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Spine pruning in 5xFAD mice starts on basal dendrites of layer 5 pyramidal neurons

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Abstract

Transgenic mice with Alzheimer's disease (AD) mutations have been widely used to model changes in neuronal structure and function. While there are clear gross structural changes in post-mortem brains of AD patients, most mouse models of AD do not recapitulate the considerable loss of neurons. Furthermore, possible connections between early subtle structural changes and the loss of neurons are difficult to study. In an attempt to start unraveling how neurons are affected during the early stages of what becomes full neurodegeneration, we crossed a mouse model of familial AD, which displays massive neocortical neurodegeneration (the 5xFAD mouse), with the fluorescent H-line YFP mouse. This novel bigenic mouse model of AD, which we have named the 5XY mouse, expresses YFP in principal neurons in the cortex such that even fine details of cells are clearly visible. Such bright fluorescence allowed us to use high-resolution confocal microscopy to quantify changes in spine density in the somatosensory cortex, prefrontal cortex, and hippocampus at 2, 4, and 6 months of age. A significant loss of spines on basal dendrites in the somatosensory and prefrontal cortices of 6-month-old 5XY female mice was found. There was no observed spine loss at 6 months of age on the oblique dendrites of the hippocampus in the same mice. These data suggest that spine loss is an early event in the degeneration of the neocortical neurons in 5xFAD mice, and a likely contributor to the cognitive impairments reported previously in this AD mouse model.

Keywords

spine density; spine type; Alzheimer's disease; 5xFAD; neurodegeneration

Introduction

The primary characteristics of pathology in Alzheimer's disease (AD) are amyloid plaques, neurofibrillary tangles (NFTs), and neuronal loss. However, not all of these pathological characteristics correlate strongly with cognitive decline, the clinical manifestation of the disease. The loss of synapses and neurons in the brain most strongly correlates with clinical scores of dementia (Selkoe 2002; Scheff and Price 2006; Akram et al. 2008; Luebke et al. 2010), while the correlation between plaque load and cognitive function is poor (Morrison and Hof 2007). Dendritic spines represent the postsynaptic component of the synapse and

play a crucial role in cognition, learning, and memory (Lamprecht and LeDoux 2004). Both spine number and morphology have been shown to relate to synaptic strength (Matsuzaki et al. 2001). Changes in either or both spine number and morphology can have profound effects on the function of individual neurons, as well as neural circuits. In several studies, the structural and functional changes of spines have also been studied in models of neurodegenerative diseases, traumatic brain injury, and stress (Halpain et al. 2005). In particular, studies of spine density in AD mouse models have revealed synaptic alterations in the forms of spine loss in various brain areas and shifts in spine morphology distribution (Knafo et al. 2009; Tackenberg et al. 2009; Dickstein et al. 2010). There have been many reported factors associated with the loss of spines in AD, such as proximity to A β plaques (Knowles et al. 1999; Spires and Hyman 2004; Tsai et al. 2004; Spires et al. 2005; Knafo et al. 2009), the presence of NFTs (Dickstein et al. 2010), increased levels of oligomeric A β (Selkoe 2002; Lacor et al. 2007), and intraneuronal A β (Takahashi et al. 2002).

The discovery of genetic mutations associated with familial AD (FAD), or early onset AD, revolutionized the ability to study the disease by enabling the creation of transgenic mouse models. The transgenic expression of mutated human proteins amyloid precursor protein (APP) and presenilins induce various degrees of AD-like pathology. Despite this, there is currently no mouse model with FAD mutations in APP and/or presenilins that recapitulates the development of amyloid plaques, NFTs, and subsequent neuronal loss (Duyckaerts et al. 2008). Most of the transgenic mouse models of FAD develop plaques, but fail to induce significant neuronal loss (Duyckaerts et al. 2008). However, the 5xFAD mouse model displays massive neuronal loss in the neocortex, as well as intraneuronal A β and extensive plaque pathology (Oakley et al. 2006). This mouse model expresses five FAD transgenic mutations in total: the Swedish, Florida, and London mutations of the amyloid precursor protein (APP) and the M146L and L286V mutations in presenilin 1 protein (PS1). A β ₄₂ is over-expressed in 5xFAD mice to a level that is about 4.5 times that detected in humans with AD (Oakley et al. 2006; Roher et al. 2009). The combination of transgenic FAD mutations induces intraneuronal A β accumulation and extracellular plaques as early as 2 months of age, and significant neuronal loss by 18 months of age (Oakley et al. 2006).

