Age and Sex-related Dendritic Changes in the Visual Cortex of the Rat

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Abstract：Visual functions undergo an age-related degradation. However, the neural mechanisms underlying these changes are not yet clear. This study was designed to investigate the influence of age and sex on the anatomy of the rat's visual cortex. Dendritic tree extent and spine density were examined in young adult rats（2－3 months）and aged male and female rats（22－24 months）using a modified Golgi-Cox staining method. A sex difference in dendritic branching of the pyramidal cells was found among young adults. However, this difference disappeared during aging, due to a reduction in branching with age for males but not for females. Moreover, the pyramidal cells of young males also have a greater spine density. Although there was a reduction in spine density with age for both sexes, this reduction was more pronounced for males, resulting in a disappearance of sex difference with age. Thus these results suggest that aging could lead to the degeneration of dendrites, which might contribute to the degradation of age-related visual functions. Also the results indicate that age-related degeneration of dendrites is more severe for males than for females.

Key words：Aged；Visual cortex；Dendrite；Sex difference

年龄与性别相关的大鼠视皮层树突形态变化

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摘要：衰老会导致视觉功能的退化，但其潜在的神经机制尚不清楚。通过改良 Golgi-Cox 染色法，测定了青年雄性、青年雌性及老年雄性与老年雌性 4 组共 20 只大鼠视皮层的树突长度和树突棘密度，以研究年龄与性别对视皮层树突形态的影响。结果显示青年雄性大鼠视皮层顶树突、基树突、树突总长度均明显高于青年雌性大鼠，但这种性别差异在老年雌雄组之间并不显著，可能是由于在雄性组之间存在着明显的年龄相关性树突长度减少而在雌性组之间并不存在。青年雄性组的树突棘密度要明显高于青年雌性组，尽管衰老导致了青年雄、雌性组的树突棘密度均明显降低，但老年雄、雌性组的树突棘密度并无显著差异，这可能是由于雄性组的年龄相关性树突密度降低程度要远大于雌性组。由此可见衰老确实能导致视皮层树突形态的退化，这可能是老年性视觉功能衰退的潜在神经机制，但这种退化可能具有一定的性别差异。

关键词：衰老；视皮层；树突；性别差异

It is well known that visual functions are impaired during normal aging（Hua，2004）. However, to understand the mechanisms responsible for such age-related visual function declines remains a critical challenge in neurobiology. Although it was once hypothesized that functional declines arised from widespread and

substantial loss of neurons, increasing evidence suggests that in most regions, including the visual cortex, the number of cortical neurons is largely preserved during aging (Peters et al, 1998; Terry et al, 1987). However, many neurons may undergo structural or neurochemical changes that alter their functions. For example, an age-related breakdown of myelinated nerve fibers has been reported in the monkey visual cortex which may account for impairments in cognition (Peters et al, 2000). An age-related loss of GABA (γ-aminobutyric acid) was also thought to be correlated with the visual function declines (Leventhal et al, 2003). For a long time, age-related changes in dendrites were of particular interest, since dendrites are the targets of the majority of synapses, and since dendrites remain plastic even into adulthood (Harris & Kater, 1994; Kolb et al, 1998). Moreover, recent studies suggest that both the extent and pattern of the dendritic arbor could influence how synaptic inputs are integrated (Yuste & Tank, 1996). Thus, age-related changes in dendritic geometry might significantly alter a neuron's physiological response properties, thereby contributing to the cognitive changes that often accompany senescence. Although several investigators have reported age-related changes in dendrites, the result is quite confused. Dendritic stability, lengthening and regression have all been reported previously, and the reported changes in spine density are also inconsistent (de Brabander et al, 1998; Markham et al, 2005; Pyapali & Turner, 1996; von Bohlen et al, 2006; Nicolle et al, 1999). However, possible age-related changes in dendrite tree extent and spine density in the rat visual cortex have not been investigated.

