

Purpose:

Aii amacrine cells (Aii GACs) function by mediating scotopic vision via connection of rod bipolar cells (Rod BCs) to cone bipolar cell pathways. The purpose of this project is to determine the effect of retinal degeneration (RD) on Aii GAC networks. We explore this in a pathoconnectome of early RD (RPC1), using a connectome of healthy retina (RC1) as control. Cells in each volume are evaluated by comparison of morphology, synaptic connectivity, and eventually network analysis.

Methods:

Tissue for RPC1 was collected from a 10-month-old transgenic p347L rabbit model of autosomal dominant retinitis pigmentosa. RC1 was collected from a wild type 13-month-old Dutch-Belted rabbit. Tissue was fixed in a mixed aldehyde solution, before subsequent dehydration, osmication, and resin embedding. Volumes were sectioned at 70nm and placed on Formvar grids. 1 section was reserved from every 30 TEM sections for computational molecular phenotyping where it was placed on a slide and probed for small molecules or proteins. TEM sections were captured at 2.18nm/px using SerialEM software on a JEOL JEM-1400 TEM. The RC1 volume has a diameter of 250µm and RPC1 has a diameter of 90µm. Both volumes were analyzed using the Viking software suite.

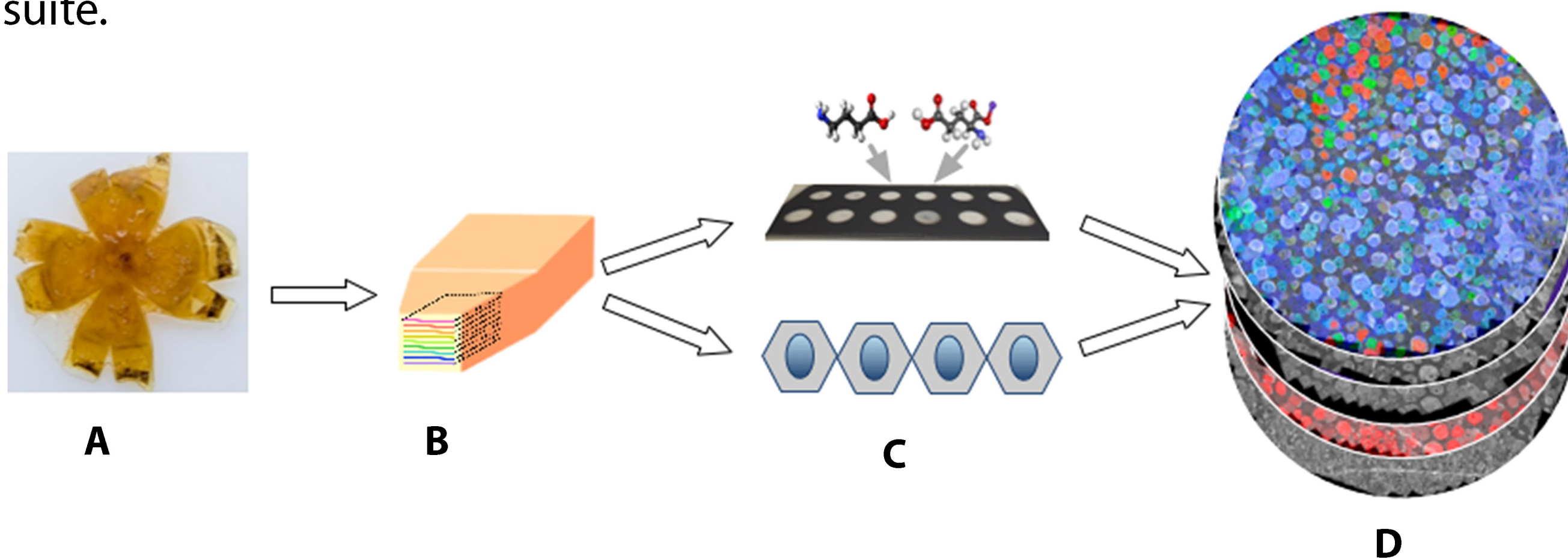


Figure 1: TEM-CMP Volume Assembly

A fixed retina tissue in aldehyde solution. **B** dehydrated and resin embedded tissue. **C** Sectioned (70nm) placed on grid for TEM and or on slide for CMP of small molecules or protein. **D** Computationally assembled volume.

