Thin, Stubby or Mushroom: Spine Pathology in Alzheimer's Disease

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Abstract: Since their first description by Ramon y Cajal at the end of the 19th century, dendritic spines have been proposed as important sites of neuronal contacts and it has been suggested that changes in the activity of neurons directly affect spine morphology. In fact, since then it has been shown that about 90% of excitatory synapses end on spines. Recent data indicate that spines are highly dynamic structures and that spine shape correlates with the strength of synaptic transmission. Furthermore, several mental disorders including Alzheimer's disease (AD) are associated with spine pathology suggesting that spine alterations play a central role in mental deficits. The aim of this review is to provide an overview about the current knowledge on spine morphology and function as well as about different experimental models to analyze spine changes and dynamics. The second part concentrates on disease-relevant factors that are associated with AD and which lead to spine alterations. In particular, data that provide evidence that Aβ oligomers or fibrillar Aβ deposits influence spine morphology and function will be presented and the contribution of tau pathology will be discussed. The review ends with the discussion of potential mechanisms how disease-relevant factors influence dendritic spines and whether and how spine changes could be therapeutically suppressed or reversed.

INTRODUCTION

A characteristic feature of neurons is their functional and structural differentiation into a signal-receiving (somatodendritic) and a signal-transducing (axonal) compartment. Dendrites can receive thousands of synaptic inputs from other neurons, which are then integrated and processed in a complex manner. Dendrites emenate from the cell body and develop a dendritic arbor specific for the respective neuron type.

Dendritic spines are postsynaptic morphological specializations at dendrites. They are formed by small protruding pieces of the dendritic membrane with the total volume ranging from less than 0.01 to 0.8 µm³ [1]. Spines are the primary site of excitatory input and about 90% of the excitatory synapses in the mature brain end on spines. Mature dendrites have up to 10 spines/µm of dendrites. In total, a typical CA1 pyramidal cell of the hippocampus has approximately 30,000 spines with 50% of them located in stratum radiatum and 40% in stratum oriens [2]. Spines may function as a micro-compartment for segregating postsynaptic responses [3]. Thus, changes in spines may not only be important for learning and memory but also for neurodegenerative processes involving changes in signal transduction such as those occurring in Alzheimer's disease.

SPINE SHAPE AND FUNCTION

Spines have various shapes but they all are essentially formed by a neck and head attached to a dendritic membrane. Three major classes based on the relative size of the spine head and neck can be distinguished [4] (Fig. 1). Thin spines have a small head and a narrow neck (<0.6 µm in diameter). Their total length is greater than the neck diameter and the head is not bulbous. Stubby spines have no obvious constriction between the head and attachment to the shaft. Mushroom spines have a constricted narrow neck and a large irregular head (>0.6 µm in largest diameter).

Each functional spine consists of postsynaptic density (PSD) usually at the head where the synaptic junction is located. PSD is an electron-dense thickening of the membrane in which hundreds of components including receptors, cytoskeletal proteins and associated signalling molecules are involved in a number of signalling pathways controlling synaptic plasticity. PSD ranges in shape from a disc (macular PSD) or a perforated annulus (perforated PSD), to a highly irregular or segmented structure. Perforated PSDs are consistently associated with more α-amino-3-hydroxy-5-methyl-4-isoxazole receptors (AMPARs) than the non-perforated PSDs. Mushroom shaped spines are more likely to have perforated shaped PSD whereas thin spines contain macular PSD [5].

Spines have an actin-based cytoskeleton. Occasionally, the larger CA3 dendritic spines have one or more microtubules within them [6]. Some pyramidal cell spines contain a structure called spine apparatus, an organelle formed by two or more disks of smooth endoplasmic reticulum (SER) separated by electron-dense material [7]. Large spines are associated with spine apparatus, whereas small spines are less likely to contain this structure. SER likely regulates the concentration of calcium in the spines. Therefore differently sized spines may have different ways of controlling calcium homeostasis [5].

