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INFLUENCE OF AGING AND NEURODEGENERATION ON DENDRITIC SPINE MORPHOLOGY

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Abstract

In neuronal circuits, excitatory synaptic transmission predominantly occurs at postsynaptic protrusions called dendritic spines. Spines are highly plastic structures capable of formation, enlargement, shrinkage, and elimination over time. Individual spine morphology is widely variable, and evidence suggests these differences in morphology are relevant to spine function. Recent reports provide evidence that spine structural plasticity underlies functional synaptic changes, including those seen in animal models of learning and memory plasticity. Conversely, impairments in cognitive functions, such as those commonly seen in aging, have recently been linked to and correlated with alterations in spine density and morphology. In addition, dendritic spine density and morphology also appear to be altered in various transgenic animal models of neurodegenerative diseases. Ultimately, an understanding of the synaptic basis of age- and disease-related cognitive impairments may lead to the development of drug treatments that can restore or protect synaptic profiles in neural circuits that mediate cognition.

Keywords

• Dendritic spine • Aging • Neurodegeneration • Cognition • Synapse • Alzheimer's disease

Abbreviations

AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid
NMDA	*N*-methyl-D-aspartate
SSTEM	serial section transmission electron microscopy
PP	perforant path
DNMS	delayed nonmatching-to-sample
EM	electron microscopy
AD	Alzheimer's disease
HD	Huntington's disease
PD	Parkinson's disease
DA	dopamine
MSN	medium spiny neuron
DLB	dementia with Lewy bodies
CJD	Creutzfeldt-Jakob disease
PrP	prion protein
Aβ	amyloid beta protein
CDR	clinical dementia rating
APP	amyloid precursor protein

1. Introduction

The vast majority of excitatory connections within neuronal circuits form synapses onto dendritic spines, which are specialized postsynaptic structures for glutamatergic neurotransmission. These tiny protrusions generally range between 0.001 and 1 μm^3 in volume, and are studded along the dendritic shafts of principal neurons in densities as high as 10 spines per mm of dendrite [1]. Spines play multiple roles in neuronal circuits; for example, the presence of spines drastically enhances the number of synaptic connections per area of dendrite, and spines also act to maintain input specificity by serving as isolated microdomains for calcium dynamics, receptor trafficking, and intracellular signaling molecules (for reviews, see [2-5]).

Spines vary widely in their morphology along a dendrite (Figure 1). Investigators have divided spines into three basic morphological subtypes; spines with no necks and a stubby-like appearance ("stubby spines"), spines with small necks and a large, often complex, and irregular heads ("mushroom spines"), spines with and thin necks and small heads ("thin spines") [6]. An additional set of structures called filopodia have long, thin protrusions with no obvious head. Filopodia, however, are most commonly seen during development and are rarely seen in the mature brain [7]. It is well established that the large majority of spines in the adult brain exhibit thin spine morphology [8].

Evidence suggests spine morphology may be a major determinant of spine stability and spine synaptic strength. For example, *in vivo* evidence from mouse neocortex has shown that a majority of mushroom-type spines remain stable across multiple days or even months [9-11], while thin spines often appear and disappear rapidly (i.e., within a day) [7, 10, 12, 13]. These differences suggest that spine subtypes have different roles maintaining synaptic stability and plasticity within neural circuits. Spine morphological differences are also reflected by distinct physiological and molecular characteristics; for example, large spines are dominated by α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid-type (AMPA) glutamate receptors and have greater glutamate-elicited excitatory postsynaptic potentials, while thin spines appear to be *N*-methyl-D-aspartate (NMDA) receptor-dominated and comparatively less sensitive to glutamate [14-16].

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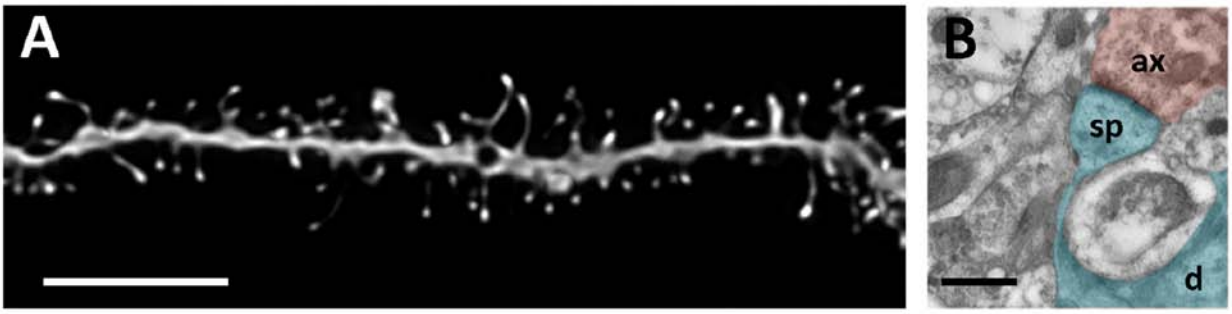