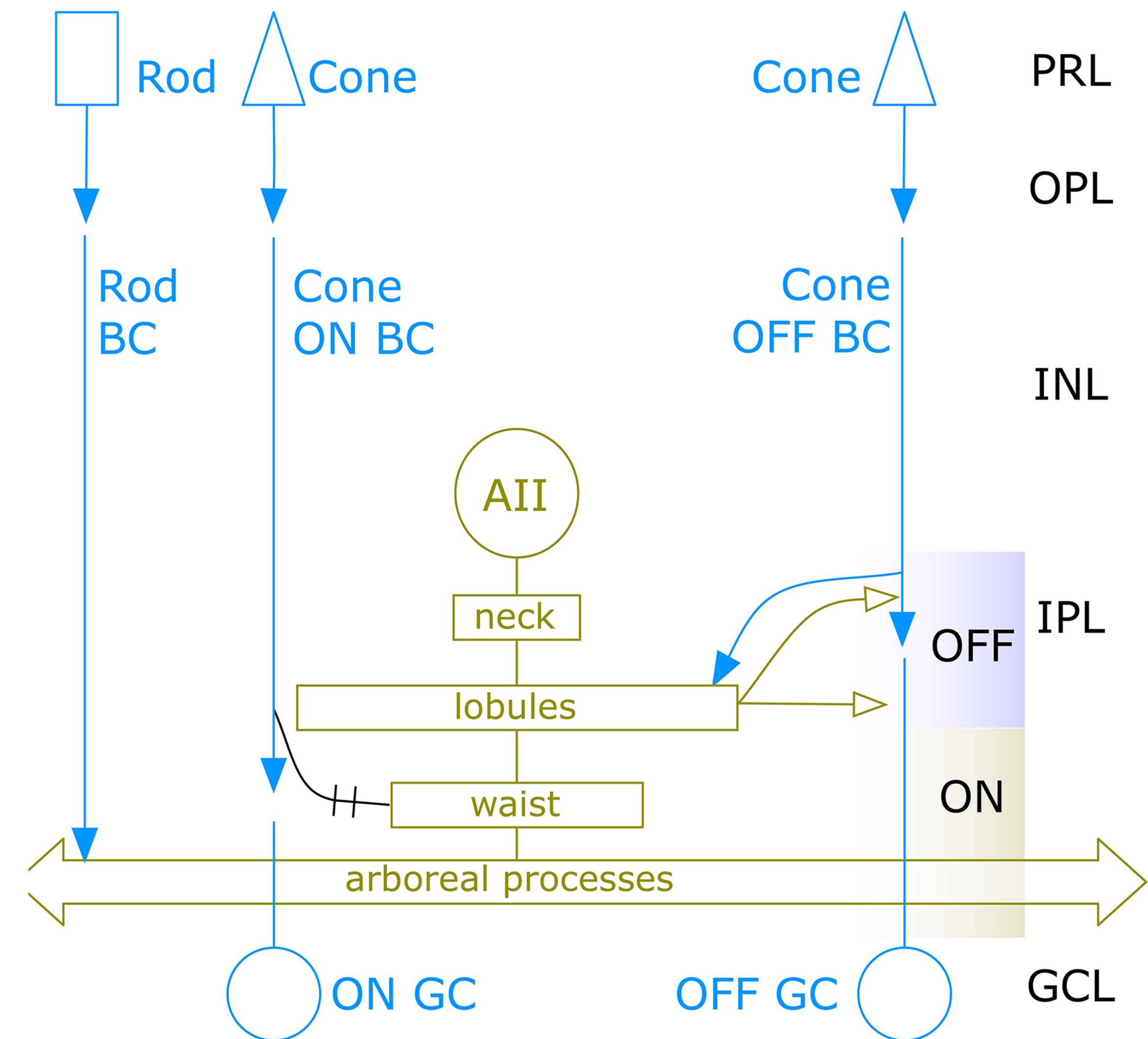


Figure 2: Aii Network Diagram

Classic Aii GAC network connectivity. Aii GAC (green), bipolar cell contacts (blue).

