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The sexual difference of aging-associated functional degradation in visual cortical cells of rats

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ABSTRACT

Function of visual cortical cells declines during normal aging. Whether there are sex-related differences in this functional degradation is still unknown. In the present study we compared the properties of adaptation, onset latency, and signal-to-noise ratio of visual cortical cells between age-matched sexes in order to investigate any sex related difference. Our results show that visual cortical cell function did not differ between young male and young female rats. However, compared with female rats in the same age, the signal-to-noise ratio, but not adaptation or onset latency, was significantly impaired in mid-aged and aged male rats. These results indicate that the functional degradation of visual cortical cells to some extent is associated with sex and therefore, could contribute for the differential degree of cognitive decline that occurs in males and females during senescence.

Visual ability declines during normal (non-pathological) aging. A large body of evidence from anatomical and morphological studies of aged retina and subcortical area failed to provide explanations for many of these aging-related deficits. Recent studies suggest that the degeneration of function in visual cortical cells contributes to the decline of visual abilities during aging [6,8,17,20]. Schmolesky et al. provided the first evidence for a significant degradation of orientation and direction selectivity in visual cortical cells of old monkeys. The decreased selectivity of cells in senescent monkeys was accompanied by a significant increase in spontaneous activity, resulting in a greatly reduced signal-to-noise ratio [17]. The same results were also observed in aged cats [6], indicating a universal mechanism is involved in different species. Additionally, in the visual cortical area, aging affects the temporal processing of aged rats and signal timing of senescent monkeys [8,20]. Together, these studies suggest that functional degradation of the visual cortex is age-related and may be physiologically implicated in declining visual ability associated with aging. On the other hand, aging may affect the cerebral cortex in differential patterns between male and female. In rats, several studies have indicated that the size of the cerebral cortex is greater in males than that in females [14,21]. Reid and Juraska [15] found that the cortex of male rats was both longer and wider than that in female rats. At the cellular level, the sexual difference in cortical thickness as well as volume of the visual cortex is due to the fact that male rats have more neurons in this area [10,15,16]. Interestingly, no sexual difference in number of visual cortical neurons in aged rats was found [22], thus indicating that male rats lose more neurons than female rats during aging. Together these studies raise an interesting question: are there any aging-related sexual differences in the functional degradation of visual cortical neurons?

In the present study, we used extracellular single-unit recordings to examine any sexual difference in degradation of visual cortical neuronal properties. Our studies were designed to investigate the sex difference of visual cortical function degradation during aging and provide insight into its mechanisms.

12 young Long-Evans rats (3–4 month old, six male and six female), 11 mid-aged rats (13–16 month old, six male and five female) and 10 aged rats (23–25 month old, five male and five female) were used. All rats were obtained from the Laboratory Animal Center, University of Science and Technology of China and given food and water *ad libitum*. Experiment methods were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal's eyes were examined regularly with an ophthalmoscope to ensure that they were free of cataracts. Only those animals with clear optics were used in present study.

Rats were anaesthetized with urethane (20%, 1.2 g/kg, i.p.), and then mounted in a stereotaxic apparatus. A craniotomy was

Abbreviations: GABA, gamma-aminobutyric acid; CFF, critical flicker frequency; PSTHs, poststimulus time histograms; GAD, glutamic acid decarboxylase; ChAT, choline acetyltransferase; AI, adaptation index.

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performed over the visual cortex (approximately 7.0–8.0 mm posterior to bregma, 3.0–4.0 mm lateral to the midline for primary visual cortex). Dura mater was dissected away and the cortex was bathed with silicone oil to prevent it from drying and reducing pulsations. Similarly, glycerine was administered periodically to prevent the cornea from drying. Body temperature was maintained at 37.0 ± 0.2 °C.

Flashing stimuli were used to investigate response properties of the cells in visual cortex. The stimulus was presented by a light-emitting diode (LED) located 1 cm in front of the eye. Twenty stimuli delivered at frequencies of 1, 2, 4 and 8 Hz were used for our adaptation studies because cells of aged rats display a critical flicker frequency (CFF) threshold about 7 Hz [8]. Extracellular action potentials of neurons were recorded with glass electrodes filled with NaCl (2 M), with an impedance of 4.0–5.0 MΩ.

Poststimulus time histograms (PSTHs) were constructed as the average number of evoked spikes following stimulus presentation. The evoked spikes of every stimulus in 20 stimuli were counted. The adaptation index (AI) for different frequencies was measured as the ratio of the average of the last two evoked response spikes in the plateau (spontaneous activity not subtracted) to the average of the first and second evoked response spikes.

