Sexual dimorphism in retinal function: endocrine modulation of sensitivity to light

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Introduction

Hormones such as estradiol have been shown to have protective effects in the retina. Studies have shown a correlation between multiple retinopathies and postmenopausal state in women.[1] Exposure to endogenous premenopausal estrogen and exogenous estrogen during and post-menopause (hormone replacement therapy - HRT) protect against age-related macular degeneration[1] and improve visual function.[2] Further, there are sexual dimorphisms in effects of gonadal hormones on the retina. Macular hole is more common in post-menopausal women than men of similar age and exogenous testosterone therapy increases the risk of central serous chorioretinopathy.[1] While these relationships have been established, how these hormones modulate the visual system, including their effect on retinal sensitivity, is not well understood. The current study examines the effects of reproductive state and sex on sensitivity to light in the green tree frog (*Hyla cinerea*). Unlike rodents, the frog retina can respond to wavelengths spanning the visual light spectrum, so it is the preferred animal model. It was hypothesized that reproductive females have increased visual sensitivity compared to non-reproductive females and reproductive males. Electroretinograms (ERGs) were used to record neural responses to varying wavelengths and light intensities. This study provides insight into underlying mechanisms that can explain the findings of previous studies showing the sexually dimorphic risk factors of post-menopausal state and the protective effects of HRT in post-menopausal women.

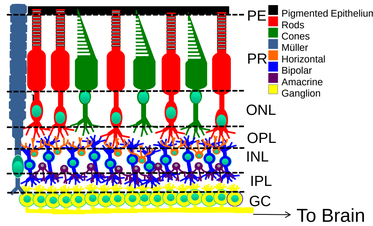


Figure 1. Architecture of the retina. Gonadal hormones likely modulate retinal processing and estrogen has protective effects in the retina.

Methodology: Electroretinograms

- Reproductive males and females and non-reproductive females were dark adapted in a light proof enclosure for at least 16 hours.
- The paralytic succinylcholine (SCC) was administered to immobilize the frog during the experiment. Frogs were kept awake throughout the procedure.
- An electrode was placed on the surface of the eye. It was used to record the electrical response of the eye after a 5ms flash of light.
- Flashes of light across different wavelengths (400nm, 450nm, 500nm, 550nm, 600nm, 650 nm) were given at increasing intensities.
- Protocol 1: The relative B-wave amplitude across different light intensities was measured. Retinal sensitivity threshold for individual wavelengths of light were measured and calculations were made based on a voltage by logarithmic intensity curve (V log(I)).
- Protocol 2: The relative B-wave amplitudes of isointensity light across the multiple wavelengths measured was calculated to determine relative spectral sensitivity.

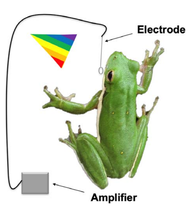


Figure 2: Standard set up for a scotopic ERG

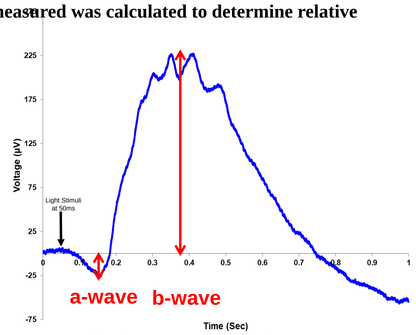


Figure 3: Graphical representation of an ERG response. The A wave is the hyperpolarization of photoreceptors in the outer nuclear layer of the retina. The B-wave is the depolarization of bipolar cells in the inner nuclear layer.

Results: V log(I) Response Curves

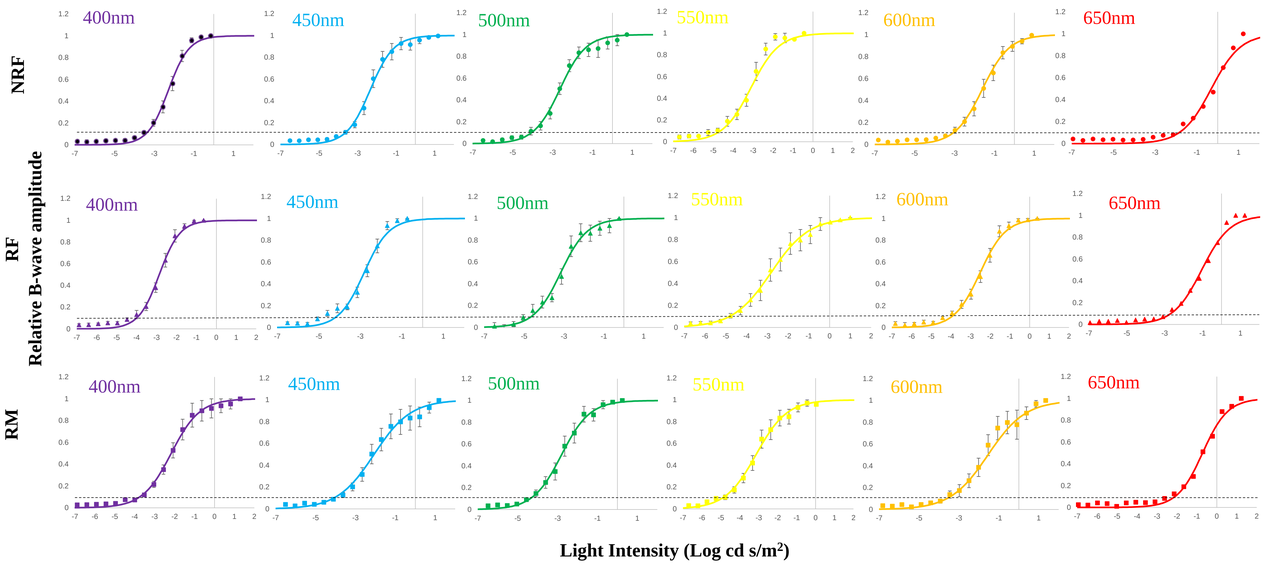
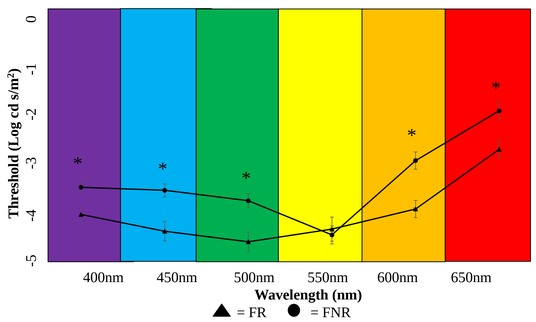


