Retinal Adaptations Following Treatment and Onset of Inherited Blindness

A large effort in neuroscience is the assessment and treatment of degenerative diseases. The underlying question can be stated as follows: what are the structural and functional changes during degeneration and how can they be corrected? An example of a neurodegenerative disease is retinitis pigmentosa (RP), a form of incurable blindness that initially targets the rod photoreceptor cells. In this disease, loss of rods initiates alterations to the overall retinal circuitry (Fariss, Li, and Milam 2000; Fei 2002; Strettoi and Pignatelli 2000). One potential impact is cone-mediated vision, which is responsible for daylight vision and acuity. Another potential impact is the synaptic connections between cones and their downstream bipolar cells. Understanding how retinitis pigmentosa alters the genetic profiles of bipolar cells may provide insights on how cone vision is affected by disease and improve genetic treatments for retinitis pigmentosa.

Though the retina's structure and function is stable after development, photoreceptor degeneration has been shown to remodel the retina and possibly deteriorate retinal function (Lund et al. 1998; Pu, Xu, and Zhang 2006; Puthussery et al. 2009; Sauve et al. 2001). In prior studies of the retina, the retina has been observed to have abnormal contacts with bipolar cells and disruptive spontaneous activity near the ganglion cells during RP (see Fig 1A for location) (Pfeiffer et al. 2020; Puthussery et al. 2009). However, recent studies suggest that the retina is able to compensate functionally to this disease (Care et al. 2020). For example, bipolar cells have been shown to be able to form new contacts with different photoreceptor cells and functionally compensate for rod loss (Care et al., 2019; Johnson et al., 2017; Shen et al., 2020)). In terms of treatments, in a slow rod degeneration model, prevention of rod loss has been shown to maintain the synaptic connections of photoreceptor cells (Koch et al., 2012; Michalakis et al., 2014; and Petersen-Jones et al., 2018). These adaptations from RP and from treatment indicate that useful vision may return to normal if rod loss is halted. It is likely that the time-dependent mechanisms following treatment and degeneration lead to retinal adaptations and also contribute to whether changes are deleterious or favorable for vision outcomes. Thus, evaluating treatment options will require measuring the adaptations across various stages of degeneration and various treatment time courses.

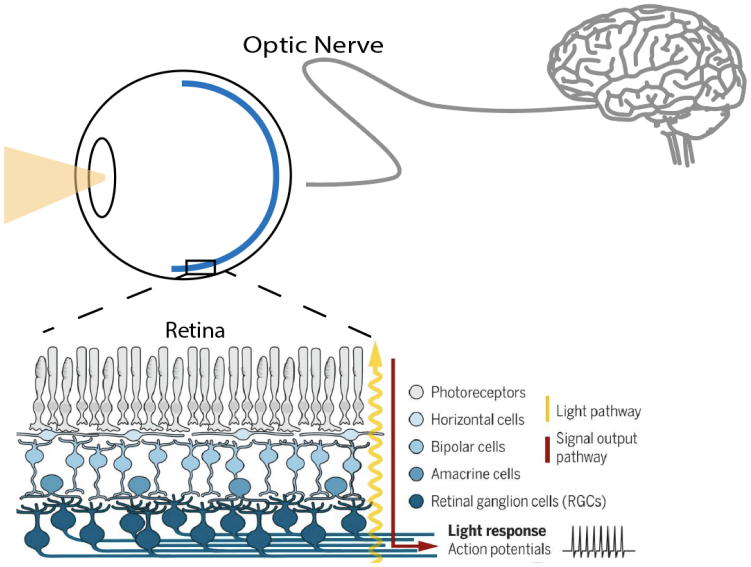

Figure 1: Diagram of Visual Transduction Process at the Retina: Physical light first travels through the retina. Light gets transformed into biological signals and gets transmitted from photoreceptors to bipolar cells and finally to retinal ganglion cells. These signals then travels to the brain where the remainder of vision occurs.

Overall, the mechanisms of adaptations in retina's bipolar cell synapses with cones during degeneration and

after treatment are unknown. In this analysis, we sought to determine whether the retina compensates for the photoreceptor degeneration and contributes to useful vision. We utilized a mouse model for retinitis pigmentosa caused by a CNGB1 mutation in the rod photoreceptors, a true cause of inherited blindness. This model can also simulate a treatment through a cre-lox recombination that prevents further degeneration progression. By conducting RNA-sequencing of bipolar cells in diseased, control, and treated mice, we find _____ (description of results). These results provide confidence in therapies for maintaining cone vision and potential target genes in therapies for retinitis pigmentosa and photoreceptor degeneration diseases.

