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 (Peikov 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 astrogial 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 developed triple transgenic animal model (3X-TgAD) 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 (APPSwes), 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 impaired long-term potentiation and deficits in spatial and long-term memory (Oddo et al., 2003a; 2003b). These changes all are manifested 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 3X-Tg-AD and non-transgenic (non-Tg) (N=4-5) were anaesthetised with intraperitoneal injection of sodium pentobarbital. Mice were perfused through the blood vessel (red) in the EC of 12 month old 3X-Tg-AD mice. In A, we can also see β-amyloid accumulation around the blood vessel (+).

Immunochemistry
The sections were incubated for 30 min in 30 % methanol in 0.1 M PB, pH 7.4. Coronal sections of the brain were cut into 40 – 50 μM thickness using a vibrating microtome.

Confocal Microscopy and Analysis
Astrocytes (n = 30-35 in the single labelling experiments) were imaged using confocal scanning microscopy (Leica SPS5 upright), recording layers at every 0.2 μm. Parallel confocal planes were superimposed and morphological analysis was carried out by Cell analyser (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.

Fig. 1 Confocal images showing the morphological changes of astrocytes at 1 and 12 months.

Global Entorhinal Cortex

Confl ict of  Interests
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References

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.

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

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 V at 9 and 12 M ( *p<0.05; **p<0.01 ).

Fig. 4 Confocal images showing the β-amyloid deposits (green) and their reposition with GFAP-positive astrocytes (red) in the EC of 12 (A) and 18 (B) month old 3X-Tg-AD mice. In A, we can also see sepi-diamidyl accumulation around the blood vessel (+).

Fig. 5 Bar graphs showing the decrease in surface (A) and volume (B) of GFAP-positive astrocytes between the age of 1, 9, and 12 months (* p<0.5 compared with age-matched control; ** p<0.01).

Fig. 6 Bar graphs showing the decrease in surface (A) and volume (B) of GFAP-positive astrocytes in the EC of 3xTg-AD mice ( *p<0.01 compared with age-matched control; ** p<0.001 ).

Fig. 7 Bar graphs showing the decrease in surface (A) and volume (B) of GFAP-positive astrocytes in the EC of 3xTg-AD mice ( *p<0.01 compared with age-matched control; ** p<0.001 ).

Fig. 8 Bar graphs showing the decrease in surface (A) and volume (B) of GFAP-positive astrocytes in the EC of 3xTg-AD mice ( *p<0.01 compared with age-matched control; ** p<0.001 ).