Cerebral Cortex March 2009;19:586–592
doi:10.1093/cercor/bhn111
Advance Access publication July 16, 2008

Widespread Changes in Dendritic Spines in a Model of Alzheimer's Disease

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The mechanism by which dementia occurs in patients with Alzheimer's disease (AD) is not known. We assessed changes in hippocampal dendritic spines of APP/PS1 transgenic mice that accumulate amyloid beta throughout the brain. Three-dimensional analysis of 21 507 dendritic spines in the dentate gyrus, a region crucial for learning and memory, revealed a substantial decrease in the frequency of large spines in plaque-free regions of APP/PS1 mice. Plaque-related dendrites also show striking alterations in spine density and morphology. However, plaques occupy only 3.9% of the molecular layer volume. Because large spines are considered to be the physical traces of long-term memory, widespread decrease in the frequency of large spines likely contributes to the cognitive impairments observed in this AD model.

Keywords: Alzheimer's disease, confocal microscopy, dementia, dentate gyrus, transgenic mice

Introduction

One of the primary pathological characteristics of Alzheimer's disease (AD) is the presence of amyloid plaques, formed by aggregated β-amyloid (Aβ) peptides. In mice, the expression of chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9), proteins that are both involved in familial forms of AD, lead to an early amyloid pathology. These double transgenic mice (APP/PS1) are often used as an animal model of AD, although they do not develop the extensive neuronal loss or neurofibrillary changes typical in AD (Irizarry et al. 1997). Nevertheless, this mouse model is used to investigate the effect of Aβ overproduction and deposition on brain circuitry.

Although Aβ plaques have been associated with changes in neurite morphology and dendritic spine density (Tsai et al. 2004; Spires et al. 2005), there is a poor correlation between plaque load and cognitive functions (Terry et al. 1991), raising doubts as to whether the accumulation of Aβ plaques in the brain induces AD. Nevertheless, morphological studies on AD models and patients have so far focused on the structural changes occurring within or proximal to plaques (Tsai et al. 2004; Spires et al. 2005).

Dendritic spines represent the major postsynaptic elements of excitatory synapses in the cerebral cortex (Gray 1959) and are fundamental to memory, learning and cognition (Lamprecht and LeDoux 2004). Dendritic spines undergo remarkable activity-dependent structural changes (Lang et al. 2004; Tsai et al. 2004). The spine head size influences spine motility and stability. Spine head size determines the probability that a spine bears a synapse (Arellano et al. 2007). Furthermore, there is a strong correlation between spine head size and the strength of the axo-spinous synapse (Zuo et al. 2005). Importantly, recent evidence indicates that spine heads are targets of oligomeric Aβ (Lacor et al. 2007). Therefore, spine head morphology may link Aβ pathology and synaptic dysfunction.

We individually injected granular neurons in the dentate gyrus with a fluorescent dye and counterstained Aβ plaques with thiflavin-s. We measured the head volume and neck length in 3 dimensions of thousands of individual dendritic spines that have been scanned by confocal microscopy. Our results show that plaques substantially affect dendrites and spines. Importantly, we also determined that plaque-free regions of APP/PS1 mice are affected. These regions are very deficient in large spines, although spine density is conserved. We suggest that widespread decreased frequency of large spines contributes to the cognitive deficits found in this AD model.

Materials and Methods

Mice

The mouse line used in this study ($N = 9$, age 12–14 months) expressed a Mo/Hu APP695swe construct in conjunction with the exon-9–deleted variant of human presenilin 1 (PS1-dE9) (Scheuner et al. 1996). Age-matched littermates served as controls ($N = 9$). The experiments were approved by the ethical institutional committee according to National Institutes of Health guidelines.

Intracellular Injections

The preparation of brains for intracellular injections is detailed in the supplemental methods. A total of 235 granular neurons from Tg– mice and 462 neurons from APP/PS1 mice were microinjected individually with Alexa594 (Invitrogen, Eugene, OR, Fig. 1*a*,*b*). After injection, plaques were counterstained with thioflavin-s. Image acquisition and processing are described in the supplemental information. All morphological measurements were performed on stacks that did not contain images of plaques (see supplemental information).

