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Astrocytes in the Entorhinal Cortex Show Early atrophy in a Triple Transgenic Animal Model of Alzheimer's Disease

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Introduction

The Entorhinal cortex (EC) is the first brain region affected by Alzheimer's disease (AD), which is a progressive neurodegenerative disease characterized by memory deficits and the most common cause for dementia. EC has dense connections with other cognitive areas such as neocortex and hippocampus, being fundamental for information transfer and integration. As the rest of the CNS, astrocytes, in addition to neurons, are key players not only in normal conditions, but also in pathological processes by controlling brain homeostasis and synaptic connectivity. Astroglia has been suggested to be involved in several neurodegenerative diseases, especially AD (Pekny et al., 2005). In fact, previous studies in our lab uncovered the co-existence of a generalised atrophic and hypertrophic astrocytes in the hippocampus of AD directly related with the presence of Aβ(Olabarria et al., 2010). However, morpho-functional astrocytic change and the contribution of astrocytes to AD pathology within the EC remain unknown. In the present study, we investigated the structural modification in astrocytes in the EC of a recent devaloped triple transgenic animal model (3xTg-AD)which mimics spatio-temporal AD pathology progression (Oddo et al., 2003a).

Material and Methods

Triple Transgenic Animal model of AD (3X-Tg AD)

This animal model is presented by Oddo et al. In 2003. These animals harbour the mutant genes for amyloid precursor protein (APPSwe), for presenilin 1 PS1M146V and for tauP301L (Oddo et al, 2003a; 2003b). These mice are recognised as relevant AD model because they show temporal- and region-specific Aβ and tau pathology, which closely resembles that seen in the human AD brain. Those mice also show imparied long-term potentiation and deficits in spatial and long-term memory (Oddo et al., 2003a; 2003b). These changes all are manifest in an age-related manner; in addition, functional deficits precede the appearance of histological markers (Oddo et al, 2003a; 2003b)

All animals were handled according to the Animal Scientific Procedures Act of 1986 under the license from the United Kingdom Home Office.

Fixation and Tissue Processing

Male 3xTg-AD and non-transgenic (non-Tg) (N=4-5) were anaesthetized with intraperitoneal injection of sodium pentobarbital. Mice were perfused through the aortic arch with 3.75 % acrolien (25ml) in a solution of 2 % paraformaldehyde and 0.1 M phosphate buffer (PB) pH 7.4, followed by 2 % paraformaldehyde (75ml). The brain sections were post-fixed in 2 % paraformaldehyde for 24 hours and kept in 0.1 M PB, pH 7.4. Coronal sections of the brain were cut into 40 – 50 μM thickness using a vibrating microtome.

Immunohistochemistry

The sections were incubated for 30 min in 30 % methanol in 0.1 M PB and 30 % hydrogenperoxidase (H_2O_2). Sections were then placed in 1% sodium borohydride for 30 minutes. Brain sections were then incubated in 0.5 % albumin bovine serum BSA in 0.1 M TS and 0.25 % Triton for 30 minutes. Sections were incubated for 48 hours at room temperature in primary antibody [mouse anti-GFAP (1:5000) for single labelling; mouse anti Aβ (1:1000) and rabbit anti GFAP (1:5000) for double labelling]. The sections were then washed with TS and incubated in secondary antibody [FITC-conjugated goat anti mouse IgG (1:100); Rhodamine-conjugated goat anti rabbit IgG (1:100)] for 1 hour at room temperature.

Confocal Microscopy and Analysis

Astrocytes (n = 30-35 in the single labelling experiments) were imaged using confocal scanning microscopy (Leica SP5 upright), recording layers at every 0.2 μm. Parallel confocal planes were superimposed and morphological analysis was carried out by Cell analyst (Chvatal et al. 2007) using digital filters (average 3x3, convolution, gauss 5x5, despeckle, simple objects removal) to determine the surface (S) and the volume (V) of the GFAP-stained cytoskeleton of astrocytes.

