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Age-related Increase in Astrocytes in the Visual Area V2 of the Cat

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Abstract.- We determined age-related changes of astrocytes in the visual area V2 of young (2–3 years old) and old (12–13 years old) cats. An immunohistochemical method was applied to demonstrate glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes. Under the microscope, the densities of the astrocytes and the diameters of the somata were calculated, and the processes of the astrocytes were also counted. Compared with the young adults, the old cats showed significantly denser astrocytes in both the cortex and medulla, accompanied with significant hypertrophy in the cell bodies, a remarkable increase in the processes, and an obvious increment in the intensity of GFAP immunoreactivity. These findings indicate a significant enhancement of astrocytic activities in the visual cortex during aging process. This enhancement might provide neuroprotective effects to the aging neurons and compensate for declining visual function in senile individuals.

Key words: Cat, visual area V2, glial fibrillary acidic protein, astrocyte, aging.

INTRODUCTION

Visual area V2, also called Brodmann area 18 in the cerebrum, receives strong projections from the primary visual cortex (V1), transfers output connections to higher loci, such as V3, V4, and V5, and also sends feedback to V1 (Gazzaniga *et al*., 2002). V2 has many important functions, such as sensitivity for contour stimuli (Hegdé and Van Essen, 2003), stereoscopic depth apperception (Qiu and von der Heydt, 2005), visual combinations of orientations (Anzai *et al*., 2007), and object-recognition memory (López-Aranda *et al*., 2009). Aging has been reported to exert severe impairment on V2 functions, for example, orientation and direction selectivities of the V2 cells show obvious degeneration, accompanied with increased visually driven and spontaneous responses (Yu *et al*., 2006). V2 has even been reported to be affected more severely than V1 in the aged, especially with respect to information processing (Wang *et al*., 2005). Such changes consequentially contribute to decline in visual function in the elderly. Although many deteriorative changes occur in the aging V2, some compensatory mechanisms might exist to suspend the impairments during cortical aging. This paper reports on age-related changes of astrocytes, which can provide neurotrophic factors to aging neurons in visual area V2 in an effort to increase our understanding of visual aging.

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MATERIALS AND METHODS

Animals and tissue treatments

Young (2–3 years old, n = 4) and old cats (12–13 years old, n = 4) were used in this study. All the subjects were healthy domestic cats (*Felis domesticus*) with complete age and health-care records during laboratory feeding. The experimental treatments met the standard of the National Institute of Health's Guide for the Care and Use of Laboratory Animals. After anesthesia and perfusion (Zhang *et al*., 2006; Hua *et al*., 2008), the brain was exposed and the visual area V2 was dissected out. Blocks of tissue were subsequently trimmed to 1 cm × 1 cm × 1 cm and fixed in 4% paraformaldehyde at 4°C overnight, followed by dehydration in graded ethanol, transparentized in xylene and embedded in paraffin. Consecutive coronal sections were cut, 6 µm in thickness.

Immunohistochemical staining

In each series, 6 sections were taken at intervals of approximately 300 µm apart for glial fibrillary acidic protein (GFAP) immuno-histochemical staining, as previously reported (Zhang *et al*., 2006). Briefly, sections were deparaffinized in xylene, hydrated through a graded series of ethanol till to distilled water. After

treatment of 3% hydrogen peroxide, sections were treated with the blocking kit of Biotin-Avidin System reagents (Vector Laboratories, USA), anti-GFAP (dilution 1:400) monoclonal antibody (Sigma), biotinylated anti-mouse IgG antibody (Sigma, diluted 1:800) and a preformed avidin-biotin-peroxidase complex. After washing in tap water, sections were incubated for 10 min in 0.05% 3,3-diaminobenzidine (DAB)/0.01% hydrogen peroxidase in phosphate buffer saline (PBS, pH 7.4) for identification of astrocytes. The neighboring sections were processed for the negative controls, *i.e.* the anti-GFAP antibody was replaced by PBS.

Quantitative analysis

Cell density calculation

The number of astrocytes was counted on each slide using a calibrator (50 μm×50 μm) under a microscope (Motic China Group Co., Ltd). Eight areas were randomly selected from the cortex and the subcortical medulla respectively in each slide, then the density (cells/mm^2) was calculated.