Data Collection

We use the CNGB1 mouse (ages 30-210 days) to model RP. In these mice, a neoloxP cassette has been inserted into intron 19 of the Cngb1 gene (cyclic nucleotide gated channel, beta-1 subunit). This cassette prevents the expression Cngb1, a critical component of phototransduction whose dysfunction leads to rod death (Biel and Michalakis 2007; Huttl et al. 2005). Mutations in this gene cause RP in humans, giving us the ability to model a genuine cause of blindness (Bareil et al. 2001).

The CNGB1 genetically rescued mice undergoing RP. The neoloxP cassette that induces RP can be removed through cre-mediated recombination. To obtain temporal control of Cre-mediated rod rescue, we have crossed these mice with CAG-CreER mice. Offspring mice (henceforth, called Cngb1neo/neo), when fed tamoxifen, express Cre in rod photoreceptors, which removes the neoloxP cassette and induces normal Cngb1 expression. Thus, tamoxifen administration halts rod death in this disease, mimicking gene therapy. This system allows us to monitor changes in bipolar cell gene expression as a function of rod photoreceptor death and following a treatment that stops RP progression. Data was collected at the Field Lab at Duke Neurobiology. A diagram of the samples in this study are listed below:

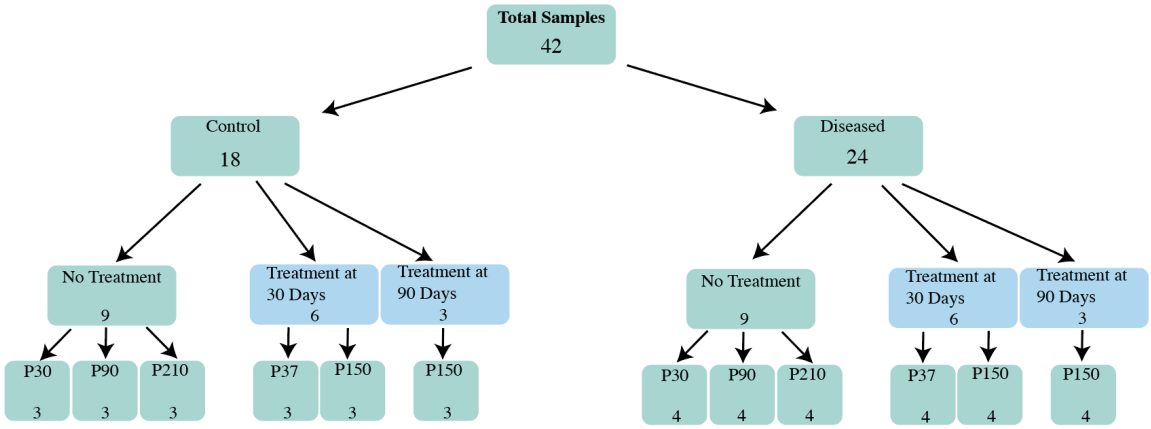

Figure 2: Diagram of Experimental Data; includes treatment, disease, and age of data collection

In an initial principle components analysis, we find that there are differences between treated, diseased, and control biological samples. This dimension reductionality device allows assessment of the similarity and between samples and across the three groups. The two principle components in Fig 3 explain a majority of the variance across the gene expression samples. Control and treated samples show high similarity with respect to the first principal components and a small within group variance. The diseased group has one biological replicate that is dissimilar from the 3 other samples in its group. Overall, this sample group separation shares that genetic differences exist in the bipolar cells of treated, diseased, and controlled retinas.

By running an unsupervised k-means clustering across all samples, gene clusters indicated that expression differences were involved in nervous system pathways. In this analysis, genes were ranked with greatest differences by their standard deviations and only the top 2000 genes were clustered. Clustered gene sets were