Results

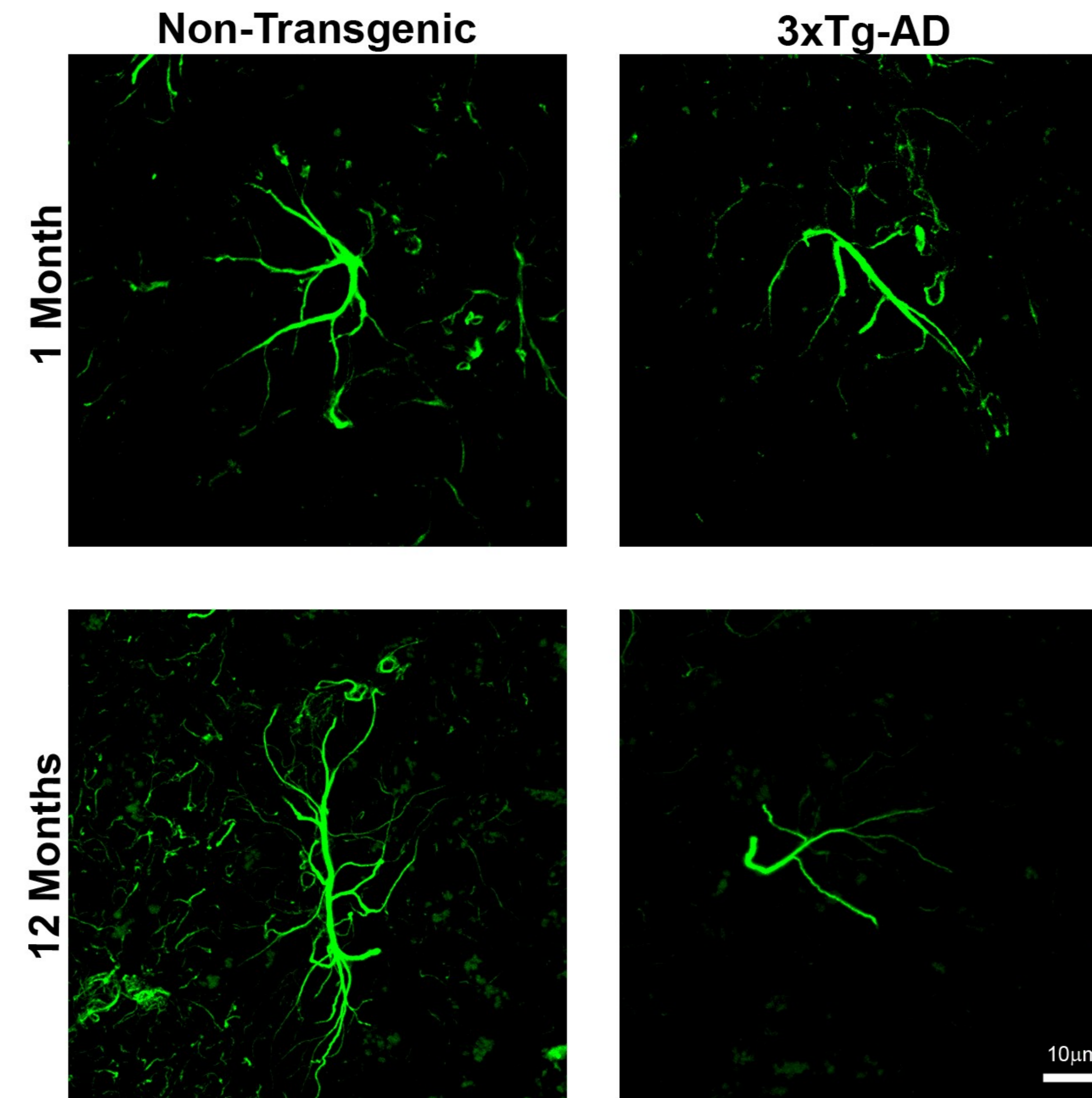### 1. General Early Astrocytic Atrophy in the Entorhinal Cortex