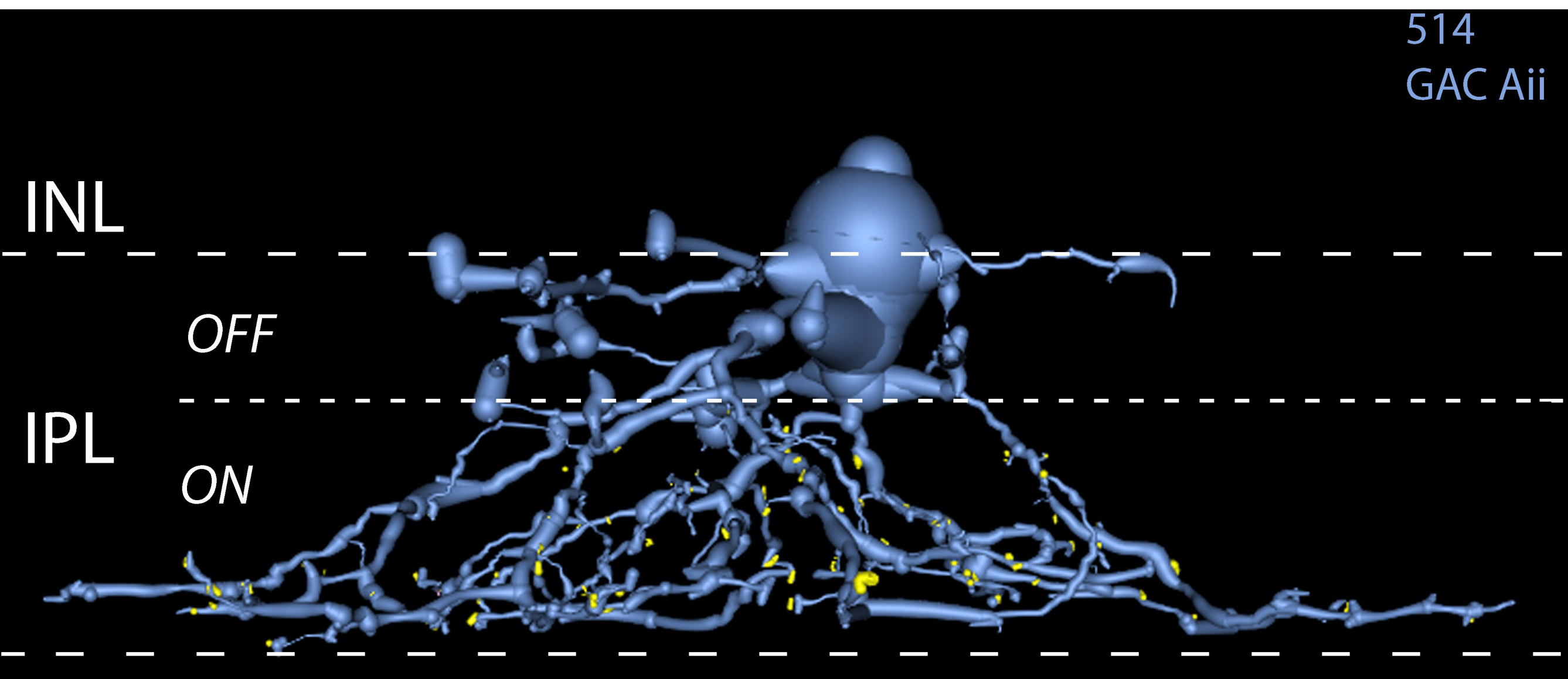


Figure 3: Morphology of RC1 Aii Amacrine Cell (514)

514 GAC Aii (blue) and gap junctions (yellow) from the healthy RC1 volume. OFF represents region of lobules and the ON layer is occupied by the arboreal processes. Arboreal processes initially branch below the waist in the transition from the OFF layer to the ON of the IPL.

