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# OFF-LAYER BRANCHES OF ON CONE BIPOLAR CELL IN EARLY RETINAL DEGENERATION

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## Introduction:

The retina is an essential part of the central nervous system, composed of specialized cells (neurons and glia) that work in concert to sense light and provide the initial signal processing required for vision. Put simply, light enters the eye through the pupil and travels to the back of the eye where it encounters the retina. In the retina, photons are absorbed by photoreceptors, which translate the light into neural signals transmitted through the following network: photoreceptors synapse with bipolar cells and horizontal cells; the bipolar cells then synapse with amacrine and ganglion cells. Horizontal cells are thought to compare signals between photoreceptors, while amacrine cells refine the signals by tuning overall retinal responses. Ganglion cell axons then collect the signals and perform final pre-processing of the visual signals before transmitting them through the optic nerve, out of the eye and into the brain for further visual processing. All of these cells connecting properly with one another within a network create the visual primitives necessary for the brain to then form complex images.

Understanding how these networks change as a result of retinal diseases is an important component to describing disease progression and also creating novel therapeutics. Retinal degeneration induces negative plasticity termed remodeling (Jones, et al., 2003, Marc, et al., 2003). Remodeling includes aberrant neurite extension from multiple cell types (rewiring) and neurons changing their receptors to that of other neuronal classes (reprogramming), both of which affect the neural networks responsible for vision in the retina. The primary retinal disease we study is retinitis pigmentosa (RP). RP is a progressive photoreceptor degeneration disorder, which initiates with the loss of rod (low-light sensing) photoreceptors and subsequentially continues with degeneration of the cone (bright-light and color sensing) photoreceptors. In this study, we are focused on the effects of photoreceptor degeneration on ON cone bipolar cells (CBbs). Bipolar cells, as mentioned above, are a key interneuron in transmitting signals from the light-sensing photoreceptors to the inner retina where they work in concert with amacrine and ganglion cells to form visual primitives. Bipolar cells have been extensively described and classified (Masland, 2012, Sigulinsky, et al., 2020). At their most basic level, bipolar cells are customarily classified into superclasses of ON (ON-BCs) and OFF (OFF-BCs) depending on their physiological responses to increases or decreases in photons, respectively. ON-BCs are then split by their input photoreceptor of cone or rod, while OFF-BCs are exclusively post-synaptic to cones. This ON or OFF specialization is further segregated by stratification within the inner plexiform layer (Lauritzen, et al., 2019, Masland, 2012, Sigulinsky, et al., 2020). Previous

analysis in healthy retina shows that ON-BCs can make small synapses in the descending axon with cells contained in the OFF-layer (Lauritzen, et al., 2019), or less frequently, simple single branch projections in the OFF-layer (Cohen and Sterling, 1990, Sigulinsky, et al., 2020). What drives the formation of these OFF-layer branches from otherwise clearly CBbs and what effects remodeling may have on these OFF-layer branches of CBbs is unknown. In this study, we compare OFF-layer branches from CBbs in a pathoconnectome of early retinal degeneration (RPC1) (Pfeiffer, et al., 2020) to the healthy retinal connectome (RC1) (Anderson, et al., 2011).

**Methods:**
Retinal tissues selected for retinal connectome one (RC1) and retinal pathoconnectome one (RPC1) were collected post-mortem from a 13 month old Dutch-belted healthy female rabbit and a male 10 month old transgenic albino New Zealand white P347L rabbit model of autosomal dominant retinitis pigmentosa(Kondo, et al., 2009), respectively. RPC1 shows signs of remodeling including rod outer-segment degeneration and aberrant neurite extension. Tissues were fixed in mixed aldehydes and subsequently osmicated, dehydrated, resin embedded, and sectioned at 70nm. Sections were placed on formvar grids, stained, and imaged at 2nm/px on a JEOL JEM-1400 TEM using SerialEM software. 1 section was reserved from every 30 for Computational Molecular Phenotyping, and probed for small molecules. Both volumes were evaluated using the Viking software suite.

**Results:**
Our previous work in the healthy, wild-type connectome (RC1) identified 145 CBbs. Eight of these CBbs morphologically show branching in the OFF-layer(Sigulinsky, et al., 2020). Of these eight CBbs, three (cell IDs: 483, 593, 5916) showed the greatest neurite extension and complexity for morphological comparison (Figure 1). Of these, CBb 593 was used for synaptic comparison.

