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Impaired Spine Stability Underlies Plaque-Related Spine Loss in an Alzheimer's Disease Mouse Model

Tara L. Spires-Jones, Melanie Meyer-Luehmann, Jennifer D. Osetek, Phillip B. Jones, Edward A. Stern, Brian J. Bacskai, and Bradley T. Hyman

From the MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, Massachusetts

Dendritic spines, the site of most excitatory synapses in the brain, are lost in Alzheimer's disease and in related mouse models, undoubtedly contributing to cognitive dysfunction. We hypothesized that spine loss results from plaque-associated alterations of spine stability, causing an imbalance in spine formation and elimination. To investigate effects of plaques on spine stability *in vivo*, we observed cortical neurons using multiphoton microscopy in a mouse model of amyloid pathology before and after extensive plaque deposition. We also observed age-matched non-transgenic mice to study normal effects of aging on spine plasticity. We found that spine density and structural plasticity are maintained during normal aging. Tg2576 mice had normal spine density and plasticity before plaques appeared, but after amyloid pathology is established, severe disruptions were observed. In control animals, spine formation and elimination were equivalent over 1 hour of observation (~5% of observed spines), resulting in stable spine density. However, in aged Tg2576 mice spine elimination increased, specifically in the immediate vicinity of plaques. Spine formation was unchanged, resulting in spine loss. These data show a small population of rapidly changing spines in adult and even elderly mouse cortex; further, in the vicinity of amyloid plaques, spine stability is markedly impaired leading to loss of synaptic structural integrity. (*Am J Pathol* 2007, 171:1304–1311; DOI: 10.2353/ajpath.2007.070055)

Dementia characteristic of Alzheimer's disease (AD) results from progressive pathology in vulnerable brain regions, including accumulation of senile plaques and neurofibrillary tangles and dramatic neuronal loss.^{1,2} This

pathology destroys the complex neural circuits needed for cognition as remaining neurons lose connections from synaptic partners that have died, change shape to accommodate space-occupying lesions, and become dysfunctional in response to neurodegenerative processes.³ The loss of synaptic structures is fundamental to this collapse of brain function because synapse loss is a strong correlate of cognitive decline in AD.^{4–6} The degree of synapse loss outstrips the amount of loss expected due to the death of neurons, indicating that networks of surviving neurons lose connectivity.⁷

Dendritic spines, small protrusions from dendrites, form the postsynaptic element of the vast majority of cortical synapses and serve to compartmentalize postsynaptic signals.⁸ Changes in spine morphology and density are postulated to be structural correlates of plasticity involved in higher cognitive functions such as learning and memory.⁹ Actin-based motility of dendritic spines and their precursors play a role in synapse formation and activity-dependent plasticity during critical periods of development in sensory cortices.^{10–13} Although spines were once believed to remain stable after development, recent studies indicate that a small population of spines remains dynamic into adulthood.^{14,15} The effect of normal aging on dendritic spine plasticity remains unknown.

Dendritic spine loss is common in many neurodegenerative disorders.¹⁶ In AD, synapses and spines are lost in the cortex and hippocampus.^{17–19} From a biochemical and genetic perspective, accumulation of amyloid- β (A β) seems to be the driving force behind disease progression²⁰; however, the relationship between A β and synapse loss remains unclear. Synapse and spine loss have been observed in several strains of transgenic mice that develop amyloid pathology, with the most pronounced loss near dense-cored amyloid plaques.^{21–24}

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Address reprint requests to Bradley T. Hyman, MGH Neurology, 114 16th St., Charlestown, MA 02129. E-mail: bhyman@partners.org.

Knowing that dendritic spines appear and disappear as part of normal structural plasticity in adult cortex, we postulated that disrupting the balance of formation and elimination by increased elimination near plaques, decreased formation near plaques, or a combination of both would result in the observed loss of spines. To investigate these possibilities, we observed the effects of plaques on spines in the living brain in the Tg2576 model.²⁵ We found that dendritic spine density and plasticity are maintained during normal aging, but in plaque-bearing Tg2576 mice, spines are lost near plaques due to an imbalance in the amount of spine formation and elimination.

Materials and Methods

Animals and Surgery

Tg2576 animals transgenic for human amyloid precursor protein containing the Swedish mutation²⁵ and nontransgenic littermate controls were used in two age groups: 8 to 10 months ($n = 4$ Tg2576, 3 control) and 18 to 24 months ($n = 14$ Tg2576, 7 control). For surgeries and imaging, mice were anesthetized with either avertin (1.3% 2,2,2-tribromoethanol and 0.8% tert-pentylalcohol, 250 mg/kg) or isoflurane (0.5 to 3% in O₂). All time-course imaging of spines was performed under isoflurane anesthesia. The gene for enhanced green fluorescent protein was introduced into primary somatosensory cortical neurons using an adeno-associated virus system as described previously.²⁴ After a 3-week incubation period to allow expression of green fluorescent protein (GFP) in neurons, methoxy X-O4 was injected (4 mg/kg i.p.; generously provided by Dr. William Klunk, University of Pittsburgh, Pittsburgh, PA) to label amyloid plaques.²⁶ The next day, a cranial window was installed as described previously.^{24,27–29} Texas Red dextran [70,000 molecular weight, 12.5 mg/ml in sterile phosphate-buffered saline (PBS); Molecular Probes, Eugene OR] was injected into a lateral tail vein to provide a fluorescent angiogram. All animal work was approved by institutional committees and conformed to National Institutes of Health guidelines.