We crossed the 5xFAD mouse line with the H-line YFP mouse line to create a novel bigenic fluorescent AD mouse model, which we call “5XY”. The 5XY mouse was designed to enable longitudinal two-photon imaging of neuronal integrity with disease progression. However, this powerful method for intravital imaging is limited in several ways. In particular, spines cannot be clearly imaged beyond 600 μ m below the pia, thus dendrites in the hippocampus are inaccessible to *in vivo* high-resolution fluorescence imaging. Secondly, spine shapes cannot be classified as clearly when compared to *ex vivo* imaging with high numerical aperture lenses. In this study we report high-resolution fluorescence imaging of individual dendritic segments of pyramidal neurons in the somatosensory cortex, prefrontal cortex, and hippocampus at 2, 4, and 6 months of age. At 6 months of age, 5XY mice showed a significant spine loss in the somatosensory and prefrontal cortices, but not in the CA1 field of the hippocampus. A total of 24,413 spines were counted allowing high significance to these results. Furthermore, low magnification imaging revealed that there is significant degradation of layer 5 pyramidal neurons in the somatosensory cortex and

prefrontal cortex. The pyramidal neurons of CA1 in the hippocampus remain structurally stable up to 16 months of age. These novel results show for the first time the selective loss of spines in the somatosensory and prefrontal cortices is an early subtle structural change in the 5xFAD mouse model of AD.

Materials and Methods

Animals

The 5xFAD mouse line (B6.Cg-Tg(APP^{Sw}FILon, PSEN1^{M146L}*L286V) 6799Vas/J; Stock number 006554; Jackson Laboratory, Bar Harbor, ME) was crossed with the H-line YFP mouse line (B6.Cg-Tg(Thy1-YFP)16Jrs/J; Stock number 003782; Jackson Laboratory, Bar Harbor, ME, USA) by The Jackson Laboratory. For this study, 2-, 4-, and 6-month-old 5xFAD-YFP (5XY; n = 9 mice) and age-matched YFP-control (Y; n = 8 mice) female mice were used. The 5XY mice expressed all five 5xFAD mutations and the YFP transgene in the same brain regions as described in the H-line mouse line (Feng et al. 2000). The Y mice expressed only the YFP transgene, and their phenotype is as described in the H-line mouse model (Feng et al. 2000). All animal work conforms to NIH guidelines and followed the Institutional Animal Care and Use Committee of Mount Sinai School of Medicine.

Animals were anesthetized with a euthanizing dose of Euthasol (100 mg/kg, i.p.), and transcardially perfused at a rate of 5 ml/min with ice cold 0.1M phosphate-buffered saline (PBS, pH 7.4) for 2 minutes followed by 4% paraformaldehyde in 0.1M PBS for 15 minutes. The brains were carefully dissected from the skull and postfixed overnight at 4°C in 4% paraformaldehyde in PBS. Brains were stored in 30% sucrose in PBS. Brains were hemisected and sectioned sagittally at 50µm on a Vibratome (Leica, Bannockburn, IL, USA) and mounted on subbed slides (Fisher Inc., Pittsburgh, PA).

