

# Loss of Spatial Memory, Learning, and Motor Function During Normal Aging Is Accompanied by Changes in Brain Presenilin 1 and 2 Expression Levels

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**Abstract** Mutations in presenilin (PS) proteins cause familial Alzheimer's disease. We herein tested the hypothesis that the expression levels of PS proteins are differentially affected during healthy aging, in the absence of pathological mutations. We used a preclinical model for aging to identify associations between PS expression and quantitative behavioral parameters for spatial memory and learning and motor function. We identified significant changes of PS protein expression in both cerebellum and forebrain that correlated with the performance in behavioral paradigms for motor function and memory and learning. Overall, PS1 levels were decreased, while PS2 levels were increased in aged mice compared with young controls. Our study presents novel evidence for the differential expression of PS proteins in a nongenetic model for aging, resulting in an overall increase of the PS2 to PS1 ratio. Our findings provide a novel mechanistic basis for molecular and functional changes during normal aging.

**Keywords** Aging · Behavior · Neuron · Cerebellum · Cognitive performance · Cognition · Motor learning · Behavior · Neurodegeneration · Calcium · Alzheimer's disease

## Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting more than five million Americans and posing a significant burden on the affected individual, caregivers, and society [1]. While the more common form of sporadic AD is believed to be of multifactorial origin and no single underlying disease-causing mutation has been identified, a number of genetic loci etiological for the rare familial form of the disease (FAD) have been identified [2]. One of the loci is the group of presenilin (PS) proteins, which form the enzymatic core of the  $\gamma$ -secretase complex [3]. Most of the almost 200 identified FAD mutations in PS are located in the gene encoding presenilin 1 (PS1) [4–6], while presenilin 2 (PS2) mutations typically cause later-onset FAD [7–9].

PS mutations have been linked to intracellular  $\text{Ca}^{2+}$  dyshomeostasis [10, 11], and we have recently proposed a novel mechanism of PS-mediated potentiation of the intracellular ryanodine receptor (RyR), which likely underlies this phenomenon [12]. This potentiation occurs via the highly evolutionarily conserved N-terminal region of PS, resulting in differential modulation of the RyR by PS1 and PS2 [13, 12, 14]. The proposed mechanism is in accordance with previous studies identifying elevated  $\text{Ca}^{2+}$  concentrations in the endoplasmic reticulum (ER) during AD [15, 16], and the critical role of RyR in regulating calcium via calcium-induced calcium release (CICR) [17]. Of note, RyR contribute to the pathologic, elevated intracellular  $\text{Ca}^{2+}$  concentrations observed in AD [18–22, 15].

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Intriguingly, similar  $\text{Ca}^{2+}$  dyshomeostasis occurs during healthy aging [23], in the absence of known mutations. This poses the question, whether molecules etiologic for FAD, such as the group of PS proteins, contribute to age-related changes in synaptic signaling and, ultimately, age-related deficits in memory and motor function.

Behavioral impairments associated with aging are generally thought to result from the accumulation of changes in synaptic structure and function at the molecular level, ultimately leading to the loss of biological function [24]. Several rodent behavioral paradigms exist that are routinely used as correlates for spatial learning and memory, motor learning, and motor function [25, 26]. This approach overcomes the difficulties associated with human studies of aging and neurodegenerative disease, where correlating cognitive function with postmortem brain pathology often is the only feasible approach.

Furthermore, it has been well established that there is a significant amount of variability in the extent of functional impairment even among animals of the same chronological age and the same genetic background [27–30]. Thus, rodent models are a useful tool to identify the anatomical, cellular, and molecular substrates underlying age-related cognitive decline [31–36].

Herein, we investigated the expression levels of PS proteins in an established preclinical model for aging and identified a strong association between PS protein expression and quantitative parameters obtained from behavioral paradigms for spatial memory and learning and motor function.

## Materials and Methods

### Animals

The animal experiments described herein were approved by the local Institutional Animal Care and Use Committees. For behavioral assays and quantification of PS expression, ten 6-month-old (6 months) and ten 24-month-old (24 months) male C57BL/6 mice were obtained from the National Institute on Aging. All animals were maintained in the institutional vivarium at ambient temperature ( $23 \pm 1$  °C), under a 12-h light/dark cycle starting at 0600 hours. Mice had ad libitum access to food and water except during the testing sessions.

### Behavioral Assays

**Spatial Learning and Memory** Spatial learning and memory were measured using a swim maze test, as described previously [37]. The performance in the test was assessed using a learning index, describing the relative improvement in test performance (path length) relative to the average performance of the 6 months cohort [37]. Briefly, mice learned the motor

components of swimming and climbing onto the platform, without learning its location in the tank during the pretraining phase. Subsequently, mice were subjected to four acquisition sessions to assess their ability to learn the location of the platform. Each session consisted of five trials during which the mouse had to swim to the platform from a different starting point in the tank.

