Effects of senescence on the expression of BDNF and TrkB receptor in the lateral geniculate nucleus of cats

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ABSTRACT

To explore the neural mechanisms mediating aging-related visual function declines, we compared the expressions of brain-derived neurotrophic factor (BDNF) and its high affinity receptor-tyrosine kinase B (TrkB) between young and old adult cats. Nissl staining was used to display neurons in each layer of the lateral geniculate nucleus (LGN). The BDNF- and TrkB receptor-immunoreactive neurons were labeled immunohistochemically, observed under optical microscope and photographed. Their neuronal density and immunoreactive intensity were measured. Results showed that the mean density of the Nissl stained neurons in each LGN layer were comparable between old and young adult cats, and their BDNF and TrkB proteins were widely expressed in all LGN layers. However, compared with young adult cats, both the density and optical absorbance intensity of BDNF- and TrkB-immunoreactive cells in each LGN layer in old cats were significantly decreased. These findings indicate that the decreased expressions of BDNF and TrkB proteins in the LGN may be an important factor inducing the compromised inhibition in the central visual nucleus and the functional visual decline in senescent individuals.

Keywords: Cat; Lateral geniculate nucleus; BDNF; TrkB; Age-related change

INTRODUCTION

Progressive recession in visual capacity, which includes decreased visual acuity, insensitiveness to visual contrast, inability to determine orientation or direction of objects and slowdown in processing visual information, can be found during the normal course of aging (Hua et al, 2006; Schmolesky et al, 2000). However, the neuronal mechanisms underlying age-related functional visual decline are still unclear (Hua et al, 2011; Zhou et al, 2011). How changes in neuronal plasticity affect age-related functional visual decline and the correlated molecular or cellular mechanisms are quite controversial (Hua et al, 2006; Liang et al, 2012b; Schmolesky et al, 2000; Zhou et al, 2013). For example, previous electrophysiological studies reported that the reactive characters of neurons in the lateral geniculate nucleus (LGN) were not significantly correlated with age and that aging might affect neuronal functions of the visual cortex, especial the advanced visual cortex (Hua et al, 2006; Liang et al, 2012a, 2012b; Zhou et al, 2011). However, these results could not rule out the possibility that both the morphology and neurotransmitter systems had changed during aging (Vidal et al, 2004), and these changes could influence the reactive characters of the visual cortical neurons to visual stimuli through synaptic connections. Other studies reported that aging was also accompanied with decreased expression of inhibitory neurotransmitters (such as GABA) in the visual cortex (Hua et al, 2008), which might induce the insensitiveness and decline in selectivity of the visual cortical neurons to visual stimuli (Hua et al, 2006; Leventhal et al, 2003; Zhou et al, 2011). Studies on neurotrophic factors found that brain-derived neurotrophic factors (BDNF) are vital in the development, survival, and lesion repair of neurons and neuronal plasticity via receptor mediated signal transductions (Ichim et al, 2012; Kwon et al, 2011; Numakawa et al, 2010). Some reports have shown that the BDNF signal system can modulate activity-dependent intracortical inhibitory (Rutherford et al, 1997) and promote the synthesis and transportation of GABA (Sánchez-Huertas & Rico, 2011; Vaz et al, 2011; Waterhouse et al, 2012). However, although a few studies

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reported that age-related changes were found in the cerebral expressions of BDNF and receptors (Erickson et al, 2010; Hayakawa et al, 2008; Luellen et al, 2007; Silhol et al, 2005), it is too early to conclude that the decreased expression of BDNF and its receptors during senescence is the reason for a decline of inhibitory neurotransmitter GABA.

Our previous immunohistochemistry results showed that the expression of GABA in the dorsal LGN (dLGN) decreased significantly during aging (Tong et al, 2006); however, whether this phenomena is correlated with the expression down-regulation of BDNF and its receptors remains unclear. To understand the cellular and molecular mechanisms of age-related functional visual decline, we explored the expression differences of BDNF and its high affinitive receptor, tyrosine kinase receptor B (TrkB), in the dLGN of young and old adult cats.

