Purpose: Ultrastructural connectomics has allowed for precise identification of neural network topologies in retina, exposing synaptic connectivity associated with specific pathways involved in neural retinal processing. In pathological degeneration retina such as retinitis pigmentosa (RP), retinal remodeling emerges as a phenomenon through a series of negative plasticity events originating from neural deafferentation initiated by photoreceptor degeneration. Early stages of remodeling include glial changes, Gliif receptor alterations (reprogramming), and rewiring of retinal networks. The connectivities initiated by these processes are currently unknown. To address this problem, we have created an ultrastructural pathoconnectome of early retinal remodeling in a rabbit model of retinitis pigmentosa, Retinal Pathoconnectome 1 (RPC1).

Approach: The tissue for RPC1 was obtained from a 10-month-old transgenic P347R, rabbit model of autosomal dominant RP. Our control connectome is RC1, which was generated from a 13-month-old Dutch-Belted rabbit. Tissue was fixed in mixed aldehydes, osmicated, dehydrated, embedded in epon resin, and sectioned at 70nm. Serial sections were placed on grids, stained, and imaged using a JEOL JEM-1400 TEM using SerialEM software. Every 30th section was reserved for computational molecular phenotyping (CMP), and probed for small molecules; glutamate, glutamine, glycine, GABA, taurine, glutathione or TEM compatible proteins GFAP and GS. The pathoconnectome volume is explored and annotated using the Vingk software suite.

Figure 1: Rewiring in Retinal Remodeling: Following photoreceptor degeneration, retinal networks enter a period of remodeling, in which gross changes have been described, but the local network impacts are still being explored. During rewiring, retinal neurons extend neurites in an aberrant fashion within the neural retina. Phase 1 is characterized by retinal degeneration. During this time, it has previously described that photoreceptors spiral neurites that extend beyond the OPL into the IPL, occasionally reaching the GCL. In phase 2, cone photoreceptors degenerate and more pronounced-neuronal sprouting occurs from all neuronal types of the retina. In phase 3, the neurites continue into microvessels. Microvessels are collections of neuronal aberrant processes from all cell classes of retinal neurons and create new areas of IFL, exhibiting ultrastructural findings consistent with active synapses, internal to the microvessels, although the partners involved are currently unknown.

Figure 2: Retinal Pathoconnectome 1 (RPC1): (A) A description of the RPC1 volume, (B) Overlay of a top-down view of the 3D rendering of all confined 30 µm photoreceptors (XRobC) and cone-contacting rod bipolar cells (XRobBCs) in the RPC1 volume on a representative TEM section from RPC1. (C) Vertical view of the 3D reconstruction of RodBC and XRobBCs in RPC1.

Figure 3: Abberant synaptology in RPC1: (A) 3D rendering of XRobC 30854 (blue) and Ali GAC 2713 (green). (B) Higher magnification of synapse indicated by a “#” indicates a gap junction between the XRobC and Ali GAC. (C) Pseudo-colored TEM image of the ribbon indicated in B. (D) Higher magnification of ribbon indicated in B. Left Pseudo-colored TEM image of gap junction indicated in B. Right Pseudo-colored TEM image of gap junction with ribbons abnormally presented in blue. Left Pseudo-colored TEM image of gap junction of XRobC and ribbons abnormally presented in blue. Right Pseudo-colored TEM image of gap junction of XRobC and ribbons abnormally presented in blue. (E) Higher magnification TEM image of gap junction in (C). Left Higher magnification TEM image of gap junction in (C). Right Higher magnification TEM image of gap junction in (C). (F) 3D rendering of gap junction annotation between XRobC 12436 (blue) and Ali GAC 25558 (green). (G) Pseudo-colored TEM image of gap junction in d. (H) Reproduced TEM image at 35000X is tilted to -10 degrees.

Figure 4: Rod BC Network Diagrams. Nodes represent individual cells: Squares (Bipolar cells and Unknown), Triangles (aGCL), Redgons (All aGAC). Lines connecting nodes are edges indicating synaptic contacts between cells. Synaptic types: Ribbon (green), Conventional inhibitory synapse (red), Gap junctions (yellow). Cells that have more than one type of contact are mixtures of the synapse type colors. The RC network (above) illustrates normal synaptic connectivity of the rod pathway in the retina. The RPC network (below) highlights the variation in synaptic connectivity observed in the degenerate retina.

Figure 5: Network alterations from rod photoreceptor stress and degeneration. Healthy circuit diagram (left) illustrates the normal connectivity of the scotopic low-light pathway. Degenerate RodBC network (right) highlights the changes (red) in the network that occur as a consequence of rod degeneration.