Visual function may also be sexually dimorphic (Seymoure & Juraska, 1997; Wang, 2006). Recently, Seymoure and Juraska (1997) reported that there may be sex difference in visual perceptual abilities between male and female rats. In our lab, Wang (2006) has also reported that the visual cortical cells of aged male rats have a significantly increased spontaneous rate and a significantly decreased signal-to-noise ratio than that of aged female rats. Although there have been some reports about the sexually dimorphic aging of dendritic morphology, few investigators have considered the sexual influence of dendrites in the visual cortex (Shors et al, 2001; Munoz-Cueto et al, 1990; Markham et al, 2005).

This study was designed to investigate how age and sex influence the anatomy of the visual cortex using a modified Golgi-Cox staining method, which is simple and has proven to be useful in several types of studies of cortical plasticity.

1　Materials and Methods

1.1　Subjects

Subjects were 20 Long-Evans hooded rats, reared in the Laboratory of the Animal Center at the University of Science and Technology of China. Rats were of the following sexes and ages: young adult (2–3 months; 5 male and 5 female) and aged (22–24 months; 5 male and 5 female). All were allowed free access to food and water. Colony! rooms were maintained on a 12 h light/dark cycle. All animal treatments were in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

1.2　Histology

All rats (n = 5 per group) were deeply anaesthetized with urethane and perfused intracardially with 0.9% saline solution. The brains were removed and prepared for processing by Golgi-Cox staining, which was described by Gibb and Kolb (Gibb & Kolb, 1998; Li et al, 2002). They were then embedded with wax, and serial coronal sections (60 μm thick) were cut perpendicularly to the pial surface of the lateral gyrus in the visual cortex (ranging from AP 1.0 to 5.0, Lateral 6.0 to 9.6 mm) (Paxinos & Watson, 1986) using a Rotary Type Microtome (American Optical). From each hemisphere of each animal, at least eight sections were randomly sampled at a regular interval of about 400 μm apart. Sections were collected on cleaned, gelatin-coated microscope slides followed by deparaffin and then Kodak developer D-76 for film for another 3 min. The sections were then washed with distilled water, dehydrated, cleared, and mounted using a resinous medium.

Slides bearing Golgi-impregnated sections were examined under light microscopy. Only neurons with the following criteria were used for quantitative analysis: (1) location of the cell soma in layers Ⅱ-Ⅳ of the visual cortex; (2) full impregnation of the neurons; (3) presence of at least three primary basilar dendritic shafts, each of which branched at least once; (4) no morphological changes attributed to Golgi-Cox staining. At least eight neurons per rat were photographed at a

magnification of $400 \times$ (BX-60, Olympus Microscope) by a person blind to the experimental groups (Jacobs et al, 1997; Kolb et al, 1998).

For each neuron, the dendritic tree, including all branches, was quantified by Sholl analysis as follows. A transparent grid with concentric rings, equivalent to 15-μm spacing, was placed over the dendrite picture, and the number of ring intersections was used to estimate the apical, basal and total dendritic length (TDL). The spine density, which was defined as the number of spines per unit length, was estimated by counting the total number of spines visible along both sides of the segment (at least 10 μm long). Spines were counted on the randomly selected second-order segment from the basilar dendrites. Approximately 2–3 segments were counted per slide and at least 6 slides were taken from each rat (Kolb et al, 1998).

1.3 Statistical analysis

Data from the neurons' spine densities and dendritic lengths were analyzed first by Two-way ANOVAs. Planned post hoc comparisons were computed using the least significant differences approach on the following groups: young males vs young females, young males vs aged males, young females vs aged females, aged males vs aged females. All analyses were conducted using the SPSS software package ($P < 0.05$ was considered significant).