Figure 1. Visualization of dendritic spines. A) Dendritic spines along a neocortical dendritic segment from a neuron labeled by intracellular injection of fluorescent dye and captured with confocal laser microscopy. Maximum spine head diameters vary from 0.14 to 0.70 µm, while spine surface areas vary from 0.32 to 2.55 µm². Scale bar = 5 µm. B) An axospinous synapse from prefrontal cortex as visualized by electron microscopy. The dendritic shaft ("d") and spine ("sp") are highlighted in blue, while the presynaptic axon ("ax") is highlighted in red. Scale bar = 500 nm.

Taken together, these observations raise fundamental questions regarding how spine morphological plasticity might underlie cognitive functions such as learning and memory [13,17-19]. There is some evidence demonstrating spine morphological plasticity with learning [20-25], as well as evidence that the converse hypothesis may be true; that is, failure of spine formation and morphological plasticity may contribute to impairments in cognition [26]. Additional support for the latter hypothesis comes from studies that report alterations of spine numbers and morphology in animal models of aging [27-32] or neurodegenerative disease [33-39], both of which are characterized by compromised cognitive function.

It is this last area of research that the current review will focus on. As the aging population grows, understanding the synaptic basis of age- and neurodegeneration-associated cognitive decline could identify rational targets for therapeutic intervention, and perhaps most importantly, prevention. Studies focused on abnormal patterns of spine morphology and altered plasticity in aging and with disease can also be informative as to how spines function in the healthy brain. The remainder of this review, therefore, will focus on three themes: 1) a brief discussion of the techniques that are commonly used to visualize dendritic spines; 2) studies that have investigated spine morphology in models of normal aging; and 3) studies focused on the relationship between dendritic spines and various models of neurodegenerative disease.

1.1 Techniques that allow visualization of dendritic spines

1.1.1 Issues regarding data bias, image resolution, and throughput

The elucidation of how age and disease affect spine morphology is made difficult by the dynamic temporal and spatial characteristics of dendritic spines. As mentioned above, spines predominantly exist as small spine heads with long, slender, necks. More often than not, these thin spine structures are approximately 100 to 400 nm in width, and are at the limit of resolution of conventional light microscopy [8]. Nonetheless, most spine data currently in the literature is based upon Golgi impregnation studies, which suffer from three limitations: 1) the Golgi stain impregnates some, but not all cells, which raises questions about sampling biases, 2) Golgi-stained neurons are very difficult to accurately quantify in the z-axis, leading to both an underestimation of spine number or density and a bias in spine sampling, and 3) Golgi does not lend itself to rigorous quantitative analysis of spine volume and other morphometric parameters.

Fluorescence-labeling strategies such as intracellular injections of dyes and genetic expression of fluorescent molecules have several advantages over traditional Golgi methodology. Intracellular injection protocols allow specific subpopulations neurons to be labeled, including neurons labeled with anterograde or retrograde tracers for circuit-level analyses [29, 40]. In addition, these labeled cells can be coupled with quantitative immunofluorescence for double- or triple-labeling studies [41]. Using genetic approaches, subsets of neurons can be engineered to express fluorescent proteins under the control of a specific gene promoter [42], allowing a level of molecular specificity that is otherwise unachievable using Golgi or electron microscopy (EM). These fluorescent techniques, when combined with confocal, two-photon, or stimulation emission-depleted microscopy enable high resolution and fully-automated reconstructions in 3-dimensions. These technical advances provide more accurate spine density numbers, and generally allow for more precise and quantitative datasets [43-45].

Nevertheless, there are drawbacks to fluorescent labeling and laser-scanning techniques. Although data deconvolution improves image resolution, particularly along the z-axis, all three laser microscopy methods suffer from the unavoidable optical aberration in the z-plane which distort spine volumes [46]. To avoid these problems, studies commonly employ serial section transmission electron microscopy (SSTEM). SSTEM remains the gold standard for spine reconstructions because it achieves the greatest resolution, the most precise volumetric measures, and unambiguous identification of spine synapses. However, this technique remains labor-intensive and extremely low-throughput (Figure 1B) [8], and thus poses experimental design issues of its own.

1.1.2 Spine plasticity as a static vs. dynamic process

A second caveat to most of the above-mentioned techniques is that they only provide a single time-point snapshot of spine structures

that are known to be morphologically and temporally plastic. For example, a majority of these techniques —including SSTEM — require fixed tissue, which prevents repeated sampling of spines after experimental manipulations. As such, perhaps the best way to interpret these data is as an indirect index of capacity for plasticity, rather than a direct measure of spine plasticity itself.

