Validating Genetic Tools to Isolate Individual ipRGC Subtypes

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Introduction

- Light regulates the mammalian vertebrate visual system to affect both image-forming and non-image forming behaviors
- Light is detected by the classic rod and cone photoreceptors, and by a newly discovered, third class of photoreceptors, intrinsically photosensitive retinal ganglion cells (ipRGCs)
- **The extent to which ipRGC subtypes differ in their gene expression and how each individual subtype contributes to visual behavior is unknown**
- *To address this gap in knowledge, it is important to develop new tools which can genetically isolate individual ipRGC subtypes*
- The Schmidt Lab has developed a new Opn4^{FlpO} mouse line (FlpO: flippase recombinase) to be used in an intersectional genetic strategy, to target subsets of ipRGCs

Objectives

- Validate the newly developed Opn4^{FlpO} mouse line as a tool in an intersectional genetic strategy to isolate, ablate, and manipulate ipRGC subsets
  - ➢ Does the line target and label **all** ipRGCs or merely a subset?
  - ➢ Does the mouse line label other non-ipRGC mouse lines?

Opn4^{FlpO} Construct

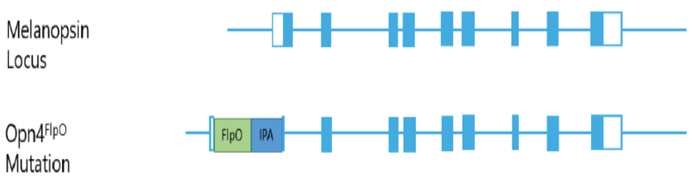

Fig 1. ipRGCs express the photopigment melanopsin (gene name: *Opn4*. The Opn4^{FlpO} line will express flippase recombinase, therefore, in all ipRGCs under a melanopsin promoter.

Visualizing cells labeled by Opn4^{FlpO} using the RCE:FRT reporter mouse line

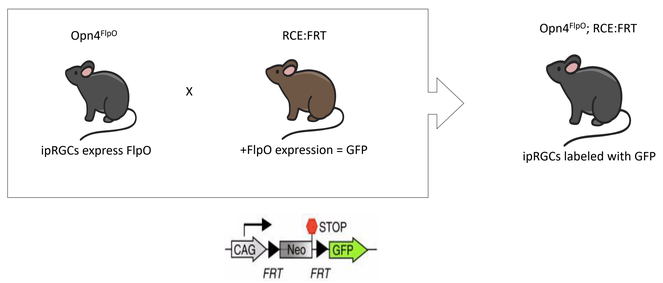

Fig 2. Mouse lines can be genetically manipulated using various tools, one of which uses FlpO and FRT sites. The Opn4$^{FlpO/+}$ line, crossed with RCE:FRT$^{GFP/+}$, produces a Opn4$^{FlpO/+}$; RCE:FRT$^{GFP/+}$ genotype to label ipRGCs with GFP.

Endogenous Labeling in Opn4^{FlpO}; RCE:FRT Mice

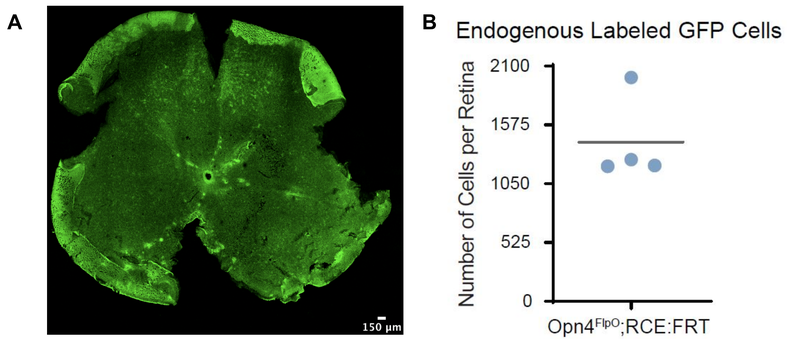

Fig 3. (A) A whole mount retina from the line with a total of 1212 GFP cells counted. **(B)** Scatter plot of the number of endogenous cells/retina for all four retinas (n=4).

GFP Immunolabeling in Opn4^{FlpO}; RCE:FRT Mice

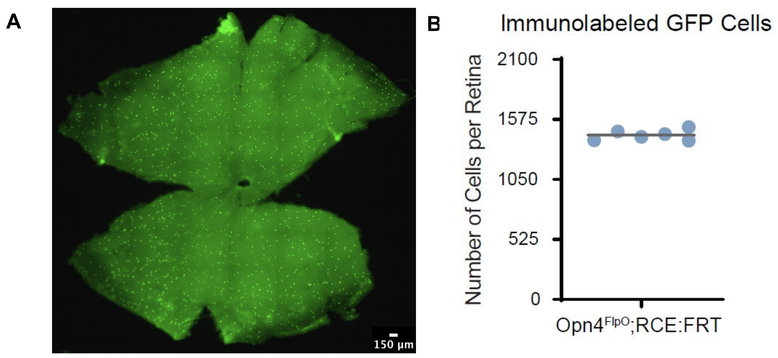

Fig 4. (A) Above is an example of an immunolabeled retina with 1446 labeled GFP cells counted. **(B)** The graph shows the number of immunolabeled cells/retina for all six retinas (n=6).