The signal-to-noise ratio was defined as the ratio between a cell's visually evoked response and its spontaneous response [17]. Visually evoked activity and spontaneous firing rate were quantified as we previously described [19]. Spontaneous firing during two periods with 20 s duration was recorded before flash stimulus and 60 s after flash stimulus while the luminance on the cornea was 0.05 l×. The spontaneous firing rate (spikes/s) was then determined by total firing of these 40 s divided by 40 s. The evoked responses were measured as the average of the first two of twenty evoked spikes to the neuron's optimal response frequency.

Finally, onset latency to visual stimuli was determined. Onset latency was defined as the first response that was twice the mean spontaneous rate and which occurred immediately after the onset of a single flashing pulse [8]. Onset latencies of all groups were obtained from first evoked response to 1 Hz flash stimulus. Neuronal activity was measured using the IGOR programming environment (Wavemetrics, Lake Oswego, OR). Evoked action potentials were digitized (National Instruments) by routines written in the IGOR programming environment (Wavemetrics, Lake Oswego, OR) and stored in a computer for off-line analysis.

Data are presented as means ± standard error of means (SEM). Statistical significance was estimated by using *t*-test analysis. We concluded there was a significant difference when $p < 0.05$.

We recorded a total of 290 neurons from young male (54 cells), young female (51 cells), mid-aged male (46 cells), mid-age female (45 cells), aged male (48 cells) and aged female (46 cells) rats. Our results showed there were no significant sexual differences in adaptation, onset latency and peak responses to flashing stimuli in all groups. However, significant sexual differences of signal-to-noise ratio were found in mid-aged and aged, but not young group.

We investigated the adaptation to flash stimuli in both sexes by using extracellular single-unit recordings. Fig. 1A depicts a representative single unit recorded at 1 Hz from an aged rat visual cortical cell. Good consistency between flashing stimuli and visual responses was observed. Fig. 1B shows the PSTHs elicited from the visual response to flashing stimuli at 1 Hz. Fig. 1C is the adaptation index of young, mid-aged and aged groups in both sexes with stimulus frequencies at 1, 2, 4, 8 Hz. Consistent with our previous studies, in both sexes the adaptation index becomes lower in older rats. Significant difference could only be observed at high stimuli frequency (8 Hz) when compared mid-aged and aged animals to young animals. However, when we compared male with female at same age, no sexual differences of adaptation were found in young, mid-aged and aged rats at all frequencies (young male: 1 Hz = 0.57 ± 0.03, 2 Hz = 0.51 ± 0.04, 4 Hz = 0.42 ± 0.04, 8 Hz = 0.30 ± 0.03; young female: 1 Hz = 0.56 ± 0.03, 2 Hz = 0.48 ± 0.07, 4 Hz = 0.39 ± 0.03, 8 Hz = 0.31 ± 0.03; mid-aged male: 1 Hz = 0.56 ± 0.03, 2 Hz = 0.42 ± 0.03, 4 Hz = 0.35 ± 0.03, 8 Hz = 0.23 ± 0.02; mid-aged female: 1 Hz = 0.55 ± 0.03, 2 Hz = 0.42 ± 0.03, 4 Hz = 0.34 ± 0.03, 8 Hz = 0.21 ± 0.02: aged male: 1 Hz = 0.56 ± 0.03, 2 Hz = 0.43 ± 0.02, 4 Hz = 0.31 ± 0.02, 8 Hz = 0.18 ± 0.02: aged female: 1 Hz = 0.57 ± 0.04, 2 Hz = 0.39 ± 0.04, 4 Hz = 0.29 ± 0.03, 8 Hz = 0.17 ± 0.02).

It has been reported that the onset latencies of V1 cells are delayed in aged monkeys [20]. The same result was also found in aged rats [19]. To test whether there was sexual difference in onset latency we compared the latencies of cells in different sexes in the young, mid-aged and aged rats. Fig. 2 shows that there were no sexual differences in onset latency of visual cortical neurons in all groups. (young male 62.03 ± 3.30 ms, young female 68.09 ± 4.27 ms, $p > 0.6$ *t*-test; mid-aged male: 67.69 ± 2.16 ms, mid-aged female: 70.86 ± 3.50 ms, $p > 0.8$ *t*-test;