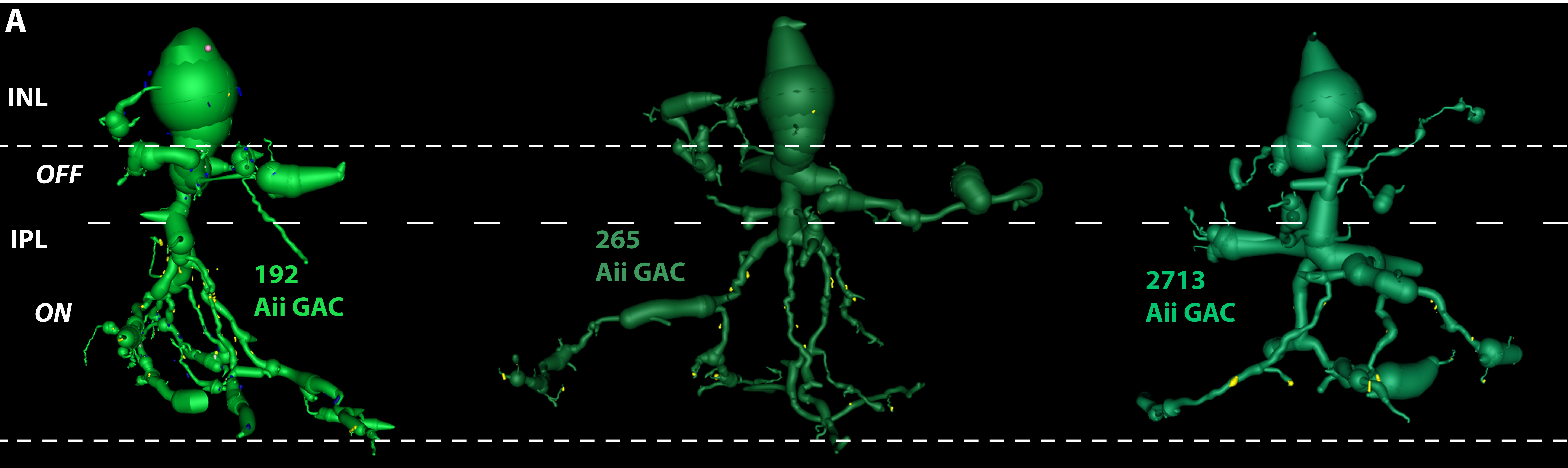


Figure 4: RPC1 Aii Amacrine Cells

3D renderings of 3 Aii GACs from the degenerate RPC1 connectome (green) and the locations of their gap junctions (yellow). 192 and 2713 are both partial cells due to the narrow diameter of the volume. 265 is completely contained within the RPC1 volume. All Aii GACs in RPC1 appear to be narrower and less complex than those found in RC1. A large portion of these morphological alterations may in part be attributed to eccentricity.