Multiphoton Imaging and Image Analysis

Anesthetized animals with a cranial window were placed in a specialized stage on a Bio-Rad 1024ES multiphoton microscope (Bio-Rad, Hercules, CA) mounted on an Olympus Optical BX50WI upright microscope (Olympus, Tokyo, Japan). An Olympus 20× dipping objective with 0.95 numerical aperture was used to collect images. A mode-locked titanium-sapphire laser (Maitai; Spectra Physics, Fremont, CA) generated 800-nm excitation, and three photomultiplier tubes (Hamamatsu, Ichinocho, Japan) collected emitted light in the following ranges: 380 to 480 nm, 500 to 540 nm, and 560 to 650 nm. To avoid photodamage, the lowest laser power that could discern all spines was used. Each dendrite observed ($n = 340$) was imaged at an initial time point using a 5× or 6× optical zoom for a final magnification of ×100 or ×120. A

z-stack of images was acquired with an interslice interval of 0.5 μm. A subset of dendrites ($n = 117$) was reimaged under the same conditions 1 hour later. Some dendrites were also imaged every minute for 10 minutes to follow rapid spine changes.

To correct for artifacts introduced by breathing and heartbeat, image stacks from the multiphoton microscope were aligned and deconvolved using Autodeblur software (AutoQuant, Watervliet, NY). Folders were coded to blind the observer, and dendrites were analyzed in green channel images. Dendrites that could be followed for more than 20 μm and had more than three spines were defined as spiny and analyzed using Image J (free software from the National Institutes of Health, Bethesda, MD). On a projection of the z-sections through the dendrite segment of interest, the outlines of the segment were traced using the three-dimensional stack as a reference to determine which structures were spines. Each spine ($n = 2008$) was labeled and assigned a morphological classification (mushroom, thin, or stubby) as described previously,^{24,30} and the length was measured from the tip to the dendritic shaft. On merged three-channel images, the distance from each spine to the nearest plaque edge (if present) was measured. Spines that were followed for 1 hour ($n = 962$) were also assigned a classification of stable, formed, or eliminated. A stable spine was present at both time points, eliminated was present at the first but not second time point, and formed was present in the second but not first time point. Dendrite segments were measured and linear dendritic spine density (spines/micron) calculated. The distance from each edge and the middle of the segment to the nearest plaque (if present) was measured and the average recorded as the segment's distance from a plaque.

The proportion of aspiny processes was estimated by sampling neurites on two-dimensional projections of multiphoton image stacks (green channel only). A template of horizontal lines every 25 μm was overlaid on each image, and processes that crossed any line were assigned as spiny if at least three spines could be identified and aspiny if not.

Immunocytochemistry

After *in vivo* experiments, animals were anesthetized with an overdose of avertin (400 mg/kg) and perfused transcardially with PBS followed by fixative (4% paraformaldehyde in PBS with 15% glycerol). Brains were stored in fixative at 4°C and then 50-μm sections cut on a freezing microtome. Sections were washed in PBS, blocked for 1 hour in 10% normal donkey serum, and then incubated overnight at room temperature in primary antibody (anti-RhoA, 1:50, sc-418; Santa Cruz Biotechnology, Inc., Santa Cruz, CA; and anti-N-methyl-D-aspartate (NMDA) NR2A subunit, 1:100, AB1555P; Chemicon, Billerica, MA). Sections were then washed with PBS, preincubated for 15 minutes with 2% normal donkey serum, and incubated in secondary antibody for 90 minutes (anti-mouse Alexa 488, 1:200, and anti-rabbit Cy3, 1:200; Molecular Probes, Invitrogen, Carlsbad, CA). Sections were

washed in PBS and then counterstained with Thioflavin S (0.05% in 50% ethanol) for 5 minutes.

Confocal images were acquired on a Zeiss LSM 510 confocal/multiphoton microscope (Carl Zeiss GmbH, Jena, Germany). Multitrack image scanning with 488 and 543 lasers separately were acquired to prevent any bleed through from one channel to the other.

Statistics

Spine density data are reported as mean of data from dendrite segments in each group \pm SD of the mean. Means were compared using analysis of variance analysis with genotype as an independent variable split by age. Data from 18- to 24-month-old Tg2576 animals were further analyzed by analysis of variance split by plaque distance category (<50 or >50 μ m). Post hoc Bonferroni-Dunn tests from these analyses of variance were used to compare individual groups. Normality of spine density data was confirmed with a Kolmogorov-Smirnov test. In the case of nonparametric data (proportions of aspiny processes, percent formation and elimination on dendrite segments, and percentage of spines with each morphology), contingency table analysis was used and χ^2 *P* values reported. For analyzing spine formation and elimination, each dendrite analyzed was grouped by whether the percent elimination (and separately formation) of spines was above or below the upper quartile of the distribution. Contingency table analysis was then used to compare control and transgenic dendrites.

Results

A viral-mediated gene transfer technique was used as described previously to fill a subset of neurons in somatosensory cortex of Tg2576 amyloid mice and nontransgenic control animals with GFP.²⁴ *In vivo* multiphoton microscopy was used to observe neurites filled with green fluorescent protein, plaques labeled with methoxy-XO4 (by i.p. injection)²⁶ and blood vessels labeled by i.v. injection of Texas Red dextran (70,000 molecular weight) (Figure 1, A–C). To assess the effects of both plaque deposition and aging on dendritic spine density and plasticity, two age groups were examined: adult (8 to 10 months old) and aged (18 to 24 months old). In nontransgenic cortex, dendritic spine density was unchanged between the adult and aged groups (Figure 1D), indicating that spines are not lost with normal aging in this brain region. In 8- to 10-month-old Tg2576 mice, very few dense plaques have developed in the cortex (only three observed in all 8- to 10-month-old mice compared with hundreds in each 18- to 24-month-old mouse). At this age, there was no significant loss of spine density compared with age-matched nontransgenic controls. However at 18 to 24 months, when amyloid pathology is prevalent, there was a 21% loss of spines on spiny dendrites (Figure 1D).

