Higher Rhodopsin Levels Enlarge Diameter of Rod Photoreceptors in Mouse Retina

Paris Chakravarty[1], Clint L Makino[2]
[1]Glendora High School, 1600 E Foothill Blvd, Glendora, CA 91741; [2]Boston University School of Medicine, 700 Albany St, Boston, MA 02118

1. Background

Rhodopsin is the visual pigment found within outer segments of rod photoreceptors [Figure 1][1]. They are light-sensitive proteins that trigger phototransduction, the conversion of light to electrical signals.

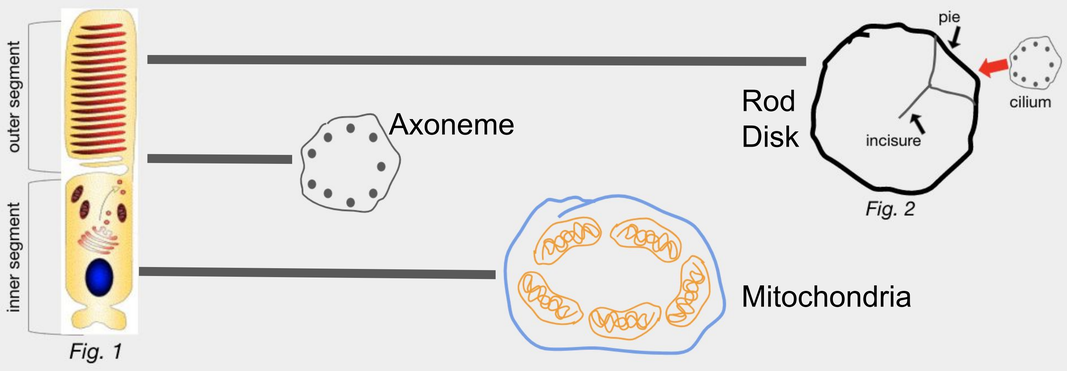


Fig. 1

Fig. 2

The outer segment is an elaborate cilium, however, the axoneme does not reach the tip. It transitions from a circular structure to a triangular "pie slice" within the rod, with an incisure that extends off into the center of the disk [Figure 2].

Mouse rod outer segments are on average 1.4 μm in diameter. Previous studies have shown that larger than normal rods can lead to the gradual degeneration of the outer segments[2]

2. Hypothesis

Rhodopsin is present in high density in disk membranes so increased levels of rhodopsin may enlarge disk diameter.

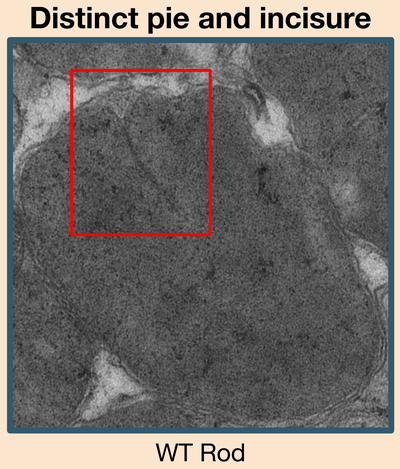
3. Methods

Electron Microscopy + Data Analysis

1. A Philips CM12 transmission electron microscope was used to view cross sections of rod outer segments from three types of mice:
   - Wild type (WT, n=111 rods) with normal levels of rhodopsin
   - Heterozygous with an additional gene for bovine rhodopsin (B+/-, n=183)
   - Homozygous for the transgene (B+/+, n=169)
2. Diameter, perimeter, circularity, and roundness were measured for rod outer segments and connecting cilia using Fiji ImageJ 2.3.0 software.
3. Parameters were compared using ANOVA and Bonferroni post-hoc test, $p \leq 0.05$ was considered significant.

