## Non-visual photoreceptors structure in the forgotten third eye

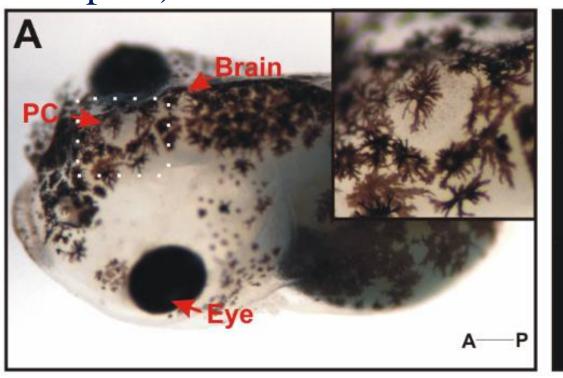
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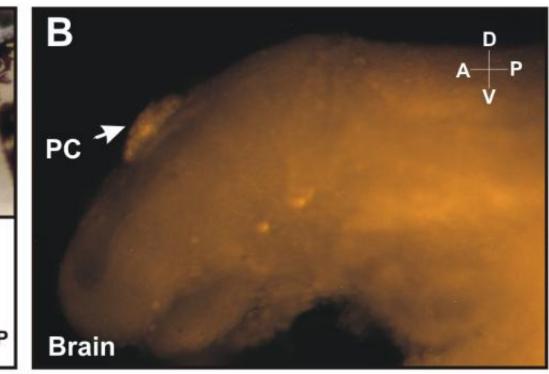
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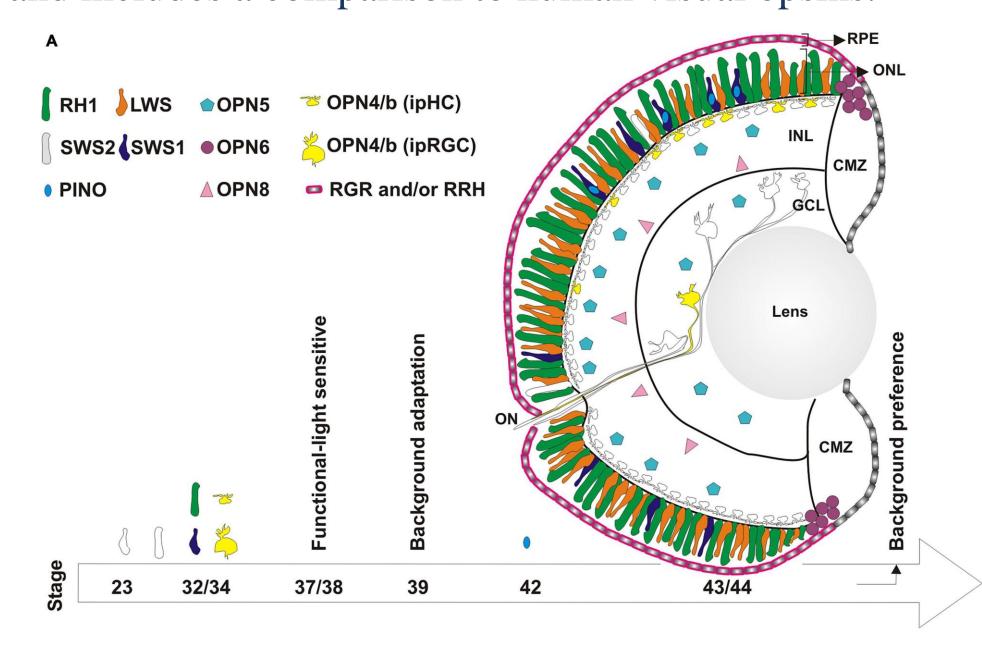
## Introduction

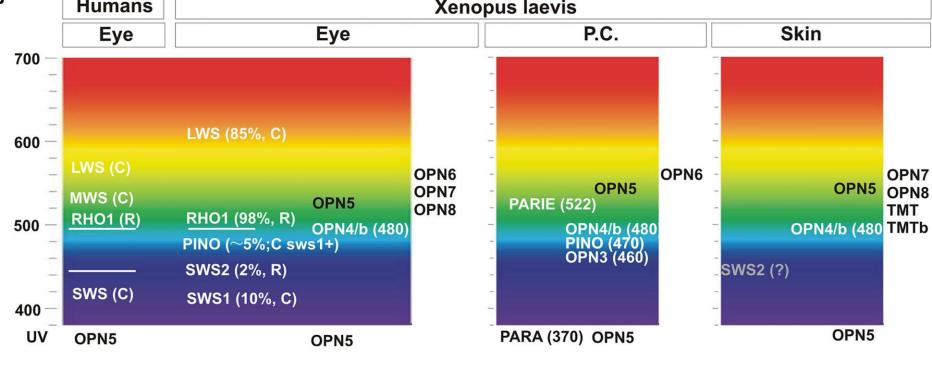
Animal Model: *Xenopus laevis* have *the third eye* (Pineal Complex)