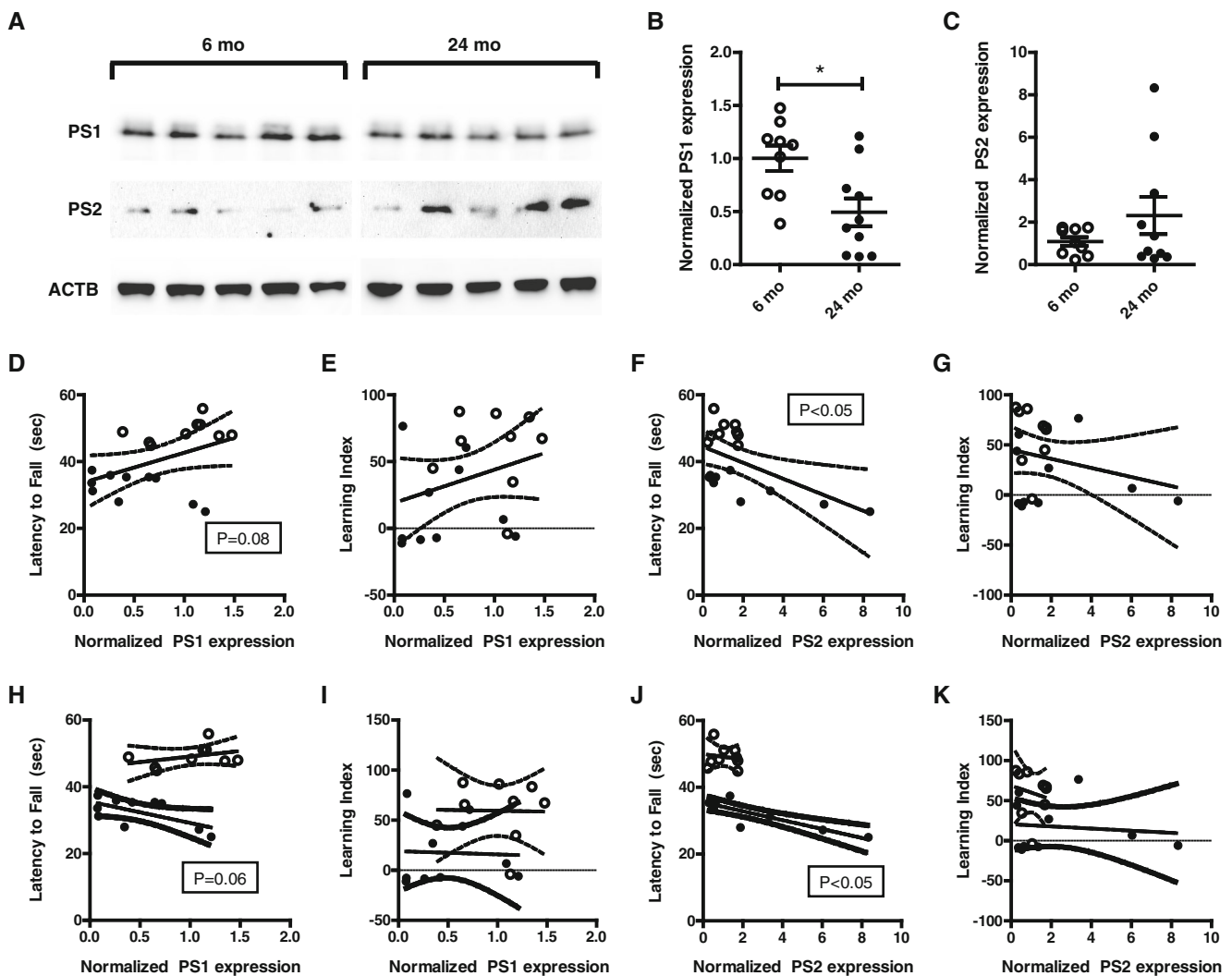
**Bridge Walking** The apparatus and protocol were as described previously [37]. Briefly, latency(s) to fall after being placed on one of four bridges (2 cm square, 2 cm round, 1 cm square, and 1 cm round), mounted 45 cm above a padded surface was measured for each mouse. The maximal latency to fall was set at 60 s. Mice were placed on each of the bridges three times, and the mean latency to fall was used as measure of performance for each bridge.

**Data** The dataset describing the behavior of this cohort of young and aged mice has previously been published by our group [37] (Fig. 1).

### Quantitative Immunoblotting

PS expression levels were quantified using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting from behaviorally characterized animals. Brains were dissected 72 h after the last behavioral testing in order to exclude potential direct effects of behavioral testing on protein expression. Forebrain, cerebellum, and olfactory bulb were separated, and total protein was extracted from frozen brain samples using CytoBuster tissue lysis reagent (EMD Millipore, Billerica, MA) in the presence of protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). We determined the total protein concentration using the method of Lowry using a commercially available kit (BioRad Laboratories, Hercules, CA).

