Decline of selectivity of V1 neurons to visual stimulus spatial frequencies in old cats

Guo-Peng HUA, Xia-Ming SHI, Jun ZHOU, Qing-Song PENG, Tian-Miao HUA

School of Life Science, Anhui Normal University, Wuhu 241000, China

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Abstract: Objective To examine whether the selectivity of visual cortical neurons to stimulus spatial frequencies would be affected by aging in cats. **Methods** *In vivo* extracellular single-unit recording techniques were employed to record the tuning responses of V1 neurons to different stimulus spatial frequencies in old and young adult cats. **Results** Statistical analysis showed that the mean optimal spatial frequency of grating stimuli that evoked the maximal response of V1 neurons in old cats was significantly lower than that in young adult cats. Furthermore, the mean high cut-off spatial frequency of grating stimuli that evoked the half amplitude of the maximal response of V1 neurons in old cats was also significantly lower than that in young adult cats. **Conclusion** These results are consistent with those reported in the V1 of old monkeys, suggesting that the age-related decline in the selectivity of visual cortical cells to spatial frequency could be generalized to all mammalian species and might contribute to visual acuity reduction in senescent individuals.

Keywords: spatial frequency selectivity; functional decline; V1 neurons; old cats

1 Introduction

Visual acuity, defined as the capacity to discriminate the fine structures of objects, is one of the most important parameters for visual quality assessment. Routine medical examination shows that visual acuity (including stereoacuity) in the senescent people is significantly decreased as compared with that in the young adults. This age-related reduction of visual acuity is not due to any special eye diseases, because normal aged people also display a decrease in visual acuity^[1-3]. Li *et al.* examined the vernier acuity of subjects in different age groups (21-75 years old) who showed normal optical media and no macular degeneration. They find that the vernier acuity of individuals older than 60 years is nota-

bly decreased, compared with that in the younger counterparts^[4]. Consistently, the threshold to detect size difference between stimuli in old people is shown to be significantly higher than that in younger subjects^[5]. The age-dependent reduction of visual acuity is generally attributed to optical factors such as corneal aging, lens transparency and elasticity decreases, and retina changes. For example, during aging, corneal thickening, lens rigidity as well as its reduced response to ciliary muscular contraction may lead to the abnormality of light focusing in the eyes^[6]. Hodos *et al.*^[7] and Fitzgerald *et al.*^[8] have also found that aged pigeons exhibit a significant reduction in visual acuity relative to young pigeons. They suggest that the decrease of visual acuity during aging is mainly correlated with deteriorations of chorioidal structures and the outer retinal sections, as well as significant loss of ganglion cells and photoreceptors especially the double-cone cells (reduced by approximately 1/3) in aging retina, while the reduction of pupillary diameter and

Corresponding author: Tian-Miao HUA

Tel: +86-553-3869669

E-mail: tianmiaohua@gmail.com

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retinal illumination decrease during aging account for only a trivial proportion of acuity loss^[7]. Recently, Zhang *et al.* have recorded the responses of visual cortical cells to stimuli with different spatial frequencies in old and young adult monkeys, and they find that the mean preferred spatial frequency and spatial resolution of V1 neurons in old monkeys are significantly lower than those in young adult monkeys^[9]. This suggests that visual cortical cells in old individuals exhibit a decreased capacity of processing fine visual signals, which may contribute to the visual acuity reduction during senescence. In the present study, extracellular single-unit recording techniques were employed to study the tuning responses of V1 neurons to different spatial frequencies of stimuli in old and young adult cats, aiming to testify whether the selectivity of visual cortical cells to spatial frequencies would change significantly during aging in cats.

2 Materials and methods

2.1 Animals Four young adult cats (2-3 years old) and 4 old cats (12-14 years old) were employed in the present study. Animals received ophthalmoscopical examination before the experiment, to confirm that their visual function had no optical or retinal problems. All the procedures were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Single-unit recording Extracellular single-unit recording was performed as previously described^[10-12]. Briefly, cats were anesthetized by intramuscular injection of ketamine HCl (20 mg/kg) and xylazine (2 mg/kg). After intubation of intravenous and tracheal cannulae, cats were immobilized in a stereotaxic apparatus with ear bars, eye bars and a bite bar. Pupils were maximally dilated with atropine (1%), and appropriate contact lenses were used to protect the corneas. Neosynephrine (5%) was administered to retract the nictitating membranes. Glucose (5%)-saline (0.9%) solution containing a mixture of urethane (20 mg/kg body weight) and galamine triethiodide (10 mg/kg body weight) was infused intravenously in each hour to maintain the state of anesthesia and paralysis. Expired pCO₂ was maintained at approximately 4%. Heart rate (approximately 180-220 pulses/min) and electrocardiography were monitored throughout the experi-