Confocal microscopy and analysis

Dendritic segments were imaged using a Leica TCS SP5 DMI inverted confocal microscope with a 63X/1.4NA Plan-Apochromat objective and an Ar/Kr laser with excitation at 514nm. Voxel size was kept consistent throughout all imaging at 0.1×0.1×0.25 µm, at 512×512 pixel resolution with a pinhole setting of one Airy unit and optimized settings for gain and offset. Tertiary dendritic segments at least 20 µm away from axonal dystrophy were randomly chosen in the basal and apical layers of the somatosensory cortex (n = 15 segments/layer/mouse), the stratum radiatum layer of the hippocampus (n = 15 segments/mouse), and the basal layer of the prefrontal cortex (n = 15 segments/mouse). Z-Stack analysis was performed in accordance to previously established methods (Dumitriu et al. 2011). Briefly, the z-stacks were deconvolved using an iterative blind deconvolution algorithm (AutoDeblur version X; MediaCybernetics, Bethesda, MD, USA). After deconvolution, the image stacks were analyzed using NeuronStudio (Wearne et al. 2005; Radley et al. 2008; Rodriguez et al. 2008) to examine spine densities and spine shape (stubby, mushroom, and thin). Mushroom spines were classified as having a head diameter was more than 0.6 µm. For presentation purposes, distracting background was digitally removed post-hoc.

Statistical Analysis

Dendritic segments from each animal were averaged for an animal mean, and tested for normal distribution using the Mann-Whitney non-parametric test. The values showed a Gaussian distribution, which allowed us to use a 2-tailed unpaired *t*-test to examine the overall effect. Confidence levels were set at 95%. Data are presented as the mean $\pm$ SEM.

Results

Neocortical spine density and type in 2-month-old mice

In the 5xFAD mouse, it has been shown that layer (L) 5 pyramidal neurons in the somatosensory and motor cortices are significantly reduced in number by 12 months of age (Jawhar et al. 2012). However, the inclusion of YFP into neurons does not alter spine number (Grutzendler et al. 2002), spine morphology, or the electrophysiology of the L5 pyramidal cells (Yu et al. 2008). We chose to examine basal dendritic segments, which are located in L5 of the somatosensory cortex, and tufted apical dendritic segments, which are found in L1, L2, and L3 of the somatosensory cortex, more simply referred to as apical dendrites for the remainder of the study. There was no statistical difference in spine density on basal dendrites between 2-month-old 5XY mice (1.90 ± 0.38 spines/ μm ; $n = 3$ mice, 3688 spines) and age-matched Y mice (1.88 ± 0.39 spines/ μm ; $n = 3$ mice, 2818 spines; $p > 0.05$, $t_{(55)}=0.255$) (Fig. 1A). Basal dendrites from 2-month-old 5XY and Y mice appeared identical, with no obvious structural differences (Fig. 1B,C). Apical spine density in 5XY mice also showed no statistical difference from the age-matched Y controls (1.33 ± 0.26 spines/ μm and 1.43 ± 0.36 spines/ μm , respectively; $n = 3$ mice/group, 4136 spines total; $p > 0.05$, $t_{(41)}=1.117$; Fig. 1D). There was a significant difference between apical spine density and basal spine density for both 5XY and Y mice, with apical density significantly lower than basal density ($p < 0.0001$). The apical dendritic structures of the 5XY and Y mice are indistinguishable from one another in processed images (Fig. 1E,F). However, apical segments can be visually distinguished from basal dendrites, appearing to have fewer spines along the main dendrite, which was supported by the analytical data.

In addition to spine density, we examined spine type distinguished based on their shape into stubby, thin, and mushroom (Peters and Kaiserman-Abramof 1970). We found that thin spines were the most abundant on basal dendrites, making up $64.94 \pm 13.35\%$ of the spine population, and apical spines, accounting for $61.74 \pm 11.01\%$ of the spine population for 5XY mice. The age-matched Y mice followed the same pattern of spine type, with $54 \pm 8.23\%$ thin spines on basal dendrites, and $52.30 \pm 9.12\%$ thin spines on apical dendrites (Fig. 2A, B). Stubby spines were the next most common spine type in 2-month old mice. Stubby spines on basal dendrites made up $34.51 \pm 13.42\%$ of the spine count in 5XY mice, and $44.40 \pm 8.54\%$ of the spine count in Y mice. Similarly, on apical dendrites, stubby spines accounted for $36.98 \pm 10.98\%$ of the spine population in 5XY mice and $45.83 \pm 12.54\%$ of spines in Y mice.