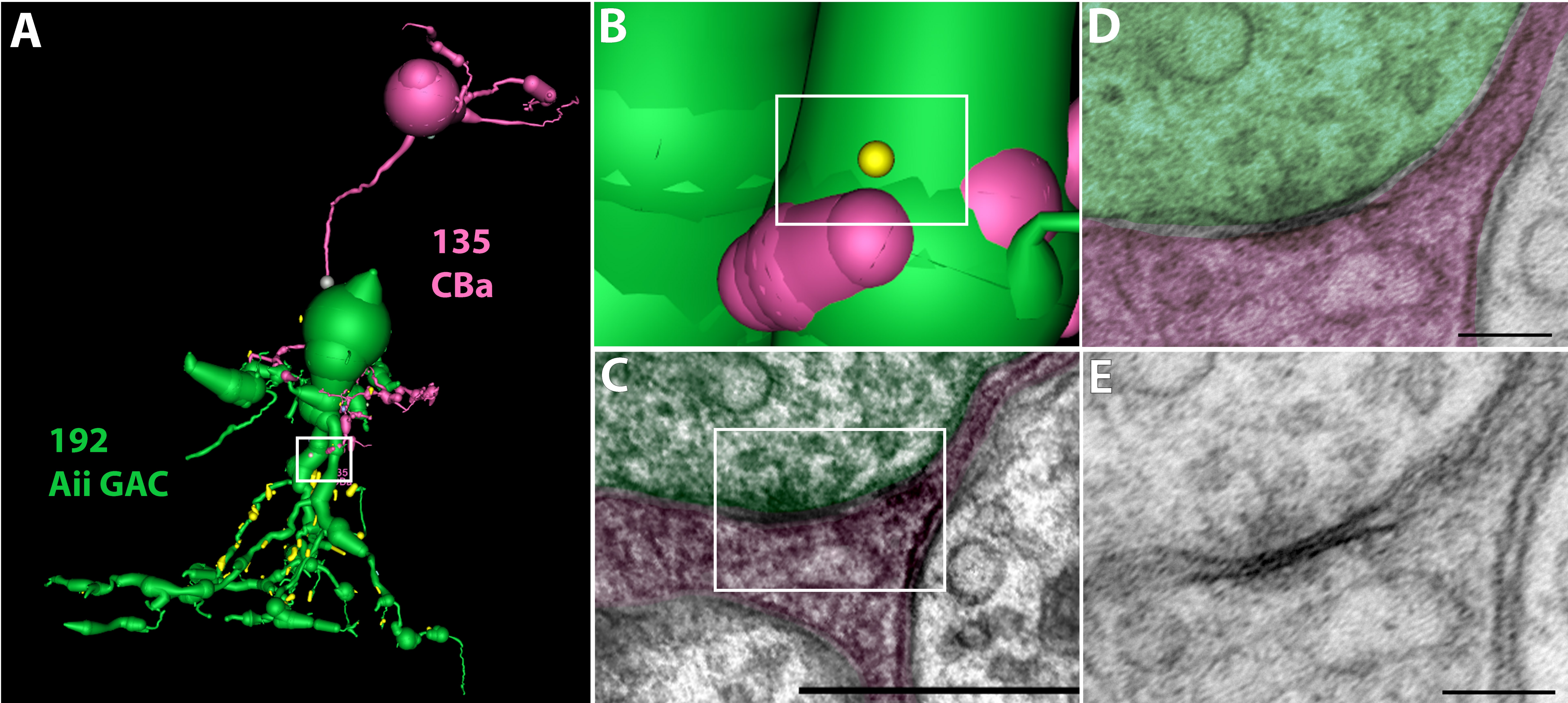


Figure 5: 192 Aii GAC Coupling with OFF BC 135

A-E 3D reconstruction of Aii GAC 192 (green) coupled with OFF BC 135 (pink) in RPC1. **B** Higher magnification of 3D rendering visualizing location of the gap junction. **C** TEM image from Viking annotation environment. Scale 500nm (res:2.18nm/px) **D and E** High resolution recapture of gap junction in 5C. **E** Recapture with -5 degree tilt at 30k (res: 0.363nm/px) Scale bar: 100 nm. Pentalaminar structure confirms gap junction.