Samples of 25  $\mu\text{g}$  total protein in SDS loading buffer (SDS, 10 %; glycerol, 10 %;  $\beta$ -mercaptoethanol, 1 %; bromophenol blue, 0.004 %; and tris(hydroxymethyl)aminomethane (Tris)-HCl, 0.5 M, pH 6.8) were heated for 10 min at 95 °C. SDS-PAGE was performed by loading samples on 4–12 % bis-tris gels (Life Technologies, Carlsbad, CA) and proteins separated electrophoretically in 3-(N-morpholino) propanesulfonic acid (MOPS) running buffer (Life Technologies, Carlsbad, CA) for 55 min at 200 V. Proteins were subsequently transferred onto nitrocellulose membranes (Pall Life Sciences, Fort Washington, NY) in transfer buffer (25 mM Tris pH 8.6, 192 mM glycine, 0.1 % SDS, and 20 % methanol) for 1 h at 900 mA. Membranes were blocked with 5 % milk, 0.2 % Tween-20 in PBS prior to incubation with primary antibodies (1:1,000, monoclonal rabbit anti-PS1, no. 3622, Cell Signaling, Danvers, MA; 1:500, rabbit anti-PS2, no. P6300-40A, US Biologicals, Salem, MA; 1:1,000, rabbit anti-nicestrin, ab62508,



**Fig. 1** Decreased cerebellar PS1 expression in 24-month-old mice correlates with motor function. **a** Representative immunoblots from five different animals for each group for PS1, PS2, and ACTB in 6- and 24-month-old cerebellum. **b** PS1 expression (normalized to ACTB) is reduced by  $51 \pm 18\%$  in cerebellum of 24 vs. 6 month old mice (open and closed circles, respectively;  $n=10$ ;  $P<0.05$ ). **c** By contrast, PS2 expression is increased by 123 %; however, this difference did not reach statistical significance. **d** We identified a trend toward a positive association between PS1 expression and bridge walking task behavior ( $P<0.08$ ). **e** There was no significant correlation between PS1 expression and performance in the swim maze test. **f** By contrast, PS2 expression was negatively correlated with bridge walking test performance ( $P<0.05$ ), i.e., higher cerebellar PS2 levels were associated with poorer behavioral performance. **g** We did not ascertain any statistically significant association between cerebellar PS2

levels and performance assessed in the swim maze test. **h** We subsequently analyzed 6 and 24 months cohorts separately and correlated the behavior of each cohort with PS expression levels. PS1 expression in 24 months cerebellum showed a trend towards a negative association with performance in the bridge walking test but did not reach statistical significance ( $P=0.06$ ). There was no association between PS1 expression and bridge walking test score in cerebellum of 6 month old mice. **i** There was no association in either cohort between PS1 expression and swim maze test performance. **j** Intriguingly, PS2 expression in the cerebellum of 24 month aged animals was highly negatively correlated with bridge walking test performance ( $P<0.05$ ), while there was no statistically significant correlation in 6 month aged mice. **k** Lastly, cerebellar PS2 expression was not correlated with swim maze test performance when analyzing cohorts separately by age. ( $*P<0.05$ )

AbCam, Cambridge, MA; 1:5,000, rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH), sc-25778, Santa Cruz Biotechnology, Santa Cruz, CA; and 1:10,000, mouse anti- $\beta$ -actin (ACTB), no. MAB1501, EMD Millipore, Billerica, CA) at 4 °C overnight. Membranes were washed three times with 2.5 % milk supplemented with 0.2 % Tween-20. Secondary antibody (1:10,000, donkey anti-rabbit IgG and sheep anti-

mouse IgG, GE Healthcare, Piscataway, NJ) was diluted in antibody incubation solution as above and membranes incubated for 1 h at room temperature. Membranes were again washed three times as above, followed by two washes in PBS supplemented with 0.2 % Tween-20, prior to rinsing in PBS. Immunoreactivity was detected with the Lumina Forte Enhanced Chemoluminescence reagent (EMD Millipore,

Billerica, MA). Membranes were imaged using a G Box Chemi XT imaging system (Synoptics, Oxford, UK).

#### Data and Statistical Analysis

Protein expression levels were quantified using the ImageJ software (NIH) using densitometry, as described previously [37]. Data was normalized against mouse ACTB and mouse GAPDH expression in the same sample, serving as endogenous control.

Prism 5 software (GraphPad Inc., La Jolla, CA) was used to perform statistical analysis. Data was compared using Student's *t* test to assess possible statistical differences between expression levels in young and aged brain. Correlation analysis between protein expression levels and behavioral measurements was performed by calculating a Pearson product-moment correlation coefficient (*r*) to evaluate the strength of the association, essentially as described previously [38, 39].