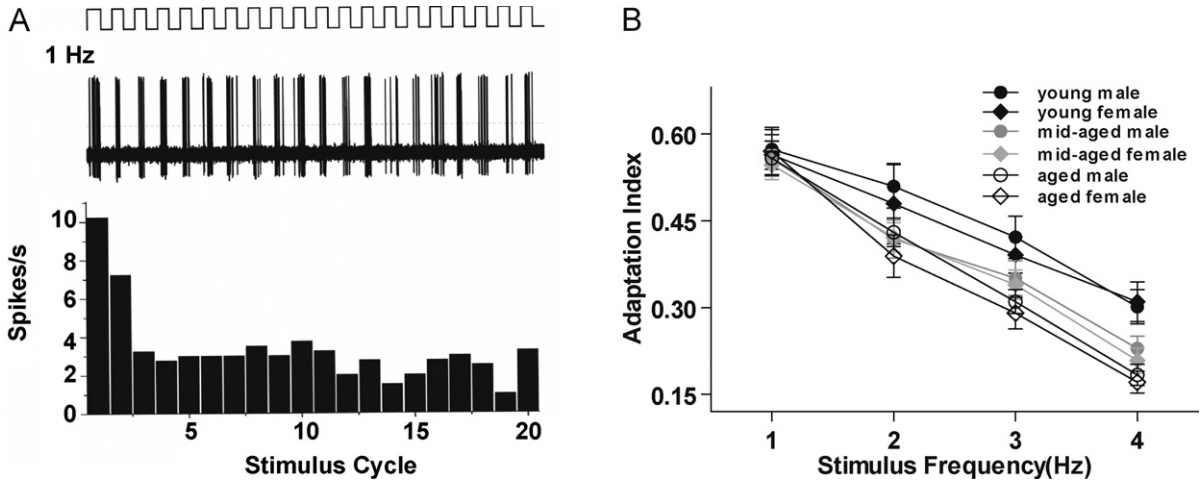

Fig. 1. (A) Visual responses to a 1 Hz flash stimulus to a primary visual cortical neuron in aged rat and the stimulus output Digital Analog Converter (DAC) wave. (B) PSTHs elicited from the visual response to flashing stimuli at 1 Hz. (C) Adaptation index (AI, average amplitude of the last two evoked response divided by average amplitude of the first and second evoked response) to flashing stimuli at different frequencies (1, 2, 4 and 8 Hz). There were no significant differences of AI between different sexes in young, mid-aged and aged groups.

aged male 100.85 ± 5.09 ms, aged female 92.05 ± 5.84 ms, $p > 0.5$ *t*-test).

The spontaneous rates and peak responses of neurons were compared in different sexes in young, mid-aged and aged rats. Sample traces of spontaneous action potential of all groups were shown in Fig. 3A. The spontaneous rate was significantly higher in aged male rats than that in aged female rats (Fig. 3B) (3.72 ± 0.52, for aged male; 2.06 ± 0.21, for aged female; $p < 0.05$, *t*-test), while no significant differences were found in young and mid-aged rats. To determine whether there is large variability in different individuals, we compared the spontaneous rate of each male and female aged rat. We found that spontaneous rates in every aged male rat were much higher than in every aged female rat (Fig. 3C). In both male and female rats, old age resulted in decreased signal-to-noise ratio (Fig. 3D). The significant decrease of signal-to-noise ratio in aged male rats (8.93 ± 0.99, for aged male; 12.17 ± 1.28, for aged female; $p < 0.05$, *t*-test) is due to a significantly increased spontaneous rate and less change of peak response rate (18.1 ± 1.5, for aged male; 15.7 ± 1.4 for aged female; $p > 0.2$, *t*-test). Although we did not observe a significant change of peak response rate (17.0 ± 1.0, for mid-aged male; 19.9 ± 1.7 for mid-aged female; $p > 0.1$, *t*-test) and spontaneous rate (2.15 ± 0.21, for mid-aged male; 1.84 ± 0.13 for mid-aged female; $p > 0.1$, *t*-test) between different sexes in mid-aged group, the signal-to-noise ratio in mid-aged male rats is significantly lower than that in mid-aged female (9.99 ± 1.27, for mid-aged male; 13.14 ± 1.27, for mid-aged female; $p < 0.05$, *t*-test).

In this study we found that adaptation and onset latency to flashing stimulus of visual cortical neurons degraded without sex difference in older rats. These results suggest that these degradations may share the same mechanisms in both sexes. The loss of myelin sheaths of axons of old animals [11–13] might cause the prolonged onset latency of visual cortical cells in aged rats. The enhanced adaptation in older rats, especially at frequencies over 4 Hz, may be the result of degradation in the number, size and surface contact area of the synaptic junctions [1] as well as the decreased density of synapses in primary visual cortex of old animals [2].