Spine Morphology

Imaris 5.0 (Bitplane AG, Zurich, Switzerland) was used to measure spine head volume and neck length. A solid surface that exactly matched the contours of the head was constructed for each spine (Isosurface module, Fig. 1*j*–*m*, Supplemental Fig. 1*b*,c). For details, see supplemental methods.

Unbiased Stereology

The number of labeled plaques was estimated by optical fractionator. The volume of plaques was estimated with the Nucleator probe (Moller et al. 1990), as described in the supplemental methods.

Statistics

For all measured morphological parameters, values were averaged to give a cell mean. Neurons from each animal were averaged for an animal mean. Normality was tested using the Kolmogorov–Smirnov test.

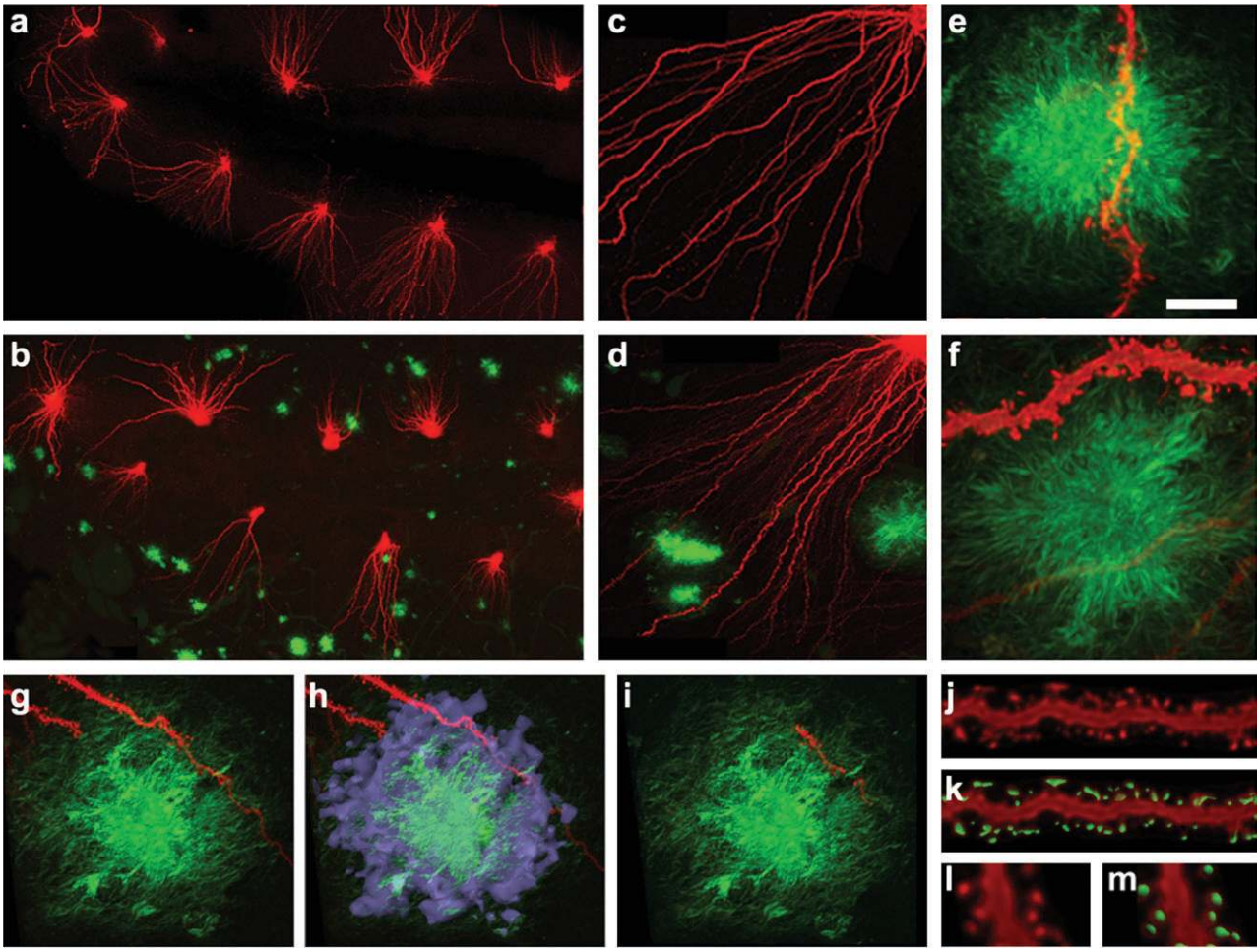

Figure 1. Intracellular injections and methodology. Panoramic confocal composite (10×) views of the dentate gyrus, showing Alexa594 injected neurons and thioflavin-s positive plaques in a Tg− mouse (*a*) and an APP/PS1 mouse (*b*). Representative projection images of granular neurons from a Tg− mouse (*c*) and from an APP/PS1 mouse (*d*). (*e*) An example of a dendrite passing within a plaque. (*f*) An example of a dendrite in contact with a plaque. (*g–i*) The method used to distinguish dendrites and spines within plaques. (*g*) A plaque suspected to contain a dendrite due to the rotation of its 3D image. (*h*) The plaque surface is marked with the aid of the IsoSurface tool of Imaris software. (*i*) The voxels outside the surface are set to zero, leaving only the dendritic segment within the plaque. This process was performed after measurements of the spine head and neck that were done blindly. (*j*, *k*) The method used for spine head volume measurements. (*j*) A solid surface that exactly matched the contours of the head was constructed for each spine (*k*). (*l*, *m*) Amplified image of a short dendritic segment with (*l*) and without (*m*) the marked spine heads. Stacks used for images (*e–m*) were taken with a 63× glycerol objective, NA 1.3, electronic zoom 3.2). Scale bar, (*a*, *b*) 150 μm, (*c*, *d*) 40 μm, (*e*) 6 μm, (*f*, *j*, *k*) 4 μm, (*g–i*) 10 μm, (*l*, *m*) 2.5 μm.