The Opn4^{FlpO} Line Labels ipRGCs

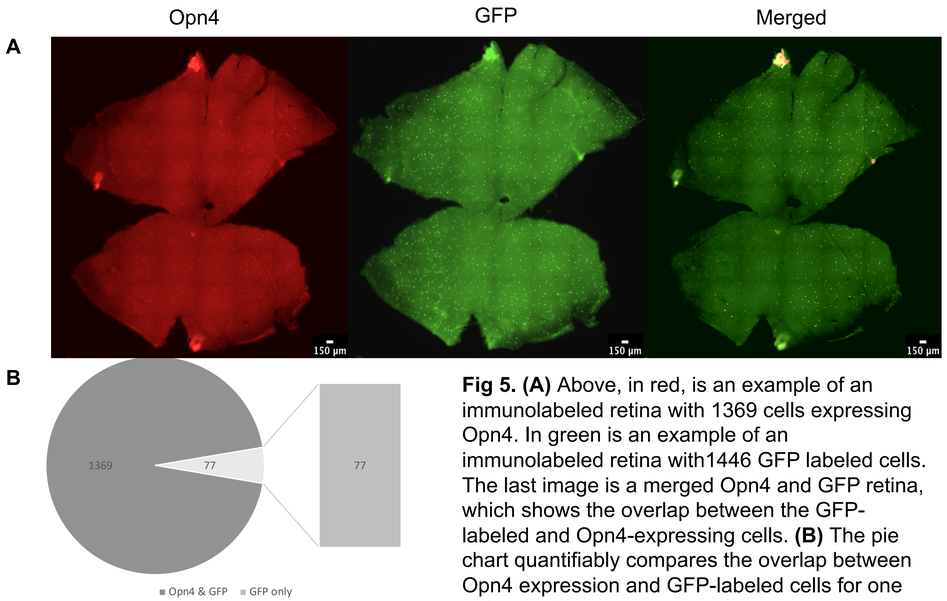

Fig 5. (A) Above, in red, is an example of an immunolabeled retina with 1369 cells expressing Opn4. In green is an example of an immunolabeled retina with1446 GFP labeled cells. The last image is a merged Opn4 and GFP retina, which shows the overlap between the GFP-labeled and Opn4-expressing cells. **(B)** The pie chart quantifiably compares the overlap between Opn4 expression and GFP-labeled cells for one retina (n=1).

Using Reporter Line, Ai65F, to Visualize Cells Labeled by the Opn4 Mouse Line

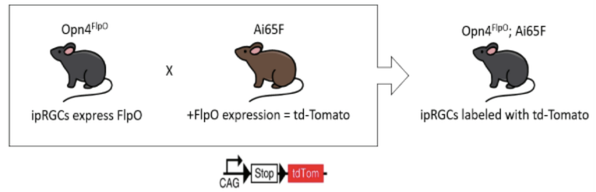

Fig 6. The Ai65F is a FlpO-dependent reporter that can be used to characterize the Opn4^{FlpO} line. When the Opn4$^{FlpO/+}$ line is crossed to the Ai65F line (ROSA26$^{td\text{-}tomato/+}$), the resulting genotype is then Opn4$^{FlpO/+}$; Ai65F, which labels ipRGCs with td-tomato.

td-Tomato Expression in Opn4^{FlpO};Ai65F Mice

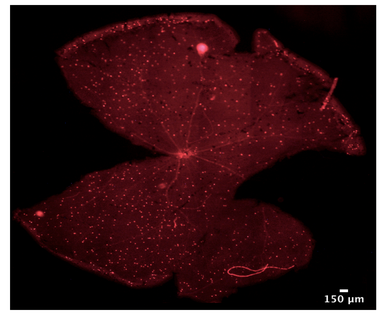

Fig 7. Above is an example of an immunolabeled retina from the Opn4^{FlpO}; Ai65F line with 1078 labeled td-tomato cells (n=3).

Conclusions

- The Opn4^{FlpO} line can be crossed with single-reporters, RCE:FRT and Ai65F, to label ipRGCs with a fluorescent protein
- The Opn4 staining confirmed that the Opn4^{FlpO} mouse line labels ipRGCs; however, not all GFP-labeled cells co-immunolabeled with Opn4
  - ➢ These cells are likely M4, M5, and M6 ipRGC subtypes
- The cross with the RCE:FRT line labels more cells than the Ai65F reporter mouse line

Future Experiments

- Investigate the identity of the non-Opn4, GFP-labeled cells
  - ➢ To do so, I will immunostain for other markers of ipRGCs, such as SMI-32
- Inject Cre and FlpO dependent viruses into the retinas of Opn4^{Cre}; Opn4^{FlpO} mice to further characterize the intersectional genetic strategy

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