## Results

### PS are Differentially Expressed in the Cerebellum of Aged Mice

As the cerebellum represents the primary anatomical substrate for motor function, we first quantified PS expression in the cerebellum of aged, 24-month-old, mice and of young, 6-month-old controls, using quantitative immunoblotting. Representative immunoblots are shown in Fig. 1a. PS1 expression was significantly reduced in the aged cerebellum ( $1.00 \pm 0.12$ ,  $n=9$  in 6 months vs.  $0.49 \pm 0.13$ ,  $n=10$  in 24 months;  $P<0.05$ ; Fig. 1b). By contrast, PS2 expression was higher in the aged cerebellum, however, the difference did not reach statistical significance ( $1.09 \pm 0.21$ ,  $n=9$  in 6 months vs.  $2.32 \pm 0.88$ ,  $n=10$  in 24 months;  $P=0.21$ ; Fig. 1c). While quantitative immunoblotting data for all experimental groups passed the D'Agostino and Pearson normality test, the coefficient of variance was greater for aged cerebellum, for both PS1 (36 vs. 84 %) and PS2 (57 vs. 120 %).

Since we measured changes in both PS expression and performance in behavioral outcome measures of motor function and spatial learning, we next tested for associations between PS expression and behavior (Fig. 1d–k; Table 1). With both age groups combined, 6 and 24 months, cerebellar PS1 expression did not correlate significantly with performance in the bridge walking test (assessed as the latency to fall (LTF)), although there was a trend toward a positive association ( $r^2=0.190$ ;  $P=0.08$ ; Fig. 1d). There was no correlation in the swim maze test (assessed by the learning index, LI; Fig. 1e). In contrast, increased cerebellar PS2 levels were weakly negatively associated with LTF ( $r^2=0.295$ ;  $P<0.05$ ). As for PS1, no statistically significant association was found for cerebellar PS2 levels and performance in the swim maze test (Fig. 1g).

Subsequently, we tested for associations in the 6 month and the 24 month groups separately. While there was no association with LTF in the young cohort, the aged cohort showed a trend towards a moderate negative association ( $r^2=0.378$ ;  $P=0.06$ ; Fig. 1h). There was no statistically significant correlation with the LI (Fig. 1i). Cerebellar PS2 expression levels in the aged cohort were highly statistically significantly, strongly negatively associated with bridge walking test performance ( $r^2=0.745$ ;  $P<0.01$ ; Fig. 1j) while no significant association was detected for the young cohort. Neither cohort showed any significant association with performance in the swim maze test (Fig. 1k).