Additionally, our results show that the spontaneous rate was increased during aging in both sexes, while a significant sexual difference was only found in aged rats. Compared with aged female rats, the spontaneous rate was significantly higher in aged male rats. Increased spontaneous activity in the visual cortex could be the result of a decline in introcortical GABAergic inhibition in aged

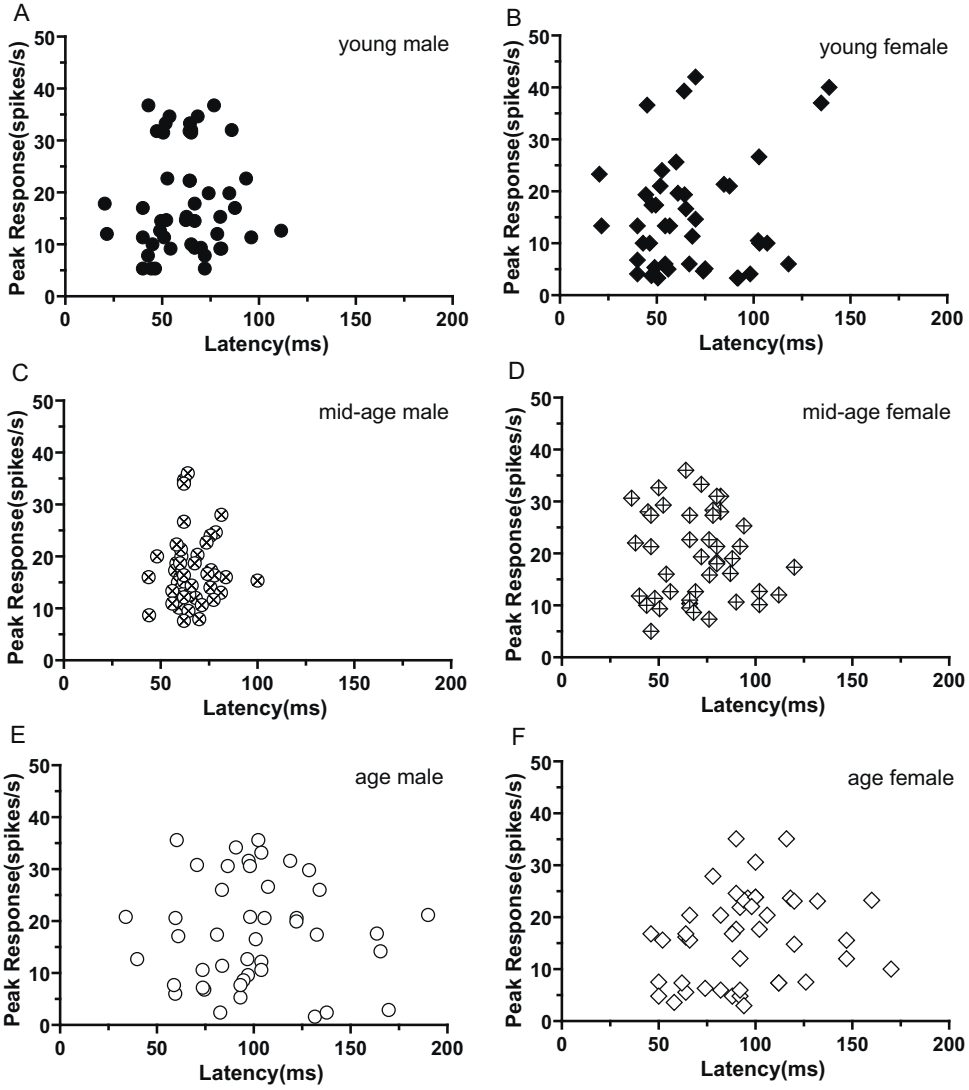

Fig. 2. Scatter plot illustrating peak visual evoked responses and onset latencies of neurons in primary visual cortex of both sexes of young (A, B), mid-aged (C, D) and aged (E, F) rats. There was no significant difference in peak response rate and latency between sexes. Response rates are in action potentials per second and latencies are in milliseconds.