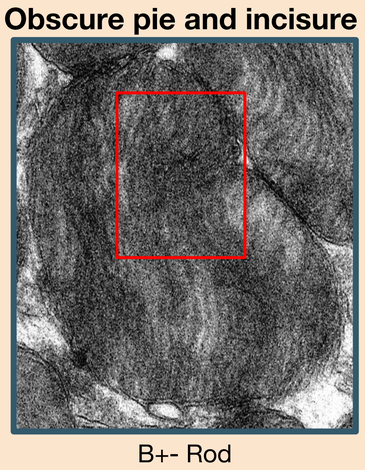
4. Results

Distinct pie and incisure



WT Rod

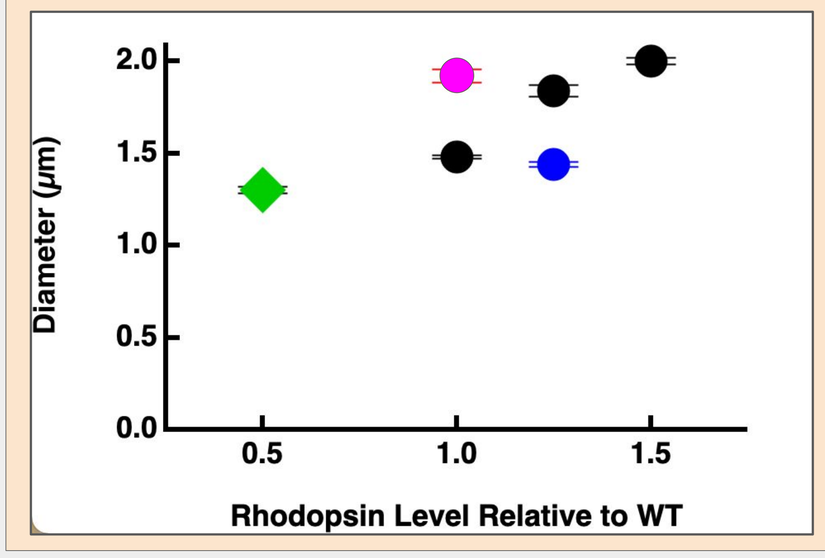
Obscure pie and incisure



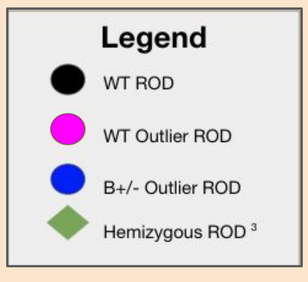
B+- Rod

No pie or incisure

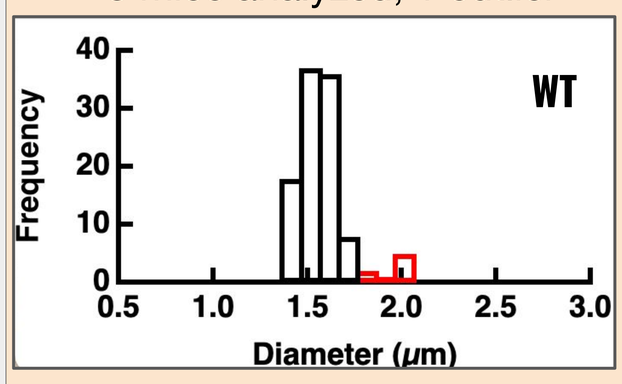
B++ Rod



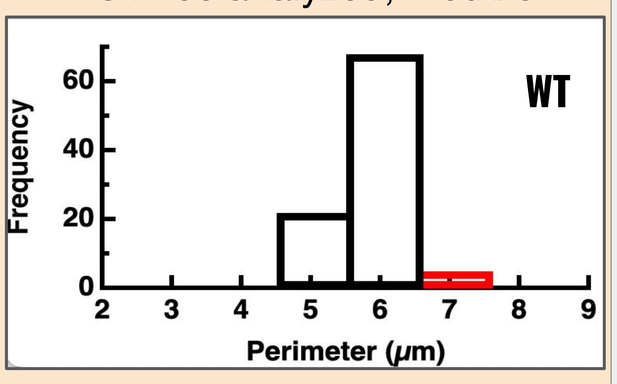
B+/- & B+/+ Rods Have Increased Levels of Rhodopsin and Larger Diameters



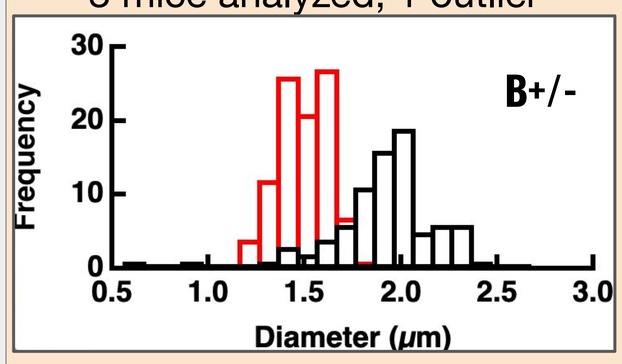
1. Majority of WT Diameters Between 1.4 and 1.6 μm
3 mice analyzed; 1 outlier