To assess whether some spiny dendrites have lost all of their spines (and therefore would be excluded from the set of spiny dendrites analyzed for spine density), we

used a stereological sampling method on images to count spiny and aspiny processes. In both adult and aged Tg2576 cortex, there was an increase in the proportion of aspiny processes compared with controls (Figure 1E). This was pronounced in aged Tg2576 cortex (increasing from 10% in control to 25% in Tg2576) and dramatically more so near plaques (40% aspiny processes within 50 μ m of a plaque edge). Because the analysis of aspiny processes indicates that many once spiny dendrites have been denuded in Tg2576 cortex, the spine density measurements are a substantial underestimate of the true amount of spine loss in these mice. Estimating spine density, taking into account the number of naked dendrites expected from aspiny process counts, reveals a loss of 23% in Tg2576 cortex distant from plaques, exacerbated to 46% loss within 50 μ m of a plaque edge (Figure 1F). Because any dendritic segments that lost spines and ultimately degenerated would not be accounted for, even this may be an underestimate.

To investigate the causes of this dramatic spine loss, dendrite segments were followed over 1 hour and the spines analyzed. Spine changes can be remarkably rapid. When imaged every minute over 10 minutes, we occasionally observed spine changes occurring on the order of minutes as has been previously reported in developing cortex¹² (Figure 2), suggesting that at least a subpopulation of spines is rapidly formed and eliminated in mature neocortex. Tg2576 and control mice were imaged and reimaged 1 hour later to evaluate rapid spine plasticity (Figure 3). Each spine followed over 1 hour ($n = 962$) was classified as stable if it was present at both time points, eliminated if it disappeared at the second time point, and formed if it was present in the second but not first time point. In control cortex, approximately 5% of the total observed spines formed over 1 hour, and 5% were eliminated, resulting in no net change of spine density. This large average amount of turnover was surprising in aged cortex over a relatively short period of time. To ensure the validity of the measurements, two control animals were analyzed using a different anesthetic (avertin). One of these was injected with GFP-adenovirus as reported here and the other with fluorescent dextrans (Alexa 488 3000 molecular weight). Both of these controls had similar amounts of turnover in an hour as the animals reported in this study, suggesting that the observed dynamics are not artifacts due to anesthetic or staining method.

The proportions of spine formation and elimination were unchanged with age in the control animals, indicating maintenance of structural plasticity with normal aging. In 8- to 10-month-old Tg2576 animals, there was no change in spine formation or elimination, in accord with the unchanged spine density at this age. In 18- to 24-month-old Tg2576 cortex, however, spine elimination was increased in 1 hour on dendrites near plaques (Figure 4). Because formation and elimination data were not normally distributed, a nonparametric analysis was performed. Dendrite segments followed over 1 hour were grouped by whether they had more or less than the upper quartile values of formation or elimination seen in control animals of the same ages (control

