analysis, as the mutant protein is expressed at the normal level with the endogenous spatial and temporal pattern. In this study, we have analyzed a KI mouse model to examine the effect of a FAD-linked PS1 missense mutation in behavioral, morphological and molecular characteristics that are affected in AD.

Our results show that mice carrying the PS1 M146V mutation targeted in the endogenous *PS1* locus exhibit impaired spatial learning and memory. At the age of 3 months, after 6 days (24 trials) of training, *PS1* KI mice (both KI/KI and KI/+ groups) displayed poorer performance in the posttraining probe trial of the water maze task. With 6 more days of training (48 trials in total), their performance improved, suggesting that the mild memory impairment can be overcome by overtraining. By the age of 9–11 months, the reduction in platform crossing exhibited by *PS1* KI/KI mice in the probe trial persisted even after overtraining. The age-dependent nature of this phenotype is especially interesting with regard to the implication of PS1 in the pathogenesis of AD where memory decline is also progressive, and suggests that *PS1* KI mice are a valuable animal model that mimics memory loss in AD.

Several previous studies using genetically modified mice expressing FAD-linked mutant PS1 have been reported. Although no memory deficit was found in the same KI line when they were tested in the Morris water maze task [13], our study is not inconsistent with this prior report. The reason is that in the previous study, using the same intensity of training (4 trials per day), performance in the probe trial was assessed after 3 days of training. In the current study, although in the training day 6 probe trial *PS1* KI mice performed significantly worse than +/+ mice, the probe trial in training day 2 failed to reveal any significant difference between the genotypic groups. In another report, homozygous KI mice from the same KI line at 3 months of age showed a subtle reduction in freezing response in the 4th-min epoch during the memory retention test period (24 h posttraining) in contextual fear conditioning, a paradigm for associative learning and memory, although their overall performance during the entire 5-min testing period was normal [16]. The subtle associative memory deficit found in this study is consistent with the mild spatial memory impairment revealed by our study. In contrast to the KI mice, transgenic mice expressing human M146L or L286V mutant PS1 do not show any significant memory deficits in the Morris water maze task [20]. The discrepancy between the findings from KI versus transgenic mice could be due to the difference in the expression of the mutant PS1. In the KI system that we used here, mutant PS1 is expressed at normal levels and under the control of the endogenous regulatory elements for transcription, translation, and cell specificity. In the transgenic mice, human mutant PS1 was expressed under the control of exogenous promoters and in the presence of endogenous mouse wild-type PS1. Therefore, although the use of different PS1 mutations between the two studies cannot be excluded as a possible cause for the different results, the absence of apparent learning deficits in the transgenic mice may be due to ectopic PS1 expression, the presence of endogenous wild-type PS1, or difference in training protocol of the water maze task.

Several mechanisms could account for the impairments in spatial learning and memory in *PS1* KI mice. One possible explanation is that the introduced mutation compromises the normal function of PS1, resulting in reduced activity of PS. This is supported by the results of genetic studies in *Caenorhabditis elegans* that have ascribed reduced activities to PS homologues bearing FAD-associated mutations [21, 22]. Moreover, a variety of FAD-linked PS mutations confer reductions in the generation of the intracellular domains of Notch and APP [4, 23, 24]. Our previous studies have shown that *PS* cDKO mice, which lack both PS in the postnatal forebrain, display learning and memory deficits, which become more severe with age [9]. Furthermore, *PS1* cKO mice also exhibit hippocampal memory impairments, though the phenotype of *PS1* cKO mice is much more milder than *PS* cDKO mice [8]. The hippocampal memory impairment exhibited by *PS1* KI mice, therefore, can be explained by reduced PS1 activity caused by the M146V mutation.

Generation of Aβ42 is selectively increased in *PS1* KI mice, whereas Aβ40 generation is unaffected. Higher concentrations of Aβ42, which is more amyloidogenic than Aβ40, in the brain of AD patients have been suggested as a pathogenic mechanism of AD. Overexpression of human APP bearing the FAD-linked mutations K670N, M671L (Swedish) or V642I (London) in transgenic mice results in high levels of Aβ40 and Aβ42 and in poor performance in the water maze task [25, 26]. Therefore, increased Aβ42 production in *PS1* KI mice could also explain the observed water maze phenotype.