In contrast to the methods that require fixed tissue, confocal or two-photon live imaging of slices or dissociated cultures allows repeated spine sampling after experimental manipulations. However, these methods leave questions as to whether spine dynamics are similarly regulated between *in vitro* and *in vivo* conditions. Two-photon microscopy combined with transgenic mice expressing fluorescent proteins in neurons has recently allowed investigators to measure spine plasticity and stability *in vivo* [5, 47], but requires surgical manipulations that may provoke inflammatory responses and alter spine plasticity [48]. Lastly, *in vivo* two-photon studies are restricted to the overlying cortex and, with the exception of few studies (e.g. [49]), have not yet been extended to deeper brain structures such as the hippocampus.

1.2 Evidence for aging-related changes in spine morphology

1.2.1 Selective spine vulnerability in the perforant path-dentate gyrus circuit

A common misconception about brain aging is that cognitive decline is a reflection of neuronal death. In fact, modern stereological methods have provided evidence that neuronal circuitry remains very much intact in the aging brain, with little evidence for gross anatomical change. For example, declines in cognitive abilities in aging animals models are not associated with reductions in neuron numbers in hippocampus [50] or neocortex [51-53], save for a single exception in area 8a of the primate prefrontal cortex [54]. With these data in hand, investigators have focused on the hypothesis that aging may be manifested by subtle losses in network connectivity via reductions in dendritic complexity, spine synapse density, and morphological plasticity [55]. Rodents and non-human primates provide excellent models for testing these hypotheses, as they show similar age-related declines in long-term memory and executive function but do not suffer from neurodegenerative diseases [56].

The strongest evidence in support of age-related synaptic morphological change in the medial temporal lobe memory system has largely come from SSTEM reconstruction studies. Geinisman and colleagues [27, 28] have shown that aged rats that are impaired in a hippocampus-dependent spatial memory task show reduced density of large, perforated synapses (synapses with a segmented or non-continuous postsynaptic density). This subtype-specific synapse loss is restricted to perforant path (PP) inputs from the entorhinal cortex onto dentate gyrus granule cell dendritic spines, and does not occur in aged animals with intact spatial memory performance. Furthermore, the loss of perforated synapses correlates with greater cognitive impairments [27]. Hara et al. [31] recently extended these observations to aging Rhesus monkeys, in which perforated synapses from the PP termination zone in the outer molecular layer correlated with performance on a delayed nonmatching-to-sample (DNMS) task. In general, spines and axospinous synapses elsewhere in the hippocampal formation appear to be maintained throughout aging, thus suggesting a selective vulnerability of large hippocampal synapses within the entorhinodentate circuit (for review, see [57]).

1.2.2 Subtle yet extensive thin spine loss in neocortex

In contrast to the limited age-related spine synapse changes seen in the hippocampus, studies focusing on neocortex have suggested more widespread changes in dendritic morphology, dendritic spine number, and spine morphology. Studies in human specimens using Golgi stains have found evidence of dendritic atrophy and dendritic spine loss in association cortex pyramidal neurons [58-60], but minimal changes in neurons from visual cortices suggesting a selective vulnerability of pyramidal neurons in association cortices. In the absence of age-related effects on dendritic morphology, Petanjek and colleagues [61] have reported increased inter-individual variability in dendritic morphology with age in human prefrontal neurons.

Naturally, animal models are more tractable with respect to experimental dissection of the effects of age on neocortical neuronal structure. Using tract-tracing and fluorescence microscopy in young and aged Rhesus monkeys, Duan and colleagues [29] demonstrated that while neurons projecting from temporal association cortex to prefrontal cortex in aged animals had relatively intact dendritic morphology, spine number and density were reduced by as much as 34 and 26%, respectively. Using unbiased electron microscopy, Peters and colleagues [62] recently demonstrated age-related axospinous synaptic loss in layer I of area 46 from aged monkeys that correlated with impairments in working memory.

However, these studies did not clarify whether the age-related spine synapse loss was selective or occurred across all spine types. In a recent study, our group demonstrated layer III pyramidal neurons show a selective loss of small, thin spines (<400 nm in diameter) in aging monkey prefrontal cortex; in contrast, there was no change in the occurrence of large spines (>600 nm) [32] (Figure 2). This selective reduction of thin spines correlated with impairments in acquisition of a DNMS task, suggesting that thin spines within dorsolateral prefrontal cortex might provide crucial support for task learning and behavioral plasticity [32]. In addition, disruption in dendritic and spine morphologies in prefrontal neurons of aged macaque monkeys has been shown using electrophysiologic [63,64] and modeling approaches [65,66], suggesting aging also influences significantly the firing and cable properties of these neurons. As these data were obtained from dendritic segments of layer III neurons, of which a majority are corticocortically-projecting neurons, they suggest age-related decrements in prefrontal functions may be the result of subtle disruptions of corticocortical circuits at the level of the spine.

