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Dendritic Spine Abnormalities in Hippocampal CA1 Pyramidal Neurons Underlying Memory Deficits in the SAMP8 Mouse Model of Alzheimer's Disease

Jaume del Valle[a,b], Sergi Bayod[a,b], Antoni Camins[a,b], Carlos Beas-Zárate[c,d], Dulce A. Velázquez-Zamora[c,d], Ignacio González-Burgos[c,d] and Merce Pallàs[a,b,*]
[a]*Facultat de Farmàcia, Unitat de Farmacologia i Farmacognòsia, Institut de Biomedicina (IBUB), Universitat de Barcelona, Nucli Universitari de Pedralbes, Barcelona, Spain*
[b]*Centros de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain*
[c]*Centro de Investigación Biomédica de Occidente, IMSS, División de Neurociencias, Guadalajara, Jal., México*
[d]*Departamento de Biología Celular y Molecular, CUCBA, Universidad de Guadalajara, Guadalajara, Jal., México*

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Abstract. SAMP8 is a strain of mice with accelerated senescence. These mice have recently been the focus of attention as they show several alterations that have also been described in Alzheimer's disease (AD) patients. The number of dendritic spines, spine plasticity, and morphology are basic to memory formation. In AD, the density of dendritic spines is severely decreased. We studied memory alterations using the object recognition test. We measured levels of synaptophysin as a marker of neurotransmission and used Golgi staining to quantify and characterize the number and morphology of dendritic spines in SAMP8 mice and in SAMR1 as control animals. While there were no memory differences at 3 months of age, the memory of both 6- and 9-month-old SAMP8 mice was impaired in comparison with age-matched SAMR1 mice or young SAMP8 mice. In addition, synaptophysin levels were not altered in young SAMP8 animals, but SAMP8 aged 6 and 9 months had less synaptophysin than SAMR1 controls and also less than 3-month-old SAMP8 mice. Moreover, while spine density remained stable with age in SAMR1 mice, the number of spines started to decrease in SAMP8 animals at 6 months, only to get worse at 9 months. Our results show that from 6 months onwards SAMP8 mice show impaired memory. This age coincides with that at which the levels of synaptophysin and spine density decrease. Thus, we conclude that together with other studies that describe several alterations at similar ages, SAMP8 mice are a very suitable model for studying AD.

Keywords: Alzheimer's disease, dendrites, learning, memory, object recognition test, SAMP8, senescence, synaptophysin

INTRODUCTION

SAMP8 mice are one of the senescence-accelerated strains of mice [1] that have a reduced lifespan and share several characteristics with aged humans such as lordosis, loss of hair, and reduced physical activity [2, 3]. Studies have also shown that SAMP8 mice present some characteristic neurodegenerative alterations, such as spongy degeneration [4], neuronal cell loss [5], and gliosis [6]. The changes in SAMP8 have been reviewed recently [7], and this strain has been described as a neurodegeneration model.

Furthermore, SAMP8 have been considered to be a sound model for investigating the pathophysiology of the early events in Alzheimer's disease (AD) [8].

*Correspondence to: Mercè Pallàs, PhD, Facultat de Farmàcia, Unitat de Farmacologia i Farmacognòsia, Universitat de Barcelona, Nucli Universitari de Pedralbes, Barcelona E-08028, Spain. E-mail: pallas@ub.edu.

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In fact, these mice have several characteristics that are also seen in AD patients, such as alterations in learning and memory [9, 10], amyloid-β accumulation [11], an increase in hyperphosphorylated tau [12], cerebral amyloid angiopathy [13], brain blood barrier alterations [14, 15], increased oxidative stress in the brain [16], alteration of the cholinergic system [17], a decreased release of neurotransmitters [18, 19], altered emotions and abnormal circadian rhythm [20], and different hippocampal protein aggregates [21]. In fact, due to the early amyloid accumulation in the brain and the spontaneous onset of AD, SAMP8 has also been proposed as an excellent model of late-onset AD [11].