ment to assess the level of anesthesia. The skull and dura over V1 (area 17) were removed under light microscope. Single-unit recordings were performed using a glass-coated tungsten microelectrode (with an impedance of 3-5 M Ω) which was driven by a hydraulic micromanipulator (NARISHIGE, Japan). The small hole over V1 was filled with 4% agar solution in saline and sealed with wax. After that, the optic discs of the 2 eyes were reflected onto a movable transparent tangent screen that was positioned 57 cm from the retina and overlapped with the CRT monitor used for stimulus presentation. The area centralis of each eye was located prior to physiological recording based on the position of the optic discs reflected onto the tangent screen.

2.3 Visual stimuli Visual stimuli were drifting sinusoidal gratings shown on a CRT monitor (resolution 1024 $\times$ 768, refresh rate 85) that was positioned 57 cm from the animal's eyes. The stimuli-generating program was written in MATLAB, using the extensions provided by the high-level Psychophysics Toolbox^[13] and low-level VideoToolbox^[14]. Once a single unit was isolated, the cell's receptive field (RF) center was carefully located by consecutively presenting a series of computer-generated light spots on the CRT. By comparing the neuron's responses to a series of stimulus package, the optimal stimulus size, orientation and motion direction and temporal frequency for each cell were determined. Then the cell's responses to different stimulus spatial frequencies (0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.6 and 2 c/deg) were recorded. Each stimulus was presented monocularly to the dominant eye and repeated 4-6 times with 3-min intervals for functional recovery of the recorded cell. Before the presentation of each stimulus, spontaneous activity (baseline response) was acquired during a 1-s period of mean luminance presentation on the screen.

The contrast of each stimulus used was set at 100%. The mean luminance of the display was 19 cd/m², and the environmental ambient luminance on the cornea was 0.1 lux.

2.4 Data acquisition and analysis After the signal was amplified with a microelectrode amplifier (Nihon Kohden, Japan) and a differential amplifier (FHC, USA), action potentials were fed into a window discriminator with an audio monitor. The original voltage traces (Fig. 1A, C) were digitized using an

Fig. 1 Responses of V₁ neurons to different spatial frequencies of stimulus in young adult (A, B) and old (C, D) cats. A and C: a representative voltage trace of the cell in response to the grating stimulus with the optimal spatial frequency (A: 0.6 c/deg, C: 0.2 c/deg). A spike above the broken horizontal line is counted as an action potential. B and D: the cell's tuning response to grating stimuli with different spatial frequencies. Black squares with error bars represent the mean firing rate (spikes/s) to different stimulus spatial frequencies. The smooth curve represents the best-fitting Gaussian function (fitting goodness: $r^2=96.1$ in B, $r^2=98.4$ in D). From the fitting curve, the optimal stimulus spatial frequency (B: 0.618 c/deg; D: 0.246 c/deg indicated by the arrow) that evoked the maximal response (B: 102.1 spikes/s; D: 169.2 spikes/s), and the high cut-off spatial frequency (B: 1.07 c/deg; D: 0.424 c/deg indicated by the arrowhead) that evoked the half amplitude of the maximal response were obtained.

acquisition board (National Instruments, USA) controlled by IGOR (WaveMetrics, USA), and saved for later analysis. Post-stimulus time histograms (PSTHs) of the responses were also obtained. As shown in the spatial frequency tuning curves (Fig. 1B, D), cell response to a drifting sinusoidal grating was defined as the mean firing rate (subtracting the baseline) corresponding to the time of stimulus modulation. The response-spatial frequency tuning curve of each cell was fitted with Gaussian function: $R(f)=R_0+A \times \exp[-(f-f_0)^2/\text{width}^2]$, where f and f_0 indicate the spatial frequency of the grating stimulus and the peak spatial frequency, respectively. A means the cell's maximum visually-evoked response, and R_0 refers to the minimum response. Cells with less than 95% goodness of fit were excluded from data statistics. The optimal spatial frequency corresponding to the peak of response and the high cut-off spatial frequency at half amplitude of the peak response were obtained from the best Gaussian fitting function (Fig. 1B, D).

Data were expressed as mean $\pm$ SD. Comparisons between

different individuals and age groups were made using one-way or two-way analysis of variance (ANOVA) and Chi-square test. $P < 0.05$ was indicated as significantly different.