Taken together, studies from rat and monkey models suggest that selective changes in spine number and morphology in specific neocortical regions is associated with functional

impairments in cognition. Data from the medial temporal lobe memory system suggest a restricted, circuit-specific vulnerability of large, perforated synapses. In contrast, studies from prefrontal cortex suggest an extensive yet selective loss of thin spines and a resilience of large, mushroom-type spines. These studies suggest that the nature of the age-related synaptic vulnerability is distinct between hippocampus and prefrontal cortex. If true, this implies therapeutics designed at increasing the presence of a single type of spine throughout the brain may not result in improved function across all cognitive domains, and may instead result in impaired cognitive functions mediated by other brain structures. This hypothesis is supported by a similar dichotomy between prefrontal/hippocampus regarding pharmacological manipulation of the protein kinase A intracellular signaling pathway to ameliorate age-related cognitive deficits [67].

1.3 Evidence for spine morphological changes in models of neurodegenerative disease

As noted in the introduction, the remodelling of synapses is a fundamental property of neuronal circuits. Neurodegenerative diseases result in synaptic dysfunction, losses of synaptic connectivity, and ultimately neuronal death, each of which may lead to functional impairments. Evidence is mounting that spine dysfunction and synapse loss may underlie the earliest symptoms of many neurodegenerative diseases including prion disease, Huntington's disease (HD), Parkinson's disease (PD), and Alzheimer's disease (AD).

1.3.1 Prion diseases

Prion diseases are fatal transmissible diseases affecting the central nervous system. In animals, prion diseases such as scrapie and bovine spongiform encephalopathy are characterized by non-viral spread of disease proteins from one species to another. In humans, prion diseases can be sporadic (Creutzfeldt-Jakob disease [CJD]), familial (familial CJD, fatal familial insomnia, Gerstmann-Sträussler-Scheinker, acquired through iatrogenic sources), or by ingestion of contaminated meat from infected animals.