Because our values had Gaussian distributions, a 2-tailed unpaired *t*-test was used to test for overall effect. When more than 2 groups were compared, a one-way ANOVA was used, followed by the Newman–Keuls multiple comparison *post hoc* test. Data are presented as the mean ± SE.

Results

Altered Spine Density in Plaque-Related Dendrites

We used APP/PS1 mice at the age of 12–14 months. Because transgenic mice of this age range show cognitive decline (Malm et al. 2007) and deficits in cortical plasticity (Battaglia et al. 2007), our working hypothesis was that these mice would exhibit substantial changes in their dendritic spines. Although the toxic effects of Aβ plaques on dendritic spines have been documented before in the neocortex (Tsai et al. 2004; Spires et al. 2005), we hypothesized that in the dentate gyrus, Aβ plaques would also have a tropic effect, given the extensive sprouting in the hippocampal molecular layer seen in an AD model (Masliah et al. 1991).

We examined 1,589 amyloid plaques and 462 APP/PS1 injected granular neurons by confocal microscopy (63×, glycerol, zoom 3.2, Fig. 1*a–d*). We encountered 52 dendrites that passed within plaques and 32 dendrites that contacted a plaque but did not pass within it (Fig. 1*e,f*). Typical plaques, positive for thiflavin-s, consisted of a core, surrounded by a diffuse ring of decreasing density (Cruz et al. 1997). The dendrites passing within plaques were always located in the diffuse peripheral ring (Fig. 1*e,g–i*).

Dendrites were categorized according to their location with respect to Aβ plaques (Fig. 2*a*). Categories included: 1) dendrites from transgene-negative (control) mice (Tg–), 2) dendrites located in a plaque-free area throughout their length (Plaque-free), 3) dendrites that passed within a plaque (Plaque), and 4) dendrites in contact with a plaque edge (part of the dendrite located within 0.2 μm from the plaque edge, as measured **3D**) but did not pass through it (In contact, see Supplemental Fig. 1*a,b*).