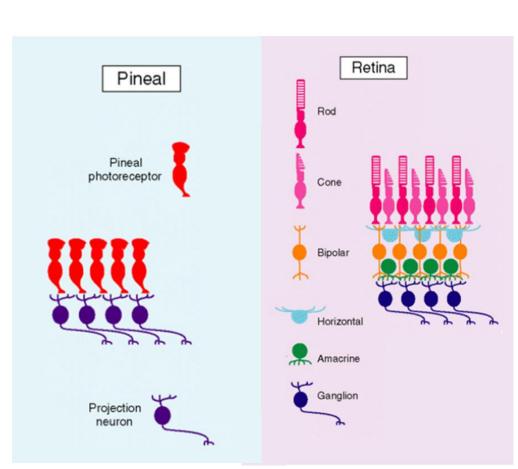


A) The developmental timeline below indicates when these opsins and associated behaviors first appear. B) Schematic compares opsin expression in the eye, pineal complex, and skin (tail or MEX cells), aligned with their peak spectral sensitivities, and includes a comparison to human visual opsins.





Knowledge gap: The cellular organization of the pineal complex





- 1) Fluorescent in-situ hybridization and slide immunohistochemistry
- 2) Whole-mount immunostaining using the Pinopsin antibody
- 3) Imaging technique and determination of the Pigmentation Index

## Result

Figure 1. Circadian variation in skin pigmentation emerges at early larval stages.

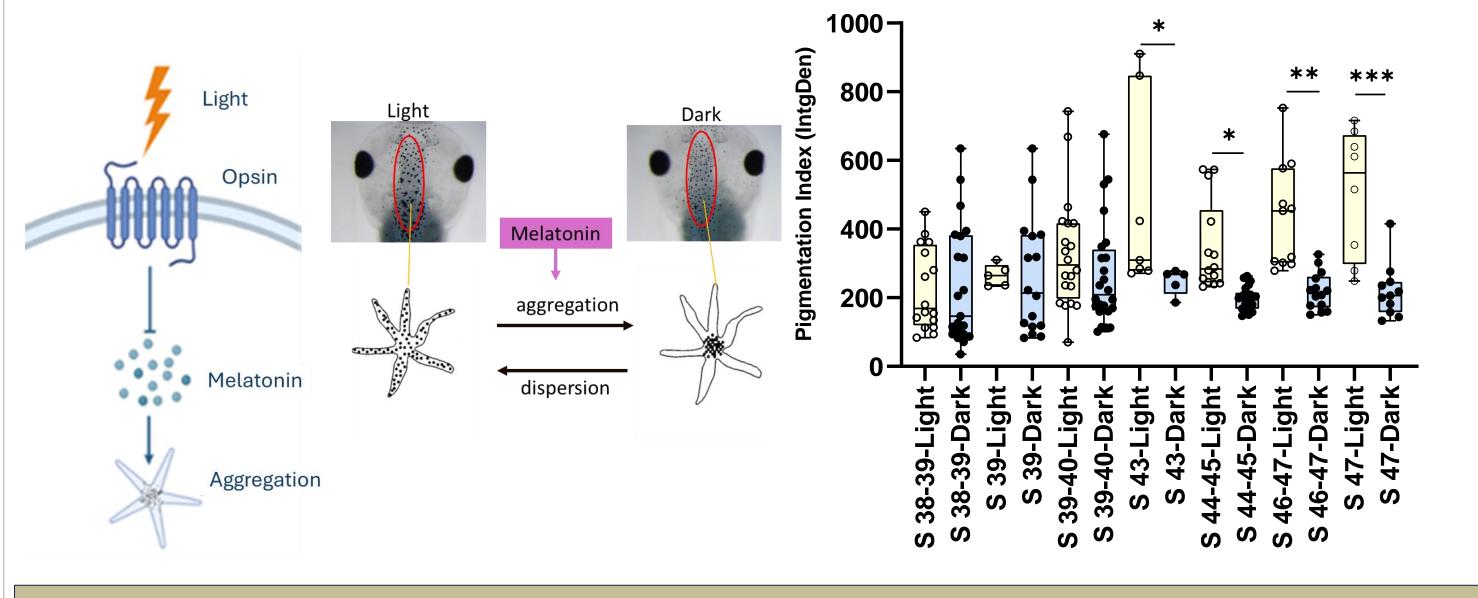


Figure 2. When do Pinopsin-expressing cells appear? Pinopsin-expressing cells emerge at stage 39/40, earlier than the pigmentation circadian response at stage 42/43

