Brief Report

Region-specific changes in the microanatomy of single dendritic spines over time might account for selective memory alterations in ageing *hAPPswe*Tg2576 mice, a mouse model for Alzheimer disease

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ABSTRACT

Tg2576 mice over-expressing human mutant APP (*h*APPswe) show progressive impairments in hippocampal plasticity and episodic memory while fronto-striatal plasticity and procedural memory remain intact. Here we examine the status of synaptic connectivity in the hippocampus and the dorsolateral striatum (DLS) of 3- and 15-month-old Tg2576 and wild-type mice through the analysis of single dendritic spines microanatomy. We found that, in each region, all mice showed a global reduction in the size of spines as a function of age. Ageing mutants, however, exhibited smaller spines with shorter necks on CA1 pyramidal neurons but larger spines with longer necks on DLS spiny neurons compared to their age-matched wild-type controls. Our findings indicate that hippocampal and DLS dendritic spines in *h*APPswe mutants undergo a different pattern of morphological changes over time and point to minor alterations in the microanatomy of DLS spines as a compensatory mechanism maintaining procedural abilities in the ageing mutants.

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Amyloid-β (Aβ) peptide aggregation in hippocampal and cortical regions has long been regarded as the main pathogenic feature of Alzheimer disease (AD). Consistent with this view, mice over-expressing human mutant APP show alterations in neuronal morphology including a massive reduction in spine density, diameter and dendritic arborization as well as the presence of dystrophic neurites with abnormal trajectories in close proximity to amyloid plaques (Alpar et al., 2006; Spires et al., 2005). Nevertheless, increasing evidence indicates that synaptic loss in APP mutants develops long before the plaques are formed thus questioning the existence of a causal link between amyloid deposits, structural abnormalities of neurons and cognitive impairments. For example, spine density, that predicts the number of excitatory synapses is decreased on CA1 (Lanz, Carter, & Merchant, 2003) or dentate gyrus (Jacobsen et al., 2006) neurons in 4-month-old Tg2576*hAPP* (Tg2576) mutants that do not show amyloid deposits or long term potentiation (LTP) alterations but exhibit a memory decline. In the CA1 region, however, genotypic differences in the number of spines disappear between 11 and 20 months of age (Lanz et al.,

2003) despite ageing mutants still display diffuse amyloid plaques, abnormal LTP and cognitive impairments (Chapman et al., 1999; Fitzjohn et al., 2001; Jacobsen et al., 2006). These observations underlines that the amount of spine loss in the hippocampus does not fully depict the status of synaptic transmission and cognition in the ageing mutants. Evidence has accumulated that not only the number but also the dimension of spines directly interferes with synaptic activity. Specifically, robust synaptic strength is a peculiarity of spines with large heads and long necks allowing accumulation of activated synaptic proteins (Alvarez & Sabatini, 2007). Spines with long necks show major concentrations of second messengers, enzymes, and structural proteins (Harris & Kater, 1994; Koch & Zador, 1993), as well as a higher number of presynaptic docked vesicles (Schikorski & Stevens, 1997). A positive correlation exists between the dimension of spine heads and the area of post synaptic density (Kopec & Malinow, 2006) while the length or volume of spine necks interferes with the control of synaptic integrity via the diffusion of transported molecules (Bloodgood & Sabatini, 2005). Interestingly, a reduction on PSD-95 and GluR1 at synapses of cultured neurons derived from APP mutants has been reported (Almeida et al., 2005). In addition, data showing that induction of bidirectional synaptic plasticity (Alvarez & Sabatini, 2007; Yuste & Bonhoeffer, 2001) or learning (Lee, Jung, Arii, Imoto, & Rhyu, 2007) can also triggers changes in spine morphology confirm the

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relationship existing between the dimension of spines and synaptic strength. Based on studies showing that APP mutant mice exhibit a progressive impairment in hippocampal plasticity and episodic-like memory (Chapman et al., 1999; Good, Hale, & Staal, 2007; Jacobsen et al., 2006; Middei, Daniele, Caprioli, Ghirardi, & Ammassari-Teule, 2006) while cortico-striatal plasticity (Middei, Geracitano, Caprioli, Mercuri, & Ammassari-Teule, 2004) and procedural learning are preserved (Janus, 2004; Middei et al., 2004), here we examine the geometric characteristics of dendritic spines on CA1 pyramidal neurons and DLS spiny neurons in 3-month and 15-month-old Tg2576 mutants.

Heterozygous transgenic male mice (Swedish mutation Lys760-Asn, Met671-Leu) under the control of hamster prion protein cosmid vector (Tg2576) and wild-type (WT) male mice were purchased from Taconic Farms Inc. (Germantown, NY, USA). Six WT and six Tg2576 mice were used for each age-point (3- and 15-months). Mice were individually housed in a temperature-controlled room (24 °C) with light–dark 12/12 cycle with food and water available ad libitum. Adequate measures were taken to minimize pain and discomfort in experimental mice, and all the experiments were carried out in accordance with the guidelines laid down by the European Community Council Directives of 24 November 1986 (86/609/EEC). Impregnation of brain tissue was performed according to a previously described protocol (Restivo et al., 2005). Briefly, the mice were anesthetized with chloral hydrate (400 mg/kg) and perfused intracardially with 200 mL 0.9% saline solution. Brains were dissected and, to warrant optimal staining of each region, the two hemispheres were impregnated separately in a Golgi-Cox solution (1% potassium dichromate, 1% mercuric chloride, 0.8% potassium chromate) at room temperature, according to the method described by Glaser and Van der Loos (1981). At the end of the impregnation periods, samples were immersed in a sucrose solution (30%) for 2 days, and sectioned coronally (100 μm) using a vibratome. Sections were mounted on gelatinized slides, stained according to the Gibb and Kolb method (1998), and covered with Permount. All successive measurements were performed by an investigator blind to the experimental condition of the sample under examination.