Spine density was significantly different among the 4 categories of dendrites ($P = 0.019$, one-way ANOVA, Fig. 2*b*).

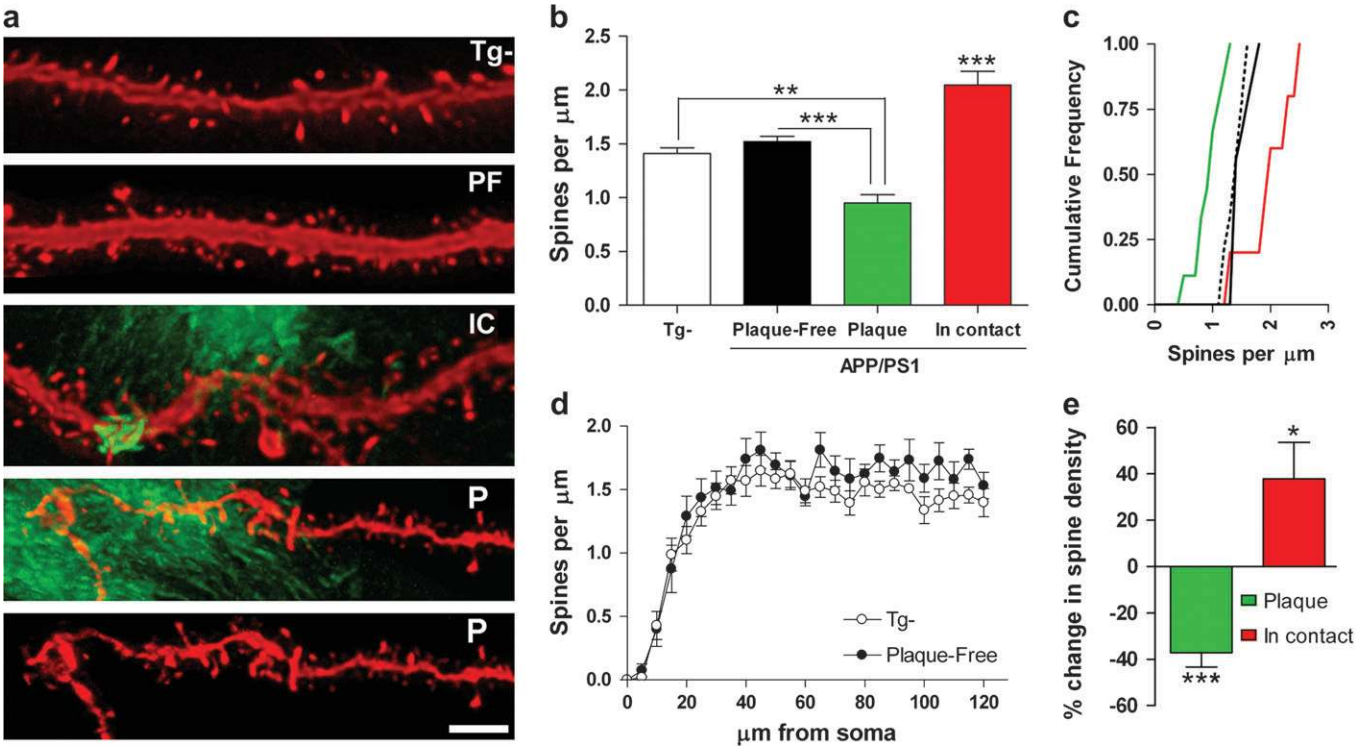

Figure 2. Pronounced changes in dendritic spine density in plaque-related dendrites. (*a*) Representative projection images of dendrites from Tg− and APP/PS1 mice (63×, glycerol). Note the variation in spine density among dendrites. Tg−, dendrite from a control mouse; PF, dendrite located in plaque-free region of APP/PS1 mouse; IC, dendrite of which a part is in contact with a plaque; P, dendrite that passes within a plaque, with and without the green channel that contains the plaque image. (*b*) Spine density was significantly decreased in Plaque dendrites and increased for In contact dendrites. Note that spine density is similar in Tg− mice and plaque-free areas. (*c*) Cumulative frequency curves show shifts in spine density of plaque-related dendrites. (*d*) Spine density as a function of distance from soma is similar in Tg− mice and APP/PS1 mice in plaque-free regions. (*e*) For each plaque-related dendritic segment, the distance of the plaque from the soma was measured, and the ratio between spine density for the segment and the average spine density for the same distance in Tg− mice was calculated. Scale bar, 3 µm. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

