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Structural motifs of excitatory synapses in the mammalian retina

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Background

Connectivity within the nervous system is precise and disruptions lead to degraded performance and disease, yet the rules that govern connectivity remain unknown. Recent efforts reveal that different types of cone bipolar cells in the neural retina show preferences in the selection and frequency of presynaptic structure types used for signal transmission (Yu *et al.,* 2023). However, it is not yet known how these differences are related to the quantity or type of postsynaptic partner in rabbit.

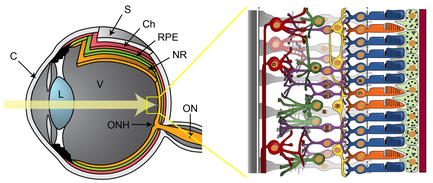

Figure 1: Anatomical view of the eye and layers of the retina. Rod (R) and cone (C) photoreceptors, located in the outer retina, are the light sensing cells. Ganglion cells (G), located in the inner retina, send visual information through the optic nerve to various parts of the brain for both image and non-image functions. Cone bipolar (CB) and rod bipolar (RB) cells reside in between these two cell types and transmit information from their respective photoreceptors to G cells. Horizontal (H) and amacrine (A) cells modulate this signal transfer in the outer and inner synaptic layers, respectively. The inner synaptic layer where CB and RB cells synapse with A and G cells was the region of focus in this study.

Methods

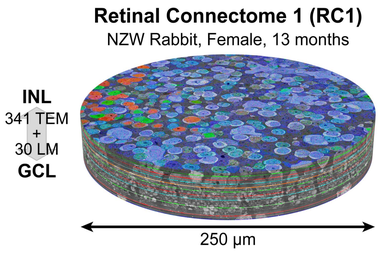

Figure 2: Creating a connectome. Retinal Connectome 1 (RC1) contains all synaptic connections of the inner synaptic layer of healthy adult rabbit retina. The dataset was constructed from transmission electron micrograph (TEM) images of serially sectioned mid-peripheral retinal tissue captured at 2 nm/px. Together with light microscopy images of sections stained and imaged for small molecules, sections are mosaicked and alignment into a volume. Annotation performed using the Viking Viewer for Connectomics to mark morphology and synapses.

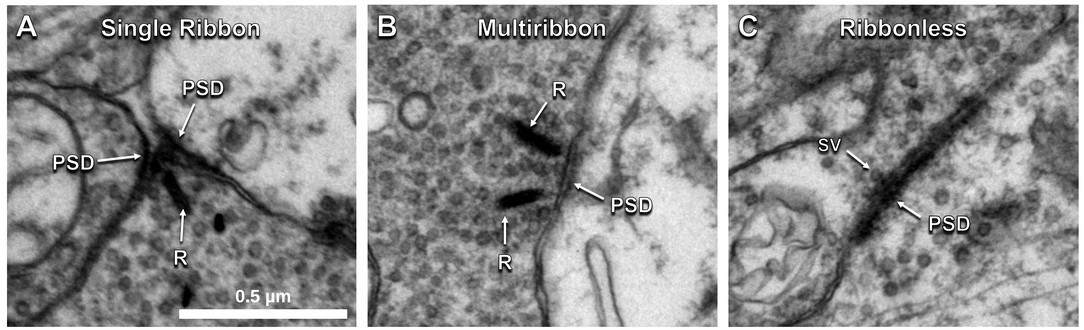

Figure 3: **Rabbit ON cone bipolar CBb6 cells use 3 excitatory presynaptic structures.** A-C)Transmission electron micrographs of single ribbon (A), multiribbon (B), and ribbonless (C) synapses formed by CBb6 ON cone bipolar cells. Single ribbons have 1 ribbon, multiribbons have >1 ribbon, and ribbonless structures lack a ribbon but have ≥2 synaptic vesicles tethered to the presynaptic membrane. Abbr: R, ribbon; PSD, postsynaptic density; SV, synaptic vesicle.