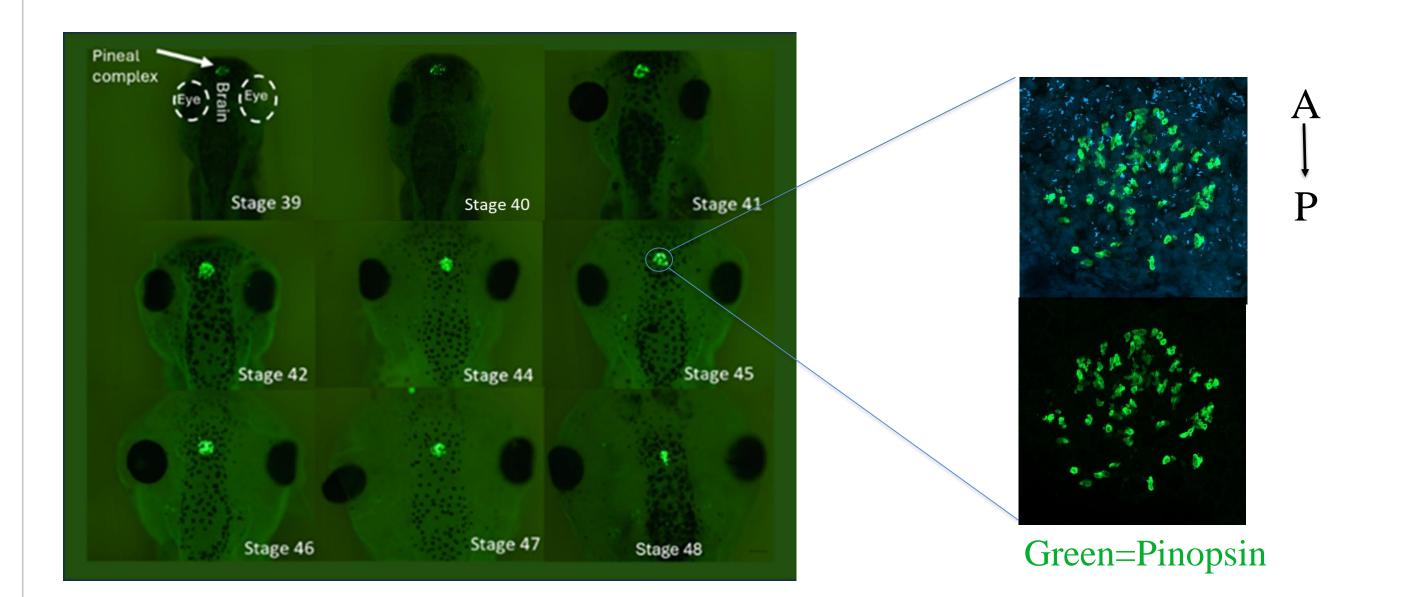


Figure 3. How are Pinopsin-expressing cells arranged within the pineal complex over development?