In Plaque dendrites, spine density was significantly lower than in other categories of dendrites (0.950 ± 0.075 spines/µm, $N = 9$, $P < 0.001$, Newman–Keuls multiple comparison test). Spine density for In contact dendrites was significantly higher than in other categories of dendrites (2.05 ± 0.13, $N = 9$, $P < 0.001$). Spine density for Plaque-free dendrites (1.521 ± 0.047, $N = 9$) was not significantly different than control (Tg–) dendrites (1.410 ± 0.052, $N = 9$).

Spine density normally changes as a function of distance from the soma. Sholl analysis can be used to examine spine density at different distances from the soma. Sholl analysis revealed that spine density for Plaque-free dendrites was similar to control (Tg–) dendrites over their entire length (Fig. 2*d*). Spine density for each segment located within or in contact with a plaque was compared with the average spine density at same distance from soma in Tg– mice. We found a decrease of 37.21 ± 6.03% in spine density within plaques ($P < 0.0001$, *t*-test) and a 37.77 ± 15.77% increase in spine density of In contact dendrites ($P = 0.01$, Fig. 2*e*). Thus, we found considerable sprouting of spines in dendrites contacting Aβ plaques, whereas there was a loss of spines in dendrites passing through Aβ plaques.

Decreased Frequency of Large Spines in Plaque-Free Areas

Spine head volume and neck length were measured in 3 dimensions in confocal image stacks. Head volume was significantly different among the 4 categories of dendrites ($P = 0.0016$, one-way ANOVA, Fig. 3*b*). Average head volume was significantly lower ($P < 0.05$, Newman–Keuls multiple comparison post hoc test) in Plaque-free spines (0.080 ± 0.004 µm^3; 9913 spines) and for In contact spines (0.076 ± 0.006; 443 spines), when compared with Plaque spines (0.107 ± 0.008; 474 spines), and control (0.103 ± 0.006; 10 677 spines).

Although the average head volumes for control spines and Plaque spines were similar, frequency analysis revealed that the within-groups distribution was substantially different between the 2 groups (Fig. 3*c*). Within plaques, there was a 36% decrease in the prevalence of small-headed spines (head volume < 0.05 µm^3) and a 40% increase in the prevalence of spines with a medium-size head (head volume 0.05–0.1 µm^3), compared with control spines (Fig. 3*c*). The prevalence of large-headed spines (large spines, head volume > 0.1 µm^3) was similar for both dendritic types (Fig. 3*c*). Our measurements show that large spines represent 23% of Tg– dentate gyrus spines but only 14.4% of the plaque-free APP/PS1 spines. This implies a decrease of 38% in the prevalence of large spines in the plaque-free regions of APP/PS1 mice. When compared with control mice, there was a 52% decrease in the prevalence of large spines in dendrites contacting plaques (Fig. 3*d*). We also divided the granular neurons into neurons that have come into contact with or passed within plaques at some point along their length and those that never contacted a plaque. An analysis of the spine head volume revealed that spines located in plaque-free regions had a similar head volume although they arise from different neuronal types (Supplemental Fig. 2).

