Program # 2977

Partial circuitry rescue by cones in retinitis pigmentosa

B.W. Jones1, K.Kinnard1, D.W. Marshak2, R.E. Marc1. 1Ophthalmology, Moran Eye Center, Salt Lake City, UT; 2 Neurobiology and Anatomy, University Texas Medical School, Houston, UT. • Univ Utah Moran Eye Center, Salt Lake City, UT • EY02576 (RM), EY06472 (DM), EY014800 Vision Core (RM)

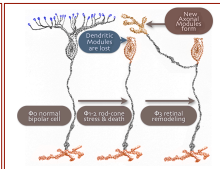
Purpose: Retinal remodeling alters neuronal connectivity and survival in forms of retinitis pigmentosa (RP) where all photoreceptors are lost. But, what are the neural sequelae found in forms of cone-sparing RP. We used *ex vivo* excitation mapping and computational molecular phenotyping (CMP; Marc and Jones 2002 J Neurosci 22: 413) to probe signaling pathways in cone-sparing RP retinas.

Methods: The hrhoG model (Chan et al., PNAS 101:9109) shows aggressive but inhomogeneous rod-cone degeneration, allowing concurrent assessment of ionotropic glutamate receptor (iGluR) drive in zones of complete rod-cone loss and islands of cone sparing. We used normal C57Bl6/J mice for comparison. We also acquired a rapid postmortem sample of human RP case (21 y.o. male). Consistent with the patient's history, the eye was rich in cones (albeit morphologically altered) and lacked rods. As a primate control, we used baboon (*Papio anubis*) retinas. Isolated eyecups or segments were incubated in a complete Ames medium augmented with 5 mM 1-amino-4-guanidobutane (AGB), an organic cation reporter that permeates glutamate-gated channels. Signaling pathways were activated with kainate (KA: 0, 0.003, .006, .012, or .025 mM) for 10 min. Characteristic small molecule signatures and AGB signals were visualized by CMP.

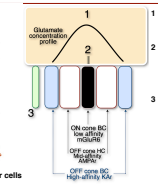
Results: KA activates iGluRs on OFF bipolar cells (BCs). Combined with CMP, this parses BC signaling pathways into distinct cone ON, rod ON and AGB-labeled cone OFF populations in normal retinas. In hrhoG retinas with complete degeneration, BC signaling was absent, consistent with prior evidence that all BCs retract dendrites and down-regulate glutamate receptor expression. But, iGluR-mediated BC signaling was preserved beneath small patches of surviving cones. In the human RP case, KA activation roughly resembled normal primate and mammalian patterns, but rod BCs were absent and the number of OFF BCs appeared to have nearly doubled from a normal level of 39% to 79%. There was no evidence of BC death.

Conclusions: Cone sparing in RP leads to nominal sparing of retinal circuitry, but the human RP case showed a remarkable increase in OFF BCs. We conclude that rod BCs, bereft of their normal contacts, switch dendrites to make novel contacts with cone pedicles and change gene expression, becoming functional OFF BCs by upregulating iGluR display. This anomalous rod BC survivor burden will lead to corrupted vision if the normal rod amacrine pathway remains intact in RP.

Commercial Relationship: BW Jones, None; K Kinnard, None; DW Marshak, None; RE Marc, Signature Immunologics F, E.



Retinal remodeling occurs in all forms of RP with the first severe neural effect being the disconnection of bipolar cells from photoreceptors early in disease. Remodeling of circuitry occurs in phases. In phase 1 of remodeling, photoreceptor stress initiates remodeling followed by phase 2 where photoreceptor death seals off the retina. Phase 3 is characterized by persistent remodeling which revises the retina. In models of RP where rods and cones die concurrently, bipolar cells lose dendrites and all iGluR/mGluR responsivity. In RP models where cones outlive rods, some bipolar cell dendrites switching targets.