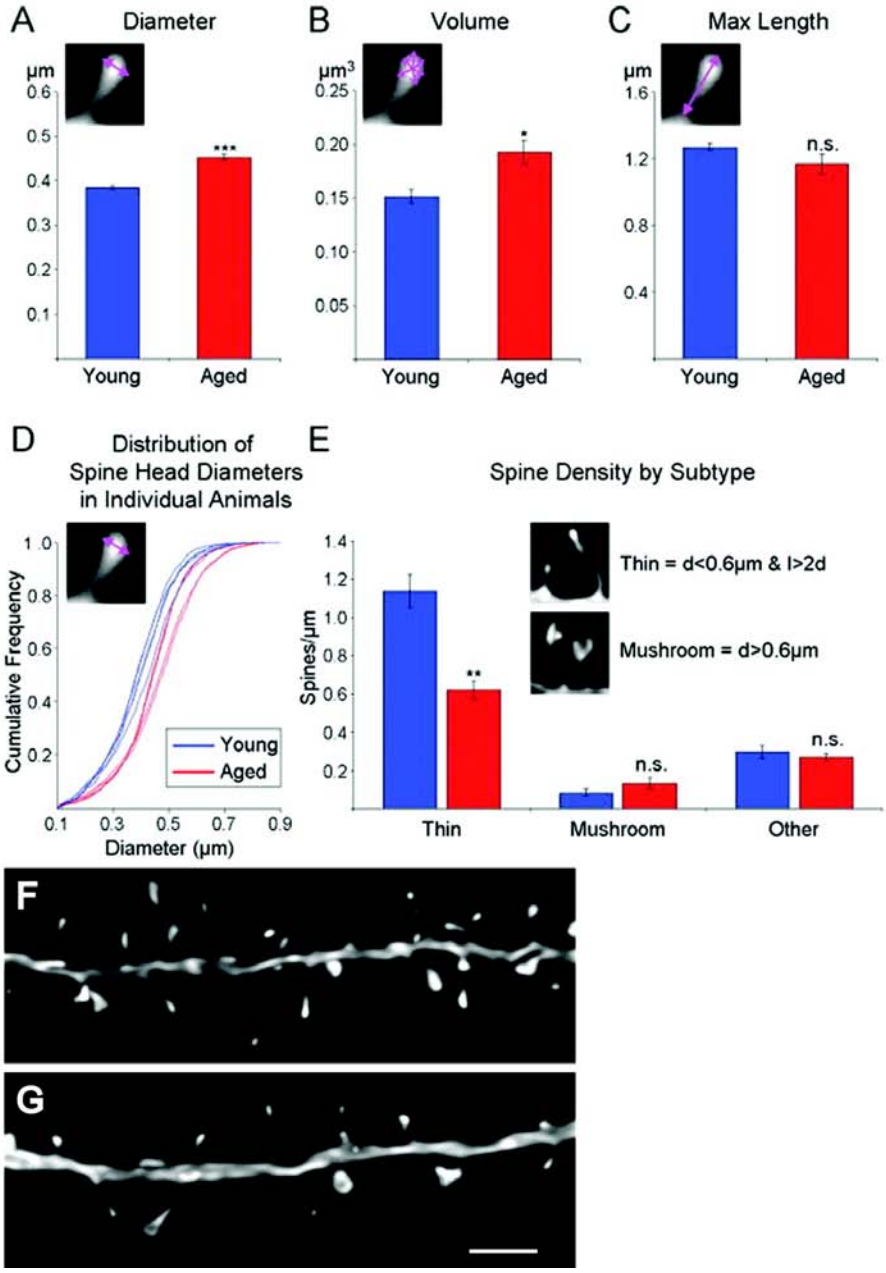

Figure 2. Age-related changes in spine density and morphology in monkey layer III pyramidal neurons from area 46. A) Age-related alterations of spines on layer III pyramidal neurons of the monkey PFC includes increased mean spine head diameter, increased B) mean spine head volume, but not C) mean spine distance from dendrite. D) Cumulative frequency plot of each spine head diameter from individual animals demonstrates a shift towards larger spines in individual animals. E) Quantitative analysis reveals age-related spine losses are comprised solely of thin spines, while mushroom spines remain unaffected by age. Representative three-dimensional reconstructions of dendritic segments from young (F) and aged (G) rhesus monkeys. Scale bar = 2 µm. (Adapted from [32]).

Genetically, prion disease is caused by mutations in the prion protein (PrP) gene. In this instance, cellular prion protein (PrP^{C}), a cell surface glycoprotein, is converted to a partially protease-resistant form (PrP^{Sc}), resulting in protein misfolding and accumulation of proteins in the brain (reviewed in [68]). The self-propagating process of PrP^{Sc} acting as a

recruiter for PrP^C and causing conformational changes that are thought to be critical for the neurotoxicity typically observed in disease pathology [69]. The pathological hallmarks of prion disease are spongiform degeneration of the brain accompanied by synaptic alterations, extensive neuronal loss, astrogliosis, dementia, and locomotor changes [70]. While the physiological role of PrP is debated, several observations suggest that in non-disease states PrP may play a role in synaptic structure, function or maintenance (for review see [71]).

Studies in mice infected with prions have shown cognitive, behavioral, and neurophysiological impairments that correlate with the loss of presynaptic terminals in the dorsal hippocampus [72,73]. As with other neurodegenerative diseases such as AD (see below), it appears that the synaptic dysfunction that occurs in prion diseases is independent of aggregated misfolded proteins. Rather it may be the soluble, non-aggregated proteins causing dysfunction as functional deficits have been shown to occur before accumulation is apparent [69]. Morphological neuronal changes and loss of dendritic spines have been observed in patients suffering from CJD [74]. Further morphological analyses in various prion mouse models have revealed dendritic abnormalities such as the emergence of varicosities and loss of dendritic spines have been well described in the terminal stage of the disease [75-79]. Moreover, during the spongiform stage that occurs in prion-induced neurodegeneration [79], spine loss has been localized to regions of the dendrite that exhibit vacuolar pathology [80]. Live *in vivo* studies by Fuhrmann et al. [79] using two-photon *in vivo* imaging techniques demonstrated an extremely slow and progressive loss of persistent mushroom spines in the somatosensory cortex over several days to weeks, with linear kinetics of 5.9 ± 0.5 spines/mm per day from the presymptomatic phase to the terminal phase of the disease with no change in the density of thin spines. Interestingly, these authors also reported an increase in new spine formation before the largest period of spine loss was observed, suggesting cellular compensatory mechanisms may fail to maintain neuronal circuitry in prion disease models.