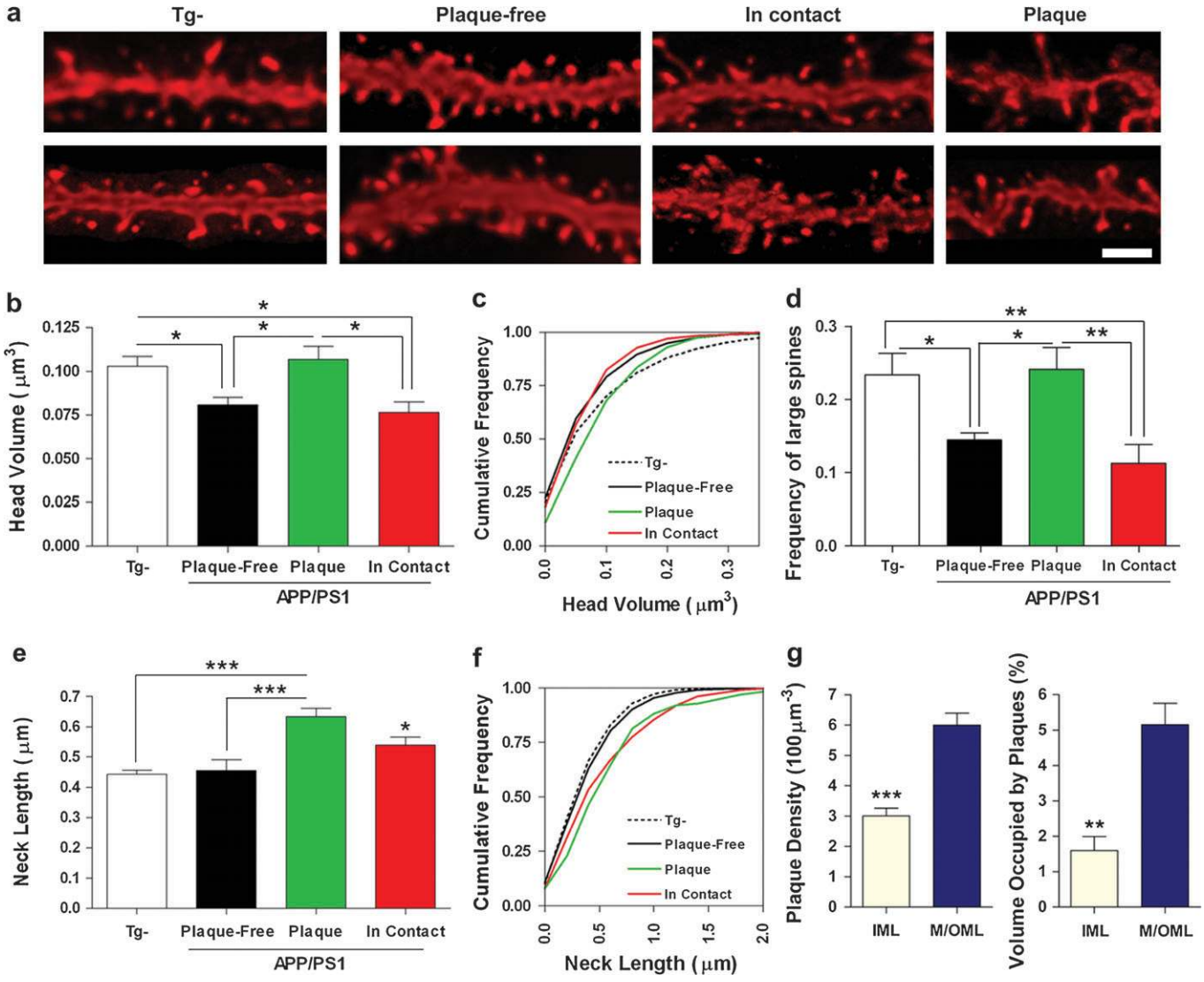

Figure 3. Changes in spine morphology occur both within and outside Aβ plaques. (*a*) Maximum projection confocal images (63×, glycerol) of representative dendrites. Note the great variation in spine morphology among groups. Scale bar, 1.5 μm. (*b*) Decreased average head volume for spines located outside plaques. (*c*) Cumulative frequency plots showing the distribution of spine head volumes. (*d*) The frequency of large spines is substantially reduced outside plaques. (*e*) Bar graphs showing that spines located within plaques have longer necks. (*f*) Cumulative frequency curves showing the distribution of neck lengths. (*g*) On the left, density of plaques in the molecular layer of the dentate gyrus. On the right, the fraction of the molecular layer volume occupied by plaques. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Significant differences in average spine neck length, as was measured manually in 3 dimensions, were found among dendritic categories ($P < 0.0001$, one-way ANOVA, Fig. 3*e*,*f*). Plaque spines had a longer neck (0.634 ± 0.028 μm, $P < 0.001$, Newman–Keuls Multiple Comparison Test) than control spines (0.443 ± 0.012) and Plaque-free spines (0.455 ± 0.035). Necks of In contact spines showed intermediate length (0.540 ± 0.026) and were significantly different from other dendritic categories ($P < 0.05$).