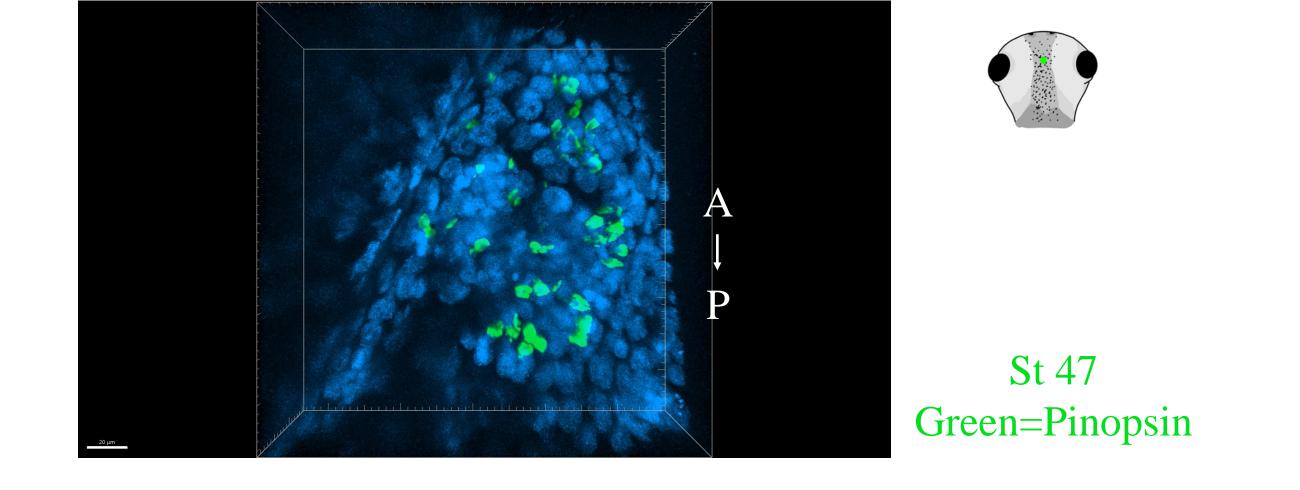


Figure 4. Are Melatonin and Pinopsin expressed within the same cells? Pinopsin and Melatonin-synthesis enzymes are colocalized within pineal cells

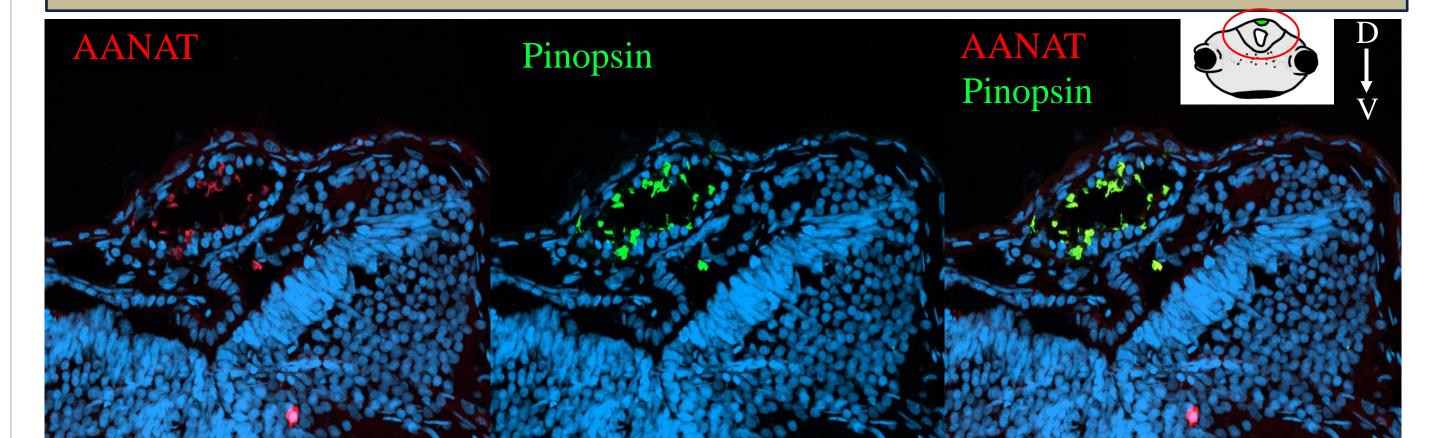
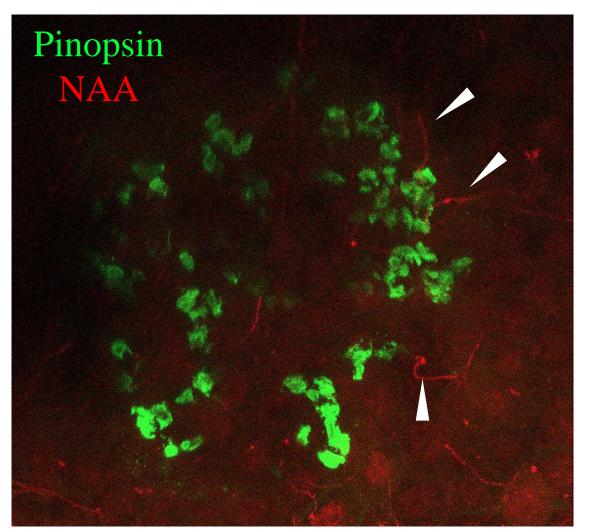
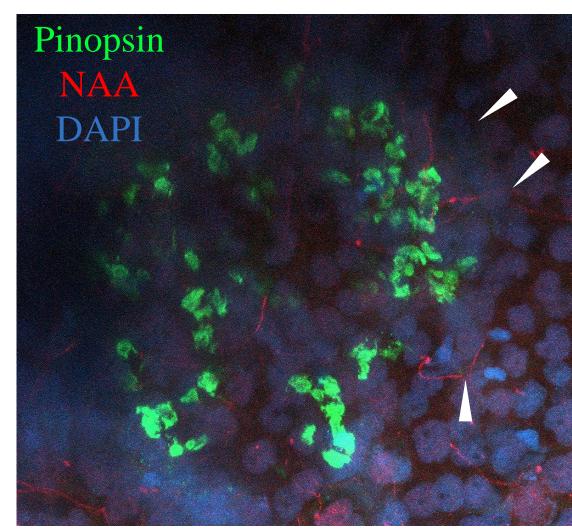
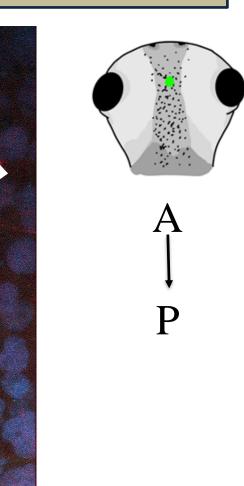