2 Results

2.1 Dendritic spine density

The dendritic spine density was influenced by both age [$F = 89.8$ (1, 16), $P < 0.001$] and sex [F $= 5.3$ (1, 16), $P < 0.05$]. Additionally, an interaction between the influences of age and sex was found on this measure [$F = 18.5$ (1, 16), $P < 0.001$]. An age-related decrease in spine density occurred among both the male group ($P < 0.001$) and the female group ($P < 0.001$). A sex difference in spine density was found only among young animals. Young males possessed higher spine density than young females ($P <$

0.01). Unlike in the young animals, no sex difference was found among aged animals ($P = 0.18$) (Tab. 1, Fig. 1).

2.2 Dendritic complexity

Apical dendritic tree extent, as estimated by the total number of Sholl ring intersections, was influenced by both age [$F = 45.1$ (1, 16), $P < 0.001$] and sex [$F = 7.6$ (1, 16), $P = 0.014$]. An interaction between the influence of age and sex was found on this measure [$F = 16.1$ (1, 16), $P < 0.001$]. Also, there was an age-related decrease in apical tree extent among males ($P < 0.001$), but not in females. A sex difference, favoring males, was found among young animals ($P < 0.01$). As with spine density, the sex difference apparent among young animals was not found in aged subjects.

A similar age-related decrease was also found in both the basilar and total dendritic tree extents [basilar $F = 32.3$ (1, 16), $P < 0.001$, total $F = 52.1$ (1, 16), $P < 0.001$]. Sex also influenced the basilar and total tree extents [basilar $F = 4.9$ (1, 16), $P < 0.05$, total $F = 8.3$ (1, 16), $P < 0.05$], with males having greater tree extent than females in young adults (basilar $P < 0.05$, total $P < 0.01$). However, the sex difference disappeared during aging (Tab. 1, Fig. 2, 3).

3 Discussion

A series of studies on human beings and animals showed that many visual functions deteriorate with age. The aged, compared with the young, demonstrated not only slower information processing and prolonged response latency, but also reduced visual acuity, contrast sensitivity and so on. These functional declines might be partially due to the degeneration of the dendritic tree. However, there is no clear model of how the dendritic extent is regulated in the aging brain as yet (Hua, 2004; Mendelson & Wells, 2002). Dendritic stability, lengthening and regression have all been reported. Although the methodological differences may account for some of the varied findings, it seems reasonable to

Tab. 1 Morphometric dendritic measurements of cortical neurons from young and aged rats

Parameter	Young male	Young female	Aged male	Aged female
Dendritic length (μm)				
Apical	591.4 ± 13.5	441.2 ± 29.9[++]	353.7 ± 17.2[***]	381.6 ± 24.2
Basilar	1271.5 ± 48.8	992.3 ± 73.2[+]	772.6 ± 65.9[***]	773.6 ± 61.9
Total	1861.9 ± 45.1	1419.6 ± 96.7[++]	1116.1 ± 72.9[***]	1155.2 ± 53.7
Spine density (number/μm)				
Basilar	0.43 ± 0.018	0.35 ± 0.005[++]	0.26 ± 0.013[***]	0.28 ± 0.009[***]

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (young vs aged); [+] $P < 0.05$, [++] $P < 0.01$, [+++] $P < 0.001$ (young male vs young female).

conclude that the age-related changes in dendrites is species, region and even layer-specific (Duan et al, 2003; Grill & Riddle, 2002).

    The present study demonstrates a considerable reduction in both spine density and the complexity of apical and basilar dendrites in the rats' visual cortex during aging process. The age-related decreases in the complexity of apical and basilar dendrites in the rats'
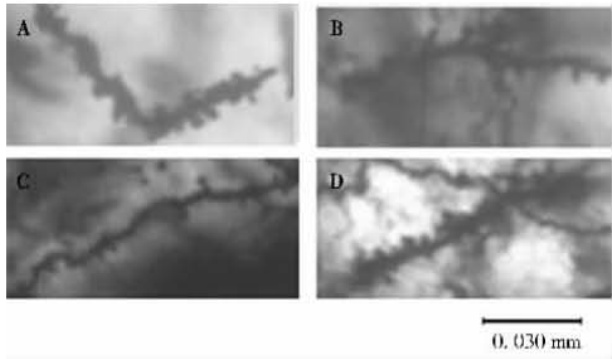