Amyloid Plaques Occupy a Small Fraction of the Molecular Layer Volume

We have described remarkable changes within and near plaques that can affect local synaptic circuits. To quantitatively determine the impact of plaques on the dentate gyrus connectivity, we immunocytochemically stained Aβ plaques in sections of APP/PS1 brains, taken from the same mice. We then determined, using unbiased stereology, the total number of plaques and their volume in the molecular layer. From this, we calculated the total volume occupied by plaques.

The estimated total number of molecular layer plaques per mouse in one hemisphere was 609.9 ± 37.14 (range 555–756 plaques/mouse, $N = 5$ mice). The density of plaques in the inner molecular layer (IML) was 3.00 ± 0.25 plaques/100 μm^3, whereas the density of plaques in the middle/outer molecular layer (M/OML) was 5.99 ± 0.39 plaques/100 μm^3 (Fig. 3*g*). This observation supports the finding that plaques in the dentate gyrus of AD brains have a strong tendency to line up in the molecular layer, approximately two-thirds of the way from the top of the hippocampal fissure (Crain and Burger 1989). The average plaque volume was 5657 ± 1488 μm^3 in the IML and 8719 ± 1109 μm^3 in the M/OML ($P > 0.05$). The estimated volume occupied by Aβ plaques was 1.59 ± 0.39% in IML and 5.15 ± 0.60% in the M/OML (Fig. 3*g*). The volume occupied by

Aβ plaques in the entire molecular layer was 3.9 ± 0.4%. These results suggest that under our experimental conditions, Aβ plaques occupy a relatively small fraction of the total molecular layer volume.

Discussion

The current study shows that plaque-free regions of the dentate gyrus in APP/PS1 mice are deficient in large spines and that dendritic spines are also affected locally by Aβ plaques. However, under our experimental conditions, plaques occupy a volume below 4% of the dentate gyrus molecular layer.

Decreased Frequency of Large Spines may Imply Loss of Memory

Dendritic spine heads bear the vast majority (90%) of excitatory synapses in the central nervous system (Gray 1959). They undergo remarkable activity-dependent structural changes (Lang et al. 2004; Knafo et al. 2005) and are targets of oligomeric Aβ (Lacor et al. 2007). Although morphological changes within Aβ plaques can affect local synaptic circuits, because plaques occupy a minor fraction of the dentate gyrus volume, widespread changes outside plaques are more likely to contribute to the synaptic and cognitive impairments found in APP/PS1 mice (Battaglia et al. 2007; Malm et al. 2007).

Large spines contain polyribosomes that locally regulate protein synthesis and are surrounded by perisynaptic astroglial processes that regulate local glutamate and Ca^{2+} levels (Ostroff et al. 2002; Haber et al. 2006; Witcher et al. 2007). Large spines persist for months in the mouse cerebral cortex (Grutzendler et al. 2002; Trachtenberg et al. 2002). Large spines bear synapses exceptionally stable (Bourne and Harris 2007). Large spines are also enriched with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (Matsuzaki et al. 2001), which make their synapses functionally stronger (Matsuzaki et al. 2001; Nimchinsky et al. 2004; Ashby et al. 2006). Moreover, large spines are formed after synaptic potentiation in the dentate gyrus (Bourne and Harris 2007) and in CA1 region (Lang et al. 2004; Matsuzaki et al. 2004). Thus, large spines were proposed to act as physical traces of long-term memory (Kasai et al. 2003; Bourne and Harris, 2007).