MATERIALS AND METHODS

Animal subjects and experimental reagents

Four young (1-3 years old, 2-3.5 kg) and four old (10-13 years old, 2-3.5 kg) adult cats were examined ophthalmoscopically prior to experimentation to confirm that no optical or retinal problems impaired their visual function. All experiment procedures were performed strictly in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals. After a deep anesthesia by ketamine HCl (40 mg/kg, im) injection, cardiac perfusion was immediately performed on the animals (0.9% saline water). When pale livers were observed, a pre-fixture with 0.1 mol/L PBS (10% formalin, 2.5% glutaraldehyde, pH 7.2-7.4, 200 mL/kg) was performed. The isolated brainstem was fixed for 2 h and the LGN was then separated from it and transferred into a fixing solution (10% formalin, 2.5% glutaraldehyde, 30% sucrose) till the tissue sank to the bottom. Every four continuous coronal frozen slices (30 μm) were selected as a group for Nissl staining, BDNF and TrkB immunohistochemistry labels and negative controls. The slice groups were separated from each other by an interval of five slices. Every cat included 10 groups of slices.

Neuronal Nissl staining

Slices were stained in 0.1% cresyl violet solution for 5 min at room temperature, rinsed with distilled water, dehydrated by gradient alcohol, transparentized by xylene and then sealed with gum. Stained slices were used to determine the layering structures of the LGN and the neuronal density of each layer.

Immunohistochemical labeling

The frozen slices were incubated in 3% H_2O_2 for 15 min at room temperature to eliminate endogenous peroxidase activity and were then rinsed with distilled water, incubated in 0.3% TritonX-100 PBS for 20 min at room temperature, incubated in solution with 5% fetal bovine serum protein for 20 min at room temperature to seal the specific reactive areas, incubated in rabbit-anti-mouse BDNF and rabbit-anti-human TrkB multiclonal antibodies (1:100, primary antibodies) for 36 hours at 4°C and then rinsed three times with PBS for 5 min, incubated in biotinylated secondary antibody (goat-ant-rabbit) for 20 min at room temperature and then rinsed three times with PBS for 5 min, incubated in streptavidin-biotin complex (SABC) working solution for 20 min at room temperature and then rinsed three times with PBS for 5 min, developed by DAB to produce colorimetric end products, dehydrated by gradient (80%, 95% and 100%) alcohol, transparentized by xylene and then sealed with neutral gum. The only difference in the treatment of the negative control slices was replacing primary antibodies with PBS. All immunohistochemistry kits and the DAB substrate were products of Boster, Wuhan, China.

Statistical analysis

Nissl stained slices and BDNF and TrkB immuno-reactive slices were observed under a microscope (Olympus BX-51). Images were collected by Image-Pro Express 6.0 software. All related morphological parameters went through quantitative analysis, including the Nissl stained neuronal densities in each LGN layer, densities of BDNF and TrkB immuno-reactive positive neurons and intensities of immuno-reactions (evaluated by average optical absorbance, where higher absorbance values indicated stronger immuno-reactions). Data analyses were performed unbiased.

Images of slices were initially acquired under 40× ocular magnification and the layering structures (layer A, layer A_1 and layer C) of the LGN were then discriminated under 100× ocular magnification. Ten view fields (50 μm×50 μm) of each layer were randomly selected under 400× ocular magnification to calculate the densities of Nissl stained neurons and BDNF and TrkB immuno-positive neurons (cells/mm^2), respectively. A typical Nissl stained neuron was characterized with royal purple Nissl bodies and a clear nucleus in soma, and a typical BDNF/TrkB immuno-positive neuron was characterized with obvious immuno-positive matter and a clear nucleus in soma. The optical absorbance values of 20 immuno-positive neurons from each slice were randomly selected by Image-Pro Express 6.0 software, and the mean value was taken as the index indicating the intensity of immuno-reactions. All data were analyzed via SPSS 13.0 software and expressed as means±*SD*, with $P<0.05$ being considered statistically significant.

RESULTS

Densities of Nissl stained neurons

A three-layer-structure of the LGN (layer A, layer A_1 and layer C) (Figure 1) was shown in the Nissl stained slices. Blue or dark blue somas in various shapes, such as oval, tapered or polygon, were visualized in each layer. The results showed that age-independently, the neuronal density of layer A was significantly higher than that of layer A_1 ($F_{(1,158)}=31.04$, $P<0.001$), and neuronal densities of layer A and A_1 were both higher than that of layer C ($F_{(1,158)}=212.04$, $P<0.001$; $F_{(1,158)}=85.72$, $P<0.001$). No differences were found in the neuronal densities of each layer between young and old adult cats ($F_{(1,238)}=1.88$, $P=0.17$, Table 1).