Dendritic spines are tiny protrusions along dendrites that constitute major postsynaptic sites for excitatory synaptic transmission. These spines are highly mobile and can undergo remodeling, even in the adult nervous system. Spine remodeling and the formation of new synapses are activity-dependent processes that provide a basis for memory formation [22, 23]. A loss or alteration of these structures has been described in patients with neurodegenerative disorders such as AD, and in mouse models for these disorders [24]. As synapse loss is strongly correlated with cognitive impairment in AD [25], synaptic damage and loss are factors that affect the degree of dementia experienced in AD patients [26].

The aim of this study was to investigate the density and types of dendritic spines in this model of AD pathology, the types of dendritic spine that is most affected, and its putative influence on memory processes using the object recognition test (ORT) paradigm.

MATERIAL AND METHODS

Animals

Twelve to fourteen male 3-, 6-, and 9-month-old SAMR1 and SAMP8 mice were housed in the University of Barcelona facility under controlled temperature and light conditions (21–24°C, 12-h light/12-h dark cycle). Sentinels from the facility were tested regularly to ensure our facility is virus- and pathogen-free. Six animals from each group were used for Golgi staining and spine studies, and six to eight animals were used in the ORT and the posterior western blots. The care and use of these animals were carried out in accordance with the policy on the use of animals in neuroscience research, published by the Society for Neuroscience. The experimental protocol was approved by the University of Barcelona's Ethics Committee and complied with the 'Principles of laboratory animal care' and the European Communities Council Directive (86/609/EEC).

Object recognition test

The test was conducted in a 90° two arm, 25 cm long, 20 cm high maze. The light intensity in the middle of the field was 30 lux. The objects to be discriminated were plastic figures (object A: 5.25 cm high, object B: 4.75 cm high). First, mice were individually habituated to the apparatus for 10 min for three days. O the fourth day, they were submitted to a 10 min acquisition trial (first trial) during which they were placed in the maze in the presence of two identical novel objects (A + A or B + B) placed at the end of each arm. A 10 min retention trial (second trial) occurred 2 h later. During this second trial, the objects A and B were placed in the maze and the time that the animal took to explore the new object (tn) and the old object (to) were recorded. A discrimination index (DI) was defined as (tn−to)/(tn + to). In order to avoid object preference biases, objects A and B were counterbalanced so that half of the animals in each experimental group were first exposed to object A and then to object B, whereas the other half saw first object B and then object A. The maze, the surface, and the objects were cleaned with 96° ethanol between animals, so as to eliminate olfactory cues.

Brain isolation and western blot analysis

Mice were euthanized one day after the last trial had been conducted, and the brain was quickly removed from the skull. The hippocampus were dissected, frozen in powdered dry ice, and maintained at –80°C until use. When necessary, tissue samples were put into ice-cold conditions and homogenized in lysis buffer containing phosphatase and protease inhibitors (Cocktail II, Sigma). The protein concentration in tissue samples was determined by the Bradford method. A total of 20 μg of protein was separated by SDS–PAGE (5–15%) and transferred to PVDF membranes (Millipore). The membranes were blocked in 5% non-fat milk in TBS containing 0.1% Tween 20 (TBS-T) for 1 h at room temperature, followed by overnight incubation at 4°C with synaptophysin (1 : 500; Abcam), GADPH (1 : 2000; Millipore), and β-actin (1 : 20000; Sigma). Membranes were then washed and incubated with secondary antibodies for 1 h at room temperature. Protein bands were visualized using a chemiluminescence detection kit (Amersham Biosciences). The band