In a recent report, mice heterozygous for the same *PS1* M146V allele and *PS1*-null allele displayed impaired associative learning and reduced adult neurogenesis in the dentate gyrus, compared to *PS1*+/- mice [16]. These results suggest that reduced adult neurogenesis in these mice may contribute to the memory impairments. We have also found a slight reduction in the number of neurons generated in the adult hippocampus of *PS1* M146V KI/KI mice at 2–3 months of age [unpubl. results]. Therefore, reduced adult neurogenesis associated with the M146V mutation could further contribute to the observed deficits in learning and memory in *PS1* KI mice. Such a mechanism is supported by our previous studies showing an essential role of PS1 in the maintenance of neural progenitor population and the control of the timing of neuronal differentiation [5, 6]. This function of PS1 is mediated primarily through the Notch signaling pathway, since PS1 is required for the proteolytic cleavage of Notch to release the functionally active intracellular domain [3, 4]. Notch signaling is reduced in neural progenitor cells lacking PS1 expression [6], and *PS1*-null and neural progenitor cell-specific *Notch1* cKO mice similarly show premature neuronal differentiation at early neurogenesis phase [6, 27]. Interestingly, it has been recently reported that Notch signaling plays an important role in hippocampal synaptic plasticity [28]. We therefore hypothesize that a possible implication of deficient adult neurogenesis in the water maze phenotype of *PS1* KI mice could be mediated by an alteration in Notch signaling.

In summary, in this study we have found that the FAD-linked PS1 M146V mutation in KI mice causes progressively more severe impairment in spatial learning and memory and increased production of Aβ42. These results suggest that *PS1* KI mice are a valuable model system for the study of memory loss in AD.

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References

1 Hutton M, Hardy J: The presenilins and Alzheimer's disease. Hum Mol Genet 1997;6:1639–1646.

2 De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Figura K, Van Leuven F: Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature 1998;391:387–390.

3 De Strooper B, Annaert W, Cupers P, Saftig P, Craessaerts K, Mumm JS, Schroeter EH, Schrijvers V, Wolfe MS, Ray WJ, Goate A, Kopan R: A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. Nature 1999;398:518–522.

4 Song W, Nadeau P, Yuan M, Yang X, Shen J, Yankner BA: Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. Proc Natl Acad Sci USA 1999;96:6959–6963.

5 Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S: Skeletal and CNS defects in presenilin-1 deficient mice. Cell 1997;89:629–639.

6 Handler M, Yang X, Shen J: Presenilin-1 regulates neuronal differentiation during neurogenesis. Development 2000;127:2593–2606.

7 Wines-Samuelson M, Handler M, Shen J: Role of presenilin-1 in cortical lamination and survival of Cajal-Retzius neurons. Dev Biol 2005;277:332–346.

8 Yu H, Saura CA, Choi S-Y, Sun LD, Yang X, Handler M, Kawarabayashi T, Younkin L, Fedeles B, Wilson MA, Younkin S, Kandel ER, Kirkwood A, Shen J: APP processing and synaptic plasticity in presenilin-1 conditional knockout mice. Neuron 2001;31:713–726.

9 Saura CA, Choi S-Y, Beglopoulos V, Malkani S, Zhang D, Rao BSS, Chattarji S, Kelleher RJIII, Kandel ER, Duff K, Kirkwood A, Shen J: Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. Neuron 2004;42:23–36.

10 Beglopoulos V, Sun X, Saura CA, Lemere CA, Kim RD, Shen J: Reduced β-amyloid production and increased inflammatory responses in presenilin conditional knock-out mice. J Biol Chem 2004;279:46907–46914.

11 Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP: Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. Nat Med 1999;5:101–106.

12 Guo Q, Sebastian L, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP: Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid β-peptide toxicity: central roles of superoxide production and caspase activation. J Neurochem 1999;72:1019–1029.