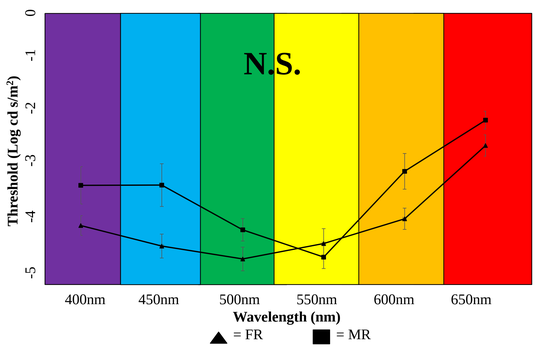
Figure 4: V logi(I) curves showing mean ERG B-wave responses for non-reproductive female (NRF), reproductive female (RF) and reproductive male (RM) groups. Each point represents the mean relative B-wave response and the curves represent the Boltzmann function fit. The dotted line represents the threshold response (10%).

Results: Spectral Sensitivity



The relative threshold for retinal sensitivity in reproductive females is significantly lower than for non-reproductive females.

Figure 5: Comparison of threshold at isointensities across different wavelengths of light in reproductive females (n=8) vs non-reproductive females (n=9). $F(5,88)=3.72$, $P<0.01^{**}$



The relative threshold for retinal sensitivity in reproductive females is not significantly lower than for reproductive males.

Figure 6: Comparison of threshold at isointensities across different wavelengths of light in reproductive females (n=8) vs reproductive males (n=10). $F(5,93)=1.81$, $P=0.119$

Conclusions

- Reproductive females display a lower threshold than non-reproductive females for perceiving all wavelengths along the entire spectrum of visible light except for 550nm.
  - Estrogen modulates retinal processing such that it increases retinal sensitivity in *Hyla cinerea.*
  - The natural environment of *Hyla cinerea* as well as their own coloring lie within close range of 550nm. Both their cones and rods may be able to perceive 550nm. Their retinas may be optimally designed to function at this wavelength and thus quite sensitive regardless of hormonal modulation.
- Reproductive females do not show a significantly lower threshold compared to reproductive males for perceiving any wavelengths along the visible light spectrum.
  - Estrogen is synthesized at the level of the retina. In males, androgens may be synthesizing estrogen in the retina, resulting in similar levels of estrogen functioning at the level of the retina in both sexes.
  - Both male and female *Hyla cinerea* display visually guided reproductive behavior. Both sexes have functional motivation for the evolutionary development of estrogen pathways in the retina.
- Clinical significance: The increased sensitivity seen in the reproductive state may be part of the mechanism underlying sexually dimorphic risk factors of post-menopausal state and the protective effects of HRT in post-menopausal women.
  - A deeper understanding of this mechanism will help in the further development of even more efficacious forms of HRT or in the development of other effective therapies for post-menopausal women.

Figure 7: the Louisiana green tree frog, *Hyla cinerea*

- Future Work
  - Enzyme Linked Immunosorbent Assay will be used to verify serum hormone levels in reproductive vs non-reproductive specimen.
  - Flourescence in-situ hybridization will be used to investigate cellular location of estrogen receptors in the retina to further understand the mechanism of estrogen modulation in the retina.
  - Human Chorionic Gonadotropin (HCG) injections will be given to reproduce reproductive state in non-reproductive specimens – retinal sensitivity will be measured to investigate similarities or differences in this laboratory-induced model.
  - Further investigation into reasons for the non-significant 500nm finding will be undertaken, including investigating which cell types are involved.

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References

1. Nuzzi, R., Scalabrin, S., Becco, A., & Panzica, G. (2018). Gonadal Hormones and Retinal Disorders: A Review. *Frontiers in Endocrinology*, 9. doi: 10.3389/fendo.2018.00066
2. Guaschino, S. et al (2003). Visual function in menopause: the role of hormone replacement therapy. The Journal of North American Menopause Society, 10(1), 53-57. doi: 10.1097/01.GME.0000030663.97769.FE
3. Klein, B. E., Klein, R., & Lee, K. E. (2000). Reproductive exposures, incident age-related cataracts, and age-related maculopathy in women: The Beaver Dam Eye Study. American Journal of Opthamology, 130(3), 322-326. doi: 10.1016/S0002-9394(00)00474-8