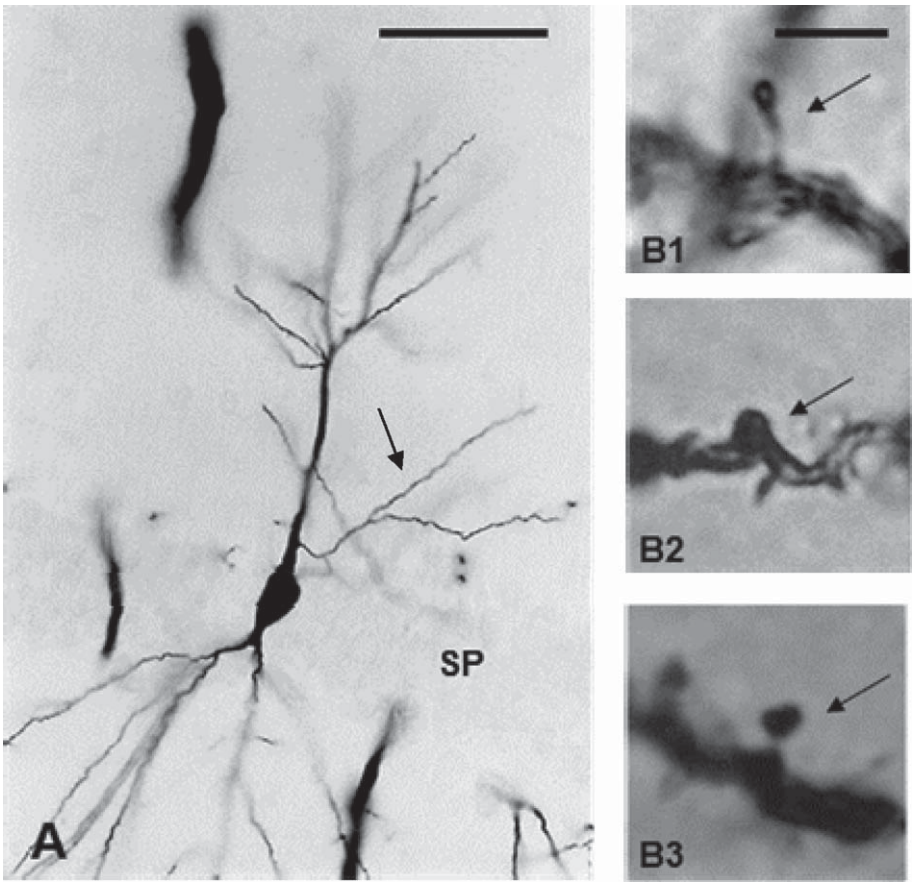

Fig. 1. A) Photomicrograph of a pyramidal neuron from the hippocampal CA1 field of a SAMR1 mouse, similar to those studied in the present work. Dendritic spines were counted in a secondary dendritic segment of 50 μm length (arrow). SP: stratum pyramidale. Scale bar: 50 μm. B) Representative thin (B1), stubby (B2), and mushroom (B3) spines (arrows), like those counted in this study. Scale bar: 2 μm.

intensities were quantified by densitometric analysis, and values were normalized to β-actin or GAPDH expression.

Golgi studies

Animals were anesthetized with 30 mg/kg intramuscular ketamine and 50 mg/kg i.p. sodium pentobarbital. Then, they were intracardially perfused with washing phosphate-buffered solution (pH 7.4; 0.01 M), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (100 ml/100 g body weight). Both solutions flowed at a rate of 5 mL/min. Each brain remained for at least 48 h in 50 mL of a fresh fixing solution. The bilateral dorsal hippocampi were dissected out and impregnated using a modification of the Golgi method [27]. Several 100 μm thick coronal slices were mounted on one slide per animal. The numerical density of spines and the proportion of thin, stubby and mushroom spines (González-Burgos, 2009) were assessed in CA1 pyramidal neurons. Spines were counted in one 50 μm segment per cell, located in the middle of one of the secondary dendrites that protrude from the apical dendrite (Fig. 1). Six CA1 pyramidal neurons were studied per animal. Counts were performed by direct observation at 2,000×, using a magnification changer coupled to a light microscope. To ensure the consistency of counting the dendritic spines, an initial "double-blind" study was performed and the reliability index was calculated (number of agreements - number of disagreements/number of agreements). Once a minimum reliability of 0.95 had been reached, the quantification of dendritic spines from the study groups was performed with a "blind" procedure.