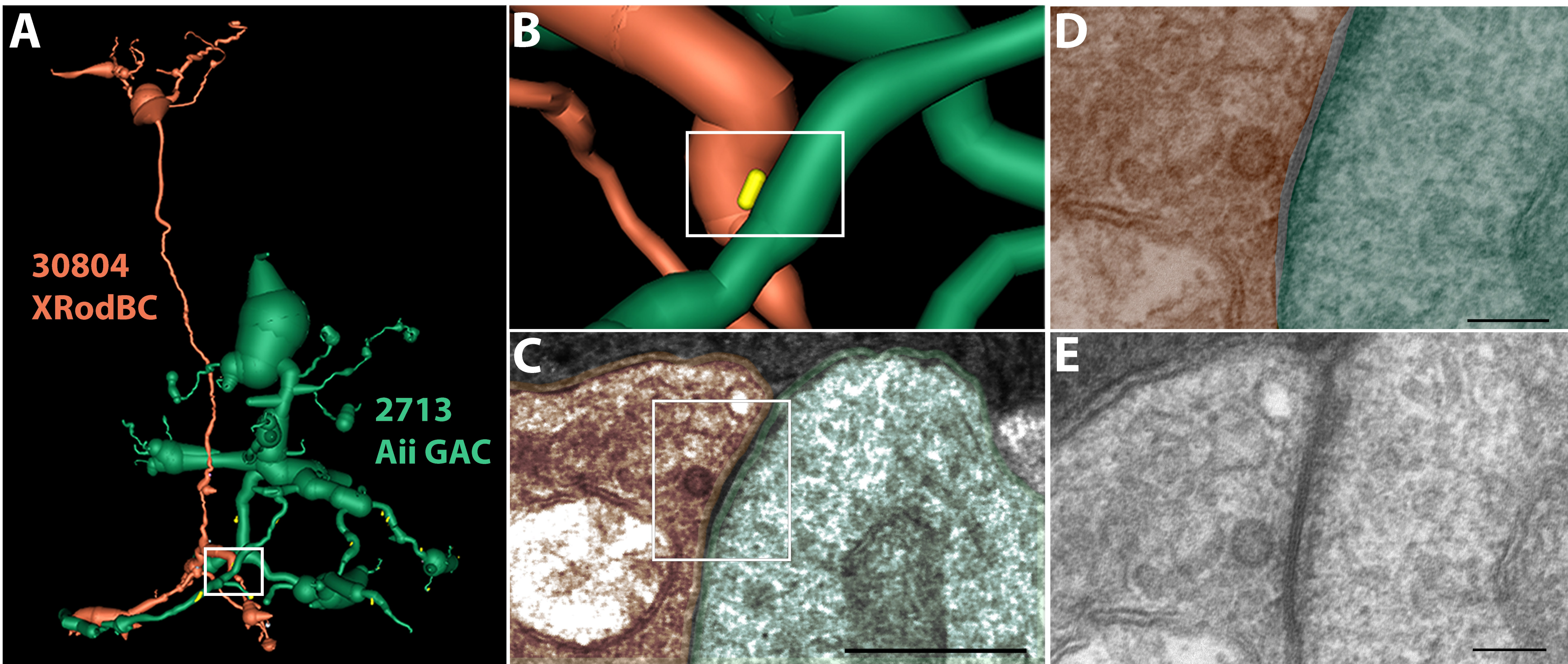


Figure 6: Aii 2713 Aberrant Coupling with XRodBC 30804

A-E 3D reconstruction of Aii GAC 2713 (green) coupled with XRodBC 30804 (orange) in RPC1. **B** higher magnification of 3D rendering visualizing location of the gap junction. **C** TEM image from Viking annotation environment. Scale 500nm (res:2.18nm/px) **D and E** High resolution recapture of gap junction in 6C. **E** Recapture with 0 degree tilt at 25k (res: 0.436nm/px) Scale bar: 100 nm. Pentalaminar structure confirms gap junction.