In each mouse, eight fully impregnated hippocampal CA1 pyramidal or DLS spiny neurons were identified under low magnification (20 × 0.5 NA). Dendritic spines were then measured under higher magnification (100 × 1.25 NA) from images acquired using a digital camera (Axiocam, Zeiss) connected to a p.c. Measurements were performed on secondary and tertiary branches of CA1 basal dendrites and of spiny neuron dendrites irrespective of their orientation. On each neuron, five dendritic spines counted on two 20 μm segments were selected according to the criteria proposed by Knafo, Libersat, and Barkai (2005): (i) considering only spines separated throughout their entire length from neighbor spines or dendrites; (ii) excluding spine-like protrusions with bifurcated heads or with heads longer than 3.5 μm; (iii) including only spines in the same z-plane as the middle of the dendrite or spines with necks clearly visible in a single z-section. Morphology was estimated measuring spine length, including a subdivision into neck length and head length, and spine head area. Spine length was defined as the distance from the dendritic shaft to the tip of the spine. Spine neck length was defined as the distance between the edge of the dendritic shaft and the bottom edge of the spine head. Head length was calculated by subtracting the neck length form the total spine length. Spine head area was defined as the total area of the spine, excluding the neck area. Measurements were scored using the public domain ImageJ software (HYPERLINK "http://.info.nih.gov/ij/"). A total of 384 neurons (96 per region and age category), 768 segments and 1920 spines were examined. Values were averaged for each neuron and means compared among groups. Kolomogorov–Smirnov tests were performed on spine morphology data to check for the distribution normality. In each ROI, two-way ANOVAs with genotype and age as main factors were performed on each variable. Post hoc comparisons were calculated where necessary using the Newman–Keuls multiple comparisons test. Differences were considered significant for $p < .05$.

Fig. 1 shows representative dendritic spines in each experimental condition. Fig. 2 shows histograms depicting the total length of spines, with a subdivision in head length and neck length, and the head area diameters. Values ($\pm SEM$) for these parameters as well as a scale diagram showing the average size of spines per genotype, age and region condition are included in Table 1. The ANOVAs performed on total length, neck length, and head area diameter of spines on CA1 pyramidal neurons revealed a significant effect of age (total length: $F_{1,188} = 418.88$, $p < .001$; neck length: $F_{1,188} = 163.14$, $p < .001$; head area diameter: $F_{1,188} = 7.92$, $p < .001$) and significant age × genotype interactions (total length: $F_{1,188} = 9.51$, $p < .005$; neck length: $F_{1,188} = 6.28$, $p < .05$; head area diameter: $F_{1,188} = 7.92$, $p < .001$). Post hoc comparisons then showed that these three variables were lower in ageing mice of each genotype relative to their young counterpart, and in ageing mutant mice relative to ageing WT mice. For the head length values, there was only an effect of age ($F_{1,188} = 312.60$, $p < .001$). The ANOVAs performed on total length and neck length of spines laying on DLS spiny neurons (Fig. 2B) also pointed to a massive effect of
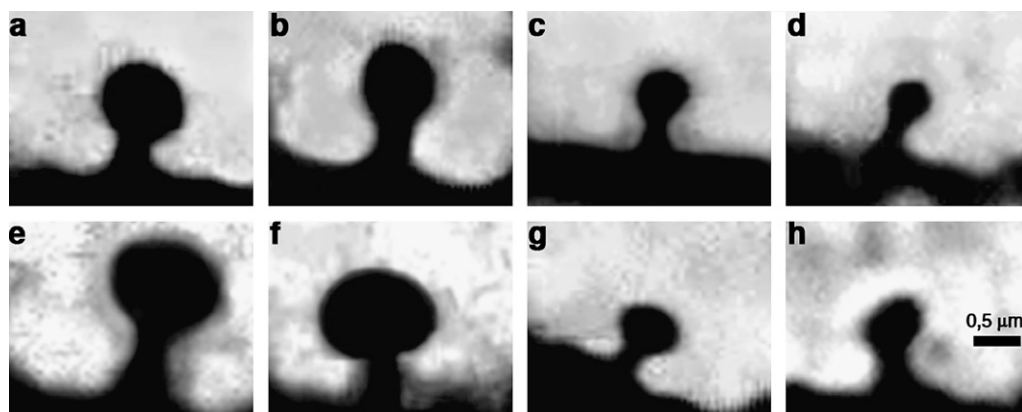

Fig. 1. Photomicrographs of Golgi-Cox stained dendritic spines, magnification 100× 1.25 NA. (Top) spines on CA1 pyramidal neuron dendrites in 3-month-old wild-type (a) and Tg2576 (b) mice, and in 15-month-old wild-type (c) and Tg2576 (d) mice. (Bottom) spines on dorsolateral striatum spiny neuron dendrites in 3-month-old wild-type (e) and Tg2576 (f) mice, and in 15-month-old WT (g) and Tg2576 (h) mice. Shown images were adjusted by an image software (Adobe Photoshop) to regulate light and contrast and enlarge the picture.