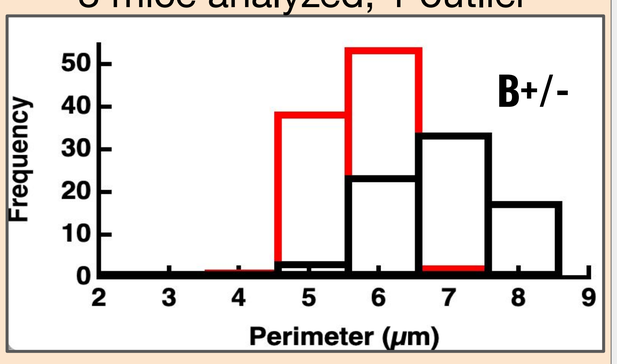
1. Majority of WT Perimeters Between 5 and 6 μm
3 mice analyzed; 1 outlier



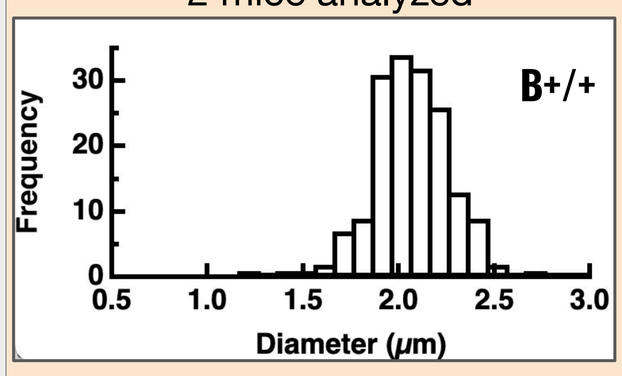
2. Majority of B+/- Diameters Between 1.5 and 2.1 μm
3 mice analyzed; 1 outlier



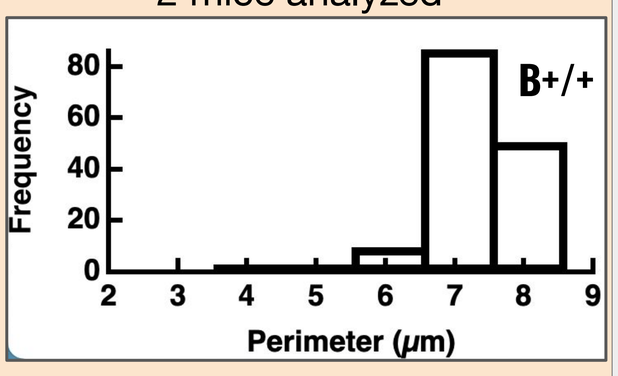
2. Majority of B+/- Perimeters Between 6 and 8 μm
3 mice analyzed; 1 outlier



3. Majority of B+/+ Diameters Between 2.0 and 2.5 μm
2 mice analyzed



3. Majority of B+/+ Perimeters Between 7 and 8 μm
2 mice analyzed



5. Discussion

Increased rhodopsin expression enlarged disk size and rod outer segment diameter and shortened incisure length in mouse. B+/- rods had a less prominent pie slice. B+/+ rarely exhibited incisures or pie slices. Instead, the transitioning axoneme formed a "hat" that protruded outward. Presumably, the larger disk also required more structural proteins that became scarce. B+/- and B+/+ undergo rod degeneration due to the insufficient supply of these structural proteins. Since larger outer segments are occasionally observed in WT mice, our results suggest that normal fluctuations in rhodopsin expression may be one cause for the slow loss of rods over the lifetime of a mammal. Inadequate transportation of essential nutrients and materials along the outer segment because of the lack of pies and incisures may have contributed to degeneration. In addition, our results support the notion that a deficiency in the expression of structural proteins disrupts rod degeneration.

6. References

1. Deretic, D. *Vision Research* 2006, 46 (27), 4427–4433.
2. Price, B. A.; Sandoval, I. M.; Chan, F.; Nichols, R.; Roman-Sanchez, R.; Wensel, T. G.; Wilson, J. H. *PLoS ONE* 2012, 7 (11).
3. Makino, C. L.; Wen, X.-H.; Michaud, N. A.; Covington, H. I.; DiBenedetto, E.; Hamm, H. E.; Lem, J.; Caruso, G. PLoS ONE 2012, 7 (5).

7. Acknowledgments

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