There are several views how mature spines are formed. According to one hypothesis spines can form de novo. A filipodium shoots out of the dendrite, forms a contact with the presynapse and then collapses to become a short 1-2 µm mature spine [1]. In another hypothesis, spines are constantly formed and stabilized by the presynaptic partner into a func-
tional spine of any morphology [7]. However, data from many experiments suggest that no general path of spine formation may exist but that spines may form by different mechanisms.

The currently accepted opinion is that the spine functions as a microcompartment for segregating postsynaptic response [3]. In many studies, a correlation between the spine neck and the postsynaptic calcium response has been shown, proposing a spine as an unique calcium compartment [8], which could be important for the regulation of the phosphorylation of synaptic proteins that is necessary for synaptic plasticity [9]. Since large spines are more likely than smaller spines to contain SER, the calcium concentration may be regulated more tightly. In addition, large spines have larger PSDs, which anchor more AMPARs making the synapses functionally stronger than thin spines that contain predominantly N-methyl-D-aspartate glutamate receptors (NMDARs). From these features it has been suggested that mushroom spines represent more stable “memory” spines whereas thin spines could be “learning” spines.

The mechanism and relationship between spine alteration and learning and memory is still unclear. Experimental models to analyze spine dynamics and changes could contribute information, which would also be important for a better understanding of disease related impairment in spine function.

**EXPERIMENTAL APPROACHES TO ANALYZE SPINE DYNAMICS AND CHANGES**

In neurobiological investigations, several approaches have been used to analyze spine density, morphology, dynamics or function. Fixed cell cultures or tissues are appropriate to explore changes in density or morphology of populations of spines after exposure to putative spine effectors. In contrast, spine dynamics, i.e. change in spine shape, spine motility and the appearance or loss of spines over time can only be analyzed in living cell or tissue cultures or in vivo.

Classically, spines can be analyzed after Golgi-staining of brains and sectioning followed by light microscopy. While this method allows determining densities and morphological features of spine populations in brain regions of humans and animals, experimental manipulations are limited. The most simple culture model for spine analysis are differentiated, dissociated cortical or hippocampal primary neurons, which permit visualization of spines by standard microscopic techniques. However, during culture preparation synaptic contacts are disrupted and have to be reformed in vitro. The artificial re-establishment of synaptic contacts can be avoided using hippocampal slice cultures, where neuronal connections remain largely intact. An additional advantage is the possibility to analyze distinct subregions which may differ in their connectivity. Spine density and morphology may be analyzed manually or automated with the latter having the advantage of avoiding a potential bias posed by the researcher. This approach is exemplified by the study of Shanhani *et al.* [10], where spines in CA1 and CA3 neurons of fixed hippocampal slices were analyzed using the algorithm-based software 3DMA-Neuron [11]. This software allows a semi-automated analysis of spine density, length, volume and shape as it divides spines into the three classes “mushroom”, “stubby” or “thin”.

One of the first live imaging studies used hippocampal primary neurons expressing GFP-actin [12]. With this approach, rapid actin-based changes in spine shape within seconds were shown. Such high spine dynamics were confirmed in a later study that reported morphological changes in less
than a minute in hippocampal slice cultures [13]. Both experiments identified actin as a key player in spine dynamics since blocking of actin polymerization by cytochalasin D arrested spine motility. Interestingly, blocking of the actin-based motor myosin had no effect. Remarkably, highest spine dynamics were seen at the spine tip where the spine is in contact with the presynaptic bouton [14] suggesting a relation between synaptic transmission and spine motility. Interestingly, activation of AMPARs blocked motility rapidly but reversibly whereas NMDAR stimulation led to long-lasting effects which required 30 min to evolve [15, 16]. Thus, these results link actin-based spine dynamics to effects of glutamate receptor dependent activation such as long-term potentiation (LTP). NMDARs are also involved in the induction of long-term depression (LTD), the weakening of a synapse, by activating the actin depolymerizing factor cofilin through calcineurin [17-19].