Given that large spines in the dentate gyrus bear stable synapses that form part of the hippocampal memory storage system (Bontempi et al. 1999), a decrease in the frequency of large spines should lead to the memory loss seen in APP/PS1 mice (Malm et al. 2007). Nevertheless, it is possible that the decreased frequency of large dendritic spines is secondary to the cognitive impairments observed in APP/PS1 mice, and those in plasticity (Fiala et al. 2002). Both memory and synaptic plasticity are disrupted in AD models and patients (Nalbantoglu et al. 1997), potentially preventing plasticity-related spine enlargement (Lang et al. 2004).

Dendritic Spines are Altered by Aβ Plaques

We report here that dendrites that pass through a plaque lose spines, whereas dendrites that contact a plaque gain spines. Dendrites located in plaque-free areas show a spine density similar to those of Tg– mice but have a decreased frequency of large spines. Decreased spine density within plaques and sprouting of spines in the vicinity of plaques have been documented in the neocortex (Ferrer et al. 1990; Tsai et al. 2004). The decreased spine density within plaques could be explained by alterations to the ratio of spine formation to elimination, leading a higher proportion of aspiny dendrites as observed in the cortex of Tg2576 mice (Spires-Jones et al. 2007).

In contrast to our observations, a recent study reported a reduction in spine density in the hippocampus of APP mice before and after the appearance of plaques (Jacobsen et al. 2006). This discrepancy could be related to the fact that a different mouse model was used in this study (APP vs. APP/PS1), as well as a different staining method (Golgi staining vs. intracellular injections), and a different method for quantification (quantification of short dendritic segments vs. full dendritic lengths). A decrease in spine density in the hippocampus of aged APP/PS1 mice described previously (Moolman et al. 2004) could be explained by including the dendrites located within plaques in the analysis, because spine density is significantly lower at these sites (see Tsai et al. 2004 and Fig. 1*b*). Other studies reported a decrease in synaptic density in AD models (Dong et al. 2007; Priller et al. 2007). However, although we quantified dendritic spines, these earlier studies quantified synapses regardless of their location (spine or dendritic shaft). Thus, the possibility that the decrease in synaptic density arises from the loss of shaft synapses cannot be excluded.

The increased spine density in the vicinity of the plaques can be explained by the release of neurotrophic factors, such as brain-derived neurotrophic factor by astrocytes that typically surround Aβ plaques (Mandybur 1989; Saha et al. 2006). It is tempting to speculate that the growth of new spines in the vicinity of the plaques is an attempt by the diseased brain to compensate for the loss of spines within them.

Aβ Plaques Protect Large Spines

Notably, the prevalence of large spines within plaques and in Tg– control mice was similar. Although we observed a 36% decrease in spine density within plaques, this decrease was mainly due to a decrease in the frequency of spines with small heads (see Fig. 3*c*). This may imply that plaques serve as a protective microenvironment for large spines. The persistence of large spines within plaques can contribute to the weak correlation between the quantity of amyloid deposition and dementia (Katzman et al. 1988). The sprouting of spines in dendrites of immediate proximity to plaques is another observation that can explain the same phenomenon. These dendrites possess predominantly small-headed spines. However, it remains to be determined whether spines within and near plaque bear synapses and if these synapses are functional.

To summarize, we suggest that the substantial alterations in dendritic spines within plaques most likely affect local synaptic connections. However, it is difficult to determine whether these changes contribute to the cognitive impairment seen in these mice. Because the plaque load and cognitive impairment in AD patients does not appear to be correlated (Terry et al. 1991), it is possible that the contribution of plaques to cognitive impairment is relatively insignificant. Therefore, we propose that the widespread changes in plaque-free regions, corresponding to more than 95% of the neuropil, may have a more important influence on the cognitive status of these mice.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

Funding

CIBERNED grant, Fundacion Caixa (BM05-47-0); The EU 6th Framework Program (PROMEMORIA LSHM-CT-2005-512012); the Spanish Ministry of Science and Technology (grant BFU2006-13395); and Juan de la Cierva Program of the Ministry of Science and Technology fellowship to S.K.

Notes

We thank Gabriel Santpere, Ana Martinez, and Gerard Muntane for genotyping the mice. *Conflict of Interest*: None declared.

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