Neocortical spine density and type in 4-month-old mice

Analysis of spine density of basal dendrites revealed no significant difference between 5XY mice (1.67 ± 0.16 spines/ μm , $n = 1754$ spines, 3 mice, Fig. 3A) and Y mice (1.70 ± 0.18

spines/ μm , $n = 1230$ spines, 2 mice; $p > 0.05$, $t_{(31)} = 0.448$; Fig. 3A). Images of 5XY basal dendritic segments (Fig. 3B) appeared structurally similar to Y basal dendritic segments (Fig. 3C). Similarly, apical spine density in 5XY mice (1.31 ± 0.23 spines/ μm , $n = 1056$ spines, Fig. 3D) was comparable to the apical spine density in Y mice (1.32 ± 0.21 spines/ μm , $n = 1003$ spines; $p > 0.05$, $t_{(27)} = 0.253$; Fig. 3D). Dendritic segments from the 5XY mice (Fig. 3E) are indistinguishable from dendritic segments of Y mice (Fig. 3F). There was a significant difference between average basal spine density and average apical spine density for both 5XY and Y genotypes ($p < 0.0001$). There was also a significant drop in overall spine density from 2 months of age to 4 months of age for both areas in both genotypes.

The distribution of stubby spines, thin spines, and mushroom spines in 5XY and Y mice was also analyzed for both basal and apical segments. Thin spines made up the largest population of spines on basal dendrites (5XY: $67.15 \pm 4.43\%$, Y: $64.66 \pm 6.34\%$, Fig. 2C), followed by stubby spines (5XY: $31.52 \pm 4.17\%$, Y: $33.28 \pm 6.89\%$), and finally mushroom spines (5XY: $1.33 \pm 1.52\%$, Y: $2.06 \pm 3.04\%$). Spine type distribution on apical dendritic segments was similar. Thin spines made up $68.09 \pm 7.28\%$ of apical spines in 5XY mice and $66.99 \pm 8.04\%$ in Y mice, stubby spines were $29.44 \pm 6.64\%$ in 5XY mice and $31.46 \pm 8.84\%$ in Y mice, and mushroom spines comprised the last $2.47 \pm 2.45\%$ of the spine population in 5XY mice and $1.56 \pm 3.30\%$ in Y mice (Fig. 2D).

Neocortical spine density and type in 6-month-old mice

Basal dendrites of the 5XY mice had significantly lower spine densities when compared to densities in age-matched Y mice (1.26 ± 0.31 spines/ μm and 1.50 ± 0.25 spines/ μm , respectively, $p < 0.001$, $t_{(37)} = 2.654$; $n = 3$ mice/group, 2874 spines total; Fig. 4A). The decrease in spine density on 5XY basal dendritic segments can be discerned when compared to the z-stacks of Y segments (Fig. 4B,C). There was no statistical difference for apical spine density between 5XY and Y mice (1.12 ± 0.26 spines/ μm and 1.20 ± 0.28 spines/ μm , respectively; $n = 3$ mice/group, 2437 spines total, $p > 0.05$, $t_{(33)} = 0.930$; Fig 4B). Dendritic segments in the apical layers from 5XY and Y mice show no obvious structural differences in the z-stack images. The significant differences between apical and basal spine densities within groups seen at 2 and 4 months of age is still seen in the 6-month-old Y mice, with basal density significantly higher than apical density ($p < 0.001$), but is abolished in the 5XY mice. There was also a significant drop in all average spine densities for both groups when compared to the corresponding densities recorded in 2-month-old mice ($p < 0.001$).