Astrocyte processes counting

The number of astrocyte processes was counted from the distinctly and completely stained cells. Ten cells were counted in the cortex and subcortical medulla respectively on each slide. Minor adjustments in the fine focus were made when necessary in order to make the images as legible as possible. However, as only the processes distributed laterally on the soma can be identified from a cross section, the process number counted in this study is a rough estimation. But the result should be unaffected since the same counting criteria were used for the both age groups.

Somatic diameter calculation

The somatic diameters of the astrocytes were directly measured (10 cells in the cortex and the subcortical medulla on each slide) from the cells with a clear border and distinct nucleus (clear vacancy in the somatic middle) using an eyepiece micrometer at a magnification of 1000×. The diameter of the astrocytic soma was estimated as: d = (a+b)/2 (a and b were the longitudinal and transversal diameters of an astrocyte respectively).

All of the measurements were made blind to the animal identity in order to avoid investigator bias.

Statistical analysis

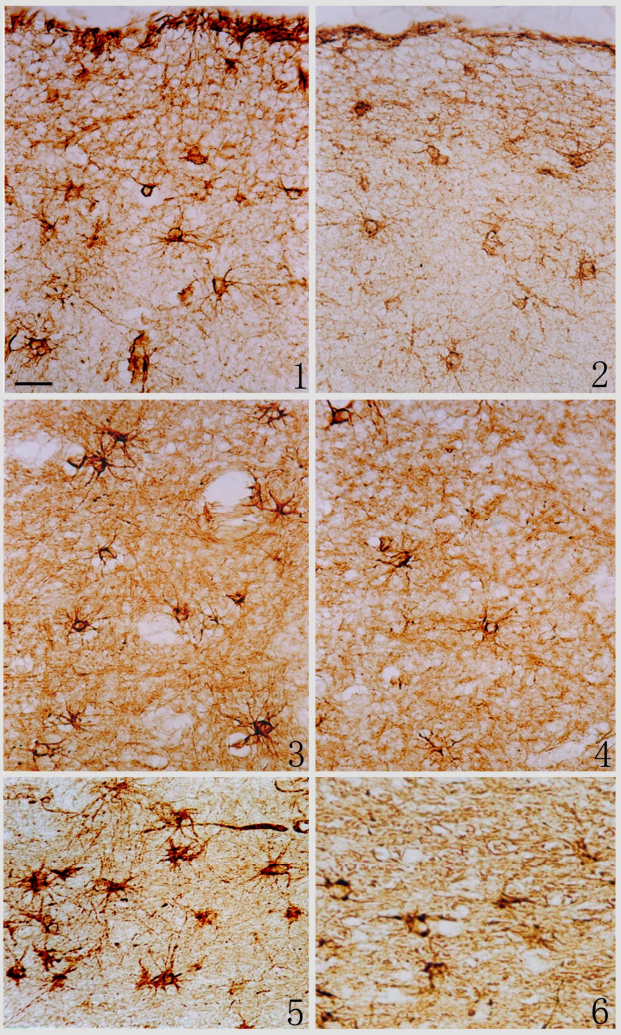
The data are expressed as the mean±standard error of the mean (SEM). Statistical comparisons were made by two-way analysis of variance (ANOVA). A P value <0.01 was considered of significance.

RESULTS

The sections showed a large amount of dark-brown, star-shaped astrocytes in the cortex (usually protoplasmic astrocytes exhibiting a large soma with short and highly ramified cellular processes) and the subcortical medulla (usually fibrous astrocytes exhibiting a small soma with long, unbranched cellular processes). Compared with the young adults, the astrocytes in old cats not only displayed significant hypertrophy and hyperplasia in number and size, but also an increased intensity of GFAP immunoreactivity. The processes of adjacent astrocytes were interwoven with each other, especially in the old sections (Figs. 1, 3, 5). Compared with the young cats, the numeric density, somatic diameter, and astrocyte processes in old cats were increased significantly by 25.9%, 10.1%, and 14.9% in the cortex, and 29.1%, 12.0%, and 28.9% in the subcortical medulla, respectively (P<0.01, Table I). The data also showed that astrocytes in the medulla were more sensitive to aging than astrocytes in the cortex. Negative controls showed no positive immunohistochemical reactions.