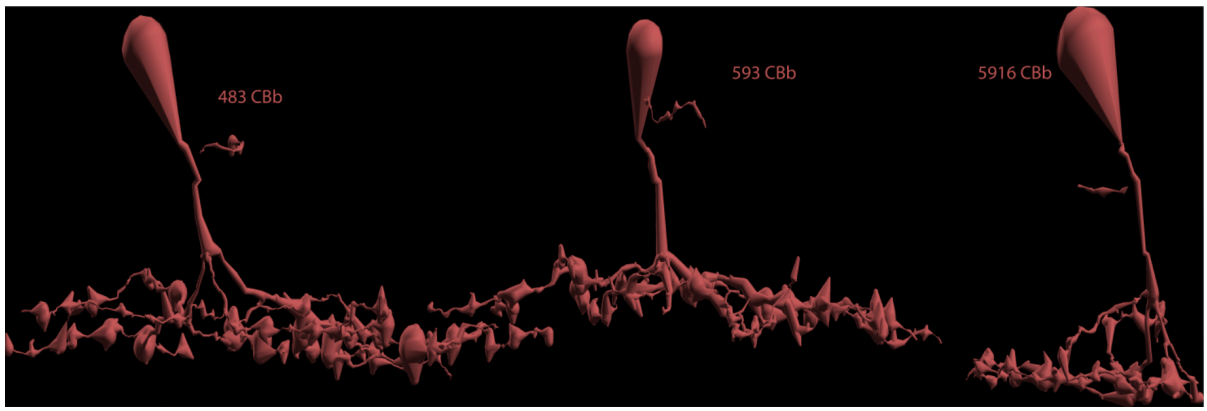

***Figure 1:*** *CBb #s 483, 593, 5916 from RC1 that have branches in the OFF-layer of the IPL.*

Our initial analysis of these branches endeavored to describe what cells these atypical branches are pre-synaptic to. Excitatory neurons of the retina (like bipolar cells) are glutamatergic and often make pre-synapses with a distinct morphology called a ribbon pre-synapse. In addition, we and others have found occasional pre-synapses made by bipolar cells which lack the traditional

ribbon structure, bipolar conventional presynapses (BCS), which are also presumed to be glutamatergic. Examples of these synapse types are seen in Figure 2.

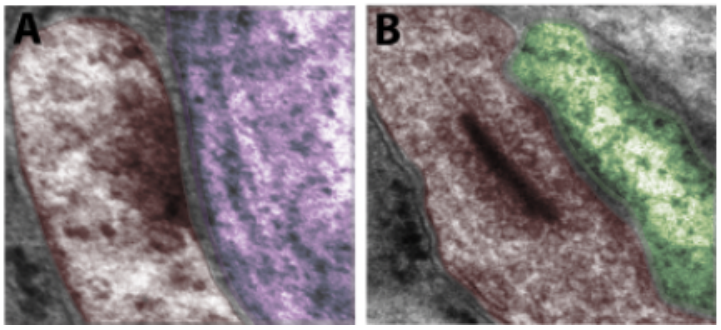


***Figure 2:*** *Glutamatergic synapse types made by bipolar cells. (A) Example pre-synaptic BC conventional cell (red) to a post-synaptic cell (purple). (B) Example pre-synaptic BC ribbon (red) to post-synaptic cell (green).*

In this OFF branch of CBb 593, we identified seven post-synaptic partner cells (or cells with densities opposing ribbon or BCS type pre-synapses), from here on referred to simply as partners. Of these seven partners, three were identified as GABAergic amacrine cells (yAC 12804, 20537, AC 596). The remaining four partners could not be conclusively identified (130710, 130710, 59696, 68613). These post-synaptic partners to CBb 593 can be seen Figure 3 below. The reason for the unidentified cells is that the morphology, metabolic profile, and/or network motifs made by the unidentified cells were insufficient to conclusively assign them to a known class. The most common reason for inadequate metrics was due to the majority of the partner not being contained fully within the connectome volume.