The distribution of spine type in each area followed the same pattern shown in the 2- and 4-month-old groups (Fig. 2E,F). On basal dendrites, thin spines made up the majority of the population 5XY ($71.26 \pm 7.56\%$) and Y mice ($70.59 \pm 7.21\%$), followed by stubby spines (5XY: $27.59 \pm 7.46\%$ and Y: 28.67 ± 7.64), and lastly, mushroom spines (5XY: $1.31 \pm 1.44\%$ and Y: $0.71 \pm 0.87\%$). Apical dendrites had mostly thin spines for both groups (5XY: $66.52 \pm 6.69\%$ and Y: $68.20 \pm 8.99\%$), succeeded by stubby spines (5XY: $32.44 \pm 6.49\%$ and Y: $29.66 \pm 8.58\%$), and mushroom spines (5XY: $1.04 \pm 1.30\%$ and Y: $2.14 \pm 1.71\%$).

Prefrontal cortex spine density in 6-month-old mice

In order to determine if spine loss is restricted to the somatosensory cortex, we examined spine density in the prefrontal cortex. Basal dendritic segments were collected from Layer 5 pyramidal neurons in the prefrontal cortex of 6-month-old 5XY and Y mice. Spine density was significantly reduced in 5XY mice compared to Y mice (1.20 ± 0.16 spines/ μm and 1.58 ± 0.2 spines/ μm , respectively; $n = 6$ mice, 2452 spines total, $p < 0.001$, $t_{(30)} = 5.942$; Fig. 5).

Spine type distribution was also analyzed for the basal dendrites (data not shown). Similar to the somatosensory cortex, thin spines were the most abundant (5XY: $68.08 \pm 8.30\%$, Y: $73.25 \pm 4.82\%$), stubby spines were the second most abundant (5XY: $29.56 \pm 7.73\%$, Y: $24.82 \pm 4.84\%$), and mushroom spines were by far the least abundant (5XY: $2.36 \pm 2.77\%$, Y: $1.93 \pm 1.54\%$).

Hippocampal spine density and type in 6-month-old mice

In contrast to younger mice, we found a significant loss of spines in the basal layers of the somatosensory cortex in 6-month-old 5XY mice. In order to determine if spine loss was an early event specific to the degeneration of the somatosensory pyramidal neurons, we examined spine density on oblique dendrites located in the stratum radiatum of CA1 hippocampal pyramidal neurons. Hippocampal spine density for 6-month-old 5XY mice (1.89 ± 0.26 spines/ μm) was not statistically different from the hippocampal spine density in 6-month-old Y mice (1.86 ± 0.25 spines/ μm , $n = 3$ mice/group, 4786 total spines; $p > 0.05$, $t_{(42)} = 0.284$; Fig. 5A). The 5XY oblique segments were visually indistinguishable from the Y oblique segments. As with the somatosensory and prefrontal cortex pyramidal neurons, we also looked at spine subtype in the recorded spine population (data not shown). Thin spines were again the most profuse spine type identified for both 5XY ($71.07 \pm 5.25\%$) and Y groups ($73.59 \pm 4.86\%$), with stubby spines the second most abundant (5XY: $27.01 \pm 5.41\%$ and Y: $24.80 \pm 4.39\%$), and mushroom spines comprising the smallest percentage of the distribution (5XY: $1.92 \pm 1.45\%$ and Y: $1.61 \pm 1.76\%$). The spine types in the CA1 pyramidal oblique dendrites followed the same general distribution seen in the somatosensory pyramidal neurons.

Macroscopic imaging of 5XY sagittal brain slices

Sagittal slices from 5XY brains of mice aged 2, 4, 6, 13, and 16 months were examined under a stereoscope at 6x magnification, with concentration on the somatosensory cortex, the prefrontal cortex, and the hippocampus (Fig. 7A–I). Imaging revealed a decline in structural integrity of L5 pyramidal neurons at 6 months of age in the prefrontal cortex and somatosensory cortex. At 6 months of age, CA1 appears structurally healthy. At 16 months of age, the prefrontal cortex and somatosensory cortex show further structural decline, while the CA1 cell bodies still appear structurally sound.