Several research groups have analyzed changes in spine morphology that may be associated with LTP. Yang et al [20] used acute hippocampal slice cultures and combined patch-clamp technique to induce LTP with two photon time-lapse imaging to analyze spine growth. They reported a rapid and persistent NMDAR dependent spine expansion after LTP induction. To study vesicle trafficking in pre- and postsynapses and the role of glutamate receptor exocytosis during LTP, dual channel time-lapse two-photon microscopy was used to simultaneously monitor receptor trafficking and spine morphology [21]. Chemical induction of LTP increased spine volume and enhanced AMPAR exocytosis whereas the density of NMDARs at the spine surface decreased. Interestingly, spine volume increased before accumulation of AMPARs on spine surface occurs, suggesting that spine expansion and glutamate receptor exocytosis follow distinct mechanisms. Contrary, in primary neurons, LTP triggered spine growth via relocation and enhanced exocytotic travelling of endosomal vesicles. Blocking endosomal transport prevented LTP-induced increase in spine volume [22].

Two photon laser scan microscopy (2PLSM) offers also the possibility of investigating spine dynamics in vivo. Since biological tissue strongly scatters light, standard confocal imaging is inappropriate for deep imaging. Application of 2PLSM is a break through in deep tissue imaging and allows imaging several hundred micrometer deep in different tissues [23-25]. By creating a cranial window in the head of mice or rats, dendrites and spines in the cortex can be analyzed by 2PLSM up to several months [26]. However, analysis in the intact brain is restricted to the cortex but via special surgical preparations, cortical tissue above the hippocampus of anesthetized mice can be removed to permit two photon imaging of fluorescence labeled hippocampal CA1 pyramidal neurons in vivo [27].

Spine stability is developmentally regulated and increases during aging as determined from the fraction of persistent spines (lifetime longer than 8 days) in mice from different ages [28]. Interestingly, spine stability varied between different brain regions, which is probably due to the different capacity for experience-dependent plasticity. During a 1-month observation period of spine turnover in the visual cortex of mice, it was found that 73% of spines were stable in young mice whereas 96% were stable in old animals [29]. In the somatosensory cortex, spine stability increased from 35% to 73% during aging [28].

The results show that spines become remarkably stable during aging which may indicate a maturation of neuronal circuits and which may provide the structural basis for long-term memory. Thus, analyzing mechanisms of spine loss in aged animals could provide important information for the pathogenesis of neurodegenerative diseases.

SPINE PATHOLOGY IN AD

AD patients show changes in many brain regions with respect to axonal and dendritic morphology and neuron loss [30]. In addition, loss of synapses and dendritic spines is one of the common abnormalities found in human AD brains. In the acoustic cortex of patients with AD many distorted, dystrophic and degenerated dendritic spines were observed, which were intermixed with some giant spines [31]. The acoustic cortex was analyzed in order to determine a potential neuropathological correlate of the communication impairment in AD patients. These results suggest that spines become deformed during AD compared to normal aged brains. Synapse loss and spine alterations in selected brain regions appear to be an early event during the development of AD since already individuals with mild AD have much fewer synapses (55%) in the stratum radiatum of the CA1 subfield [32]. The reduction in synapses correlates well with tests for cognitive impairment indicating that spine and synapse alterations are structural correlates of cognitive decline in AD. Interestingly, a significant reduction in the number of dendritic spines was also observed in CA1-3 pyramidal neurons in patients with Down’s syndrome before onset of AD [33]. Spine density was then further reduced in Down’s patients with associated AD. However, it should be taken into account that neurons undergo morphological changes also during “normal” aging. The changes include a reduction in dendritic arborisation, dendritic lengths and a decrease in spine number, most likely representing a change in synaptic density [34]. Thus, it appears that spine alterations and loss of synapses are a common feature during normal aging and are pathologically enhanced during the course of AD. It will be interesting to know which molecular factors cause spine impairment in the disease and how these factors interact in order to develop therapeutic approaches to prevent spine pathology and age-related neuronal dysfunction.

FACTORS INVOLVED IN SPINE PATHOLOGY

An important role of Aß as a main factor in the pathogenesis of Alzheimer’s disease is widely accepted and is also supported by familial mutations that affect Aß production [35]. Aß is produced by proteolytic cleavage from the amyloid precursor protein (APP) and aggregates to amyloid plaques, which constitute histopathological hallmarks of AD although the amount of plaques doesn’t directly correlate with the degree of cognitive dysfunction in patients. Instead, elevated levels of soluble Aß peptides [36, 37] and the number of neurofibrillary tangles (NFTs) [38] correlate with cognitive decline.