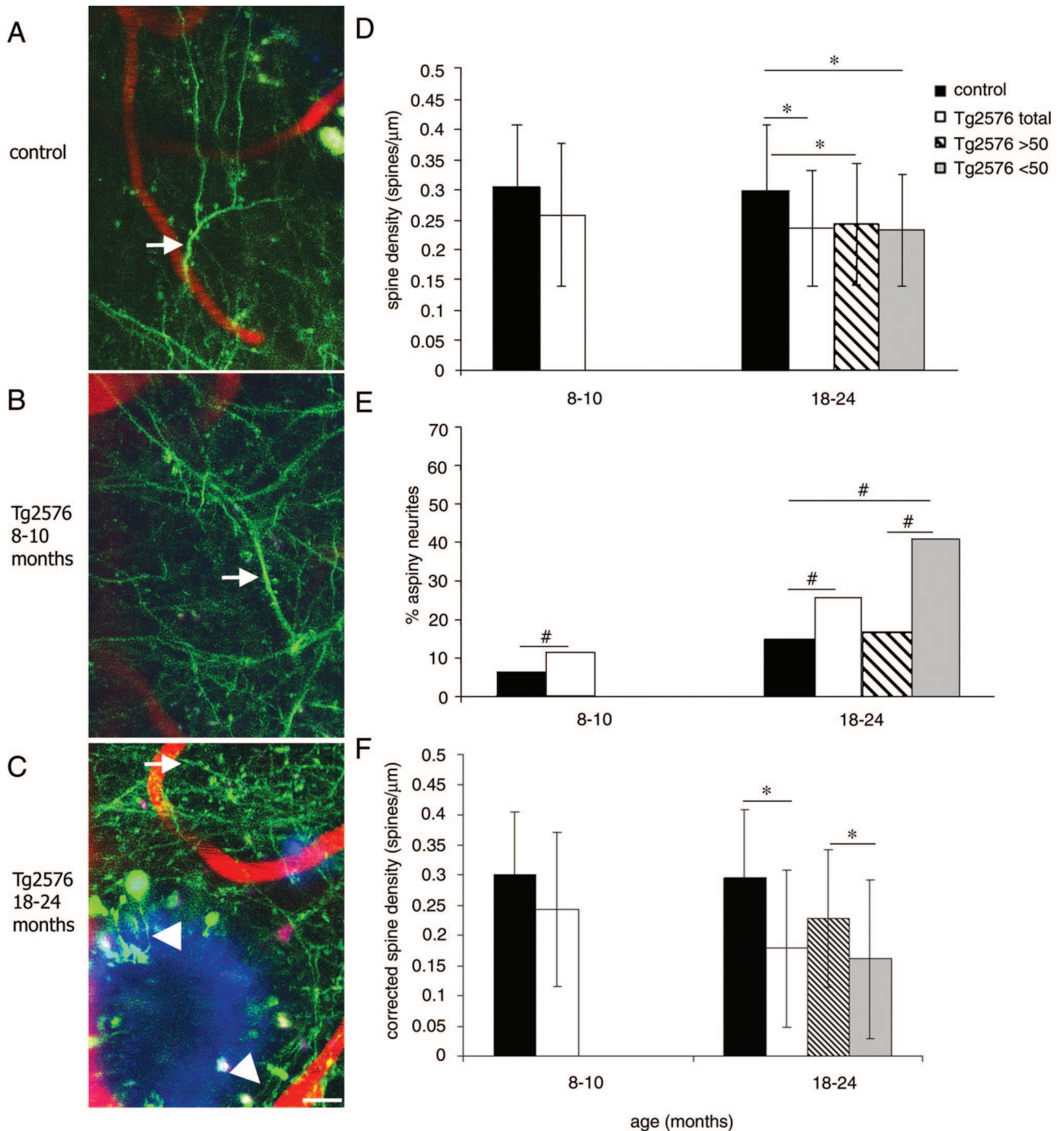


Figure 1. Spine density *in vivo* is maintained in normal aging but decreased in aged Tg2576 cortex. Examples of spiny dendrites (**arrows**) and aspy processes (**arrowheads**) filled with GFP (green) are presented in control cortex (**A**), 8- to 10-month-old Tg2576 cortex (**B**), and 18- to 24-month-old Tg2576 cortex (**C**). Vasculature is labeled with Texas Red. Aspy processes are more often associated with plaques (blue). **D:** Spiny dendrites in nontransgenic animals have 0.284 ± 0.123 spines/ μm at 8 to 10 months of age and 0.296 ± 0.111 spines/ μm at 18 to 24 months, showing maintenance of spine density with normal aging. In Tg2576 mice, there is no significant loss of spines at 8 to 10 months, before amyloid deposits are common in the cortex. At 18 to 24 months, dendritic spine density is significantly lower within 50 μm of plaques (Tg2576 < 50) and distant from plaques (Tg2576 > 50). **E:** At 8 to 10 months in Tg2576 cortex, there are already more aspy neurites present than in control animals. At 18 to 24 months, the proportion of aspy neurons is increased with a more pronounced effect near plaques, indicating that many previously spiny dendrites have been stripped of spines. **F:** Because only spiny processes were used to determine spine density, the loss of spine density in (**D**) is probably an underestimate. Taking into account the proportion of neurites that have lost their spines, spine loss is even more pronounced in Tg2576 cortex (see Results for discussion). * $P < 0.05$. Bonferroni-Dunn post hoc tests; # $P < 0.05$. χ^2 P values from contingency analysis. Data are presented as mean \pm SD. Scale bar = 10 μm .

median, 0% formation and 0% elimination at both ages; upper quartiles: 8 to 10 months, 11.1% formation and 9.1% elimination; 18 to 24 months, 9.8% formation and

14.3% elimination). Elimination near plaques in 18- to 24-month-old animals was significantly increased compared with control animals (contingency table $\chi^2 P =$

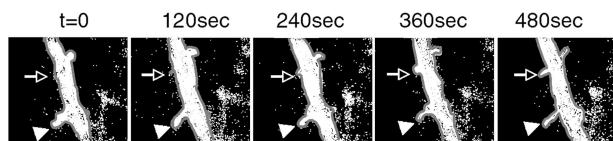


Figure 2. Rapid spine changes. Following a dendritic segment (outlined in gray) at short time intervals revealed that spine changes can occur on the order of minutes. **Arrowheads** point to a stable spine and **arrows** to a small spine that forms over the course of 8 minutes. Scale bar = 5 μm .

0.01). This high rate of elimination without a compensatory increase in formation results in observed spine loss at this age. In fact, near plaques, almost no new spines were observed, suggesting either that spine formation was completely inhibited or that new spines were quite unstable and collapsed before the second imaging session.

As observed previously, the morphology of dendritic spines is not altered in Tg2576 cortex compared with control animals with approximately 25% mushroom-shaped spines, 55% thin spines, and 20% stubby spines.²⁴ We also examined the morphology of the small subset of spines that are formed or eliminated to determine whether different types of spines are dynamic in Tg2576 cortex. In control animals, the vast majority of spines that were formed or eliminated were thin (~80%). This concurs with other studies showing that transient spines are thin.^{14,15} In Tg2576 cortex, even at 8 to 10 months of age, this begins to be disrupted ($\chi^2 P = 0.0351$ Tg2576 versus control), only 31% of eliminated spines are thin. The change in morphology of dynamic spines is more dramatic at 18 to 24 months ($\chi^2 P < 0.0001$), particularly near plaques. Instead of having thin morphology, more dynamic spines are stubby in these mice (Table 1). When divided by morphological classification, the numbers of dynamic spines are small, but nonetheless, the change in the morphological class of spines that change over time is interesting because spine morphology affects the postsynaptic integration of signal.³¹

To investigate the role of changes in regulation of actin cytoskeleton near plaques, we examined the interaction

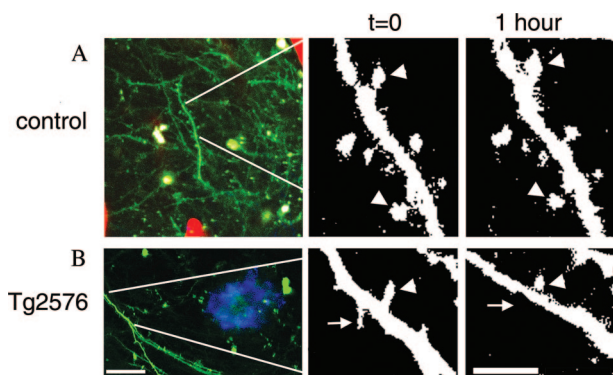


Figure 3. Observations of spine changes over 1 hour. Spiny dendrites were observed in control (A) and Tg2576 (B) animals at an initial time point and then again 1 hour later. Dendrites were filled with GFP (green), plaques were labeled with methoxy X-O4 (blue), and blood vessels filled with Texas Red to provide an angiogram. Images from the green channel were analyzed, and the number of stable spines (**arrowheads**), new spines, and eliminated spines (**arrow**) per dendrite segment was counted. Scale bars: 20 μm (left); 5 μm (right).