Discussion

We have made a novel bigenic FAD mouse model called the 5XY model. This is a fluorescent derivative of the 5xFAD mouse, an AD model that has an aggressive pathological phenotype, with intraneuronal A β and extracellular plaques appearing around 3

months of age, and massive neuronal loss in the neocortex by 12 months of age (Oakley et al. 2006; Jawhar et al. 2010). Using high-resolution confocal microscopy, we have found a selective loss of a unique subset of spines in cortical pyramidal neurons in young 5XY mice. Specifically, at 6 months of age, spines of basal dendrites, but not apical dendrites, of L5 neurons were decreased. Furthermore, this loss did not extend to equivalent structures of neurons in the hippocampus at the same age. It is known that the 5xFAD mouse model has significant neuronal death in the neocortex, but not in the hippocampus or prefrontal cortex (Oakley et al. 2006; Jawhar et al. 2012). Using intravital two-photon fluorescence microscopy we have imaged gross changes in neocortical pyramidal neurons (Crowe and Ellis-Davies, accepted), however such studies cannot image spines at high resolution nor in deeper parts of the brain of living mice. Thus, we studied changes in spine density with age in the bigenic 5XY mouse model in fixed brain tissue using high-resolution confocal imaging (Dumitriu et al. 2011) of the somatosensory cortex, prefrontal cortex, and CA1 regions.

Intraneuronal A β accumulation has been reported in several mouse models of AD, including the 5xFAD mouse. The accumulation of intraneuronal A β was shown to correlate with neuronal loss in both the 5xFAD mouse model (Oakley et al. 2006; Jawhar et al. 2012) and the APP_{SL}PS1KI_{M233T,L235P} mouse model (Breyhan et al. 2009; Christensen et al. 2009; Christensen et al. 2010). In the 5xFAD mouse model, it was discovered that positive staining for intraneuronal A β was found at 12 months of age in L5 pyramidal neurons of the cerebral cortex, an area shown to have significant neuron loss, but not in the hippocampus, in which there is no significant neuron loss (Jawhar et al. 2012). While there was no reported neuronal loss for the whole of the frontal cortex in 12-month old 5xFAD mice, our stereological images of the prefrontal cortex show a similar decline in the structural integrity of this area compared to the somatosensory cortex, which was shown to have profound neuronal loss (Fig. 7). These data suggest that while the overall numbers of neurons in the frontal cortex do not show a significant decrease, a more specific count of the Layer 5 neurons from these regions may reveal a significant loss.

There have been several studies of the synaptic changes in FAD mouse models showing significant reductions in spine number and changes in spine morphology (Anderton et al. 1998; Falke et al. 2003; Giannakopoulos et al. 2009; Knafo et al. 2009; Tackenberg et al. 2009; Dickstein et al. 2010; Luebke et al. 2010), including many studies that have focused on how amyloid plaques effect nearby spines (Spires and Hyman 2004; Knobloch and Mansuy 2008). In vivo studies have also shown that amyloid plaques have a local toxicity to surrounding neurites (Tsai et al. 2004; Bittner et al. 2012). However, plaques in other AD mice have been shown to occupy less than 5% of the molecular layer volume, which lead to the proposal that the overall changes in spine density may be a more significant contribution to cognitive deficits (Merino-Serrais et al. 2011). We chose to study changes in density with age in the total population of spines in order to determine if overall spine loss was specific to areas known to undergo neuronal loss in the 5xFAD mouse model. As reported in the previous studies, 5XY mice had a very low occurrence of plaques (Oakley et al. 2006), thus precluding any significant close juxtaposition of deposits near basal and apical dendrites in the somatosensory cortex at 4 months of age. Furthermore, at 2 months of age, there was no evidence of plaque deposits in the areas studied. Therefore, we examined randomly selected

dendritic segments in the somatosensory cortex, prefrontal cortex, and hippocampus in 2-, 4-, and 6-month old 5XY mice. At 6 months of age, spine densities on basal dendrites in the somatosensory and prefrontal cortices are significantly reduced (Figs. 4 and 5), while spine density in the hippocampus remains stable (Fig. 6). These data suggest that the neocortical areas are more vulnerable to the increasing pathology in the 5xFAD mouse model than the hippocampus.