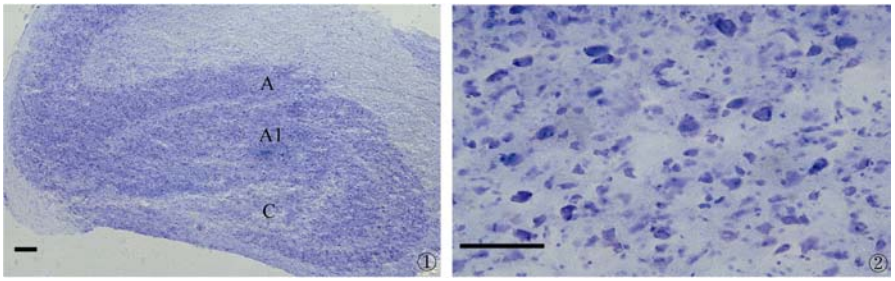

Figure 1 Nissl stained laminar organization (left, layer A, A_1 and C) and neurons (right) in the LGN of cats (Scale bar=50 μm)

Densities of BDNF and TrkB immuno-positive neurons

Immunohistochemical staining results showed that BDNF and TrkB immuno-positive neurons with brown or dark brown somas and their fiber distributions were found in every layer of the LGN in young and old adult cats (Figure 2). Age-independently, the densities of BDNF immuno-positive neurons in layer A and A_1 were both significantly higher than that of layer C ($F_{(1,158)}$=27.12, P<0.001; $F_{(1,158)}$=40.06, P<0.001, Table 1), whereas, those between layer A and A_1 were comparable ($F_{(1,158)}$=1.33, P=0.25). However, although no differences were found in the densities of BDNF immuno-positive neurons in each layer among cats at similar ages ($F_{(3,236)}$=1.11, P=0.35), those in old adult cats were significantly lower than those in young adult cats ($F_{(1,238)}$= 125.39, P<0.001, Table 1).

Age-independently, the densities of TrkB immuno-positive neurons in layer A and A_1 were both significantly higher than that of layer C ($F_{(1,158)}$=28.16, P<0.001; $F_{(1,158)}$=29.92, P<0.001, Table 1), whereas, those between layer A and A_1 were comparable ($F_{(1,158)}$=0.02, P=0.89). However, although no differences were found in the densities of BDNF immuno-positive neurons in each layer among cats at similar ages ($F_{(3,236)}$=0.22, P=0.88), those in old adult cats were significantly lower than those in young adult cats ($F_{(1,238)}$= 208.54, P<0.001, Table 1).