of RhoA and the NR2A subunit of the NMDA receptor, because dissociation of RhoA from ionotropic glutamate receptors leads to spine collapse *in vitro*.³² We found that in sections from Tg2576 mice with plaque pathology, RhoA and NR2A colocalize in dendritic spines both near and far from plaques (Supplemental Figure 1; see <http://ajp.amjpathol.org>), thus implying that changes in RhoA activity are not responsible for actin depolymerization and spine collapse near plaques.

Together, these results show a profound disruption in structural plasticity of spines associated with plaque pathology in the intact mammalian brain. The disrupted ratio of spine formation to elimination accounts for the spine density loss and increased percentage of aspiny neurites observed in Tg2576 cortex.

Discussion

Based on the intriguing loss of spines, particularly near plaques, in the Tg2576 mouse model of amyloid pathology,²⁴ we have investigated the structural plasticity of dendritic spines to determine the causes of spine loss. In the healthy brain, spine plasticity is a normal part of neuronal function. During development, an initial overproduction of spines occurs followed by activity-dependent pruning as circuits develop.^{12,13} These young spines are dynamic, with changes occurring on the scale of tens of minutes in barrel cortex.¹² In young adult mice (up to 3 months of age), approximately 20% of spines are eliminated in 1 day, balanced by the formation of new spines (implying a transient population of 40%), whereas 60% of spines persist for more than 8 days, indicating a large proportion of very stable spines.¹⁵ In more mature mice (6 months old), the proportion of transient spines decreases to 20%, whereas 80% remain stable long-term.¹⁴ Few studies have been done looking at spine changes in the aging mammalian brain. Age-associated spine changes vary by brain region. For example a decrease in spine density in subiculum has been reported in aging primates but other hippocampal areas maintain spine density in rodents.³³ In one study of dendrites observed 18 months apart in mouse barrel cortex, 74% of spines were found to be stable over the entire 18 months, 19% formed, and 26% were eliminated.³⁴ To our knowledge, ours is the first study of rapid spine plasticity in the aging brain. We observed no change in spine density or dynamics with normal aging, indicating maintenance of plasticity with normal aging.

Dendritic spine loss is common in several neurodegenerative diseases, and in AD the loss of synaptic apparatus seems particularly important because of the strong correlation between synapse loss and cognitive decline. Several studies in different mouse models of amyloid pathology have shown spine loss.²¹⁻²⁴ We found that spine density is decreased from control levels in plaque-bearing cortex, with a more dramatic loss near plaques, and that this is due to a disruption in the balance of spine formation and elimination. These effects were not present before amyloid deposition into plaques. It is important to note that this suggests that no major change in spine