Fig. 1   Photomicrographs of basilar dendrite spine in the visual cortex

A: Young male; B: Young female;
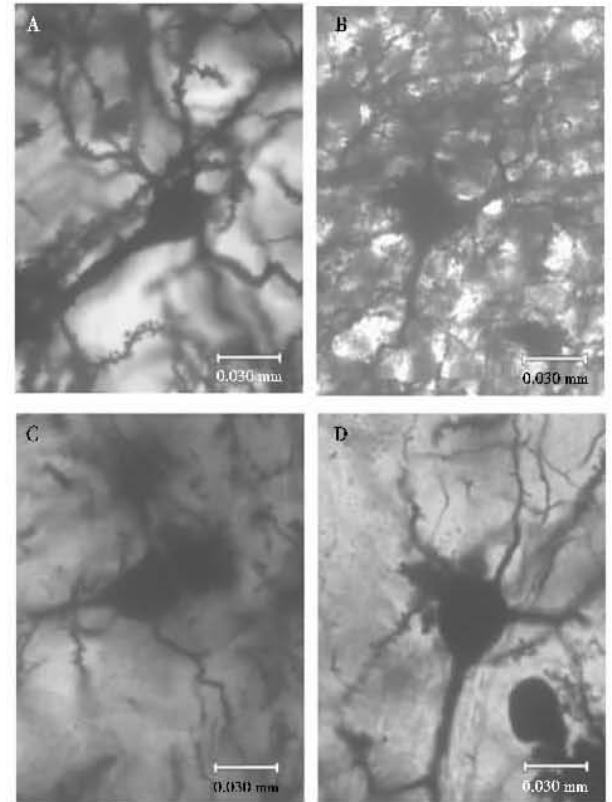C: Aged male; D: Aged female. *n* = 5, each group.

Fig. 2   Photomicrographs of Golgi-Cox stained pyramidal neurons in the visual cortex

A: Young male; B: Young female;
C: Aged male; D: Aged female. *n* = 5, each group.

visual cortex mirrors the results of previous studies on aged rodent parietal and medial frontal cortexes (Wong et al, 2000; Grill & Riddle, 2002). Although the potential mechanism is not clear yet, the age-related decline in protein and mRNA expression of dendrite-specific microtubule-associated protein (MAP2) might be related to the dendritic retraction (Shimada et al, 2006). The members of the neurotrophin family of growth factors, as well as several other neurotrophic factors, have been demonstrated to regulate dendritic growth in the developing brain. For example, recent studies of the developing rodent cortex revealed promotion
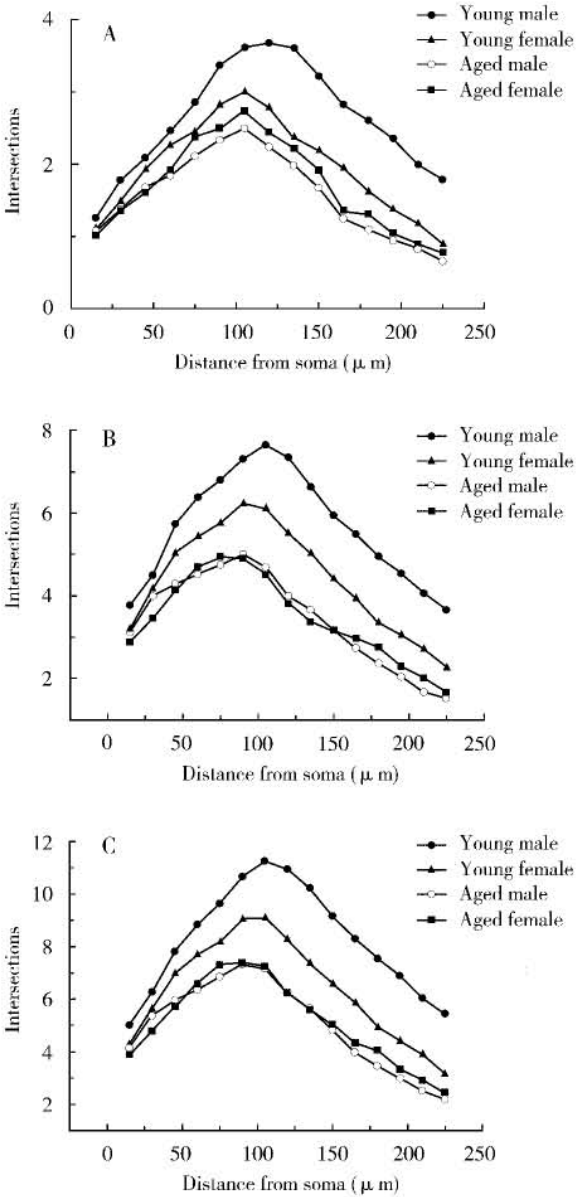