### PS Are Differentially Expressed in the Forebrain of Aged Mice

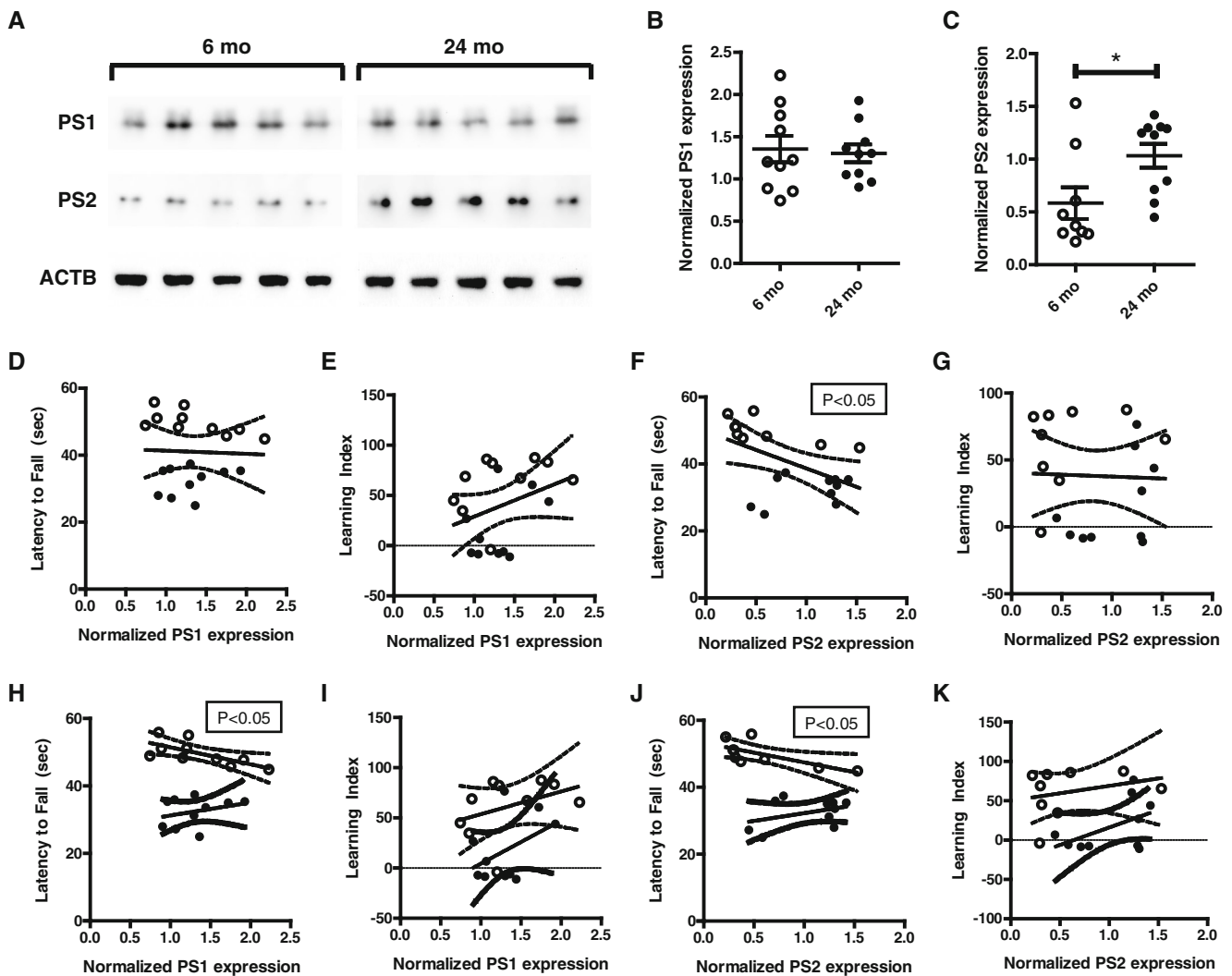
Subsequently, we focused on CNS regions other than the cerebellum that contribute to the generation of motor function and spatial learning and memory and determined PS1 and PS2 expression levels in the forebrain. Representative immunoblots are shown in Fig. 2a. Forebrain PS1 expression was similar between 6 and 24 months mice ( $1.35 \pm 0.16$  vs.  $1.30 \pm 0.10$ ,  $n=10$ ;  $P=0.79$ ; Fig. 2b). By contrast, PS2 expression was significantly increased in the forebrain of aged mice ( $0.58 \pm 0.15$ ,  $n=9$  vs.  $1.03 \pm 0.11$ ,  $n=10$ ;  $P<0.05$ ; Fig. 2c). Expression data was normally distributed, as assessed by the D'Agostino and

**Table 1** Statistical parameters of correlations of cerebellar PS expression with measurements of behavioral paradigms for spatial memory and learning and motor function

	Swim maze test (LI)			Bridge walking test (LTF)		
	6 months	24 months	Combined	6 months	24 months	Combined
PS1						
$r^2$	0.000	0.002	0.090	0.135	0.378	0.190
<i>P</i>	0.96	0.91	0.20	0.33	0.06	0.08
PS2						
$r^2$	0.032	0.013	0.066	0.021	0.745	0.295
<i>P</i>	0.65	0.75	0.29	0.71	<0.01	<0.05

*P* and  $r^2$  values are given for the Pearson product-moment correlation coefficient calculation

*LI* learning index, *LTF* latency to fall



**Fig. 2** Increased forebrain expression of PS2 in 24 months mice correlates with age-related loss of motor function. **a** Representative immunoblots from five different animals for each group showing PS1, PS2, and ACTB expression in the forebrain of 6 and 24 months mice. **b** PS1 expression in the forebrain is similar between 6 and 24 months mice. **c** By contrast, PS2 expression was statistically significant higher in 24 months compared with 6 months mice ( $n=10$ ;  $P<0.05$ ). **d** Correlation analysis did not reveal any statistically significant association between PS1 forebrain expression and bridge walking test performance. **e** Similarly, PS1 expression and learning index did not correlate in the forebrain of mice. **f** By contrast, forebrain PS2 expression was negatively associated with bridge walking test performance ( $n=10$ ;  $P<0.05$ ). **g** There was

no correlation between forebrain PS2 expression and performance in the swim maze test. **h** When analyzing young and old cohorts separately, we identified a negative association between performance in the bridge walking test in 6 months mice ( $n=10$ ;  $P<0.05$ ) that was lost in 24 months mice. **i** There was no association for either cohort with the learning index as a paradigm for spatial learning and memory. **j** Expression levels in the forebrain of 6 months mice showed a negative association with bridge walking test performance ( $n=10$ ;  $P<0.05$ ), which was absent in the 24 months cohort. **k** There was no association in either cohort between forebrain PS2 expression and performance in the swim maze test. (\* $P<0.05$ )

Pearson normality test. The coefficient of variance was similar for all experimental groups, ranging from 25 to 48 %.

We did not identify any statistically significant association between PS1 levels with behavioral performance in either the bridge walking or the swim maze test (Fig. 2d, e; Table 2). By contrast, forebrain PS2 expression was weakly negatively associated with LTF ( $r^2=0.265$ ;  $P<0.05$ ; Fig. 2f); there was no statistically significant correlation with the LI as a correlate for swim maze behavior (Fig. 2g).