In several APP transgenic mouse lines cognitive deficits and amyloid plaques are found in the absence of substantial
neurodegeneration [39, 40]. This observation led to the hypothesis that alteration of neuronal connectivity by Aβ may precede neurodegeneration for which other factors beside Aβ, e.g., tau protein, are required. Whether amyloid plaques or lower molecular weight Aβ oligomers (ADDLs) are responsible for spine and synapse alterations is still a matter of debate. Evidence for plaque related spine alterations is given by the finding that within a radius of 40 μm around plaques in a plaque-forming mouse model (PS1/APP mouse line) dendrites show varicosity formation, sprouting and spine loss. Similar changes were seen in human post-mortem AD brain [41]. In another plaque-forming mouse line, Knafo et al [42] found differential effects in plaque-near and plaque-free regions. Whereas dendrites crossing plaques showed a strong reduction in spine density and an increase in spine length, the volume of spine heads was only decreased in plaque-free regions. This suggests that both, amyloid plaques and ADDLs, affect spines. ADDLs may weaken synapses without causing a complete loss of synaptic contacts. In vivo multiphoton imaging revealed no obvious pathology at ages before plaques appear but increased spine elimination combined with stable spine formation after onset of plaque formation. This resulted in spine loss in plaque-bearing mice [43]. Interestingly, a study by Jacobsen et al. [44] in the same mouse line reported decreased spine density and impaired LTP long before plaques appeared. While it may not be excluded that both plaques and ADDLs contribute to synaptic alterations in AD, more studies focus now on oligomer-induced pathology. For example, several in vitro studies showed that soluble ADDLs caused a decrease in spine density [45, 46], impaired LTP [47, 48] and were responsible for alterations in spine morphology like an elongation of spines [49]. Support for ADDL-mediated spine pathology comes also from a study in which plaque-bearing APP transgenic mice having memory deficits were treated with an antibody against Aβ [50]. In these mice, memory improved although plaque levels remained unchanged. Another study using a different antibody and another mouse line came to similar results [51]. The data suggest that memory impairment is not only caused by plaques but also by more soluble Aβ species. Besides its effect on spine density and morphology, Aβ appears also to affect the motility of spines. Using two photon time lapse imaging of hippocampal neurons, Shrestha et al. [46] observed a strong decrease in spine motility in ADDL-treated cultures.

Besides amyloid plaques, AD is also characterized by the formation of neurofibrillary tangles composed of hyperphosphorylated and aggregated tau protein. The influence of tau modification on neuronal connectivity is still a matter of debate. In hippocampal primary neurons overexpressed human tau caused a loss of spines [52]. In transgenic mice expressing tau mutants that differ in their aggregation propensity, tau appeared to contributed to spine loss [53]. This is in contrast to a study from Shahani et al [10] in which human tau or a hyperphosphorylation-mimicking tau mutant (PHP tau) were overexpressed using viral infection in hippocampal slice cultures. While massive neurodegeneration, as evidenced by a loss of the majority of PHP-tau expressing neurons in the CA3 and dentate gyrus region, was observed, no alteration of spine density or morphology of the remaining neurons occurred. This suggests that spine alteration and neurodegeneration are affected differentially and that tau may not have a major role in spine changes that are observed early during disease progression.

Currently, most data suggest that increased levels of Aβ - either in an oligomeric or plaque-like form - is the major factor that is responsible for disrupting synaptic connections in AD (see Table 1). This is also supported by the finding that mutations of the presenilins, which are found in familial forms of AD (FAD), shift the processing of APP to the amyloidogenic form of Aβ (Aβ42) [54]. Thus, presenilins may affect spines also by increasing the levels of Aβ. However, it cannot be excluded that disease-related factors affect spines also by other mechanisms.

