The Effects of Aging on Rod Bipolar Cell Ribbon Synapses

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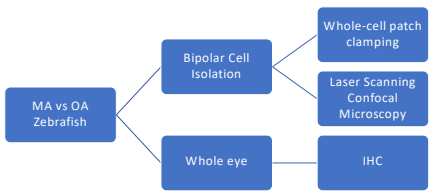
Introduction

The global health concern posed by age-related visual impairment highlights the need for further research focused on the visual changes that occur during the process of aging. To date, multiple sensory alterations related to aging have been identified, including morphological and functional changes in inner hair cochlear cells, photoreceptors, and retinal ganglion cells. While some age-related morphological changes are known to occur in rod bipolar cells in the retina, their effect on these cells and on their connection to other cells via ribbon synapses remain elusive. The primary aim of the current study was to investigate age-related changes in the function and morphology of rod bipolar cell ribbon synapses synaptic ribbons from zebrafish retinas.

Methods



Middle-aged (18-month-old) and older-aged (36-month-old) zebrafish were raised in a controlled light-dark cycle. After dark adaptation, retinas were dissected for analysis. Retinal bipolar cells were isolated following established protocols, and whole-cell patch-clamp recordings were performed to measure calcium currents under voltage clamp conditions. Laser-scanning confocal microscopy was used for fluorescence imaging with consistent acquisition parameters. Additionally, immunohistochemistry was conducted on harvested eyes to visualize neuronal and ribbon structures in zebrafish retinas, focusing on the central retina in the group analysis.

Results

1. Retinal bipolar cells isolated from older-aged zebrafish exhibit changes in the numbers and length of their synaptic ribbons

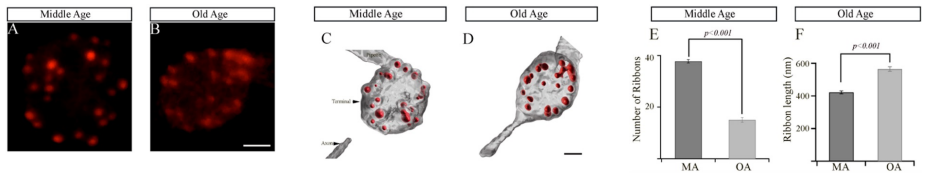


Figure 1. A-B. Representative 2D projections of synaptic terminals in middle-aged (MA; A) and older-aged (OA; B) zebrafish bipolar cells. Synaptic ribbons were labeled using TAMRA-RBP peptides and visualized via confocal microscopy (scale bar, 2 µm).
C-D. Three-dimensional (3D) reconstructions of zebrafish bipolar cells with labeled synaptic ribbons (TAMRA-RBP in red) (scale bar, 5 µm).
E-F. Average ribbon numbers (C) and ribbon lengths (D) in bipolar neurons from middle-aged (MA) and older-aged (OA) zebrafish.

2. Altered Bipolar Cell Ribbon Synapse Morphology in Older-Aged Zebrafish

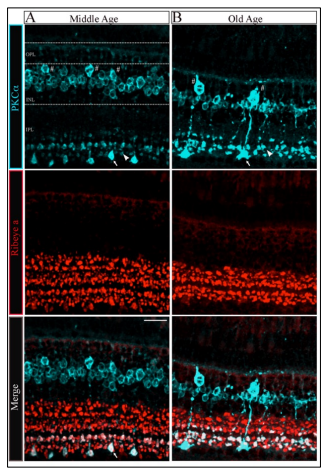


Figure 2. A-B. Transverse retinal sections from middle-aged (MA; left panels) and older-aged (OA; right panels) zebrafish were immunostained with fluorescent antibodies for rod bipolar cell marker PKCα (cyan, top panels) and ribeye a (red, middle panels). The merged images (bottom) show overlay of PKCα and ribeye a labeling. Maximal intensity projections are presented, with INL and IPL positions indicated (scale bar, 20 µm). Larger (arrow) and smaller (arrowhead) ribbon terminal morphologies in PKCα are observed in MA and OA. # Denotes the pear-shaped soma characteristics specific to Mb1 rod bipolar cells with larger terminals.

3. Altered Calcium Response in Ribbon Synapses of Older-Aged Zebrafish Mb1 Bipolar Cells

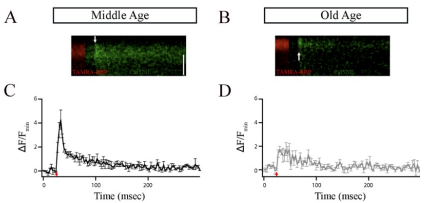


Figure 3. A -B. The x-t plots depict Cal520LA fluorescence intensity (green staining, right section of each plot) over time (horizontal axis) at a single ribbon location. RBP-TAMRA fluorescence (red staining, left section) indicates ribbon position, while the top region represents extracellular space. White arrows indicate depolarization timing.
C-D. Spatially averaged Cal520LA fluorescence over time at Mb1 bipolar cell ribbons in middle-aged (MA; C) and older-aged (OA; D) zebrafish. Average intensity (± SEM) in each horizontal pixel row during three separate 10-ms depolarizations with similar calcium currents (41 ± 4 pA; n=3 cells; three fish). Fluorescence intensity was normalized by baseline fluorescence (Fmin) by averaging over all pixels, with the red arrow denoting the onset of the 10-ms depolarizing stimulus.

Conclusion

We showed that zebrafish of advanced age acquired changes in their synaptic ribbon structure and local calcium dynamics, thereby providing valuable insight into the morphological and functional alterations in the aging retina, specifically in rod bipolar cells and their ribbon synapses. These findings suggest that while normal aging may not significantly impact the deterioration of vision, there may be more complex visual changes occurring that contribute to the visual impairment observed in human adults. The subtle changes we observed may have significant implications for disease models in which such alterations may be amplified, potentially resulting in visual impairments. The present study contributes to the growing knowledge of aging-associated changes in the visual system and will facilitate further studies to explore the implications of calcium regulatory mechanisms for age-related visual disorders.

References

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Acknowledgments

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