Subsequent subgroup analysis revealed a moderate negative association of forebrain PS1 expression with LTF in the brain of 6 months mice ( $r^2=0.476$ ;  $P<0.05$ ; Fig. 2h), which was absent in the 24-month-old forebrain ( $r^2=0.088$ ;  $P=0.41$ ; Fig. 2h). We did not identify any significant correlations between forebrain PS1 expression and performance in the swim maze test (Fig. 2i). Similarly, PS2 expression was associated moderately, negatively with LTF in 6 months forebrain ( $r^2=0.486$ ;  $P<0.05$ ; Fig. 2j) but not in 24 months forebrain

**Table 2** Statistical parameters of correlations of forebrain PS expression with measurements of behavioral paradigms for spatial memory and learning and motor function

	Swim maze test (LI)			Bridge walking test (LTF)		
	6 months	24 months	Combined	6 months	24 months	Combined
PS1						
$r^2$	0.148	0.191	0.124	0.476	0.088	0.020
$P$	0.28	0.21	0.13	<0.05	0.41	0.87
PS2						
$r^2$	0.077	0.238	0.001	0.486	0.150	0.265
$P$	0.47	0.15	0.89	<0.05	0.27	<0.05

$P$  and  $r^2$  values are given for the Pearson product–moment correlation coefficient calculation

LI learning index, LTF latency to fall

( $r^2=0.150$ ;  $P=0.27$ ; Fig. 2j). PS2 expression was not correlated with the LI in our subgroup analysis (Fig. 2k).

### Regional Differences in Nicastrin Expression in the Aged Brain

While PS function as associated proteins of ion channels and thereby regulate intracellular calcium signaling, they are also part of the  $\gamma$ -secretase complex controlling the posttranslational processing of transmembrane proteins. In order to determine if the age-related changes in PS expression levels could be attributed to changes in  $\gamma$ -secretase concentration, we measured the concentration of nicastrin, one of the four constitutive components of the complex.

In the cerebellum, nicastrin levels were reduced by 57 % overall; however, this reduction did not reach statistical significance ( $n=10$ ,  $P=0.13$ ; Supplemental (Suppl.) Fig. 1a, b). Nicastrin expression showed a trend toward a weak association with performance in the bridge walking test ( $r^2=0.171$ ;  $P=0.08$ ; Suppl. Fig. 1d). Intriguingly, when performing subgroup analysis, we found that nicastrin expression is not correlated with LTF as an outcome measure for motor coordination in the bridge walking test ( $r^2=0.018$ ;  $P=0.73$ ; Suppl. Fig. 1e). By contrast, we found a statistically significant, strong positive association between nicastrin expression and performance in the bridge walking test in the aged cerebellum ( $r^2=0.422$ ;  $P<0.05$ ; Suppl. Fig. 1e). While our combined analysis revealed a trend toward a weak positive association between nicastrin expression and performance in the swim maze test ( $r^2=0.164$ ;  $P=0.08$ ; Suppl. Fig. 1f), there were no statistically significant associations when analyzing young and aged mice separately (Suppl. Fig. 1g). However, young mice showed a trend toward a strong positive association between cerebellar nicastrin levels and the LI ( $r^2=0.363$ ;  $P=0.08$ ; Suppl. Fig. 1g); this trend was completely absent in the old cohort ( $r^2=0.030$ ;  $P=0.63$ ; Suppl. Fig. 1g).

By contrast, nicastrin expression in the forebrain of aged mice was increased, compared with the young group although this difference was not statistically significant ( $n=10$ ;  $P=0.11$ ; Suppl. Fig. 2a, b). Forebrain nicastrin levels were statistically

significantly and moderately negatively associated with performance in the bridge walking test ( $r^2=0.244$ ;  $P<0.05$ ; Suppl. Fig. 2c). Of note, neither the young nor the aged mice alone showed a correlation between nicastrin expression in the forebrain and LTF in the bridge walking test that reached statistical significance (Suppl. Fig. 2d). There was no statistically significant or biologically relevant association between forebrain nicastrin levels and swim maze test performance (Suppl. Fig. 2f, g).

### Discussion

Herein, we present evidence for the differential expression of PS proteins in a nongenetic model for aging. Both in cerebellum and forebrain, PS expression levels are differentially affected in aging, correlating strongly with deficits in motor function and overall increasing the PS2 to PS1 ratio. These findings provide a novel mechanistic basis for a loss in brain function, particularly with respect to spatial memory, learning, and motor function during normal aging, given our previous studies that had identified the isotype-specific, differential control of brain RyR by PS at the molecular level. The present data resulting from behavioral in vivo studies significantly advance this mechanistic concept toward identifying the change in the PS2 to PS1 ratio during brain aging and the resulting changes in intracellular  $Ca^{2+}$  signaling as causative for the age-related decline in brain function.