**POTENTIAL MECHANISMS OF AD-RELATED SPINE PATHOLOGY**

The mechanism of LTP and how Aβ may induce spine pathology is schematically depicted in Fig. (2). Amyloid β induced changes in density and morphology of spines may occur via direct binding of ADDLs to excitatory synapses. In support of this hypothesis, ADDLs colocalized with PSD-95, a marker protein for excitatory synapses, in hippocampal primary neurons [55] and bound to or near NMDARs [49]. Since NMDARs are essential for synapse function and LTP, Aβ-induced alterations such as a decrease in NMDAR density via increased endocytosis [56] could explain memory failure in AD. This is consistent with studies where NMDAR antibodies and a NMDAR antagonist were reported to block ADDL binding [57]. Although other NMDAR antagonists failed to block binding, they protected synapses and spines from ADDL induced toxicity [58]. Besides NMDARs also AMPARs may be targets of ADDLs. Hsieh et al. [59] showed that Aβ reduces AMPAR currents in hippocampal slice cultures. The surface density of AMPAR was decreased by increased endocytosis, along with a general decrease in spine density.

As discussed previously, high spine motility appears to be coupled to the dynamic state of the actin cytoskeleton. Therefore, alterations of spine morphology and motility found after ADDL exposure may be caused by an effect on actin binding proteins. In fact, Aβ has been shown to down-regulate the levels of the actin stabilizing protein drebrin A [60]. Similar results were obtained in mature hippocampal primary cultures [49]. This fits to data where it has been shown that drebrin A is decreased in brains of AD patients [61-63]. In other studies it has been shown that the actin depolymerizing factor coflin becomes activated by Aβ [64]. Blocking coflin and calcineurin both prevented Aβ-induced spine loss in hippocampal slices [45]. Therefore, ADDLs may mimic a blockade of NMDARs either by their inactivation, reduced calcium influx or increased NMDAR-triggered activation of calcineurin, which downstream activates coflin. The effect of Aβ on dendritic spines could be blocked by Rolipram in hippocampal slice cultures [46]. Rolipram, a phosphodiesterase inhibitor, stimulates the cAMP/PKA/CREB pathway that is involved in LTP and prevented Aβ induced inhibition of CREB. Indeed, active CREB levels are decreased in AD patients [65, 66]. Interestingly, CREB is also essential for the formation of spines in culture [66] and becomes inactivated during LTD. These
**Table 1.** Disease-Relevant Factors and Models for Analysis of Spine Changes

<table>
<thead>
<tr>
<th>Factor</th>
<th>Model</th>
<th>Effect on Spines</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Amyloid plaques</td>
<td>APP/PS1 mice</td>
<td>Dendrites sprout and develop varicosities; spine loss near plaques</td>
<td>[41]</td>
</tr>
<tr>
<td>Amyloid plaques</td>
<td>APP/PS1 mice</td>
<td>Reduction of density and increase in length of spines near plaques; decrease in spine head volume in plaque-free regions</td>
<td>[42]</td>
</tr>
<tr>
<td>Amyloid plaques</td>
<td>APP mice</td>
<td>Spine loss in plaque-bearing mice</td>
<td>[43]</td>
</tr>
<tr>
<td>Soluble Aβ</td>
<td>APP mice</td>
<td>Spine loss and impaired LTP before plaque appearance</td>
<td>[44]</td>
</tr>
<tr>
<td>Aβ oligomers</td>
<td>Hippocampal slice cultures</td>
<td>Progressive spine loss after exposure to soluble Aβ oligomers</td>
<td>[45] [46]</td>
</tr>
<tr>
<td>Aβ oligomers</td>
<td>Acute hippocampal slices</td>
<td>Inhibition of LTP, induction of LTD</td>
<td>[47] [48]</td>
</tr>
<tr>
<td>Aβ oligomers</td>
<td>Hippocampal slice cultures</td>
<td>Reduced spine motility after exposure to Aβ oligomers</td>
<td>[46]</td>
</tr>
<tr>
<td>Aβ oligomers</td>
<td>Differentiated hippocampal primary neurons</td>
<td>Reduction in density and increase in length of spines after Aβ oligomer exposure</td>
<td>[49] [55]</td>
</tr>
<tr>
<td>Aβ</td>
<td>APP/PS1 mice</td>
<td>Decrease in drebrin A levels in spines</td>
<td>[60]</td>
</tr>
<tr>
<td>Tau</td>
<td>Virus-mediated expression of tau constructs in hippocampal slice cultures</td>
<td>Spine density and morphology is stable against hyperphosphorylation mimicking tau mutants</td>
<td>[10]</td>
</tr>
<tr>
<td>Tau</td>
<td>Hippocampal primary neurons</td>
<td>Tau redistribution to somatodendritic compartment causes spine loss</td>
<td>[52]</td>
</tr>
<tr>
<td>Tau</td>
<td>Tau transgenic mice</td>
<td>Tau aggregation increases spine loss</td>
<td>[53]</td>
</tr>
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**Fig. (2).** Schematic representation showing the mechanisms of LTP (left) and the pathways affected by ADDLs (right). See text for details.