Figure 5. Are Pinopsin-expressing cells in the pineal complex projection neurons?

Pinopsin-expressing cells and projection neurons are not the same cells

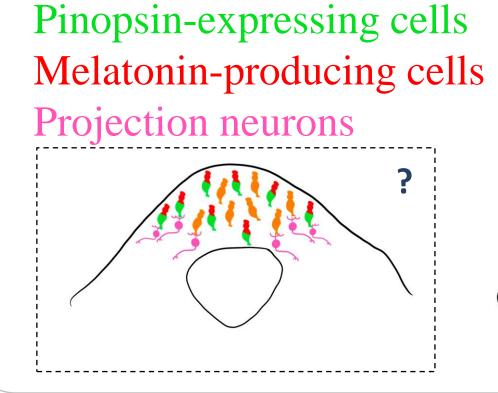


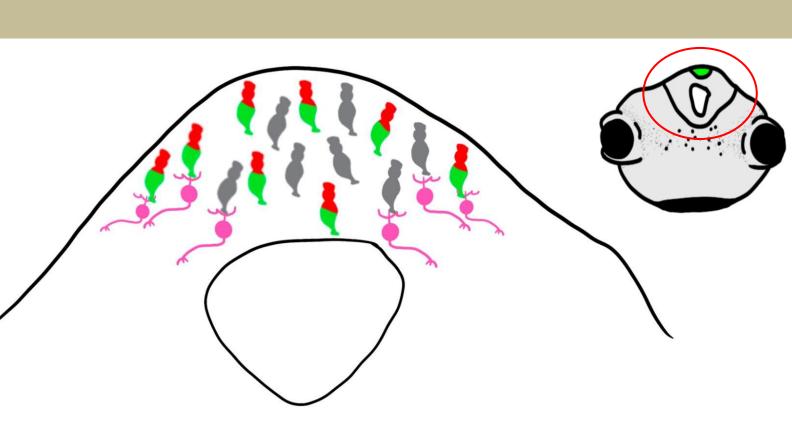




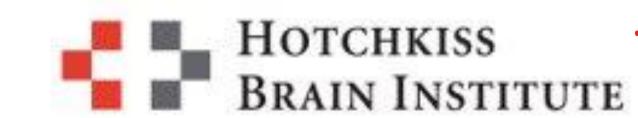
Conclusions and Future Directions: A summary of the data is illustrated in a schematic model showing the cellular organization of the Pineal Complex (figure 6), known as a third eye. I will utilize the IMARIS software and process the 3-D fluorescent images to characterize the maturation of opsin and non-opsin expressing cells over development (St 42-St 48). I will also use endothelial markers in the future to shed light on the whole pineal complex cellular structure.

Figure 6. Model of the Pineal Complex cellular structure











deferences:

Bertolesi, Journal of Photochemistry and Photobiology, 2020 Bertolesi, Journal of Frontier in Neuroanatomy, 2022