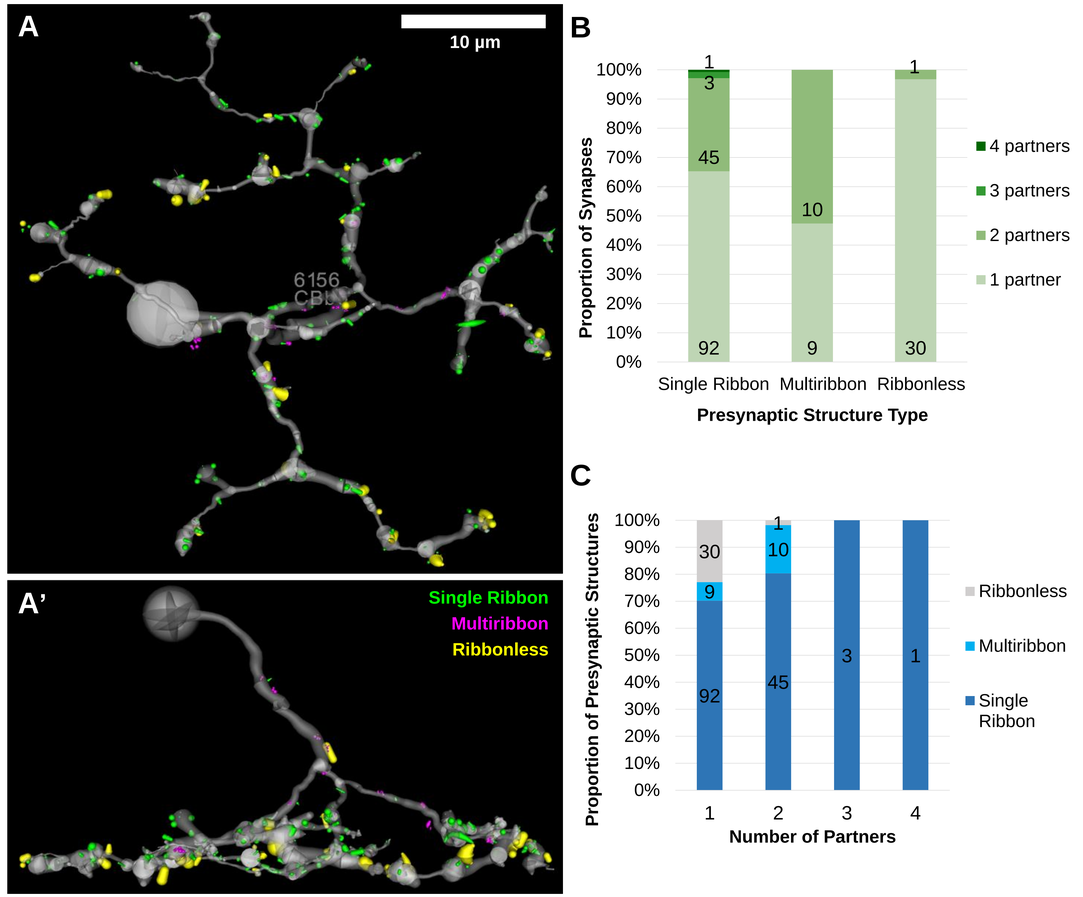

Figure 4: Distribution of excitatory presynaptic structures on CBb6 cell 6156. A) Top down (XY) view of the 3D rendered axonal arbor of CBb6 cell 6156 (gray) and presynaptic structures of its excitatory output synapses: single ribbon (green), multiribbon (magenta), and ribbonless (yellow). Structures scaled by a factor of 2 for visualization. A') Side (XZ) view. B) The proportion of synapses of each presynaptic structure type with 1, 2, 3, or 4 postsynaptic partners. Multiribbon synapses of 6156 trended towards having a greater number of output partners, with a greater proportion of 2 partner outputs (dyads) than 1 partner outputs (monads). C) 3 partner and 4 partner outputs were only found opposing single ribbon presynapses.