When the somatosensory cortex, prefrontal cortex, and hippocampus were examined in a more macroscopic setting, it appeared that the structural integrity of the somatosensory cortex and the prefrontal cortex was degraded by 16 months of age, while the hippocampus remained comparatively structurally intact (Fig. 7). A recent study of the 5xFAD mouse model revealed significant loss of Layer 5 pyramidal neurons in the somatosensory cortex, but no apparent neuronal loss in the hippocampus (Jawhar et al. 2010). We used macroscopic imaging of the 5XY model at various ages as a qualitative representative example of the disease progression reported previously. The loss of spines in the somatosensory cortex and the prefrontal cortex is likely an early event in the degeneration of the neurons in these areas, and appeared to be specific to neurons that undergo degeneration. Changes in spine density in brain samples older than 6 months of age were not determined in this study, as capturing individual dendritic segments becomes increasingly difficult as increases in axonal dystrophy and neuronal atrophy decreased the imaging clarity and individual spines could not be reliably recorded.

We believe the loss of spines in the prefrontal cortex and somatosensory cortex contributes to the significant behavioral deficits reported in previous studies of the 5xFAD mouse model. A recent study of the 5xFAD mouse revealed changes in behavioral tests dependent on the neocortex before synaptic deficits in the hippocampus (Jawhar et al. 2012). At 6 months of age, it was reported that 5xFAD mice have decreased basal excitatory transmission and deficient long-term potentiation (LTP) in the hippocampus, indicating either a change in *N*-methyl-D-aspartate (NMDA) or 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) receptor density and/or spine density. We observed no change in spine density in the hippocampus that would account for these electrophysiological and behavioral changes, suggesting changes in excitatory receptors may occur in the hippocampus at 6 months of age. A loss of AMPA receptors is not the likely mechanism responsible for the decreased LTP, as we observe no change in spine type in the 5XY mouse. AMPA receptor density positively correlates with spine size, thus a loss of AMPA receptors would cause a decrease in mushroom spines and an increased occurrence of small spines (Matsuzaki et al. 2001; Matsuzaki et al. 2004). The LTP results found in 6-month-old 5xFAD mice were consistent with previous observations of impaired NMDA-dependent LTP in CA1 of transgenic FAD mice (Snyder et al. 2005; Dewachter et al. 2009). It has been shown that A β monomers and oligomers bind to excitatory synaptic terminals but not inhibitory ones (Lacor et al. 2004; Lacor et al. 2007). Specifically, it was recently shown that these A β species bind to NMDA receptors containing the NR2B subunit, leading to effects on the downstream signaling cascades and significantly impaired LTP (Dewachter et al. 2009). Additionally, NMDA responses to glutamate are independent of spine size, unlike AMPA receptors (Sobczyk et al. 2005). Therefore, observations of LTP deficiencies could

be due the deregulation of NMDA receptors, which would not necessarily correlate with changes in spine type or density.

While there were some electrophysiological changes in the hippocampus of the 6-month-old 5xFAD mouse (Kimura and Ohno 2009), there were significant deficits in the reconsolidation of memory in the same study. The reconsolidation of memory is a task that is dependent on the neocortex (Wang and Morris 2010). Thus, the inability to reconsolidate memories points to a disruption in neocortical circuits. We believe that the spine loss in the prefrontal and somatosensory cortices in 6-month-old 5XY mice is part of the underlying cause of the reconsolidation deficits previously observed (Jawhar et al. 2010). The disruption of the hippocampal circuit, while involved in the pathology of AD in humans, seems to be less significant to cognitive decline than the disruption of corticocortical connections in the neocortex (Albert 1996). It is the degeneration of the human neocortex that most directly relates to clinical dementia (Morrison and Hof 1997; Hof and Morrison 2004; Morrison and Hof 2007). In 5XY mice, we observed a significant loss of basal spines in the neocortex at 6 months of age, while the density in the hippocampus remained unchanged. Our results suggest that such specific spine loss is an early change in neuronal populations that eventually undergo profound neurodegeneration.