Fig. 3   Sholl analysis of apical (A), basilar (B) and total dendrite (C) from young male, young female, aged male and aged female rats (*n* = 5, each group)

of dendritic growth by brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4), as well as by insulin like growth factor-1 (IGF-1) (McAllister et al, 1995; Niblock et al, 2000). As the levels of several neurotrophic factors decreased, including BDNF and IGF-1, it was reasonable to propose that age-related declines in trophic support lead to dendritic regression in some neural regions, and thus to functional deficits (Silhol et al, 2005; Katoh-Semba et al, 1998; Sonntag et al, 1999). While the measurement of spine density might be underestimated using a light microscope, due to a lack of spines on the backside of the dendrite, the result is still believable because we used the same criteria during the assessment; also the young groups have higher spine density (Jacobs et al, 1997). Unlike the changes in dendritic tree extent, this study indicates an age-related decrease in spine density in both the male and female groups. The extent of the age-related decreases in the spine density in the visual cortex is consistent with that in the anterior cingulate cortex, which might be due to a decrease in the expression of the synapse-related proteins, such as synaptophysin and PSD-95 (Markham & Juraska, 2002; Shimada et al, 2003). Once again, the study confirms the hypothesis that changes in dendritic length and dendritic spine density are independent processes (Kolb et al, 1998).

In addition to age, our study indicates that the neuroanatomy of the visual cortex consistently varies with sex. The results of this study indicate that young adult males have greater dendritic spine density and arborization on both the apical and basilar dendrites compared to females on pyramidal cells in layers Ⅱ – Ⅳ of the visual cortex. However, the sex difference disappears during aging. Although several studies have obtained the same result in other regions, the neural mechanisms are still unknown (Markham et al, 2005; Markham & Juraska, 2002). It is hypothesized that sex differences in dendritic structure might result from the differences in afferent connections. In addition, circulating levels of gonadal steroids during adulthood have also been shown to play a contributing role. During aging, the female rats continue to secrete moderate levels of ovarian hormones, while the serum levels of testosterone in male rats are significantly lowered. This may be the reason why males experience more dramatic changes in the brain during aging than females (Leranth et al, 2003; Huang et al, 1978; Markham et al, 2005; Miller & Riegle, 1978).

In summary, the data presented here suggests that age and sex can lead to significant dendritic changes in visual cortical pyramidal neurons. The age and sex-related degeneration of dendrites may not only contribute to the functional degradation of cells during aging but could also explain the sex difference of visual function. The future challenge will be to continue to elucidate which layers and which neuronal populations show alterations within visual cortex. In addition to increasing the breadth of studies, it is also critical to elucidate how the dendrites of neurons are altered. Such detailed analyses will facilitate studies of the functional significance of dendritic changes and of the regulatory mechanisms associated with age-related dendritic plasticity.

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