### PS Expression in the Aged Brain

Most studies on PS expression have focused either on developmental expression patterns [40] or on the differential expression in preclinical models for or clinical samples of AD [41–43]. By contrast, our study was based on a preliminary report describing reduced PS1 levels and increased PS2 RNA and protein levels in the cortex of 15 months compared with 6 months mice [44]. However, this study did not address any regional expression differences or functional/behavioral consequences of the change in PS expression, and there is no

published report on the expression of PS protein in senescent mice older than 15 months. Furthermore, PS expression is directly associated with the expression levels of acetylcholinesterase [45]. Given the well-documented age-related changes of the cholinergic system [46], differential changes in PS expression levels during aging are likely to occur. We, therefore, quantified PS expression in forebrain and cerebellum of young (6 months) and aged (24 months) C57BL/6 mice. These animals were behaviorally characterized using established experimental paradigms to determine age-related deficits in spatial learning and memory and motor function. We have previously shown that correlating regional protein expression levels with functional data obtained from behavioral testing represents a powerful tool to study the function of proteins controlling neuronal signaling during normal aging and age-related disease of the CNS [39, 37].

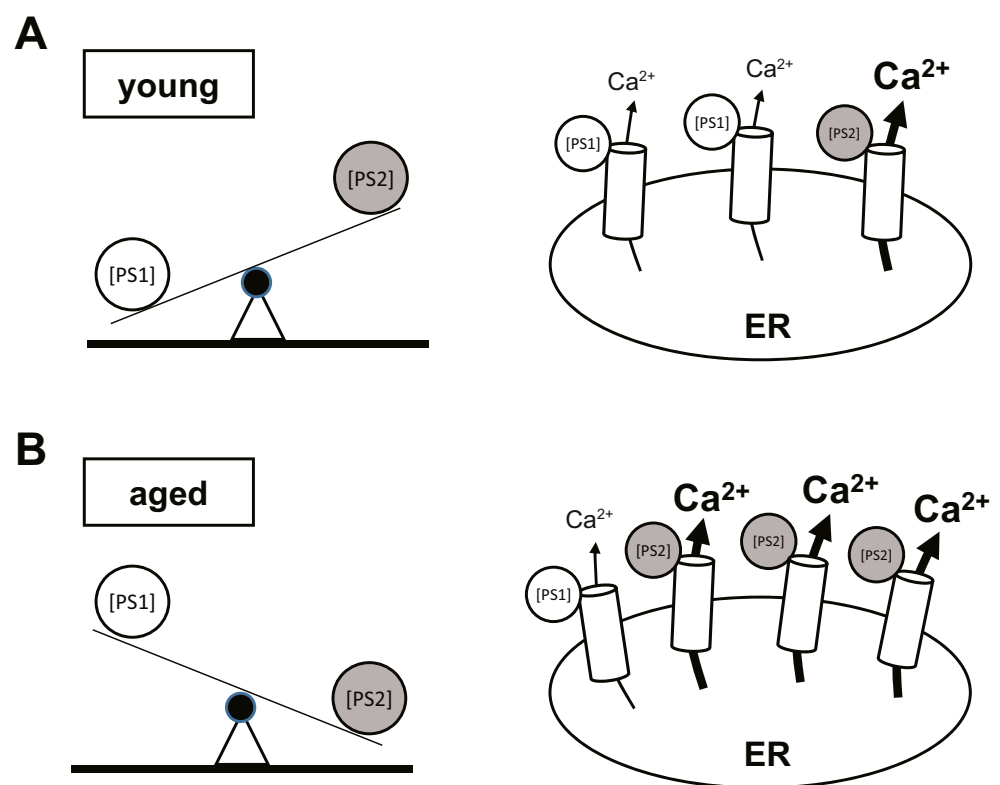
#### Functional and Behavioral Measures of Neuronal and Synaptic Aging Correlate with Changes in PS Protein Expression

To our knowledge, the present study is the first to describe a correlation between PS expression levels and the behavioral consequences of synaptic dysfunction in a nongenetic model for aging. In order to quantify behavioral phenotypes, we chose paradigms for spatial learning and motor function,

which we tested in our two cohorts of mice, i.e., 6 and 24 months. The swim maze test and the bridge walking test are well-established behavioral testing paradigms for the assessment of learning and motor function in mice [28, 47]. While cortex and hippocampus are the two primary brain regions involved in spatial learning and memory and determinant for swim maze test performance of rodents [28, 47], motor function as required in the bridge walking test are critically dependent on cortical and cerebellar networks [48, 49]. Given the complex motor control by both cortical and cerebellar structures, changes in synaptic protein expression levels in the forebrain are likely to affect both of these systems, as described by us previously [37].