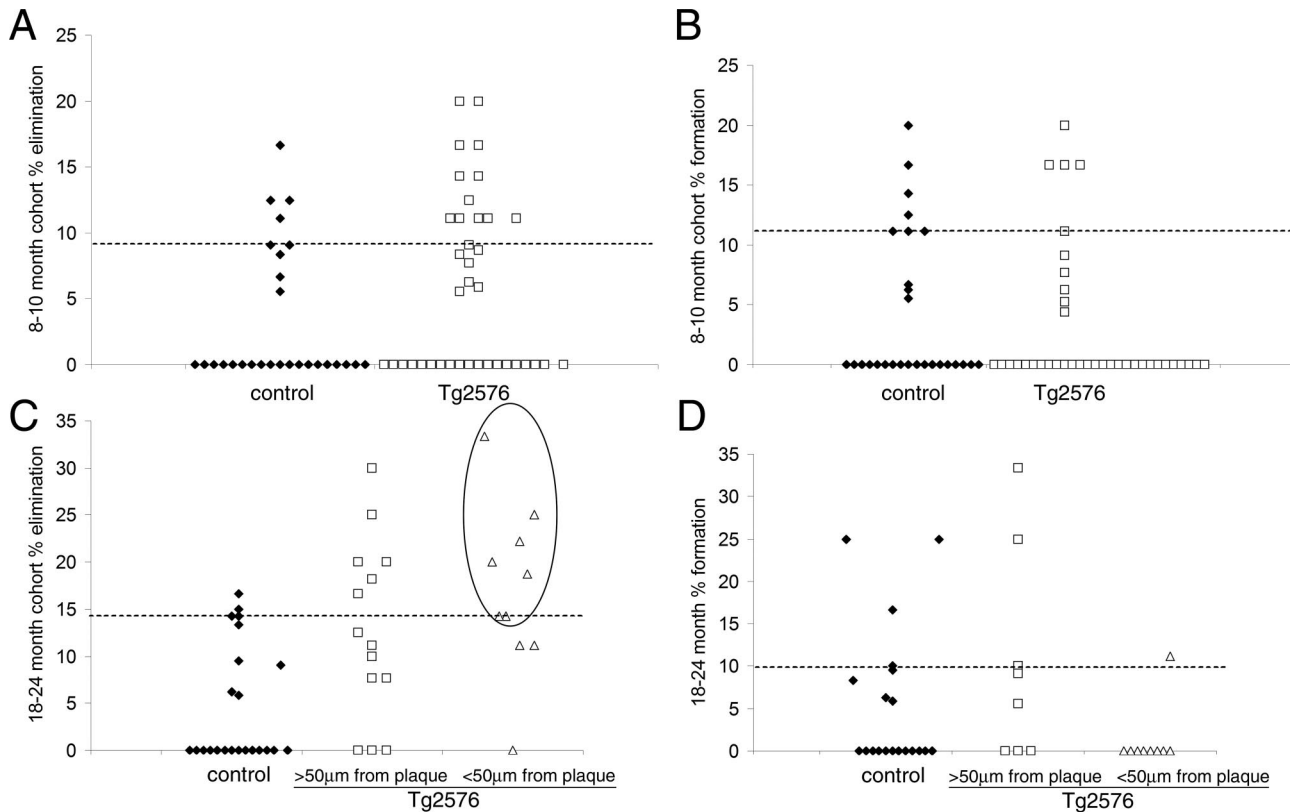


Figure 4. Dendritic spine stability is decreased in aged Tg2576 cortex. Histograms showing the spine plasticity of each spiny dendrite segment separately reveal that in 8- to 10-month-old animals, before substantial plaque deposition, there was no difference in the percentage of spines eliminated (**A**) or formed (**B**) over 1 hour between control and Tg2576 animals (upper quartile value marked with a dotted line, median control value 0%). In control animals, most dendrite segments have completely stable spines; ie, there is no formation or elimination. This is also the case in 8- to 10-month-old Tg2576 animals, but in aged animals with a significant plaque burden, there is a significant increase in spine elimination near plaques, with a larger proportion of dendrites undergoing more elimination than the upper quartile of the distribution (**C**, circled points, $\chi^2 P = 0.01$ contingency table analysis). In particular, over 90% of dendrites within 50 μm of a plaque undergo some spine elimination (median 14.3% elimination). Spine formation (**D**) remains normal (median 0% in control and Tg2576 near plaque, $\chi^2 P = 0.6$), indicating impairment of spine stability in aged Tg2576 cortex.

number occurred at this early age, despite evidence of oligomeric A β -induced changes in cognitive function³⁵ and of A β -induced changes in PSD-95-positive puncta in primary neurons from APP-transgenic mice in culture.³⁶ Loss of dendritic spines in the dentate gyrus of Tg2576 mice, even before plaque deposition, correlated with loss of long-term potentiation and behavioral deficits in a study by Jacobsen et al,³⁷ suggesting that spine loss has functional implications in these mice and that soluble amyloid contributes to spine loss. Our results suggest

that in cortex, plaques may act as a source of soluble A β that induces local spine loss.

Our data show an impairment of spine stability in plaque-bearing Tg2576 cortex, which may partially explain functional synaptic integration deficits observed in this model.³⁸ This result may also have implications for the inability of Alzheimer's patients to remember new information because spine plasticity is implicated in learning and memory.⁸ Being such plastic structures even in the adult brain, dendritic spines are also an

Table 1. Spine Morphology

Stability	Morphology	8 to 10 months		18 to 24 months		Tg2576 (distant from plaque)	Tg2576 (near plaque)
		Control	Tg2576	Control	Tg2576 (total)		
Formed	%Mushroom	0 (0)	0 (0)	8.3 (1)	16.7 (1)	25 (1)	0 (0)
	%Thin	90 (9)	81.8 (9)	75 (9)	33.3 (2)	50 (2)	0 (0)
	%Stubby	10 (2)	18.2 (2)	16.7 (2)	50 (3)	25 (1)	100 (2)
Eliminated	%Mushroom	11.1 (1)	13.6 (3)	8.3 (1)	4 (1)	0 (0)	0 (0)
	%Thin	77.8 (7)	31.8 (7)	75 (9)	48 (12)	61.5 (8)	33.3 (3)
	%Stubby	11.1 (1)	54.5 (12)	16.7 (2)	48 (12)	38.5 (5)	66.7 (6)
Stable	%Mushroom	25.1 (64)	21.8 (69)	27.1 (57)	18.9 (31)	23.9 (21)	12.7 (8)
	%Thin	54.9 (140)	53.1 (165)	56.7 (119)	51.8 (85)	53.4 (47)	54 (34)
	%Stubby	20 (51)	26.2 (83)	16.2 (34)	29.3 (48)	22.7 (20)	33.3 (21)

Values are reported as percentage spines in category (number of spines).

interesting therapeutic target with the potential to recover after treatment.

Why do amyloid plaques disrupt spine dynamics? Dense plaques are space-occupying lesions in the brain that perturb the trajectories of neurites and induce dystrophic morphology of neurites in contact with them.^{3,39–42} These space-occupying changes undoubtedly contribute to loss of spines that come into very close proximity to plaques; however, our observed effects of plaques on spine density and plasticity extend beyond the immediate vicinity, implying that a toxic environment near plaques contributes to spine changes. We propose that plaques act as a source of diffusible oligomeric A β , which has been shown to target synapses in cultured neurons⁴³ and disrupt cognition when injected into the brain *in vivo*.³⁵ Recent work in cultured neurons strongly suggests that exposure of neurons to A β causes endocytosis of glutamate receptors from the postsynaptic density in dendritic spines, which ultimately may destabilize the spines.^{44,45} Involvement of A β in synaptotoxic processes is further supported by a study showing that clearing A β using passive or active immunotherapy prevents synapse loss in transgenic mice.⁴⁶ Although A β may be the most parsimonious explanation, other plaque-associated molecules may potentially be involved in synaptotoxicity. For example, the immediate vicinity of plaques also contains reactive oxygen species,⁴⁷ activated astrocytes and microglia,⁴⁸ and multiple potentially bioactive compounds including Apolipoprotein E⁴⁹ and the axonal sprouting pathway molecules Nogo and the Nogo receptor.⁵⁰

Actin cytoskeleton rearrangements underlie morphological plasticity of dendritic spines¹¹; thus, changes in actin regulatory systems are a key target for examining effects of plaques on dendritic spines. Recent work indicates that actin polymerization and depolymerization by small regulatory proteins controls activity-dependent regulation of spine morphology. In particular, Rho family GTPases including RhoA are thought to regulate spine dynamics downstream of neurotransmitter activation of postsynaptic receptors.⁵¹ In cultured neurons, RhoA is active when bound to ionotropic glutamate receptors (NMDA receptor NR2A subunit and GluR1) at the postsynaptic density, and detachment from these receptors leads to actin depolymerization and spine collapse.³² We did not observe any changes in RhoA and NR2A colocalization, arguing against this pathway as the critical mediator of spine stability near plaques.