Results are in agreement with a recent study from Shankar et al. [47], in which ADDLs induced LTD after 300 pulses at 1 Hz, whereas no LTD was observed as a result of this stimulus in the absence of Aβ. Similarly, in acute slice preparations from APP transgenic mice (ArcAβ mice) a severe impairment of LTP was observed [48]. Taken together, the data suggest that Aβ may harm excitatory synapses by inducing a LTD signalling pathway accompanied by LTD-mediated spine retraction [67, 68].

It remains to be shown in how far receptors other than NMDARs are involved in Aβ mediated spine changes. In addition, the downstream events that finally regulate spine dynamics and shape, e.g. a potential involvement and interactions of different cytoskeletal elements, are largely unknown. This information might be very helpful to develop effective therapeutic strategies.

**THERAPEUTIC APPROACHES**

Current data suggest that spine pathology in Alzheimer’s disease is mainly caused by Aβ. Thus, treatment strategies based on a reduction of Aβ, an increase of Aβ clearance or an inhibition of oligomerization appear to be most promising and have been covered in other review articles [69].

Besides addressing Aβ, factors that positively influence spine dynamics could also have therapeutic potential. A candidate could be the brain derived neurotrophic factor (BDNF). BDNF, which signals through its receptor TrkB, has been shown to modulate structural plasticity and spine development in different types of nerve cells including mature hippocampal neurons [70, 71]. BDNF and its receptor are expressed in brain regions exhibiting a high degree of plasticity, such as the hippocampus [72]. During cortical development levels of BDNF rise, which may be important for spine maintenance in adult brains [73]. Spine development was strongly dependent on protein synthesis linking it to LTP [74]. Application of small molecules that function as
ligands for neurotrophin receptors (for review see [75]) may provide a new therapeutic strategy to positively influence spine dynamics and stabilize spines against disease-related loss.

Levels of sex hormones, such as testosterone and estradiol decrease with aging, which has been suggested to contribute a risk for cognitive decline and dementia [76, 77]. It has been shown that testosterone is required to maintain spine density in the CA1 region of the hippocampus in gonadectomized male rats [78] suggesting that it stabilizes spines. Also the steroid estradiol and neurosteroids such as pregnenolone sulfate increase spine strength in hippocampal neurons[79, 80]. At least in the case of estradiol, also increased spine density and involvement of NMDARs were observed [79, 81]. Thus, administration of sex hormones or affecting steroid signalling may constitute a neuroprotective strategy by stabilizing spines during neurodegeneration.

Recent data indicates that also microtubules may play an important role in regulating dendritic spine plasticity, especially in mushroom spines. This is suggested by the finding that knock-down of a microtubule plus-end binding protein in hippocampal primary neurons reduced spine number and microtubule-altering drugs affected BDNF-induced increase of spine density [82]. It is possible that microtubule-stabilizing agents such as taxol may prevent Aß-induced spine loss during AD.

CONCLUSIONS

Many data show that spine morphology and dynamics are closely linked to learning and memory performance and that the spine loss observed in neurodegenerative diseases is causally linked to the devastating cognitive decline. Spines are regulated by multiple processes including NMDAR-dependent processes and actin dynamics. During AD, Aß appears to have a key role in causing spine loss via multiple mechanisms. Attacking Aß in its effects on spines or supporting spine dynamics may be effective in preventing AD-related spine loss, thus slowing down or even preventing disease progression.

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