Statistical analysis

The *n* used for statistical analysis was the number of animals ($n = 6$–8, per experimental group) and the quantitative data are expressed as the mean ± standard

error (S.E.M.). The statistical analysis was performed using the one-sample *t*-test, the student's unpaired *t*-test, or analysis of variance (One-way ANOVA), followed by a Bonferroni post hoc test when appropriate. The differences were considered to be statistically significant if $p < 0.05$.

RESULTS

Object recognition test

During the sample phase of the task, all groups spent the same percentage of time exploring each object, i.e., all animals explored objects similarly both (data not shown). However, while SAMR1 mice showed novel object preference in all age groups during the test phase as their DI was positive and different from zero, 6- and 9-month-old SAMP8 mice showed a DI that was not different from zero, which indicates no preference between the two objects (Fig. 2). A comparison between young 3-month-old SAMP8 mice and 6- and 9-month-old SAMP8 mice show significant differences. In addition, 6- and 9-month-old SAMP8 mice had a significantly lower DI than SAMR1 age-matched mice. This indicates that from 6 months onwards, SAMP8 animals present memory impairment. No differences were found between the 3-month-old mice from either strain, which indicates that no memory differences exist at this point.

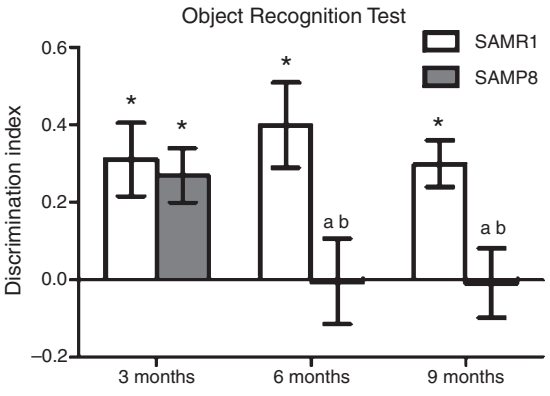

Fig. 2. Discrimination index in the object recognition test. All ages and groups except 6 and 9-month-old SAMP8 mice exhibited a positive discrimination index different from zero $*p < 0.01$, one-sample *t*-test versus zero). Six- and nine-month-old SAMP8 mice had a significantly lower discrimination index than aged-matched SAMR1 mice ($^{a}p < 0.05$ versus age-matched SAMR1, $^{b}p < 0.001$ vs. 3-month-old SAMP8, student's *t*-test). Data is presented as mean ± SEM.

Synaptophysin levels

Levels of synaptophysin, a marker of neurotransmission, were determined in all groups. The synaptophysin levels of 3-month-old SAMR1 mice are expressed as 100% of synaptophysin and all the other groups are referred to this standard. Figure 3 shows that there were no differences between the two strains at three months of age. However, 6- and 9-month-old SAMP8 mice had lower synaptophysin expression in the hippocampus than age-matched SAMR1 mice (Fig. 3).

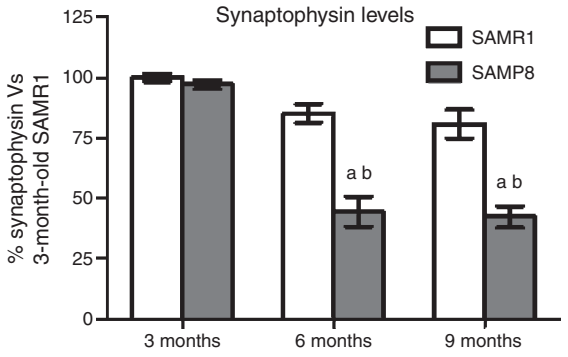

Fig. 3. Percentage of synaptophysin levels compared to 3-month-old SAMR1 mice (100%). Six- and nine-month SAMP8 animals had lower synaptophysin expression than age-matched SAMR1 mice ($^{a}p < 0.001$ versus age-matched SAMR1, ANOVA followed by Bonferroni's post-test). Synaptophysin levels decreased with age in SAMP8 mice ($^{a}p < 0.001$ versus age-matched SAMR1, $^{b}p < 0.001$ versus 3-month-old SAMP8, ANOVA followed by Bonferroni's post-test). Data is presented as means ± SEM.