13 Pak K, Chan SL, Mattson MP: Presenilin-1 mutation sensitizes oligodendrocytes to glutamate and amyloid toxicities, and exacerbates white matter damage and memory impairment in mice. Neuromolecular Med 2003;3:53–64.

14 Pak KJ, Chan SL, Mattson MP: Homocysteine and folate deficiency sensitize oligodendrocytes to the cell death-promoting effects of a presenilin-1 mutation and amyloid β-peptide. Neuromolecular Med 2003;3:119–128.

15 Stutzmann GE, Caccamo A, LaFerla FM, Parker I: Dysregulated IP_3 signaling in cortical neurons of knock-in mice expressing an Alzheimer's-linked mutation in presenilin1 results in exaggerated Ca^{2+} signals and altered membrane excitability. J Neurosci 2004;24:508–513.

16 Wang R, Dineley KT, Sweatt JD, Zheng H: Presenilin 1 familial Alzheimer's disease mutation leads to defective associative learning and impaired adult neurogenesis. Neuroscience 2004;126:305–312.

17 Sun X, Sato S, Murayama O, Murayama M, Park JM, Yamaguchi H, Takashima A: Lithium inhibits amyloid secretion in COS7 cells transfected with amyloid precursor protein C100. Neurosci Lett 2002;321;61–64.

18 Irizarry M, McNamara M, Fedorchak K, Hsiao K, Hyman B: APPSw transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. J Neuropathol Exp Neurol 1997;56:965–973.

19 Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, et al: Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21;383–421.

20 Janus C, D'Amelio S, Amitay O, Chishti MA, Strome R, Fraser P, Carlson GA, Roder JC, St George-Hyslop P, Westaway D: Spatial learning in transgenic mice expressing human presenilin 1 (PS1) transgenes. Neurobiol Aging 2000;21:541–549.

21 Levitan D, Doyle TG, Brousseau D, Lee MK, Thinakaran G, Slunt HH, Sisodia SS, Greenwald I: Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 1996;93:14940–14944.

22 Baumeister R, Leimer U, Zweckbronner I, Jakubek C, Grunberg J, Haass C: Human presenilin-1, but not familial Alzheimer's disease (FAD) mutants, facilitate *Caenorhabditis elegans* notch signalling independently of proteolytic processing. Genes Funct 1997;1:149–159.

23 Moehlmann T, Winkler E, Xia X, Edbauer D, Murrell J, Capell A, Kaether C, Zheng H, Ghett, B, Haass C, Steiner H: Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of their effect on Aβ 42 production. Proc Natl Acad Sci USA 2002;99:8025–8030.

24 Schroeter EH, Ilagan MX, Brunkan AL, Hecimovic S, Li YM, Xu M, Lewis HD, Saxena MT, De Strooper B, Coonrod A, Tomita T, Iwatsubo T, Moore CL, Goate A, Wolfe MS, Shearman M, Kopan R: A presenilin dimer at the core of the γ-secretase enzyme: insights from parallel analysis of Notch 1 and APP proteolysis. Proc Natl Acad Sci USA 2003;100:13075–13080.

25 Hsiao K, Chapman P, Nilsen S, Ekman C, Harigaya Y, Younkin S, Yang F, Cole G: Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996;274:99–102.

26 Moechars D, Dewachter I, Lorent K, Reverse D, Baekelandt V, Naidu A, Tesseur I, Spittaels K, Haute CV, Checler F, Godaux E, Cordell B, Van Leuven F: Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. J Biol Chem 1999;274:6483–6492.

27 Yang X, Klein R, Tian X, Cheng H, Kopan R, Shen J: Notch activation induces apoptosis in neural progenitor cells through a p53-dependent pathway. Dev Biol 2004;269:81–94.

28 Wang Y, Chan SL, Miele L, Yao PJ, Mackes J, Ingram DK, Mattson MP, Furukawa K: Involvement of Notch signaling in hippocampal synaptic plasticity. Proc Natl Acad Sci USA 2004;101:9458–9462.