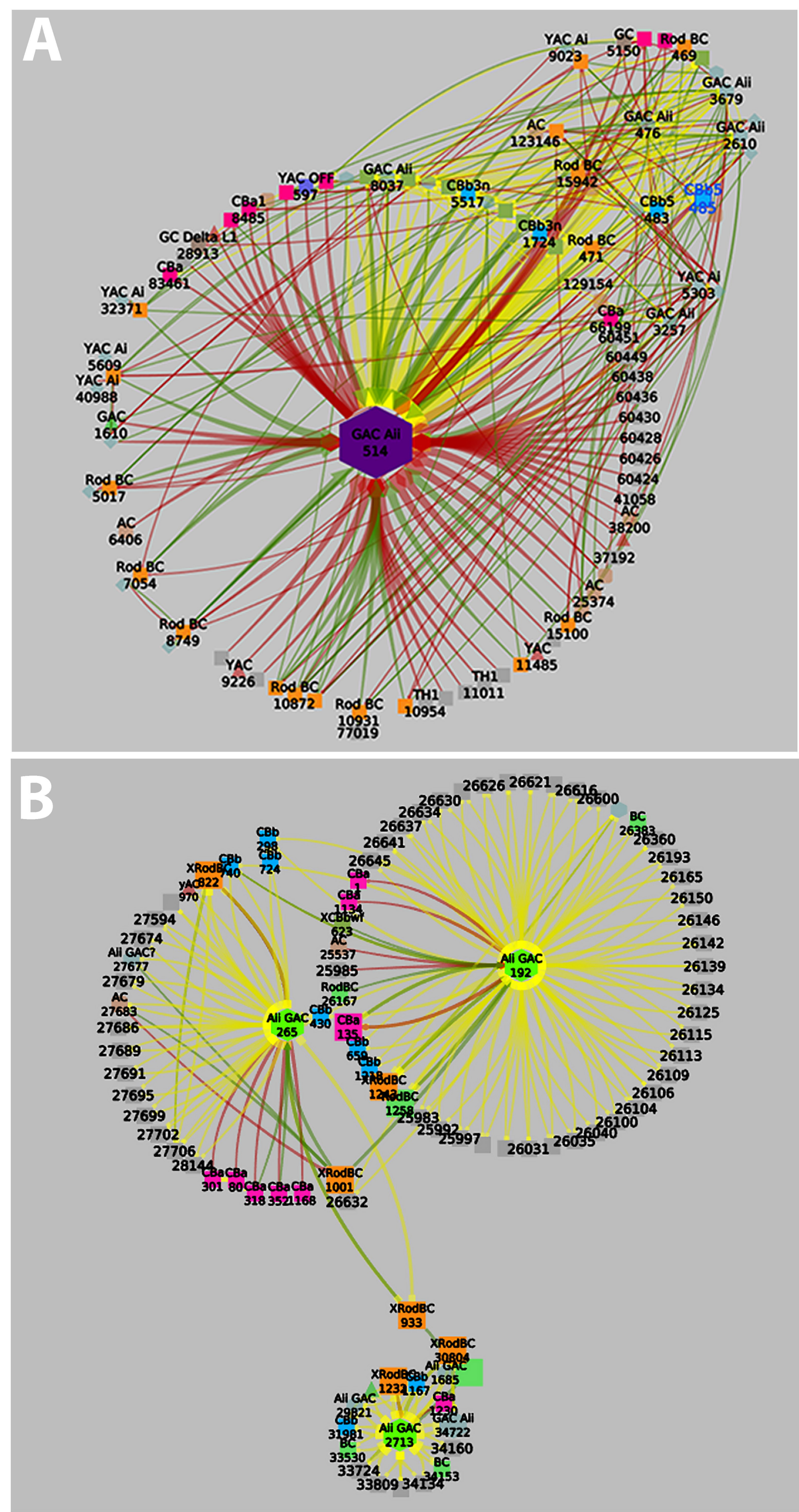


Figure 7: Aii Connectivity Network in the RC1 and RPC1

Nodes represent individual cells and lines represent edges (synapses) between cells. Synapse types: Ribbon (green), conventional inhibitory synapses (red), and gap junctions (yellow). **A** Network connectivity of representative Aii GAC 514 from healthy retinal connectome RC1. Note the lack of coupling between 514 and CBa (pink) or Rod BCs (orange). **B** Network of Aii GACs in pathological retinal connectome RPC1. There is clear network corruption. 192 couples with CBa cell 135, as shown in fig 5. In addition, more extensive RodBC coupling is present. 2713 couples with XRodBCs 1232 and 30804. 265 couples with XRodBCs 822 and 933. 192 couples with XRodBC 1243 and RodBC 1258. The color gray represents cells that have not been identified in the volume.