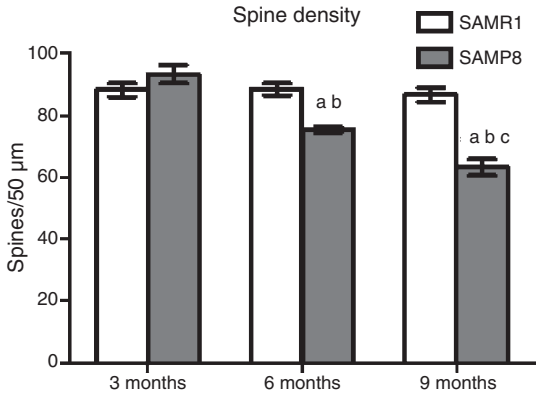

Fig. 4. Spine numerical density in CA1 pyramidal neurons of SAMP8 and SAMR1 mice. Six- and nine-month SAMP8 animals had fewer spines than age-matched SAMR1 animals ($^{a}p < 0.001$ versus age-matched SAMR1, ANOVA followed by Bonferroni's post-test). The spine number diminished with age in SAMP8 mice ($^{b}p < 0.001$ versus 3-month-old SAMP8, $^{c}p < 0.01$ versus 6-month-old SAMP8, ANOVA followed by Bonferroni's post-test). Data is presented as means ± SEM.

A time-course analysis of synaptophysin levels was also conducted, showing that SAMP8 animals present lower synaptophysin levels at 6 and 9 months of age than 3-month-old SAMP8 animals. Although SAMR1 mice seem to show less synaptophysin at 9 months than at 3 months, no statistical differences were seen. This observation indicates that there are fewer synapses in SAMP8 from 6 months onwards than in age-matched SAMR1 animals. It also shows that the loss of synapses starts as early as 6 months, compared to young individuals.

Dendritic spines

Dendritic spine density in SAMR1 mice was similar in all age groups ($F = 0.170$, $p < 0.845$) (Figs. 4 and 5). In contrast, SAMP8 mice showed significant differences in spine density ($F = 42.061$, $p < 0.001$). Both 6-month-old ($p < 0.001$) and 9-month-old ($p < 0.001$) SAMP8 mice had less dendritic spines than 3-month-old SAMP8 mice. In addition, 9-month-old SAMP8 mice had less dendritic spine density than 6-month-old SAMP8 mice ($p < 0.01$) (Figs. 4 and 5). SAMR1 and SAMP8 mice had similar numbers of dendritic spines at 3 months of age. However, at 6 ($p < 0.001$) and 9 ($p < 0.001$) months of age, SAMP8 mice had less spines than SAMR1 mice (Figs. 4 and 5).

The proportional density of thin, stubby, and mushroom spines of SAMR1 mice was the same in all age groups (data not shown). In contrast, the proportional density of thin spines varied in SAMP8 mice ($F = 19.291$, $p < 0.001$). Nine-month-old SAMP8 mice had less thin spines than both 3-month-old ($p < 0.01$) and 6-month-old ($p < 0.001$) SAMP8 mice. There were no differences in the proportional density of stubby or mushroom spines among all ages (Table 1). The thin, stubby, and mushroom spine proportional density was no different in the two strains at 3 and 9 months of age (data not shown), whilst there were more thin spines ($p < 0.01$) and less mushroom spines ($p < 0.01$) in 6-month-old SAMP8 mice than in the age-matched SAMR1 (Table 2).

Table 1
Spine types in SAMP8. Results are presented as mean ± SEM

Months-old	3	6	9
Spine Proportion (%)			
Thin	40.7 ± 1.0	43.2 ± 0.9	35.6 ± 0.5[a,b]
Stubby	19.9 ± 0.6	18.3 ± 0.7	21.8 ± 1.3
Mushroom	34.9 ± 0.8	33.6 ± 0.7	36.8 ± 1.2

[a] $p < 0.001$ versus 3-month-old SAMP8, [b] $p < 0.001$ versus 6-month-old SAMP8, ANOVA followed by Bonferroni's post-test.