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Figure 1.

Spine density in the basal and apical layers of the somatosensory cortex in 5XY and Y mice at 2 months of age. A. Basal spine density of 5XY and Y mice were not statistically different ($p > 0.05$). B,C. Maximum projections of 5XY and Y deconvolved dendritic segments. Segments were visually similar between genotypes. D. Apical spine density of 5XY and Y mice were not statistically different. E,F. Maximum projections of the apical dendritic segments. As with basal dendrites, apical segments were indistinguishable between genotypes. Scale bar = 4μm.

Figure 2.

Spine type distribution in the somatosensory cortex of 5XY and Y mouse lines. Stubby spines (pink), thin spines (yellow), and mushroom spines (orange) were determined using NeuronStudio. The average percentages of each type of spine out of the total population of spines in the basal layer and apical layer were determined for each genotype at A,B) 2 months of age, C,D) 4 months of age, and E,F) 6 months of age. $p > 0.05$

Figure 3.

Spine density the basal and apical layers of the somatosensory cortex in 5XY and Y mouse lines at 4 months of age. A. Basal spine density of 5XY and Y mice were not statistically different ($p > 0.05$). B,C. Maximum projections of 5XY and Y deconvolved dendritic segments. Segments were visually similar between genotypes. D. Apical spine density of 5XY and Y mice were not statistically different. E,F. Maximum projections of the apical dendritic segments. As with basal dendrites, apical segments were indistinguishable between genotypes. Scale bar = $4\mu\text{m}$.

Figure 4.

Spine density decreases in the basal layers, but not the apical layers of the somatosensory cortex in 5XY and Y mouse lines at 6 months of age. **A.** Basal spine densities of 5XY mice were significantly lower than spine densities in Y mice ($p < 0.001$). **B,C.** Maximum projections of 5XY and Y deconvolved dendritic segments. Segments from 5XY mice were visually distinct from Y mice. **D.** Apical spine density of 5XY and Y mice were not statistically different ($p > 0.05$). **E,F.** Maximum projections of the apical dendritic segments. As with basal dendrites, apical segments were indistinguishable between genotypes. Scale bar = $4\mu\text{m}$.

Figure 5.

Spine density in the prefrontal cortex of 5XY and Y mouse lines at 6 months of age. A. Basal spine densities of 5XY mice were significantly lower than basal spine densities in Y mice ($p < 0.001$). B,C. Maximum projections of 5XY and Y deconvolved dendritic segments. Segments from 5XY mice were visually distinct from Y mice. Scale bar = 4μm.

Figure 6.

Spine density in the CA1 oblique dendrites of 5XY and Y mouse lines at 6 months of age.

A. Oblique spine densities on CA1 pyramidal neurons were not statistically different between 5XY and Y mice ($p > 0.05$). B,C. Maximum projections of 5XY and Y deconvolved dendritic segments. Segments from 5XY mice were not visually distinct from Y mice. Scale bar = 4 μ m.

Figure 7.

Decline in structural integrity in the 5XY mouse line brain. Images of 2-, 6-, and 16-month-old 5XY mice were taken at low (6x) magnification. A–C. The somatosensory cortex showed a progressive decline in neuronal structure with age. D–F. The prefrontal cortex followed a similar pattern of degradation as the somatosensory cortex. G–I. The hippocampus did not show the same profile of neuronal decay as the somatosensory and prefrontal cortices. The pyramidal neurons in CA1 appeared to maintain their structural integrity.