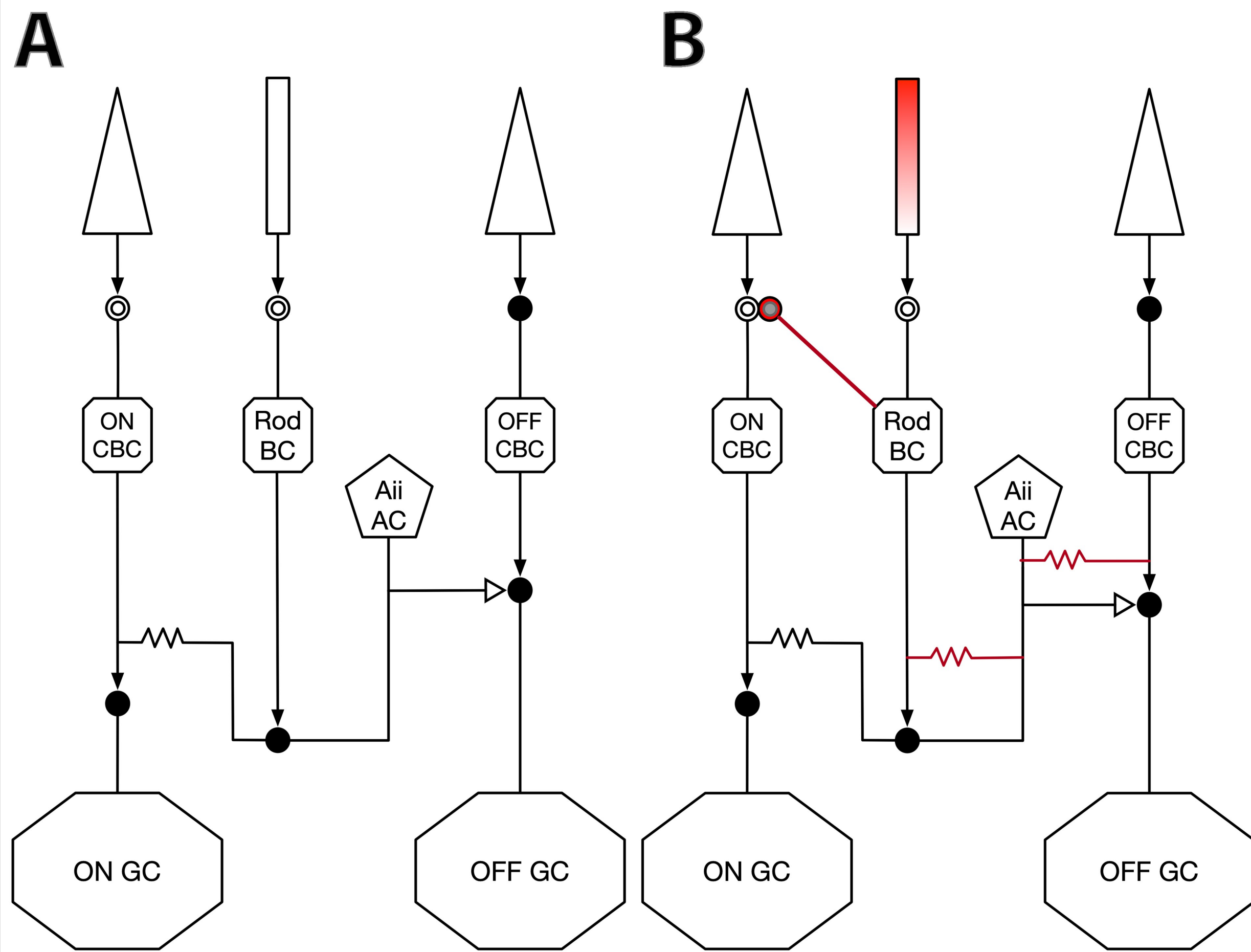


Figure 8: Normal vs. Corrupted Rod Circuitry Pathway

A Healthy circuit diagram illustrating normal connectivity of the scotopic pathway. **B** Circuit diagram demonstrating alterations observed in RPC1. Aberrant connectivity found within the scotopic pathway is highlighted in red. Notably, where there is a gap junction between the Aii and the Rod BC as well as coupling between the Aii GAC and the OFF CBC. These changes occur as a consequence of rod degeneration.

Results:

In this study, Aii GACs from RPC1 were compared to RC1. Initial results indicate no distinct difference in the morphology other than arbor size and lower complexity, which are likely due to eccentricity differences between volumes. However, in RPC1, we observe multiple instances of Aii GAC coupling with Rod BCs in the ON region of the IPL and a single instance of Aii GAC coupling with an OFF-BC in the OF layer. In contrast, Aii GACs do not couple with OFF BCs and Rod BCs never form gap junctions in healthy retina.

Conclusions:

Coupling between Aii GACs and Rod BCs and OFF BCs in RPC1 is a unique change in retinal network topology occurring in early RD. Further exploration of network changes as a response to RD is warranted, as many therapeutic interventions currently in development rely upon maintenance of inner retinal circuitry. Prior research demonstrates Rod BCs extend dendrites towards cones and change their receptor expression as rods degenerate. Therefore, knowing the network changes involving Aii ACs and their associations with bipolar cells is crucial to understanding how photoreceptor degeneration affects inner retinal visual processing.

Related Projects:

Jessica Garcia

555 - B0017

OFF-layer Branches of ON Cone Bipolar Cells in Early Retinal

Selena Wirthlin

548 - B0010

Comparative anatomy and connectivity of the Aii amacrine cell in mouse and rabbit retina

Crystal L. Sigulinsky

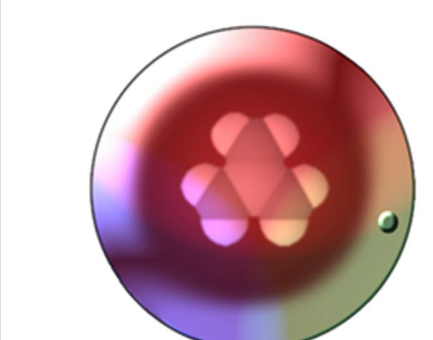
Coupling architecture of the Aii/ON cone bipolar cell network in degenerate retina
536-6441
(11:15 am)

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Disclosure: REM is a Principal of Signature Immunologics