3 Results

A total of 199 cells from 4 old cats and 166 cells from 4 young adult cats were included in this study (Table 1). Cells in the 2 age groups were recorded at the same range of depth from the pia surface of the brain to avoid laminar bias. All cells had RFs within 8 degrees from the fovea. There was no significant difference in the eccentricity distribution of cells between old and young adult cats [$\chi^2(7)=11.422$, $P=0.121$]. The proportions of simple cells and complex cells were comparable between the 2 age groups [77 simple cells, 122 complex cells in the old group; 71 simple cells, 95 complex cells in the young adult group; $\chi^2(1)=0.624$, $P=0.429$]. Since simple and complex cells showed similar age-related changes in both optimal stimulus spatial frequency [Age effect: $F(1,$

Table 1. The mean optimal spatial frequency and cut-off spatial frequency of V1 neurons in each old and young adult cat. All values were expressed as mean±SD.

	Old cats				Young adult cats			
	OC ₁	OC ₂	OC ₃	OC ₄	YC ₁	YC ₂	YC ₃	YC ₄
CN	67	82	31	19	78	18	16	54
OSF(c/deg)	0.27±0.19	0.24±0.25	0.29±0.18	0.26±0.16	0.48±0.29	0.52±0.29	0.46±0.33	0.43±0.20
CSF(c/deg)	0.45±0.32	0.39±0.41	0.48±0.29	0.44±0.27	0.80±0.49	0.87±0.50	0.77±0.55	0.73±0.35

CN: cell number. OSF: optimal spatial frequency. CSF: high cut-off spatial frequency. OC₁₋₄ represents the 4 old cats, and YC₁₋₄ represents the 4 young adult cats.

361)=64.747, $P < 0.0001$; Interaction of age and cell type: $F(1, 361)=0.217$, $P=0.641$] and cut-off spatial frequency [Age effect: $F(1, 361)=68.792$, $P < 0.0001$; Interaction of age and cell type: $F(1, 361)=0.265$, $P=0.473$], data from the 2 types of cells were combined for analysis.

As shown in Fig. 2A, most of the cells (70.8%) in old cats responded preferentially to stimulus whose spatial frequency was less than 0.4 c/deg, whereas in young adult cats, a majority of cells (65.1%) responded preferentially to stimulus whose spatial frequency was larger than or equal to 0.4 c/deg. ANOVA analysis indicated that in either group, there was no significant difference in the mean optimal spatial frequency of V1 neurons between different individuals [in old cats: $F(3, 195)=0.613$, $P > 0.5$; in young adult cats: $F(3, 162)=0.538$, $P > 0.5$]. However, the mean optimal spatial frequency of V1 neurons in each old cat was significantly lower than that in any individual young adult cat [Age effect: $F(1, 357)=44.582$, $P < 0.0001$; Interaction between age and subject: $F(3, 357)=0.563$, $P=0.581$]. A comparison of average optimal spatial frequency of V1 neurons between old cat group [(0.26±0.21)

c/deg] and young cat group [(0.47±0.27) c/deg] also showed a significant age difference [$F(1, 363)=65.777$, $P < 0.0001$].

The high cut-off stimulus spatial frequency that evokes the half amplitude of the cell's maximal response was also compared between the 2 age groups. The majority of cells (67.8%) in old cats had a cut-off spatial frequency smaller than 0.4 c/deg, while in young adult cats, more than 50% of cells (54.2%) had a cut-off spatial frequency larger than or equal to 0.6 c/deg (Fig. 2B). ANOVA analysis showed that the mean cut-off spatial frequency within either group exhibited no significant individual difference [in old cats: $F(3, 195)=0.636$, $P > 0.5$; in young adult cats: $F(3, 162)=0.522$, $P > 0.5$]. However, the mean cut-off spatial frequency of V1 neurons in each old cat was significantly smaller than that in any individual young adult cat [Age effect: $F(1, 357)=46.325$, $P < 0.0001$; Interaction of age and individuals: $F(3, 357)=0.743$, $P=0.527$]. Besides, the averaged cut-off spatial frequency of V1 neurons in old cats [(0.43±0.35) c/deg] was significantly different from that in young adults [(0.78±0.46) c/deg] [$F(1, 363)=69.936$, $P < 0.0001$].

Fig. 2 The percentage distributions of cells with different optimal spatial frequencies (A) and high cut-off spatial frequencies (B) in old and young adult cats.