1.3.2 Huntington's disease

HD is a neurodegenerative genetic disorder caused by an expanded CAG repeat in the huntingtin gene which translates into an lengthened polyglutamine track in the expressed protein. The neuropathological features of HD include the formation of aggregates of mutated huntingtin protein leading to neuronal death in the striatum and cerebral cortex; in HD patients, these neurobiological changes result in cognitive, psychiatric and motor impairments (reviewed in [81]). In the early stages of the disease, there is a decrease in striatal volume without changes in neuron number which correlates with motor disturbances, indicating that early neurobiological dysfunctions prior to neuron death can initiate HD symptomatology. In support of this hypothesis, many studies have shown that HD causes alterations in dendritic morphology and synapse number. In humans, degenerative changes of striatal medium spiny neurons including truncated dendritic arbors, dendritic varicosities, and decreased spine densities occur in severe grades of HD [82]. Similar changes have also been reported in several mouse models of HD; for example, Golgi studies in mice expressing the full huntingtin gene showed morphologic abnormalities that included a significant decrease in the number of dendritic spines and a thickening of proximal dendrites in striatal and cortical neurons [83]. These mice were symptomatic but had not reached a stage in which neuronal loss could be observed. Another mouse model of HD, R6/2 mice which express exon 1 of the huntingtin gene with different CAG repeat lengths, exhibits different ages of HD-like symptom onset and dysmorphic neurites in pyramidal neurons of the frontal and anterior cingulate neocortex. These alterations were characterized by significant retraction of apical dendrites as well as cytoplasmic vacuoles and plasma membrane blebs of the soma [84]. Recently, Spires et al. [85,86] reported decreases in dendritic spine density and dendritic spine length in striatal medium spiny neurons and anterior cingulate cortex pyramidal neurons in the R6/1 mouse model. While there was no change in the proportion of spine type, there was a decrease in the length of both mushroom and thin spines. These changes in spine morphology might reflect a loss of excitatory input into these brain regions and could potentially account for the cognitive deficits observed in HD [85,86].

1.3.3 Parkinson's disease

Selective degeneration of neurons in the nigrostriatal pathway and an extreme reduction in the striatal concentration of dopamine (DA) are the key pathological hallmarks of PD. PD is an age-related neurodegenerative disorder that affects as many as 1-2% of persons aged 60 years or older and symptoms include motor deficits such as resting tremor, rigidity, bradykinesia/akinesia and a postural reflex impairment [87]. Cognitive impairment, thought to occur due to the presence of limbic and neocortical Lewy body inclusions made of misfolded α-synuclein proteins, is also associated with PD and is found in 30–80% of affected individuals.

The main cell type affected by the loss of DAergic innervation in PD is the medium spiny neurons (MSNs) of the caudate, putamen, and nucleus accumbens that together make up the neostriatum. These neurons account for approximately 90% of striatal neurons and receive DAergic inputs predominantly onto the necks of dendritic spines [88]. The clinical symptoms of PD and dementia with Lewy bodies (DLB) suggest synaptic dysfunction as a potential early mechanism of disease pathology, likely due to the aggregation of α-synuclein in presynaptic terminals [89]. Indeed, numerous studies have demonstrated a reduction in presynaptic protein markers such as syntaxin and postsynaptic markers such as PSD95 and drebrin in DLB and PD patients compared to controls [89-91]. Previous Golgi stain studies in postmortem materials from PD patients showed severe pathological changes in striatal MSNs such as a reduction of total dendritic length, a decrease in dendritic segments, losses of dendritic spines, and several types of dendritic varicosities and swollen perikarya [92-96]. In a more recent extensive postmortem study by Stephens et al. [97], there was a 27% decrease in spine density in both the caudate nucleus and putamen of individuals suffering from PD. In addition,

the size of the dendritic trees (i.e., length and arborization) and the number of dendritic branches was also significantly reduced in the caudate nucleus and putamen from the brains of PD cases [97]. In patients with DLB, there is an almost complete loss of spines in frontal cortical neurons as well as in MSNs of the caudate nucleus compared to controls [89,95]. Rodent models of PD have revealed similar results in MSNs; for example, in a 6-hydroxydopamine rat model of PD there was a 19% decrease in spine density in the prefrontal cortex and basal ganglia [98, 99]. In a nonhuman primate model of PD, there was significant loss of striatal neurons with dopamine denervation with as much as a 50% loss in the sensorimotor striatum, the most affected region [100]. Taken together, it appears that he accumulation of a-synuclein has a detrimental effect on the glutamatergic transmission in the striatum along with denervation of DAergic systems, together which lead to PD- like symtomology and potentially cognitive decline. These initial events may cause retraction of spines, reductions in dendritic arborization, and ultimately the neuronal pathology observed in PD and DLB associated with PD.

1.3.4 Alzheimer's disease

As with the neurodegenerative disorders discussed above, AD also results in pervasive and significant changes in dendritic spine density and structure in addition to overt neuronal death [101]. AD is the most common form of dementia and accounts for approximately 80% of cases [102]. The neurodegeneration that occurs in AD affects neuronal circuits of the perforant path as well as long corticocortical projections that link association cortices [55]. While AD leads to a loss of principal neurons across multiple cortical areas, local interneuron populations remain unaffected [103-106], suggesting neuronal vulnerability to AD is selective and restricted to pyramidal neuron subtypes. The pathological changes that occur in AD have been attributed to both the presence of extracellular amyloid plaques composed of amyloid beta protein (Aβ) and intracellular neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau proteins.