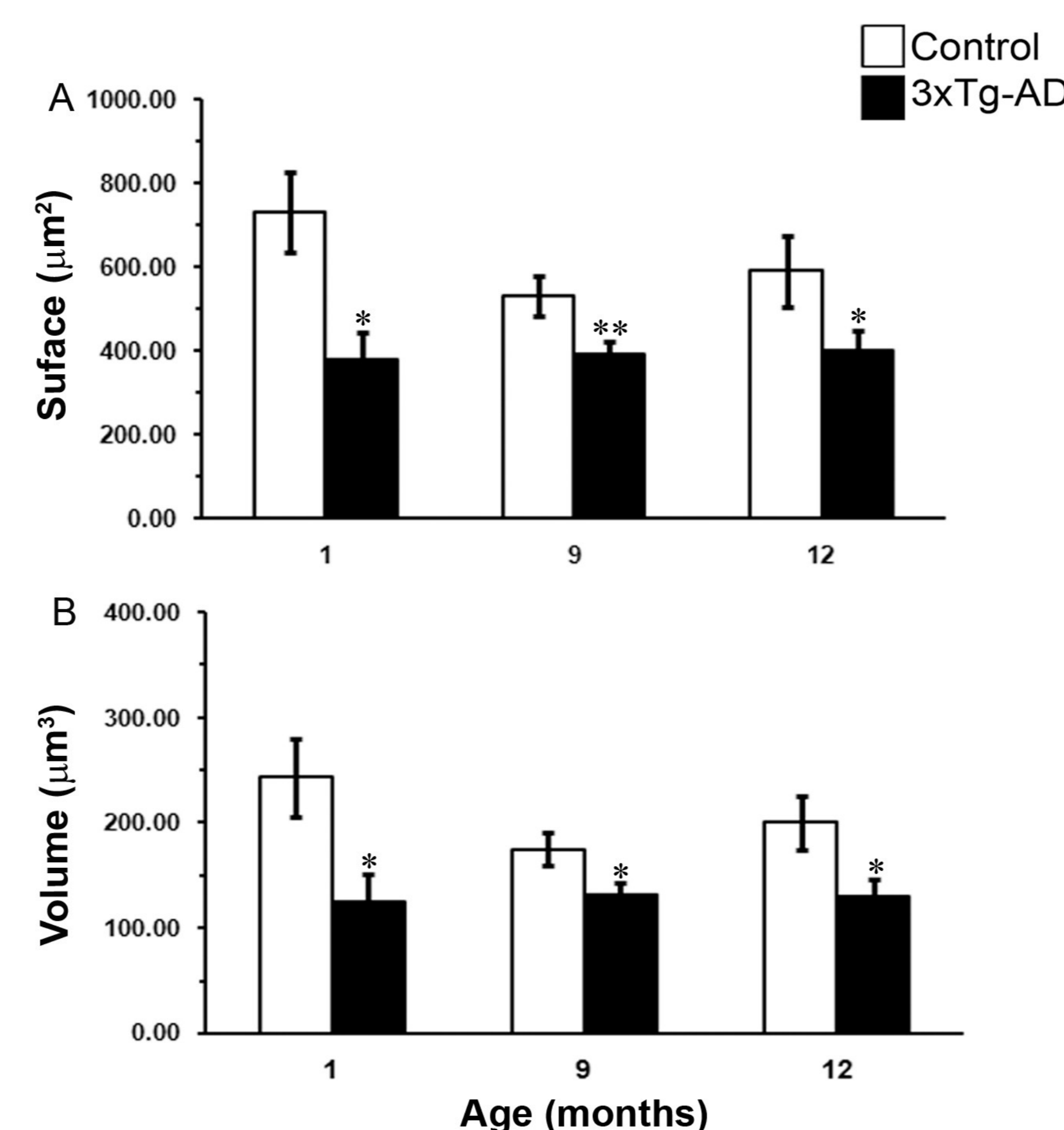Fig.1 Confocal Images Showing the morphological changes of astrocytes at 1 and 12 months.