4 Discussion

The present study examined the selectivity of V1 neurons to different spatial frequencies of stimuli in both old and young adult cats, and revealed that the mean optimal spatial frequency of V1 neurons in old cats was significantly lower than that in young adult cats. Furthermore, the mean high cut-off spatial frequency of V1 neurons in old cats was significantly lower than that in young ones. These findings are consistent with a previous study on the V1 neurons of old and young adult monkeys^[9], suggesting that age-dependent degradation of selectivity of visual cortical neurons to spatial frequencies of visual stimuli could be generalized to non-primate mammalian species with well-developed visual system. This age-related functional degradation of visual cortical cells may contribute to the visual acuity decline in the aged individuals.

Mechanisms underlying functional degradation of visual cortical cells have been frequently debated in recent years^[10,11,15-17]. The imbalance between intracortical excitation and inhibition has been suggested to mediate the functional decline of visual cortical cells in the senescent individuals, as supported by the following experimental findings. First, it has been reported that the responsiveness (including the spontaneous activity and visually-evoked response) of visual cortical cells in the old mammalian animals is significantly enhanced compared with that in the young adult animals, while the signal-to-noise ratio of visual cortical cells in the old animals is significantly decreased compared with that in young adults^[9,10,15,18]. Second, administration of GABA and GABA_A receptors by iontophoresis can significantly improve the function of V1 neurons in the old macaque monkeys, and application of antagonists of GABA_A receptors exert a much weaker effect in old than in young monkey cortex^[17]. Third, recent studies using immunohistochemistry examination of the visual cortex indicate that the proportion of GABAergic neurons and the ratio of GABAergic to Glutamatergic neurons in the visual cortex of senescent animals are significantly decreased compared with those of young adults^[19,20].

Most V1 neurons have a square or bar-shaped RF with an excitatory sub-area in the center, called classic RF, and an inhibitory sub-area in the surrounding^[21]. Therefore, V1 neu-

rons usually respond preferentially to bar stimuli with specific width and length. In this study, we found that the mean optimal spatial frequency and high cut-off spatial frequency of V1 neurons in old cats were significantly smaller than those in young adult cats. This result indicates that V1 neurons in the old individuals exhibit a larger classic RF than do the neurons in young about counterparts, which may result from a reduction of intracortical inhibition, since decreased intracortical inhibition can lead to shrinkage of inhibitory sub-area in the surrounding of RF, thus increasing the corresponding excitatory sub-area or classic RF. However, the enlargement of classic RF during aging may not necessarily originate from the visual cortex. Some investigations have shown that the decline of visual acuity in the senescent individuals might be mainly attributed to a combination of several anatomical changes of the aging ocular media^[1,7,8,22,23], such as decrepitude of the cornea and choroid, decreases in pupil diameter and retinal illumination, increases of lens rigidity and opacity, as well as loss of retinal cells. Hodos *et al.*^[7] and Fitzgerald *et al.*^[8] found that the decrease in visual acuity is mainly correlated with the deterioration of choroidal structures and loss of photoreceptors especially the double-cone cells. Another important factor that might affect the visual acuity is age-related accumulation of lipofuscin in the retinal pigment epithelium, which exerts high phototoxic and oxidative damifications to lysosomal stability, lipid peroxidation and protein oxidation, culminating in retinal cell death^[23,24].

In summary, aging could lead to a decreased selectivity of visual cortical cells to the spatial frequencies of visual stimulus, which may contribute to the decline of visual acuity in old individuals. However, further studies are needed to clarify the complex underlying mechanisms.

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老年猫初级视皮层细胞对视觉刺激空间频率调谐反应的选择性下降

化国鹏, 施夏明, 周俊, 彭青松, 华田苗

安徽师范大学生命科学院神经生物学实验室, 芜湖 241000

摘要: **目的** 探讨猫的初级视皮层(V1)细胞对视觉刺激空间频率的选择性是否受年龄影响。 **方法** 采用在体细胞外单细胞记录技术分别记录青、老年猫V1神经元对不同空间频率光栅刺激的调谐反应。 **结果** 数据统计分析结果显示, 诱导老年猫V1神经元出现最大诱发反应幅度的平均最优刺激空间频率(optimal spatial frequency, OSF)相较于青年猫的OSF显著降低。此外, 老年猫V1神经元达到半幅反应的平均高频截止空间频率(cut-off spatial frequency, CSF)也显著小于青年猫的CSF。 **结论** 该结果与在老年猴初级视皮层的研究报道一致, 表明年龄相关的视皮层细胞对刺激空间频率的选择性下降可能是哺乳动物中普遍存在的现象, 也可能是导致老年性视敏度下降的重要原因。

关键词: 空间频率选择性; 功能性下降; 初级视皮层细胞; 老年猫