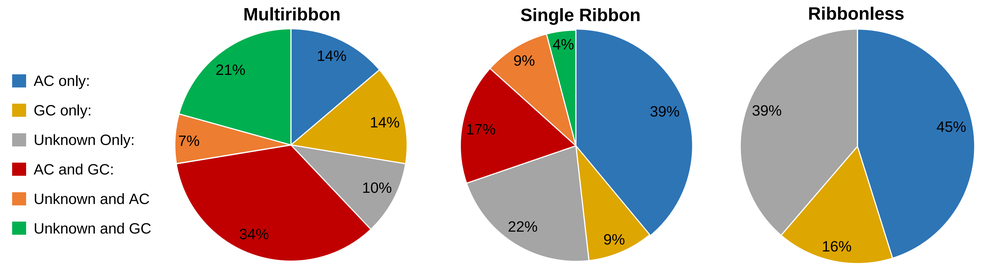

Figure 5: Partner classifications by presynaptic structure type. Both amacrine cells (AC) and ganglion cells (GC) are partners of CBb6 6156. However, single ribbon and ribbonless structures appear biased towards AC only targets at individual synapses, while multiribbon synapses appear biased towards having mixed AC and GC targets at individual synapses. Since ribbonless synapses did not form any dyads in this cell, they have significantly fewer categories.

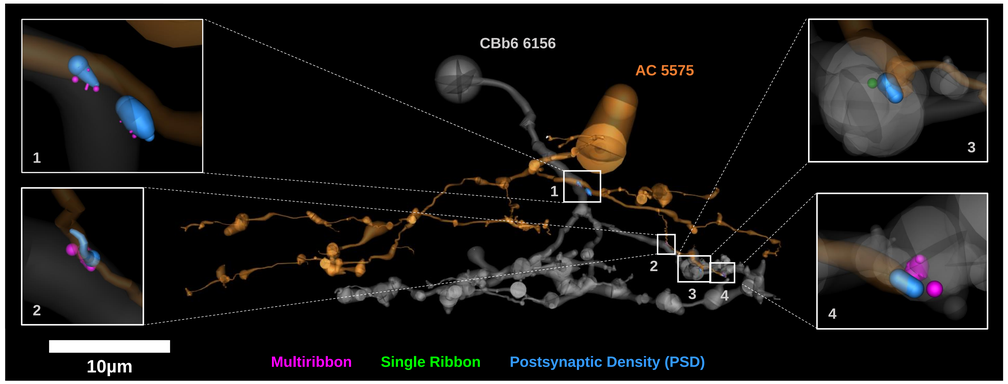

Figure 6: Partner-specific presynaptic motifs. 3D rendering of CBb6 6156 (grey) and postsynaptic partner glycinergic AC 5575 (orange) and their associated synaptic structures: single ribbons (green), multiribbons (magenta), and postsynaptic densities (blue). The fact that four of the five synapses in this partnership were multiribbons supports the idea that postsynaptic partner type plays an important role in presynaptic structure selection with a high level of specificity. Synaptic regions are boxed in white and enlarged for visualization.

Conclusions & Future Directions

It is hypothesized that presynaptic structure types may differ in the strength of neurotransmitter release (ribbonless < single ribbon < multiribbon), but the findings here are inconsistent with such scaling of output to the number of postsynaptic targets. The data suggests that target type relationships may be more important than the number of targets in determining presynaptic structure type in CBb6 cells. Future efforts will incorporate size differences of postsynaptic structures and presynaptic ribbon size, as well as compare across bipolar cell classes, in order to provide further insight on the connectivity rules underlying excitatory synapses in retina.

Related Works:

- "Species-specific connectivity in the Aii connectome" - Crystal Sigulinsky, Minisymposium: Advances in retinal connectomics: Tues, April 25, 8:45 am
- "Müller cell connectomics in health and disease" - Rebecca Pfeiffer, Minisymposium: Advances in retinal connectomics: Tues, April 25, 9:05 am
- "Mitochondrial transfer between inner retinal neurons" - Selena Wirthlin, Poster 3885 – B0165, Wed, April 26, 10:30 am - 12:30 pm, 4:15 - 5:15 pm

Works Cited:
Yu WQ, Swanstrom R, Sigulinsky CL, Ahlquist RM, Knecht S, Jones BW, Berson DM, Wong RO. 2023. Distinctive synaptic structural motifs link excitatory retinal interneurons to diverse postsynaptic partner types. *Cell Reports*. 2023 Jan 31;42(1):112006. PMID: 36680773; PMCID: PMC9946794.

Support & Disclosures

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