Fig. 2 Bar graphs showing the decrease in surface (A) and volume (B) of GFAP-positive astroyctes in the EC at the ages of 1, 9, and 12 months (* p<0.5 compared with age-matched control; ** p<0.01).
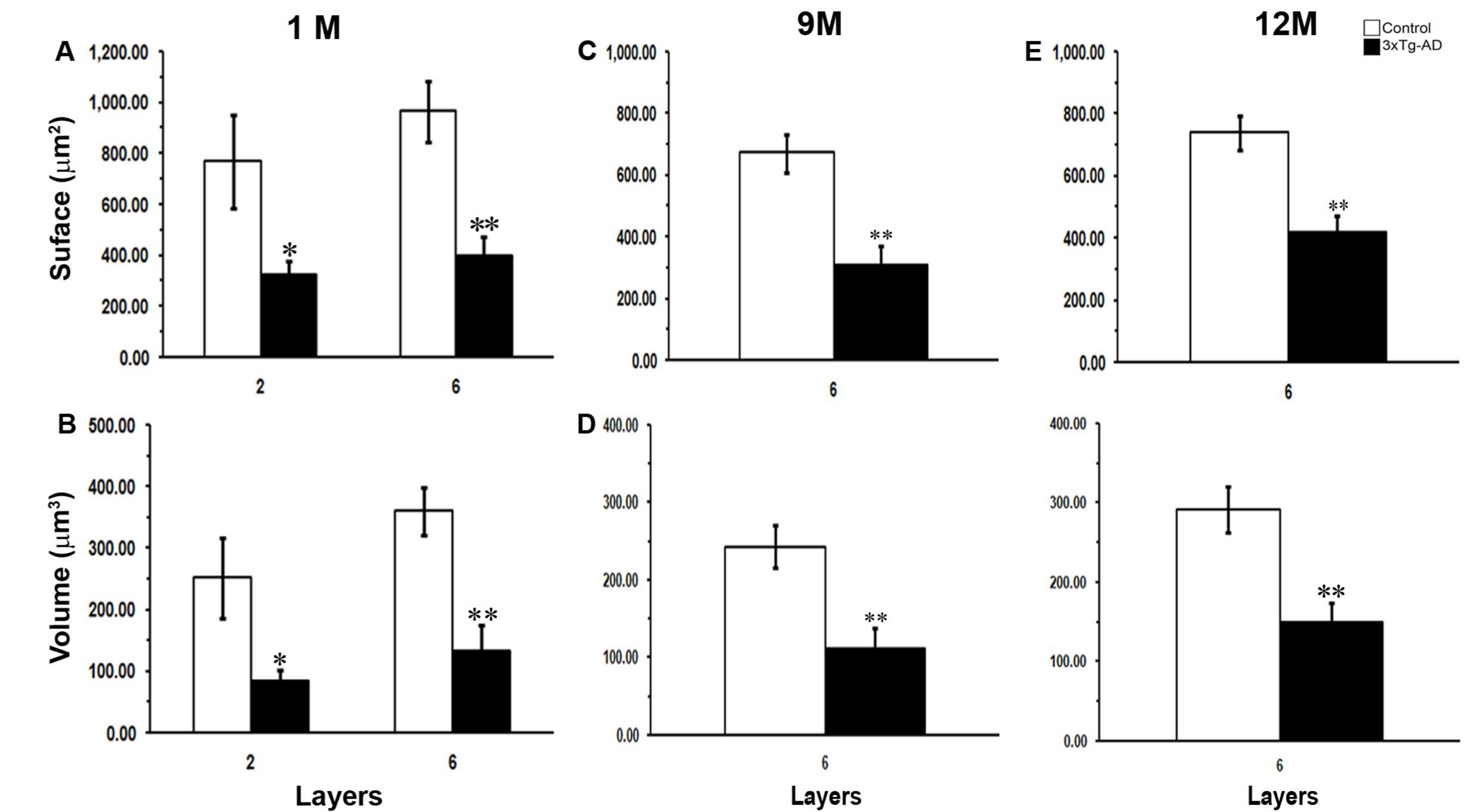
2. Astrocytic Atrophy is Layer Specific

Fig.3 Bar graphs showing the GFAP-positive surface (s) and volume (v) at the age of 1 month (A and B), 9 month (C and D), and 12 months (E and F) in specific layers. The s and v are significantly reduced in layer II and VI at 1 M but in layer VI at 9 and 12M (*p<0.5; **p<0.01)