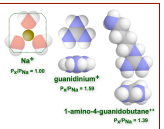


1 There aren't enough cone pedicles to accommodate all rod BCs.

2 There aren't enough ribbons to accommodate rod BC dendrites and ON cone BCs already "own" the ribbon contacts.

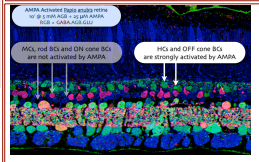
3 Ectopic contacts are too far from the ribbon to activate mGluR6 receptors.

Each cell type expresses receptors matched to concentrations prevailing at a given distance from the ribbon. If rod BC mGluR6 receptors are not activated, rod BCs should be persistently depolarized. How could such cells function in vision?

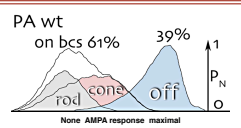


Excitation mapping uses the reporter molecule AGB to track the excitation histories of neurons in vivo and ex vivo.

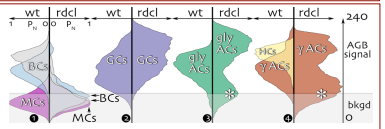
When non-selective cation channels are opened, AGB flows into neurons where it is detected immunochemically revealing endogenous drives in chains of neurons.



By combining excitation mapping and CMP, we can visualize the neurons excited by ligands or light pathways *ex vivo*. Simultaneous imaging of GABA (red) and glutamate signals (blue) demonstrate the basic stability of the baboon retina for such analysis. Adding the AGB (green) channel shows that characteristic sets of neurons (HCs, OFF BCs, Acs and GCs) are activated by AAMPA drive.

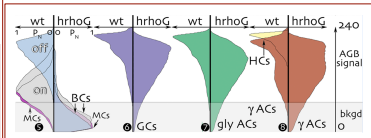


Using CMP to segment images, we can extract separate excitation histograms for each class of bipolar cell allowing us to discriminate OFF bipolar cell classes from ON bipolar cell classes. Additionally, we can segment the ON bipolar cell classes into rod and cone classes allowing us to elucidate each subtypes response profiles to AMPA. In primates, rodents & rabbit, OFF cone bipolar cells comprise about 30-40% of all bipolar cells.

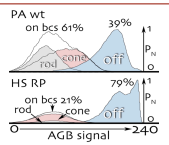


These butterfly plots have response profiles of wild type retinas (wt) on the left and the rdcl mouse (rdcl) retinas on the right as a model for retinal degeneration and remodeling. The responsivity as measured by AGB concentration in the cell of cell classes increases as one goes up in the plot and histograms encode probability.

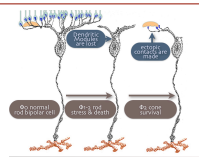
In remodeling retinas, amacrine & ganglion cells continue to encode signals via iGluR receptor complexes. Conversely, bipolar cells possess no endogenous iGluR- or mGluR6-transduced cation currents. Bipolar cells (BCs), Müller cells (MCs), ganglion cells (GCs), glycinergic amacrine cells (gly Acs), horizontal cells (HCs), GABAergic amacrine cells (γ ACs).



In remodeling retinas, amacrine and ganglion cells continue to express kainate-activated iGluR receptor complexes. Conversely, OFF bipolar cells possess no such iGluR transduced cation currents. They are silent.



After CMP segmentation, the incidence of OFF-like iGluR expressing bipolar cells doubled in cone-sparing RP, while the incidence of remnant rod bipolar cells drops nearly tenfold. However, there appears to be no detectable bipolar cell death.



Therefore, we conclude that most rod bipolar cells make new contacts and begin to express iGluRs leading to significant implications for retinal wiring.

Summary: In RP where rod & cones die concurrently, all BCs engage in dissassembly of dendritic modules, repression of GluR expression, supernumerary axon formation and neuronal death.

In models of RP where cones outlive rods, rod BCs engage in disassembly of dendritic modules, retargeting to cone pedicles, mGluR6 to iGluR phenotype switching and probable corruption of cone pathway signaling.

Cones may rescue rod bipolar cell, but not their function.