Many studies in various animal models of AD and humans affected with AD have demonstrated that synapse loss and alteration in synaptic structures is a strong correlate of cognitive decline in AD [107]. Whether the spine loss observed in AD is a result of soluble or fibrillar aggregates of Ab, hyperphosphorylated tau, or both, is still unresolved. Studies from our laboratory comparing patients with clinical dementia rating scores (CDR) of 0 and 3 have demonstrated that neurons in AD undergo morphological alterations and show significant spine alterations, as assessed by immunoreactivity to NR1 and GluR3 receptor proteins (Figure 3) (P. Hof, unpublished data). Such changes in the expression or distribution in these synaptic proteins are indicative of changes in the molecular composition of dendritic spines. Other groups have also observed significant reduction in spine density as well as a decrease in overall dendritic area in AD patients when compared to age-matched controls [108-112]. Electron microscopy studies have corroborated these data and found significant synaptic loss in the brains of patients with early onset AD compared to mild cognitively impaired and non-demented individuals [113].

Mouse models that overexpress mutated forms of the amyloid precursor protein (APP) have demonstrated the detrimental effect of Aβ plaques on neuronal morphology including attrition of apical dendritic arbors, aberrant sprouting, and curvature of dendritic processes [34,35,114-117]. In regards to spine pathology, studies in PSAPP and Tg2576 mice have demonstrated significant spine loss from cortical pyramidal neurons located close to or within Aβ plaques and in areas that are devoid of amyloid pathology [34, 117]. Spine loss has also been reported in the CA1 field, the dentate gyrus, and somatosensory cortex [33, 35, 118-125]. In contrast, studies in the dentate gyrus in APP/PS1 mice showed spine reduction in dendrites that pass through plaques and an increase in spine density in dendrites touching plaques with no changes in spine density in area devoid of plaques [37]. Such differences in reported data may be a result of studies in different brain regions, different cell types, different transgene expression, and method of quantification. Nevertheless, while changes in spine density, type, and length are evident,

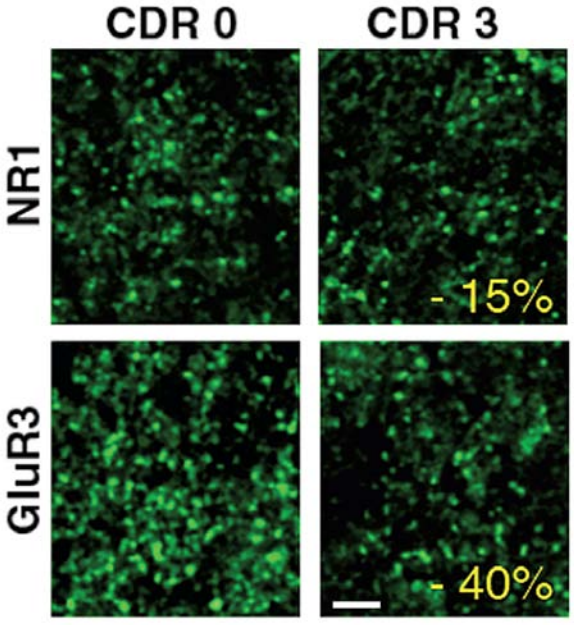

Figure 3. Evidence for spine alterations in patients affected with AD compared to non-demented controls. NR1 and GluR3 glutamate receptor subunit protein immunoreactivity is significantly reduced in CDR 3 cases compared to CDR 0 cases. Scale bar = 10 µm.

there are no data on whether certain spines are more vulnerable to degradation than others. In a recent study Tackenberg *et al*. (2009), using organotypic hippocampus slice cultures of APP_{SDL} mice, found a reduction of spine number and spine length in APP mice compared to controls with a decrease in the number of mushroom spines and an increase in the number of stubby spines. The changes observed here were attributed to the presence of soluble Aβ as treatment of the cultures with a g-secretase inhibitor abolished spine loss [124]. In regards to plaque proximity, one study has shown a decrease in spine head volume in plaque-free spines and in-contact spines compared to spines traversing a plaque and controls in the dentate gyrus [37]. Further analysis of spine characteristics were in agreement with other studies indicating an overall loss of large spines [37, 124]. The mechanism by which Aβ mediates these changes is still uncertain. It is possible that Aβ mediates these changes through many pathways including the inhibition of NMDA receptor activity, calcineurin activity, GSK3β activity, tau phosphorylation, and activation of caspases [124, 125].