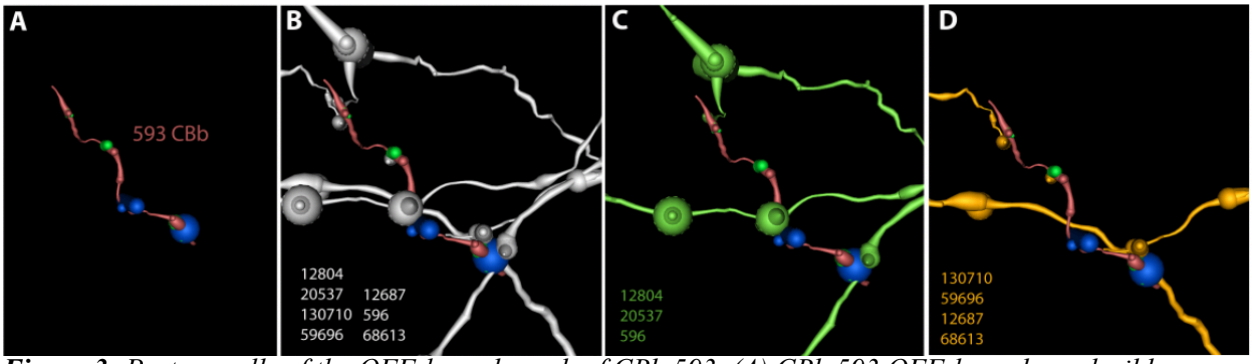


***Figure 3:*** *Partner cells of the OFF-layer branch of CBb 593. (A) CBb 593 OFF-layer branch ribbon synapses and BCS locations on branch. (B) 593 ribbon and BCS partner cells of any type. (C) Location of synapses with amacrine cell partners. (D) Location of synapses with unknown synaptic partner cells.*

To understand the relative contribution of excitatory output of the OFF-layer branch versus the total synaptic output of the CBb, we compared the number of pre-synaptic structures in the OFF-layer branch of RC1 CBb 593 to the total number made by the entire cell (Table 1). CBb 593 had a total of 213 ribbon synapses and 109 BCS. The branch in the OFF-layer had only 9 ribbon synapses and 3 BCS. From this we calculated the ribbon synapses in the OFF-layer branch which made up only 4.22% of the total ribbon synapses in the cell. Additionally, BCS in the OFF-layer branch made up only 2.75% of the total BCS in the entire cell.

| 593 | Ribbon | BC Conventional |
|---|---|---|
| Total | 213 | 109 |
| OFF-Layer Branch | 9 | 3 |
| % OFF Branch | 4.22% | 2.75% |

| 430 | Ribbon | BC Conventional |
|---|---|---|
| Total | 177 | 71 |
| OFF-Layer Branch | 20 | 13 |
| % OFF Branch | 11.30% | 18.31% |

***Table 1:*** *Table of synapses in cell 593 and 430 comparing the number of synapses in the OFF-Layer branch and entire cell.*

In our pathoconnectome (RPC1), there are currently 28 identified CBbs and of these, CBb 430 demonstrates the most complex branching in the OFF-layer (Figure 4). The same approach of evaluating post-synaptic partners of RC1 CBb OFF-layer branches was used to evaluate CBb 430. Here, we also identified seven synaptic partners that fit the criteria of being post-synaptic to either a ribbon synapse or BCS (Figure 5). Three of these cells were identified as amacrine cells (glycinergic AC 31482, and amacrine cells of unknown neurochemistry 32248 and 31942). The other four were identified as potential ganglion cells (29201, 32230, 32319, 32971) based on their lack of presynaptic structures indicating they only received input synapses within the IPL (amacrine cells, in contrast, are both pre- and post-synaptic in the IPL).