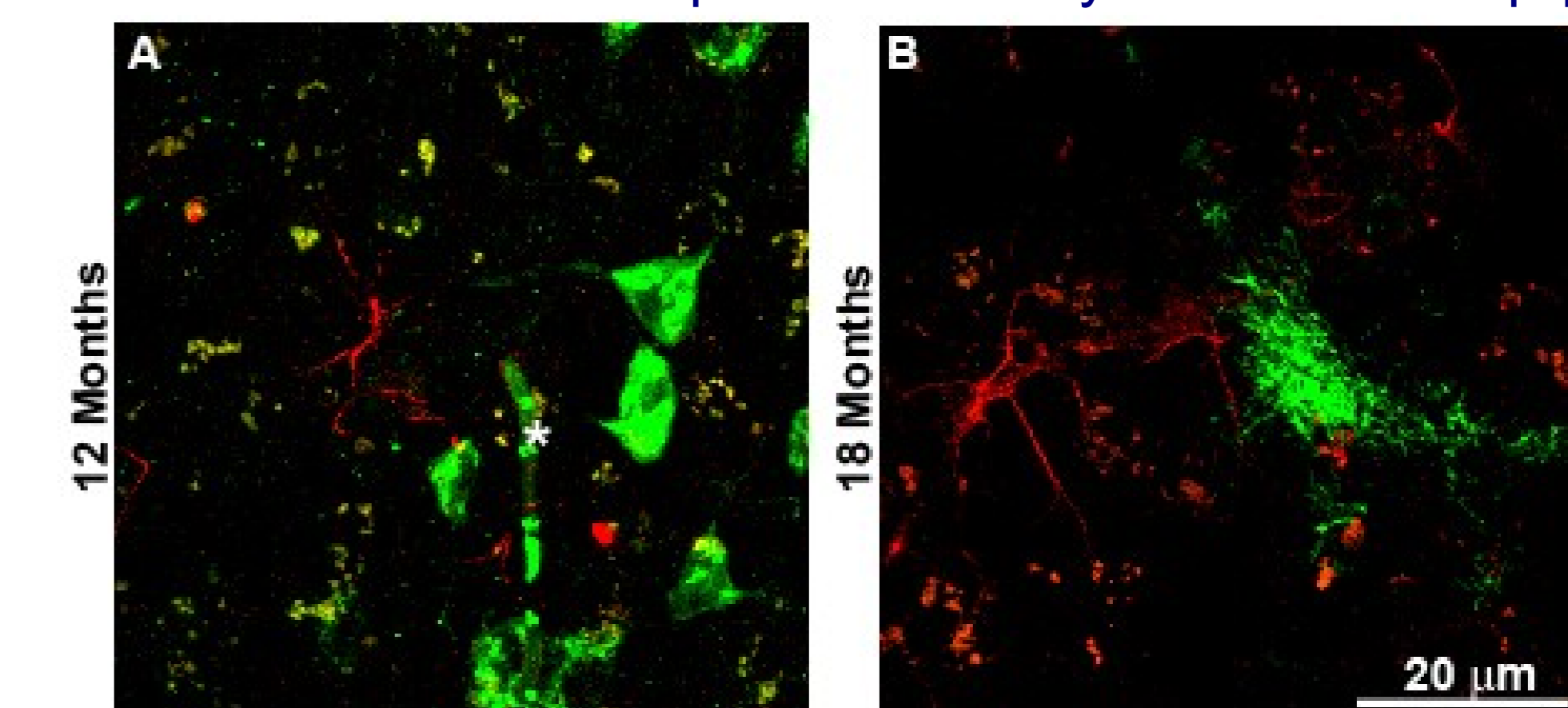### 3. Rare Presence of GFAP-positive Astrocytes around the β-plaques

Fig.4 Confocal images showing the β-amyloid despoits (green) and their replationship with GFAP-positive astrocytes (red) in the EC of 12 (A) and 18 (B) month old 3xTg-AD mice. In A, we can also seeβ-amyloid accumulation around the blood vessel (*).

Summary and Functional Implication

1. We revealed a significant reduction in the surface and volume of GFAP-positive astrocytes, which suggests an early cytoskeleton atrophy in the EC of 3xTg-AD mice.

2. The atrophy occurred at the age of 1 month and is sustained through life (12 months).

3. There are only few GFAP-positive astrocytes around the plaques, suggesting that the astrocytic atrophy may not be related to β-amyloid toxicity.

4. The morphological changes in astrocytes may compromise the extracellular glutamate balance, leading to altered EC local and inter-regional connectivity.

References

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Acknowledgement