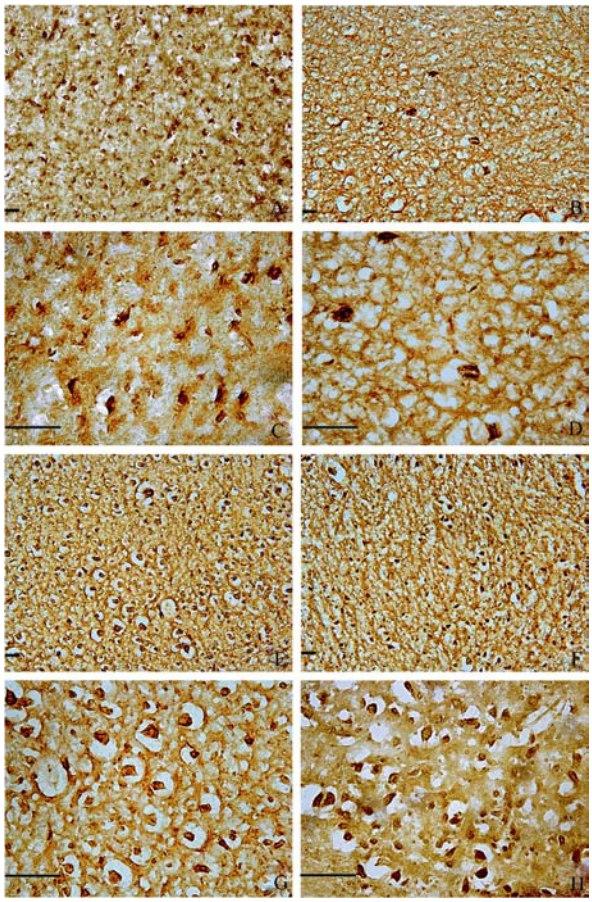

Figure 2 BDNF immuno-positive neurons (A, B, C, D) and TrkB immuno-positive neurons (E, F, G, H) in layer A of the LGN in young (A, C, E, G) and old (B, D, F, H) adult cats (Scale bar=50 μm)

Immuno-intensities of BDNF and TrkB immuno-positive neurons

The mean optical absorbance values of the BDNF immuno-positive neurons in the LGN of old cats were significantly lower than those in young cats ($F_{(1,78)}$=18.74, P<0.001), specifically, the mean absorbance values of layer A, A_1 and C were decreased by 26.4%, 20.3% and 36.3%, respectively. The mean absorbance values of the TrkB immuno-positive neurons in the LGN of old cats were also significantly lower than those in young cats ($F_{(1,78)}$=28.46. P<0.001), specifically, the mean optical absorbance values of layer A, A_1 and C were decreased by 25.6%, 26.5% and 35.3%, respectively (Figure 3).

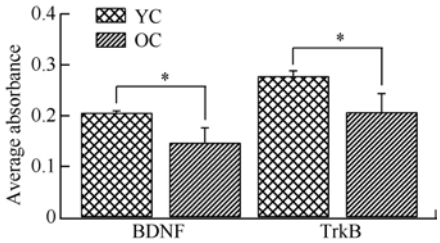

Figure 3 Mean optical absorbance values of BDNF and TrkB immuno-positive neurons in the LGN of young and old adult cats (*: P<0.01)

DISCUSSION

Vision is the most important way for higher animals, including

Table 1 Densities of BDNF, TrkB immuno-positive neurons in the dLGN of young and old cats

Animal subjects	Lamina organization of LGN		
	A	A_1	C
Densities of Nissl stained neurons (cells/mm^2)			
YC1	3 510.0±587.8	2 650.0±462.5	2 030.0±336.8
YC2	2 510.0±481.8	2 430.0±432.2	1 590.0±159.5
YC3	2 550.0±259.3	2 440.0±320.4	1 880.0±312.0
YC4	2 850.0±406.2	2 510.0±587.7	1 920.0±234.8
Mean	2 855.0±592.7$^{\#}$	2 507.5±451.4$^{\#}$	1 855.0±307.1$^{\#}$
OC1	2 700.0±434.6	2 380.0±482.6	1 980.0±278.1
OC2	2 810.0±481.8	2 430.0±503.4	1 680.0±193.2
OC3	2 930.0±490.0	2 390.0±296.1	1 750.0±222.4
OC4	2 690.0±486.4	2 020.0±297.4	1 830.0±231.2
Mean	2 782.5±465.7$^{\#}$	2 305.0±425.4$^{\#}$	1 810.0±250.9$^{\#}$
Densities of BDNF immuno-positive neurons (cells/mm^2)			
YC1	2 320.0±315.6	2 640.0±430.0	2 040.0±350.2
YC2	2 400.0±461.9	2 280.0±423.7	2 000.0±326.6
YC3	2 320.0±253.0	2 120.0±379.5	2 160.0±337.3
YC4	2 360.0±397.8	2 440.0±461.9	1 840.0±337.3
Mean	2 350.0±353.0**	2 370.0±451.1**	2 010.0±344.8**
OC1	1 640.0±295.1	2 000.0±377.1	1 280.0±253.0
OC2	1 760.0±337.3	1 840.0±386.4	1 520.0±367.6
OC3	1 960.0±350.2	1 800.0±282.8	1 080.0±270.0
OC4	1 560.0±295.2	1 920.0±367.6	1 240.0±227.1
Mean	1 730.0±343.6**	1 890.000±350.7**	1 280.0±316.4**
Density of TrkB immuno-positive neurons (cells/mm^2)			
YC1	2 280.0±369.5	2 520.0±329.3	2 000.0±326.6
YC2	2 360.0±397.8	2 280.0±388.7	2 040.0±266.7
YC3	2 320.0±168.7	2 160.0±386.4	2 120.0±423.7
YC4	2 400.0±533.3	2 480.0±454.1	1 880.0±379.5
Mean	2 340.0±379.5**	2 360.0±410.6**	2 010.0±350.8**
OC1	1 600.0±266.7	1 760.0±279.7	1 200.0±188.6
OC2	1 800.0±339.9	1 560.0±227.1	1 600.0±233.0
OC3	1 920.0±367.6	1 670.0±259.7	1 120.0±315.5
OC4	1 640.0±350.2	1 960.0±380.2	1 280.0±253.0
Mean	1 740.0±345.5**	1 737.5±311.2**	1 300.0±289.3**