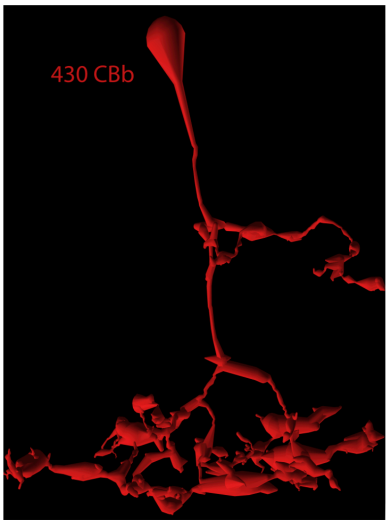


***Figure 4:*** *Morphology of CBb 430*

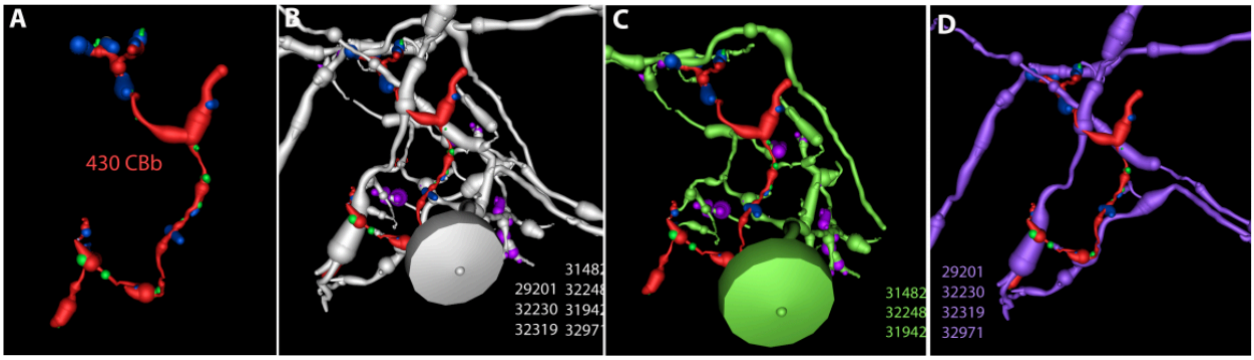


***Figure 5****: Partner cells of the OFF-layer branch of CBb 430. (A) Locations of all CBb 430 OFF-layer branch ribbon synapses and BCS. (B) All partner cells of 430 ribbons and BCS. (C) Location of synapses with amacrine cell partners. (D) Location of synapses with ganglion cell partners.*

We also identified the contribution of excitatory pre-synaptic structures from the OFF-layer branch of 430 relative to the total pre-synaptic structure of the entire cell (Table 1). Of the total 177 ribbon synapses in CBb 430, 20 were in the OFF-layer branch, contributing 11.30% of the ribbon synapses in the entire cell. There's a total of 71 BCS in CBb 430 and 13 are found in the OFF-layer branch, constituting 18.31% being found in the OFF-layer branch. This data indicates

a higher relative contribution of the excitatory synapses originating in the OFF-layer branch than was observed in CBb 593 from RC1 which can be seen in Table1.

Ultimately our goal is to understand how these OFF-layer branches contribute to the larger retinal network, and what may be altered by retinal degeneration. Therefore, we utilized a network diagram that visualized a 2-hop network (Figure 6). A 2-hop network allows us to not just look at the immediate post-synaptic partners of the CBb OFF-layer branch of interest, but also the cells that are subsequent partners downstream in the network. In the healthy retina (RC1), we see 593's OFF-layer branch is pre-synaptic to GABAergic amacrine cell 20537 and this cell also has input from other ON BCs 16026 and 5536. In the pathological retina (RPC1), we see CBb 430 is pre-synaptic to GABAergic amacrine cell 32385 and this cell also has input from another ON BC 617, which is consistent with the same network seen in RC1 along with the amacrine cell synaptic network. In RPC1 we did however find presumptive ganglion cells post-synaptic partners. Out of these partners, GCs 32695, 29192, and 29201 also show inputs from other CBbs indicating the connections from 430 are still with the correct type of cells, though in an odd lamination. GCs 32230 and 32971 are potentially more complicated because at this stage of evaluation demonstrate only Cba (OFF-bipolar) type inputs. This calls into question whether these GCs are potentially ON-OFF ganglion cells or if the OFF-layer branch from CBb 430 is now making synapses inconsistent with other CBb neurons. Further evaluation of these volumes will be required to find the answer.

