
Profiling the Visual System of *Lymnaea stagnalis* as a Novel Model for Investigating Photoreceptive Behaviours and Retinal Processing in Invertebrates

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Introduction: To navigate the external world, animals perceive and react to light using complex visual system machinery. To date, diverse animal models have been employed to explore visual system function and potential mechanisms in both physiological and pathophysiological states. While invertebrate models have been instrumental in elucidating visual properties, few have been established to investigate photoreceptive behaviors and retinal processing. As well, our current understanding of the cellular and molecular mechanisms that underlie photoreception across the animal kingdom is limited, highlighting the need to establish new animal models for studying the fundamentals of vision.

Method: In this study, we capitalized on valuable features of *L. stagnalis*, along with recent transcriptomic work, to establish a platform for assessing visual system function through anatomical and histological evaluations of the *L. stagnalis* eye, functional analysis of phototaxis behaviors, and phylogenetic assessments of core molecules involved in phototransduction.

Results: First, to understand the laminar retinal organization in the *L. stagnalis* eye and determine whether rhodopsin-positive cells were present and distributed throughout discrete retinal layers, we employed TEM and histological screens to uncover for the first time the presence of rhodopsin-positive sensory photoreceptor cells that may be associated with light sensitivity. Next, to characterize the functional outputs of photosensation, we created a novel neurobehavioral test to assess snail phototaxis *in vivo* using DeepLabCut software. By extrapolating locomotory features such as trajectory length, speed, acceleration, and tortuosity, neurobehavioral assessments revealed that most animals in a cohort exhibit positive phototaxis behaviors. Lastly, to elucidate the molecular basis of phototransduction in *L. stagnalis*, we conducted transcriptomic mining of the *L. stagnalis* CNS transcriptome, identifying three novel putative rhodopsin-like genes. Using phylogenetic assessments and AlphaFold2 structural predictions, we revealed the evolutionary conservation and structural similarity of *L. stagnalis* rhodopsin-like proteins to higher-order animal rhodopsins. Our transcriptome mining further uncovered a rich repertoire of genes for both vertebrate Gt-coupled and invertebrate Gq-coupled phototransduction signaling pathways in the CNS. This allowed us to predict the signaling pathways underlying photosensation in *L. stagnalis*.

Conclusion: Taken together, this study offers valuable insights into the conservation of photoreception processes and distinctive visual mechanisms in *L. stagnalis*, setting the stage for additional exploration of this model organism in vision research. The importance of this study lies in establishing *L. stagnalis* as a crucial model for understanding vision sciences, laying the groundwork for future investigations into the molecular and evolutionary facets of photosensitivity and phototaxis behaviors.