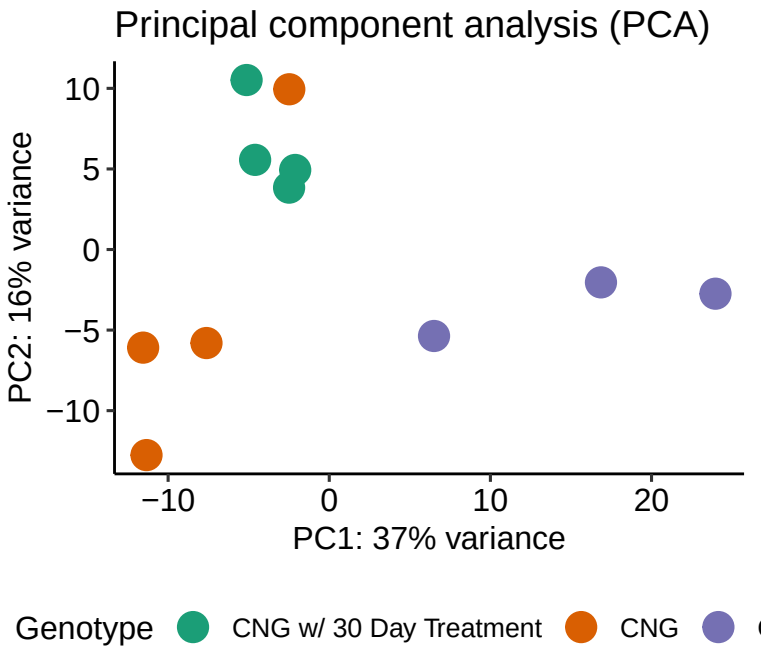

Figure 3: Principal Components Analysis of P30 Treated, P210 Diseased, and P210 Control Animals

then analyzed for which biological pathways they were concentrated in and most were found to be involved in nervous system development and processes. These results indicate that changes in bipolar cells from disease and treatment across sample groups are associated with nervous system development.

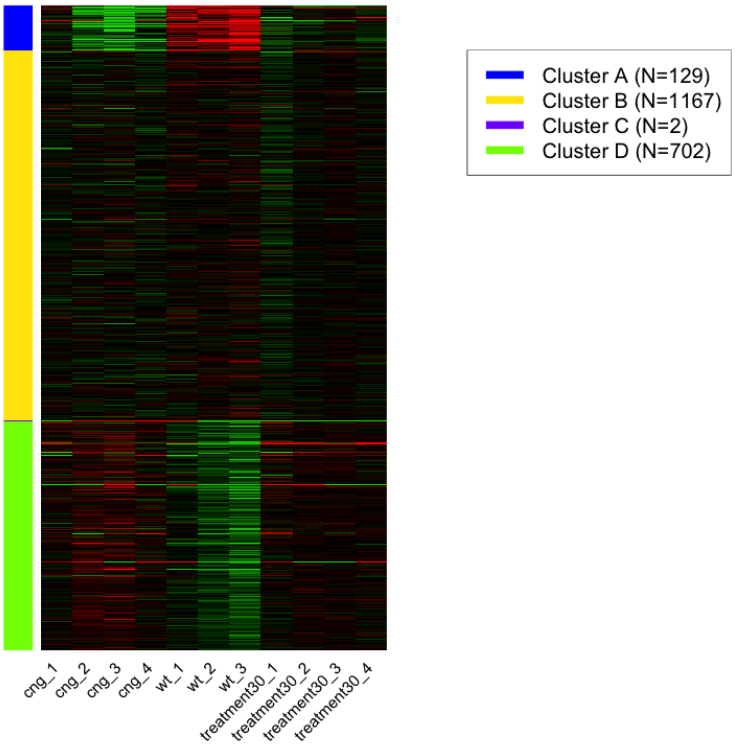

Figure 4: K-Means Clustering of Genes across P30 Treated, P210 Diseased, and P210 Control Samples

Given there are genetic differences observed in animals with RP and those that are treated, it would be useful to study exactly what genes become affected and how they contribute to the biological processes and mechanisms of blindness. From a preliminary analysis of these genes, it is likely these genes are involved in visual system. A more involved analysis involving the effect of treatment time and disease progression should better reveal the mechanisms of disease and treatment.

Appendix

Cluster	adj.Pval	Genes	Pathways
A	0.000	24	Response to inorganic substance
	0.000	35	Regulation of transcription by RNA polymerase II
	0.000	26	Response to organic cyclic compound
	0.000	35	Transcription by RNA polymerase II
	0.000	24	Negative regulation of transcription by RNA polymerase II
	0.000	27	Positive regulation of transcription by RNA polymerase II

Cluster	adj.Pval	Genes	Pathways
	0.000	27	Response to abiotic stimulus
	0.000	18	Response to metal ion
	0.000	29	Positive regulation of RNA biosynthetic process
	0.000	29	Positive regulation of nucleic acid-templated transcription
B	0.000	294	Nervous system development
	0.000	214	Generation of neurons
	0.000	221	Neurogenesis
	0.000	202	Neuron differentiation
	0.000	214	Regulation of transport
	0.000	240	Cell development
	0.000	227	Establishment of localization in cell
	0.000	174	Neuron development
	0.000	178	Cell-cell signaling
	0.000	160	Neuron projection development
D	0.000	122	Cellular protein localization
	0.000	122	Cellular macromolecule localization
	0.000	139	Nervous system development
	0.000	118	Establishment of localization in cell
	0.000	114	Cellular catabolic process
	0.000	121	Cell development
	0.000	121	Catabolic process
	0.000	105	Neurogenesis
	0.000	111	Regulation of multicellular organismal development
	0.000	98	Intracellular transport

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