In order to study the possible correlations between PS isoform expression and performance in behavioral paradigms, we have not performed an outlier analysis, despite the variation seen in, e.g., forebrain expression of PS isoforms (Fig. 2c). Each sample was tested on immunoblots multiple times, and the protein concentrations were validated prior to each experiment. Furthermore, there was no difference in the expression levels of the endogenous control, mouse  $\beta$ -actin (data not shown). We, therefore, can exclude technical problems as a reason for the observed variation. Rather, we hypothesize that individual differences in PS protein levels, and synaptic proteins in general, are responsible for subsequent age-related impairments in learning and motor function. This hypothesis is corroborated by the strong associations of PS2

**Fig. 3** Mechanistic diagram summarizing the effects of aging on PS expression. In the young brain, RyR receptors are predominantly modulated by PS1. By contrast, there is significant modulation of RyR by PS2 in the aged brain, resulting in increased intracellular  $\text{Ca}^{2+}$  release [12]. The cellular environment during aging, characterized by increased levels of oxidative stress and mild synaptic dysfunction, favors PS2 binding to the RyR. Targeting the PS/RyR interaction may represent a feasible strategy for pharmaceutical intervention for age-related synaptic dyshomeostasis



expression in the forebrain with performance in the bridge walking test (Fig. 2f, j).

### Potential Mechanisms of PS-Mediated Loss of Brain Function During Aging

PS perform several important functions in neuronal physiology and cellular signaling. Most commonly, PS form part of the  $\gamma$ -secretase complex, which controls the posttranslational proteolytic processing of single-pass transmembrane proteins including amyloid precursor protein (APP), and thereby is critical for cellular health and development [50]. Mutations in the encoding PS genes result in a change of cleavage products, most importantly an increase of  $\beta$ -amyloid<sub>1–42</sub> (A $\beta$ <sub>42</sub>) peptide, resulting in FAD pathology [51, 52]. Additionally, it has been proposed that PS act as ER-localized Ca<sup>2+</sup> leak channels [53]; however, this hypothesis remains controversial and recent evidence strongly points against this function [54]. We have identified a novel function of PS, i.e., the direct modulation of intracellular RyR through the evolutionarily conserved N-termini of both PS1 and PS2 [13, 14, 12]. RyR are the molecular substrate of calcium-induced calcium release, a primary mechanism of enhancing neuronal calcium signals, and abnormal calcium homeostasis has been hypothesized as key causative mechanism of neuronal aging [55, 56, 21]. We here report changes in PS expression in the aged forebrain and cerebellum that result in a lower PS1 to PS2 ratio, thereby increasing intracellular Ca<sup>2+</sup> release by RyRs in an isotype-specific manner (Fig. 3) [12]. Elevated RyR-mediated intracellular Ca<sup>2+</sup> release has been described in models of AD [18–22, 15], as well as during healthy aging in the absence of known mutations [23], thus directly implicating PS proteins as potentially causative pathologic modulators of Ca<sup>2+</sup> signaling in these physiological states. Given this evidence, it is likely that differential modulation of RyR-mediated intracellular calcium signaling and homeostasis by PS [13, 14, 12] and the changes in the molecular makeup of these protein–protein interactions underlies or contributes to age-related deficits in motor function and learning. Other physiological functions of PS, most notably their role as a core component of the  $\gamma$ -secretase complex, may, however, contribute in parallel to the involvement of PS in normal brain aging and age-related disorders of the CNS. Notably, PS expression is affected by sex, steroid hormones, and cellular levels of oxidative stress, which are increased during neurodegenerative diseases and aging [57, 58, 44, 59, 45]. Little is known, however, regarding the expression of PS during aging or correlation with performance in behavioral paradigms of the other subunits of the  $\gamma$ -secretase complex. Expression of nicastrin in the developing and adult rat brain with PS1 expression in both cell bodies and dendrites in all brain regions [60]. Conditional inactivation of nicastrin in the forebrain has been shown to cause progressive memory

impairment and age-related neurodegeneration [61] and to restrict amyloid deposition in an AD mouse model [62]. In the present study, we identified a differential expression pattern of nicastrin during aging.

### Conclusion

This is the first study correlating cerebellar and forebrain expression levels of the group of PS proteins with performance in behavioral paradigms in a nongenetic model for aging. In both structures, we identified changes in PS expression that lower the PS1/PS2 ratio, likely resulting in increased intracellular Ca<sup>2+</sup> release under oxidative conditions that favor PS2 binding. Based on our observations, it is likely that PS proteins contribute to both synaptic dysfunction and loss of motor function during “healthy” aging. Thus, PS are a potential drug target not only for neurodegenerative diseases with a genetic PS involvement but also for age-related motor deficits. Of therapeutic relevance, targeting PS may overcome the poor outcomes associated with targeting the cholinergic system, which have yielded only short-term effects on PS1 [45].

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