The effect of tau on dendritic spine pathology is still not fully understood. Whether tau alone, or tau in concert with Aβ cause alterations in neuronal and spine properties remains a mater of debate. Moreover, it is still unclear if mutations in tau have the same effect on neuronal pathology as non-mutated tau. This is of importance since to date no mutations on tau have been implicated in AD. *Ex-vivo* studies in organotypic hippocampal slices expressing AD-relevant tau constructs found no effect of tau expression on spine density and morphology even though there was apparent tau hyperphophorylation and accumulation with the cell [126]. Recently, Rocher *et al*. [36] examined changes in dendrites and spines in the rTg4510 tau mouse model that harbours the P301L mutation. These authors reported that mutated tau expression severely altered dendritic shafts with significant morphological alterations, including loss or atrophy of the apical tuft, reduced dendritic complexity and length. Moreover, mutations in tau resulted in a ~30% reductions in spine density in cortical pyramidal neurons [36]. Studies from our lab have examined the effect of wild-type tau on dendritic spines. We have found that with age, the hTau mouse model, which expresses all 6 isoforms of human tau and no mouse tau, exhibits significant alterations in apical dendritic architecture from prefrontal neurons of 3 month mice versus 12 month mice (Figure 4) with a reduction in spine volume and a shift from mushroom to thin spines [39]. It is evident that changes in spine density and spine type occur during the pathogenesis of AD and contribute to the cognitive deficits that are associated with disease progression. Whether it is the exposure of neurons to soluble or fibrillar Ab or hyperphosphorylated tau that causes synaptic dysfunction still remains to be elucidated.

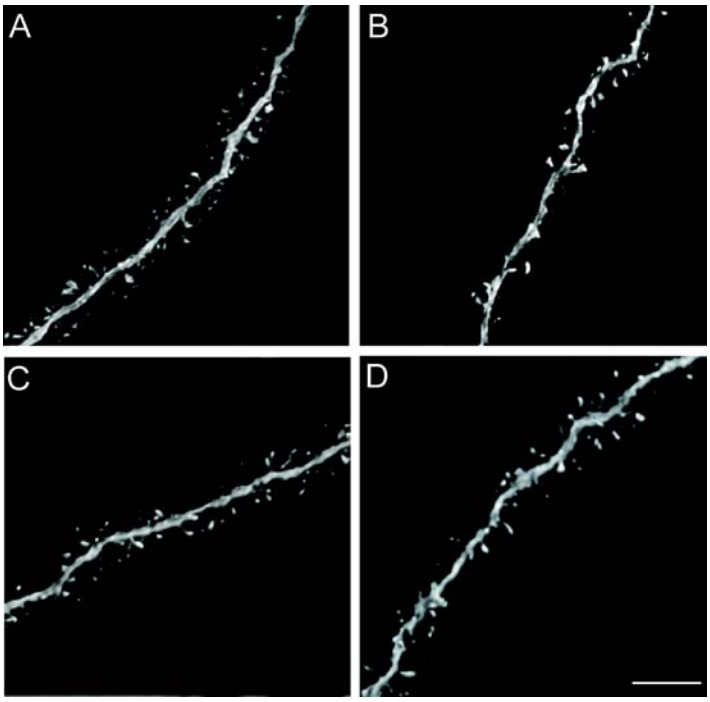

Figure 4. Representative three-dimensional reconstructions of dendritic segments from AD mouse models. Dendritic segments from 24-month-old Tg2576 AD model mice A) wt, B) transgenic and from 12-month-old htau model mice C) wt, and D) htau. Scale bar = 5 µm. (Adapted from [39]).

2. Conclusions

Changes in spine density, morphology, and synapse density are important for addressing the ultimate and proximate causes of cognitive impairment that occur during normal aging and in neuropathological disorders. The data summarized in this review support the hypothesis that age-related alterations in spine plasticity processes can drive some of the cognitive impairments commonly seen in the elderly. Furthermore, animal models of neurodegenerative disease have documented early spine pathology, suggesting that in some disease models synaptic dysfunction precedes some of the more common pathologies linked to these disorders.

An important and open question is to what extent aging neuronal circuits retain the capacity for experience-induced structural plasticity. Recent studies give us reason to be optimistic; for example, aged rats retain the capacity for spinogenesis in dentate neurons in response to environmental enrichment [127], and we have shown aging female monkeys maintain estrogen-induced spinogenesis in PFC neurons [30]. A main focus of future research should be to corroborate and extend these data to models of neurodegenerative

disease with the hopes of slowing or ultimately preventing disease progression. An important consideration should be the restoration of spines and synapses within the context of spine size, as evidence has demonstrated functional differences and differential vulnerability depending on spine size. The data presented here provide compelling evidence of aging-related vulnerable and resilient spine populations, which differ between brain structures and also between disease models. The knowledge gained by understanding the mediators of spine vulnerability and resilience should help pave the way for therapeutics aimed at synaptic preservation or regeneration in both normal aging and in disease states.

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