DISCUSSION

Astrocytes are a major type of glial cells in the central nervous system with a number of active roles (Kirchhoff *et al*., 2001), including protection of the synapses by many encircling processes (Kirchhoff *et al*., 2001), providing nutrients to the nervous tissue by forming the blood-brain barrier (BBB) (Abbott *et al*., 2006), maintaining homeostasis of the extracellular ion concentration or propagating inter- and intra-cellular ion waves over long distances (Filosa *et al*., 2004; Bernardinelli *et al*., 2004; Edwards and Gibson, 2010), secreting or



Figs. 1-6. Photomicrographs of GFAP-immunoreactive sections of visual area V2 in the old and young adult cats. **1, 2** Superficial layer; **3, 4,** deep layer; **5, 6,** subcortical medulla. Animals in the old group (Figs. 1, 3, 5) showed significant hypertrophy, hyperplasia and stronger GFAP immunoreaction when compared with those in young adult group (Figs. 2, 4, 6). Scale bar=20 μm.

absorbing neurotransmitters (Wang and Bordey, 2008), and repairing traumatized nervous tissue (Faulkner *et al*., 2004). GFAP is a special marker widely used for labeling astrocytes. It has been reported that GFAP-immunoreactive astrocytes are rather sensitive to aging effects, exhibiting significant proliferation, hyperplasia, and enhanced immunoreactive intensity in many regions (Sabbatini *et al*., 1999; Zhang *et al*., 2006).

Table I.- Astrocytic parameters in the visual area V2 of the young and old cats (means ± SEM).

	Young cat	Old cat
Number of cortical astrocytes /mm^2	144.3±50.8	194.7±61.1*
Number of medullary astrocytes /mm^2	165.3±52.5	233.3±79.8*
Somatic diameter of cortical astrocytes (μm)	8.0±1.4	8.9±1.2*
Somatic diameter of medullary astrocytes (μm)	7.3±1.8	8.3±1.3*
Process number/ cortical astrocyte	7.4±1.6	8.7±1.7*
Process number/ medullary astrocyte	5.4±1.6	7.6±1.5*

*P<0.01 vs. old cat

This study provided evidence that the normal aging process leads to significantly increased GFAP activity in the visual area V2, including an increase in the number, somatic size, cell processes, and immunoreactive intensity of GFAP-immunoreactive astrocytes. These findings are similar to the findings reported in the aging cerebellum or other brain regions (Sabbatini *et al*., 1999; Zhang *et al*., 2006), indicating that an age-related enhancement of GFAP activity is a common phenomenon in the central nervous system. An age-related increase in astrocytes might exert effects on aging neurons. Despite no significant neuron loss in the aging visual cortex (Hua *et al*., 2008), neurons in many cerebral areas undergo obvious shrinkage during aging (Smith *et al*., 1999), and we speculate that proliferation of astrocytes might be triggered by neurodegenerative changes. This kind of proliferation might fill in the interstice caused by neuronal shrinkage, provide neurotrophic factors for aging neurons (Schmalenbach and Muller, 1993) and eliminate metabolic wastes in nervous tissues (Theodosis *et al*., 2008). The basis for the age-related increased expression of GFAP in astrocytes is the focus of considerable research. The most interesting feature is the intrinsic modification in the CNS, such as degeneration of neighboring dendrites or entire neurons (Niquet *et al*., 1996). Since trophic interactions exist between astrocytes and neurons (Araque *et al*., 2001; Kirchhoff *et al*., 2001), it is

suggested that the hypertrophy and hyperplasia of astrocytes may slow down the neuronal aging process and subsequently delay visual decline in the elderly.

In summary, our study has provided the first evidence that the visual area V2 undergoes a significant age-related increase in astrocytes, which might exert a protective effect on aging neurons and compensate for the decline in age-related visual function.

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