YC1-4 and OC1-4 represent four different young and four different old adult cats, respectively. $^{\#}$:P>0.05; **: P<0.001.

humans, to acquire environmental information. Age-related functional visual decline can therefore directly lower life quality. Understanding neuronal networks and the molecular and cellular mechanisms underlying age-related functional visual decline is helpful in delaying the effects of senescence on vision and stimulating visual ability in seniors (Zhang et al, 2008a).

In higher animals and humans, the processing of visual information includes three different levels, that is, the retina, LGN and visual cortex from low to high. Previous electrophysiological studies indicate that aging may change the reactive characters of visional neurons to visional stimuli, e.g., the ability to determine the locations of visional stimuli and confirm moving directions, sensitivity to contrasts and selectivity in dimensional and temporal frequencies (Fu et al, 2013; Hua et al, 2006; Leventhal et al, 2003; Liang et al, 2010;

Schmolesky et al, 2000; Wang et al, 2005; Yang et al, 2009; Zhang et al, 2008b; Zhou et al, 2011). However, although these studies did not show significant influences of senescence on the characteristics of neurons on the LGN (Spear et al, 1994; Zhou et al, 2013), it is still possible that age-related changes are happening at the molecular or cellular level in the LGN. Our earlier work found that GABA expressions in the LGN of old cats were significantly lower than those in young adult cats (Tong et al, 2006). We assume that the functional effects of decreasing GABA may be compensated by downstream pathways or other factors, and therefore senescence may influence the function of LGN at the cellular level.

Tong et al (2006) reported that during the course of aging, the neuronal densities of the LGN maintained relatively stable, while the expression of GABA was significantly decreased; however, the underlying mechanisms for this remain unclear. Studies on neurotrophic factors showed that BDNF and its receptor (including high affinitive Trk receptor and low high affinitive p75 receptor) introduced signal transductions play vital roles in the development, survival and synaptic plasticity of neurons (Ichim et al, 2012; Kwon et al, 2011; Numakawa et al, 2010; Wong et al, 2013), including GABAnergic neurons (Cheng & Yeh, 2005; Jiao et al, 2011; Rutherford et al, 1997; Sakata et al, 2009; Waterhouse et al, 2012), and may be directly involved in the synthesis and transportations of GABA (Sánchez-Huertas & Rico, 2011; Vaz et al, 2011). However, whether the age-related GABA decrease is also correlated with the abnormal BDNF signal transduction still needs further evidence.

In the present study, no differences were found in the neuronal densities of each LGN layer, indicating relative stable neuronal quantities during the aging course, which is consistent with previous research (Tong et al, 2006). However, both the densities and intensities of the immuno-activity of BDNF and TrkB immuno-positive neurons in each LGN layer of old adult cats were significantly lower than those in young adult cats, which is consistent with results observed in old rats and the hippocampus of patients with Alzheimer's disease (Chapman et al, 2012; Erickson et al, 2010; Luellen et al, 2007; Tapia-Arancibia et al, 2008), indicating that the expressions of BDNF and TrkB decrease and the BDNF-TrkB signal transductions decline during the course of aging.

In summary, the present study indicated that senescence could induce the decreased expression of BDNF and TrkB receptors in each LGN layer, and the BDNF-TrkB signal transduction decline could decrease the expression of GABA. The weakening inhibitory effects of nerve endings on synaptic connections could influence the sensitivity and selectivity of visual neurons to visual stimuli and then induce age-related functional visual decline.

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