Table 2
Spine types in 6-month-old SAMR1 and SAMP8. Results are presented as a mean ± SEM

Strain	SAMR1	SAMP8
Spine Proportion (%)		
Thin	38.7 ± 1.1	43.2 ± 0.9[a]
Stubby	19.0 ± 0.61	18.3 ± 0.7
Mushroom	38.2 ± 1.6	33.6 ± 0.7[a]

[a] $p < 0.01$ versus age-matched SAMR1, ANOVA followed by Bonferroni's post-test.

DISCUSSION

The structural plasticity of dendritic spines, including the spine number and morphology, are crucial to learning and memory in the cerebral cortex [28]. Dendritic spine loss is observed in the hippocampus and throughout the cortex, which are the main areas affected by AD [29]. Although there is a loss of synapses in the aging brain [30], the density of dendritic spines is severely decreased in AD. This decline, rather than the amyloid burden or tau hyperphosphorylation, is the hallmark of AD that best correlates with cognitive decline [31]. In addition, it has been reported that several presynaptic terminal proteins are affected in multiple brain regions of patients with AD [32]. Taking all this into account, it has been proposed that the loss of dendritic spine density is indeed a critical event in the pathophysiology of AD [33].

Studies in AβPP transgenic mice have shown functional deficits and synaptic loss before the onset of amyloid-β plaque formation [34] and neurofibrillary tangle formation [35]. Furthermore, several studies have characterized alterations in the dendritic spine density in mouse models of AD, such as a significant decrease in spine density of the dendrites in the hippocampus of the J20 and AβPP/PS1 mice [26] and in the CA1 subzone both in Tg2576 and AβPP/Lo mice [36]. However, although synaptic plasticity alterations have been described in SAMP8 mice [6], no focus on the dendritic spine state has been made.

In this study, we used modified Golgi staining to compare the spine density of SAMP8 animals with that found in the genetically related control strain SAMR1 at 3, 6, and 9 months of age. We found that spine density is reduced in SAMP8 mice at 6 months of age, which is also the age when the amyloid burden is increased [11] and some other protein aggregates are found [21], and pretty similar to the age when tau hyperphosphorylation is increased [12]. Likewise, spine density was also reduced in SAMP8 mice at 9 months of age.