On a technical note, recent work suggests that measurements of spine turnover by two-photon microscopy in layer I of neocortex are influenced by use of a “thin skull” preparation or a cortical window, with the latter associated with increased turnover.⁵² Several important differences between this study and the observations of Xu et al⁵² should be noted: we examine dendritic spines in layers II and III of cortex, further from possible disrupting influences due to skull manipulation (thin skull preparations allow primarily layer I visualization). Second, our data compare spine turnover on dendrites within 50 μ m of a plaque to dendrites within the same animal and within the same field of view that are more than 50 μ m

from a plaque as well as to dendrites from control animals. Any technical issues associated with surgery and imaging would be equally applicable to the entire visualized field. Third, loss of spines in AD mouse models has been observed both in *in vivo* studies^{23,24} as well as in postmortem analyses.^{21,22} Thus we do not think that the relative differences in spine turnover seen near a plaque compared with 50 μ m from plaques are likely explained by a methodological issue.

In summary, these results show amyloid-induced alterations in structural plasticity of dendritic spines, which lead to spine loss. In control animals, spine dynamics were preserved with age, and dynamic spines were usually thin. In the Tg2576 cortex after plaque formation, spine formation continued at a normal pace, but spine stability was decreased, resulting in enhanced elimination. Furthermore, the morphology of dynamic spines changed from mainly thin to favor stubby spines, which may contribute to changes in neuronal function.³¹ These data support the idea that altered structural plasticity contributes to neuronal dysfunction in AD.

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References

1. Hyman BT, Van Hoesen GW, Damasio AR, Barnes CL: Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science* 1984, 225:1168–1170
2. Braak H, Braak E: Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl)* 1991, 82:239–259
3. Spires TL, Hyman BT: Neuronal structure is altered by amyloid plaques. *Rev Neurosci* 2004, 15:267–278
4. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R: Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol* 1991, 30:572–580
5. DeKosky ST, Scheff SW, Styren SD: Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration* 1996, 5:417–421
6. Masliah E, Mallory M, Hansen L, DeTeresa R, Alford M, Terry R: Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci Lett* 1994, 174:67–72
7. Coleman PD, Yao PJ: Synaptic slaughter in Alzheimer's disease. *Neurobiol Aging* 2003, 24:1023–1027
8. Harris KM: Structure, development, and plasticity of dendritic spines. *Curr Opin Neurobiol* 1999, 9:343–348
9. Matus A: Growth of dendritic spines: a continuing story. *Curr Opin Neurobiol* 2005, 15:67–72
10. Majewska A, Sur M: Motility of dendritic spines in visual cortex *in vivo*: changes during the critical period and effects of visual deprivation. *Proc Natl Acad Sci USA* 2003, 100:16024–16029
11. Matus A: Actin-based plasticity in dendritic spines. *Science* 2000, 290:754–758
12. Lendvai B, Stern EA, Chen B, Svoboda K: Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex *in vivo*. *Nature* 2000, 404:876–881
13. Stern EA, Maravall M, Svoboda K: Rapid development and plasticity of layer 2/3 maps in rat barrel cortex *in vivo*. *Neuron* 2001, 31:305–315
14. Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, Svoboda K: Transient and persistent dendritic spines in the neocortex *in vivo*. *Neuron* 2005, 45:279–291

15. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K: Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 2002, 420:788–794
16. Fiala JC, Spacek J, Harris KM: Dendritic spine pathology: cause or consequence of neurological disorders? *Brain Res Brain Res Rev* 2002, 39:29–54
17. Catala I, Ferrer I, Galofre E, Fabregues I: Decreased numbers of dendritic spines on cortical pyramidal neurons in dementia. A quantitative Golgi study on biopsy samples. *Hum Neurobiol* 1988, 6:255–259
18. Davies CA, Mann DM, Sumpter PQ, Yates PO: A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *J Neurol Sci* 1987, 78:151–164
19. de Ruiter JP, Uylings HB: Morphometric and dendritic analysis of fascia dentata granule cells in human aging and senile dementia. *Brain Res* 1987, 402:217–229
20. Selkoe DJ: Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid β -protein. *Ann NY Acad Sci* 2000, 924:17–25
21. Lanz TA, Carter DB, Merchant KM: Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. *Neurobiol Dis* 2003, 13:246–253
22. Moolman DL, Vitolo OV, Vonsattel JP, Shelanski ML: Dendrite and dendritic spine alterations in Alzheimer models. *J Neurocytol* 2004, 33:377–387
23. Tsai J, Grutzendler J, Duff K, Gan WB: Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. *Nat Neurosci* 2004, 7:1181–1183
24. Spires TL, Meyer-Luehmann M, Stern EA, McLean PJ, Skoch J, Nguyen PT, Bacskai BJ, Hyman BT: Dendritic spine abnormalities in amyloid precursor protein transgenic mice demonstrated by gene transfer and intravitral multiphoton microscopy. *J Neurosci* 2005, 25:7278–7287
25. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G: Correlative memory deficits, $A\beta$ elevation, and amyloid plaques in transgenic mice *Science* 1996, 274:99–102
26. Klunk WE, Bacskai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT: Imaging $A\beta$ plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. *J Neuropathol Exp Neurol* 2002, 61:797–805
27. Bacskai BJ, Kajdasz ST, McLellan ME, Games D, Seubert P, Schenk D, Hyman BT: Non-Fc-mediated mechanisms are involved in clearance of amyloid- β in vivo by immunotherapy. *J Neurosci* 2002, 22:7873–7878
28. Lombardo JA, Stern EA, McLellan ME, Kajdasz ST, Hickey GA, Bacskai BJ, Hyman BT: Amyloid- β antibody treatment leads to rapid normalization of plaque-induced neuritic alterations. *J Neurosci* 2003, 23:10879–10883
29. Skoch J, Hickey GA, Kajdasz ST, Hyman BT, Bacskai BJ: In vivo imaging of amyloid- β deposits in mouse brain with multiphoton microscopy. *Amyloid proteins: methods and protocols*. Edited by EM Sigurdsson. Totowa, Humana Press, 2004, pp 349–364
30. Spires TL, Molnar Z, Kind PC, Cordery PM, Upton AL, Blakemore C, Hannan AJ: Activity-dependent regulation of synapse and dendritic spine morphology in developing barrel cortex requires phospholipase C- β 1 signalling. *Cereb Cortex* 2005, 15:385–393
31. Majewska A, Tashiro A, Yuste R: Regulation of spine calcium dynamics by rapid spine motility. *J Neurosci* 2000, 20:8262–8268
32. Schubert V, Da Silva JS, Dotti CG: Localized recruitment and activation of RhoA underlies dendritic spine morphology in a glutamate receptor-dependent manner. *J Cell Biol* 2006, 172:453–467
33. Burke SN, Barnes CA: Neural plasticity in the ageing brain. *Nat Rev Neurosci* 2006, 7:30–40
34. Zuo Y, Lin A, Chang P, Gan WB: Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron* 2005, 46:181–189
35. Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH: Natural oligomers of the amyloid- β protein specifically disrupt cognitive function. *Nat Neurosci* 2005, 8:79–84
36. Almeida CG, Tampellini D, Takahashi RH, Greengard P, Lin MT, Snyder EM, Gouras GK: β -Amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol Dis* 2005, 20:187–198
37. Jacobsen JS, Wu CC, Redwine JM, Comery TA, Arias R, Bowlby M, Martone R, Morrison JH, Pangalos MN, Reinhart PH, Bloom FE: Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2006, 103:5161–5166
38. Stern EA, Bacskai BJ, Hickey GA, Attenello FJ, Lombardo JA, Hyman BT: Cortical synaptic integration in vivo is disrupted by amyloid- β plaques. *J Neurosci* 2004, 24:4535–4540
39. Knowles RB, Wyart C, Buldyrev SV, Cruz L, Urbanc B, Hasselmo ME, Stanley HE, Hyman BT: Plaque-induced neurite abnormalities: implications for disruption of neural networks in Alzheimer's disease. *Proc Natl Acad Sci USA* 1999, 96:5274–5279
40. Knowles RB, Gomez-Isla T, Hyman BT: $A\beta$ associated neuropil changes: correlation with neuronal loss and dementia. *J Neuropathol Exp Neurol* 1998, 57:1122–1130
41. D'Amore JD, Kajdasz ST, McLellan ME, Bacskai BJ, Stern EA, Hyman BT: In vivo multiphoton imaging of a transgenic mouse model of Alzheimer disease reveals marked thioflavine-S-associated alterations in neurite trajectories. *J Neuropathol Exp Neurol* 2003, 62:137–145
42. Dickson TC, Vickers JC: The morphological phenotype of β -amyloid plaques and associated neuritic changes in Alzheimer's disease. *Neuroscience* 2001, 105:99–107
43. Lacor PN, Buniel MC, Chang L, Fernandez SJ, Gong Y, Viola KL, Lambert MP, Velasco PT, Bigio EH, Finch CE, Krafft GA, Klein WL: Synaptic targeting by Alzheimer's-related amyloid β oligomers. *J Neurosci* 2004, 24:10191–10200
44. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, Greengard P: Regulation of NMDA receptor trafficking by amyloid- β . *Nat Neurosci* 2005, 8:1051–1058
45. Roselli F, Tirard M, Lu J, Hutzler P, Lamberti P, Livrea P, Morabito M, Almeida OF: Soluble β -amyloid1-40 induces NMDA-dependent degradation of postsynaptic density-95 at glutamatergic synapses. *J Neurosci* 2005, 25:11061–11070
46. Buttini M, Maslah E, Barbour R, Grajeda H, Motter R, Johnson-Wood K, Khan K, Seubert P, Freedman S, Schenk D, Games D: β -Amyloid immunotherapy prevents synaptic degeneration in a mouse model of Alzheimer's disease. *J Neurosci* 2005, 25:9096–9101
47. McLellan ME, Kajdasz ST, Hyman BT, Bacskai BJ: In vivo imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. *J Neurosci* 2003, 23:2212–2217
48. Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC: Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. *Neurobiol Aging* 2004, 25:663–674
49. Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, Sartorius LJ, Mackey B, Olney J, McKeel D, Wozniak D, Paul SM: Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2000, 97:2892–2897
50. Park JH, Gimbel DA, GrandPre T, Lee J-K, Kim J-E, Li W, Lee DH, Strittmatter SM: Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid- β plaque deposition. *J Neurosci* 2006, 26:1386–1395
51. Schubert V, Dotti CG: Transmitting on actin: synaptic control of dendritic architecture. *J Cell Sci* 2007, 120:205–212
52. Xu HT, Pan F, Yang G, Gan WB: Choice of cranial window type for in vivo imaging affects dendritic spine turnover in the cortex. *Nat Neurosci* 2007, 10:549–551