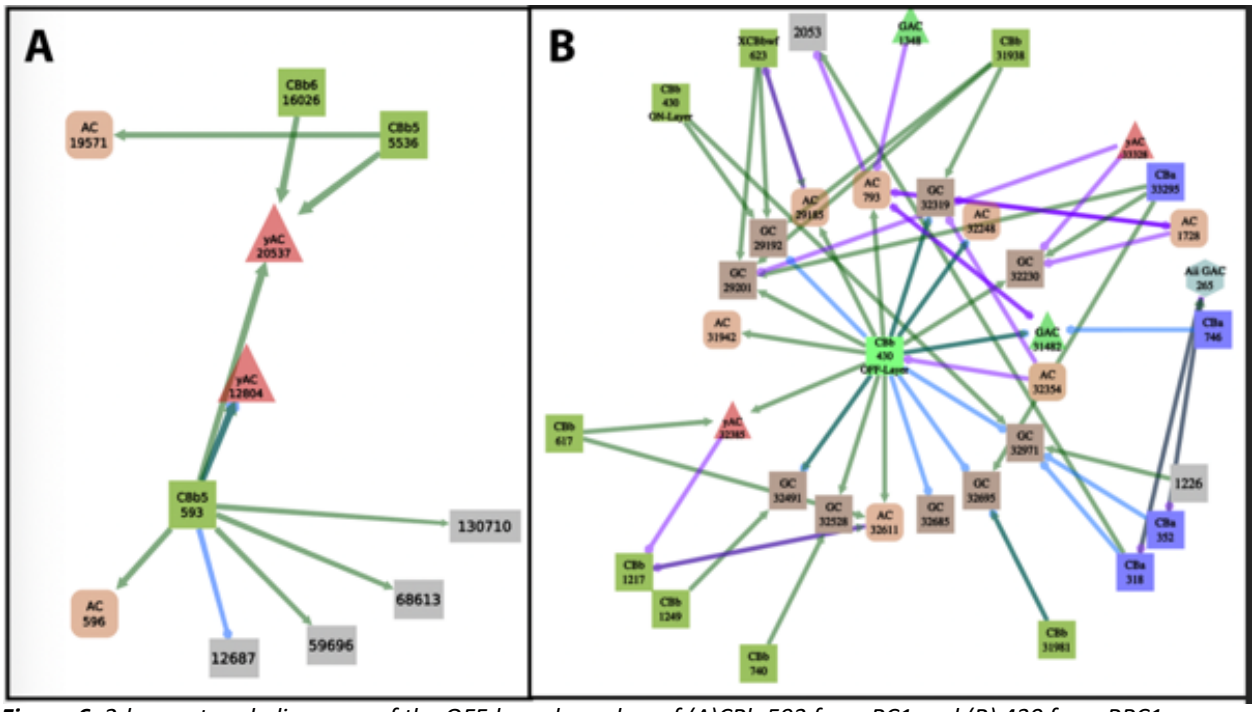


***Figure 6**: 2-hop network diagrams of the OFF-layer branches of (A)CBb 593 from RC1 and (B) 430 from RPC1.*

**Discussion:**

Morphologically we see there are differences in the ON-Cone Bipolar Cells OFF-layer branch between CBb 593 in healthy retina compared to CBb 430 in diseased retina. Branch 430 is more complex and has a longer sprouting branch compared to 593. Additionally, this complexity is found in conjunction with cell 430 having more synapses and subsequently, synaptic partners which leads us to further questions. Are these OFF-layer branches just an out of place ON bipolar cell branch? Namely, do they behave exactly the same as the rest of the ON cone bipolar cells but are simply an aberrant branch in an incorrect layer? Or are they behaving with OFF bipolar cell like phenotypes? Is it actually crossing into that OFF circuitry? In RPC1 we are actually seeing this crossover between ON and OFF through this OFF branch but we have to do more exploration to understand whether or not the ganglion cells being synapsed with normally collect input from both ON and OFF channels, or this is truly an ON cone BC making connections consistent with that of an OFF cone BC.

GC 32230 is post synaptic to ON-BC 430 and OFF – BC 33295 which indicates possible change in cell identity or a previously undescribed crossover motif. What this could mean as far as how degeneration is affecting networks and what potential corruption is happening in this network is still an unanswered question. Further investigation on synaptic partners could lead us to better understand how this OFF-layer branch effects the overall network in the retina, and how degeneration effects the role of this OFF-layer branch. Combined, these data will allow us to have a more complete view of what the impact of early photoreceptor degeneration is on the downstream networks contained within the retina.

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