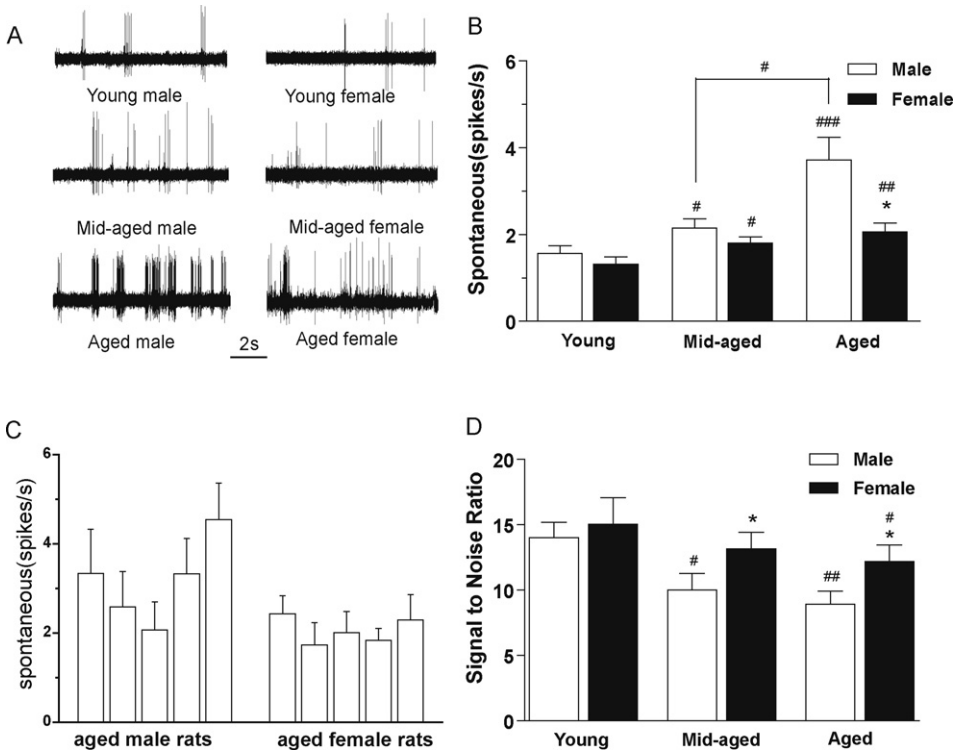

Fig. 3. Spontaneous rate and signal-to-noise ratio for different groups. (A) Sample traces of spontaneous action potential in different groups. (B) Spontaneous rates increase in both sexes during aging. However, the significant sexual difference was only found in aged rats. (C) Each individual aged male rats presented higher spontaneous rates than each individual aged female rats. (D) Signal-to-noise ratios were reduced in aged animals of the same sex. There were significant differences in signal-to-noise ratios between male and female rats in mid-aged and aged groups. #Significant difference compared with young group in same sex. *Significant difference between different sexes in the same age. $^{*}p < 0.05$; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *t*-test.

rats. Schmolesky et al. [17] suggest that a decrease in GABAergic inhibition in aged monkey cortex could account for much of the decline in function they observed, including the increased activity of spontaneous rate. Leventhal et al. [7] showed that GABA and agonists of GABAa receptors significantly reduce the spontaneous rate of V1 neurons in old macaque monkeys, but not young macaque monkeys. More remarkably, recent work shows that the density of GABAergic neurons, but not total number of neurons in aged cat's visual cortex is significantly decreased when compared to young cats [5], suggesting that the loss of cortical GABAergic inhibition is concurrent and might contribute to cortical functional degradation during aging. Therefore, we assume that the sex difference of spontaneous rate in aged rats may be due to sex difference in GABAergic system degradation. Previous studies show that the GABAergic system is different in male and female [9]. Neurons expressing GABA-immunoreactivity were found to have sex and age related differences in the rat bed nucleus [18]. In mammals, the activities of main synthesis enzyme glutamic acid decarboxylase (GAD) and main catabolism enzyme choline acetyltransferase (ChAT) of GABA are differentially affected by aging in male and female mice. There is more early impairment of GAD and ChAT activity in male mice than in female mice [3]. The sexual difference in GABAergic system degradation during aging could be the result of hormonal differences. Sex steroids have been hypothesized to preserve neural function and promote neuronal survival. Evidence indicates that estrogen serves a neuroprotective function [4], therefore, estrogen may delay the death of GABAergic neurons in old females.

Proper brain function requires that stimuli evoke reliable responses that are easily discernable from background activity. Signal-to-noise ratios, measured by the ratio between the cell's visually evoked response and the cell's spontaneous response (could be considered as signal and noise, respectively), reflect function of detection capability of the neurons in visual cortex. Observed signal-to-noise ratios were higher in female than in male of mid-aged and aged rats suggesting that functional degradation of cortex in females, at least to some degree, was slower than in males. This phenomenon could result in sexual differences in cognitive function during senescence.

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