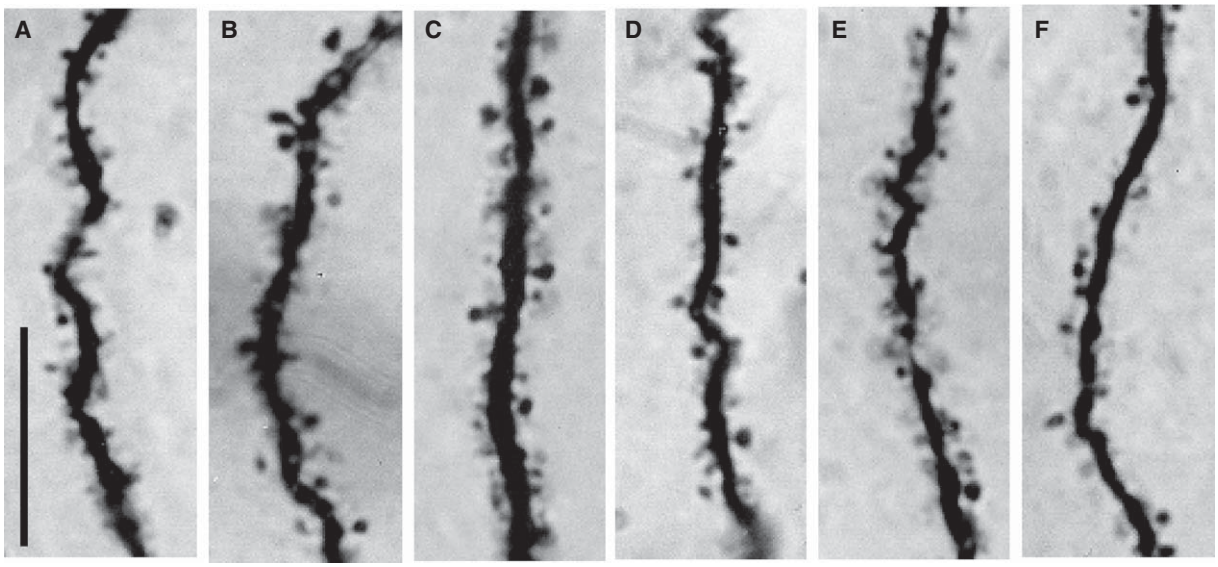

Fig. 5. Representative images of Golgi stained CA1 dendrites used to quantify and characterize dendritic spines in all groups studied. A) 3-month-old SAMR1, B) 3-month-old SAMP8, C) 6-month-old SAMR1, D) 6-month-old SAMP8, E) 9-month-old SAMR1, F) 9-month-old SAMP8. Scale bar: 10 μm.

SAMP8 and SAMR1 differed in terms of changes in the proportion of the various spine morphologies. In SAMR1 mice, studies and comparisons of the three ages showed similar proportional densities of thin, stubby, and mushroom spines. In contrast, the proportion of thin spines was less in SAMP8 mice at 9 months of age than at both 3 and 6 months of age. This suggests that the learning capabilities of SAMP8 mice decrease, since thin spines have been related with information acquisition (learning) [37–39]. This is in agreement with the finding that SAMP8 mice were inefficient at resolving the ORT at 9 months of age. However, 6-month-old SAMP8 mice had more thin spines than SAMR1 animals at this age, which suggests that compensatory plastic changes takes place in the development of the SAMP8 hippocampus over time. This is consistent with the fact that mushroom spines were proportionally less in SAMP8 mice than in SAMR1 animals, at the same age. The morphophysiological properties of mushroom spines are closely related to memory storage [37–39], which suggests that although SAMP8 mice could be particularly sensitive to novel stimuli, their capacity to retain and store incoming information would be limited, at least at 6 months of age. This would be in agreement with our behavioral findings, which revealed that the discrimination index in the ORT was significantly lower in SAMP8 than in SAMR1 mice from 6 months onwards. Thus, both dendritic spine reduction and plastic changes in the types of dendritic spines in SAMP8 mice could be closely related with the behavioral inefficacy seen in SAMP8 mice in comparison with control SAMR1 mice.

Synaptic vesicle protein synaptophysin is a marker of synapses [40] and indicates the strength of synaptic transmission. We found that changes in the levels of this protein in SAMP8, which result in a significant age-related reduction in protein levels. Moreover, the content of synaptophysin in SAMR1 is similar at different ages, which confirms the analysis of dendritic spines. Intriguingly, synaptophysin levels start to decrease at 6 months of age in SAMP8 animals, which is the age when their spine density is also reduced.

In this study, we compared performance in the ORT as a measure of memory alterations in SAMP8 mice using age-matched control SAMR1 mice. The ORT is thought to critically depend on the entorhinal cortex, hippocampus, and frontal cortex and is considered a test of short-term memory [41]. In AD, alterations in these brain structures as well as impairment in short-term memory have been widely reported in the literature. Our results show that from 6 months onwards SAMP8 mice show an impaired memory. At this age, when their levels of synaptophysin and spine density are decreased. Interestingly, 9-month-old SAMP8 mice show the same poor performance in ORT with the same decreased synaptophysin levels, but with less spine density than 6-month-old SAMP8. In contrast, SAMR1 animals show no alterations in short-term memory, synaptophysin levels, or spine density

from 3 to 9 months of age. These facts can be related with the persistence of spines in SAMR1 which have a stable density throughout the animals' lives. This stability allows the memory circuits to be consolidated for a long time.

Several studies in the literature established that amyloid-β oligomers and plaques in AD patients or experimental models first induce tau phosphorylation, and then produce cytoskeletal collapse and neuritic degeneration [42]. We demonstrated that in a *bona-fide* murine model of senescence and AD pathology, such as SAMP8 strain, synaptic, dendritic, and memory alterations appear from 6 months onwards. This reinforces the growing interest in SAMP8 as a model of AD.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1380).

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