Connectomics Analysis of Rod-Cone Interaction Networks

JS Lauritzen, JR Anderson, CB Watt, BW Jones, RE Marc • Moran Vision Institute, University of Utah, Salt Lake City, UT[1]

Grants NIH EY02576 (RM), EY015128 (RM), EY014800 Vision Core (RM), Research to Prevent Blindness (RM), Research to Prevent Blindness CDA (BWJ)

Purpose: Transitions between scotopic and photopic switch seem smooth, but psychophysical dissection reveals that it is underpinned by mutual rod-cone suppression processes (1,2). The neural architecture supporting these processes has resisted discovery.

Methods: Multiple amacrine cell (AC) networks connecting 70 rod bipolar cells (RBCs) and > 100 cone bipolar cells (CBCs), 30 A_{II} ACs (A_{II}) and 20 A_I ACs (A_I) were traced in the ultrastructural rabbit retinal connectome RC1, annotated with the Viking viewer, and explored by 3D rendering and graph visualization of connectivity (Anderson et al. 2011. The Viking Viewer. J Microscopy). RC1 contains embedded small molecule signals, enabling complete cellular classification independent of network identity. We use the MacNeil et al., 2004 bipolar cell classification scheme as follows: ON Cone BC, CBb [3-6]; OFF Cone BC, CBa[1-2].

Results: Multiple GABAergic AC (γAC) pathways connect rod and cone BCs. (1) Certain wide-field γACs are reciprocal feedback elements at every CBb they encounter, but also collect RBC input enabling rod suppression of cone signals. Every rod BC receives inhibitory input from γACs driven directly by CBbs. (2) Instances of ON glycinergic AC (GAC) > rod BC inhibition also exist. (3) Ribbon input signals from rod BCs to A_{II}s are differentially distributed to patches of coupled CBbs and CBas, which drive wide-field γACs responsible for within channel (ON & OFF) inhibitory motifs, consistent with rod signal suppression of cone signals while maintaining ON-OFF antagonism.

Conclusions: The mammalian retina appears to use ACs to create a winner-take all architecture for rod and cone bipolar cells. When cone responsivity exceeds rods, multiple inhibitory networks further suppress the rod pathway output, and vice versa. At least four synaptic chains support this process:

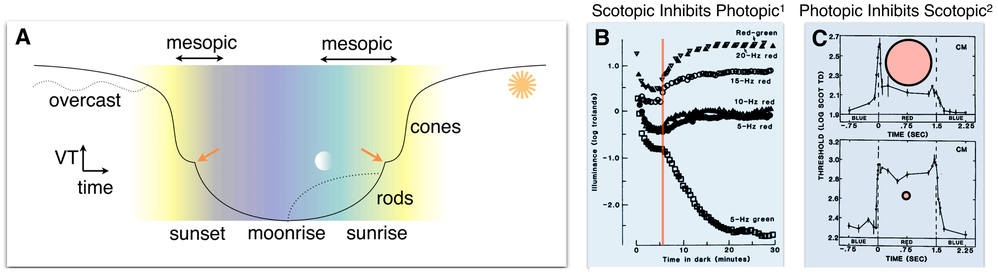
CBb > γAC > RBC
CBb > GAC > RBC
RBC > A_{II} > Coupled CBb > γAC > Coupled CBb
RBC > A_{II} > Coupled CBa > γAC > Coupled CBa

Commercial Relationship:JS Lauritzen, None; CB Watt, None; BW Jones, None; RE Marc, Signature Immunologics.

References.

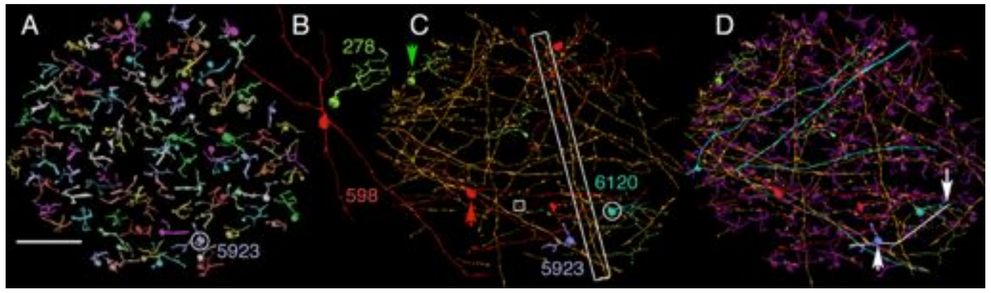
1. Buck, Stefurak, Moss & Regal.1984 Vision Res 24: 543
2. Goldberg, Frumkes, Nygaard. 1983 *Science* 221:180.
3. Copenhagen, Hemila, Reuter. 1990 *J Gen Physiol* 95, 717.
4. Yang, Wu. 2004 Brain Res 1029, 155.
5. Wu. 1991 *J Neurophysiol* 65, 1197.
6. Maltenfort, Heckman, Rymer. 1998 *J Neurophysiol* 80, 309.

Figure 1. Mesopic Vision & Rod-Cone Suppression



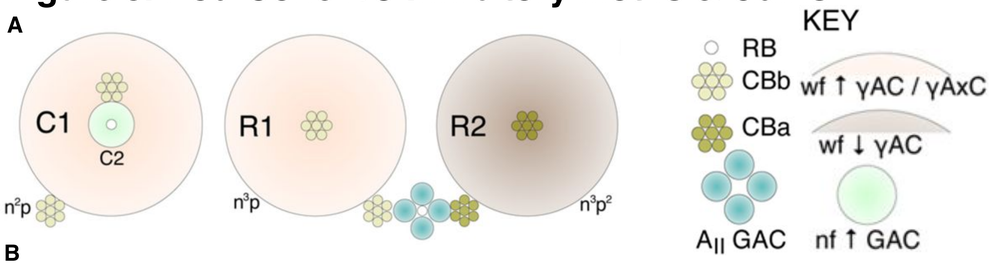
A. Visual thresholds (VT) rise and fall across daily lighting conditions, creating many hours of mesopic conditions. Note the "rod-cone break point" (arrows) **B.** As the rod system takes control, cone-mediated VT rises (red line). **C.** Exposure to disks of red light during scotopic conditions increases scotopic VT.

Figure 2. Prevalent Rod-Cone BC Suppression Networks



A. Renderings of all rod BC axon terminals in volume RC1. Rod BC 5923 is circled. Each different color is a single rod BC terminal. **B.** Wide-field γAC 598 (red) and narrow-field GAC 278 (green) engaged in motif types C1 and C2 respectively. **C.** A field of γAC (orange) and GAC (green) processes, γAC 598 (red, up arrow) and GAC 278 (green, down arrow) that provide cross-channel inhibition to every rod BC encountered. ON cone CBb5 6120 is circled. The smallest and largest inhibitory distances mediated by γACs are shown in the square and rectangle, respectively. **D.** The inhibitory field of processes superimposed on the rod BC field (magenta). Motif C1 γAC process 32477 (white process, arrows) spans rod BC 5923 and CBb5 6120. Scale 0.1 mm for A,C,D and 69 µm for B.

Figure 3. Rod-Cone BC Inhibitory Motifs & Gains

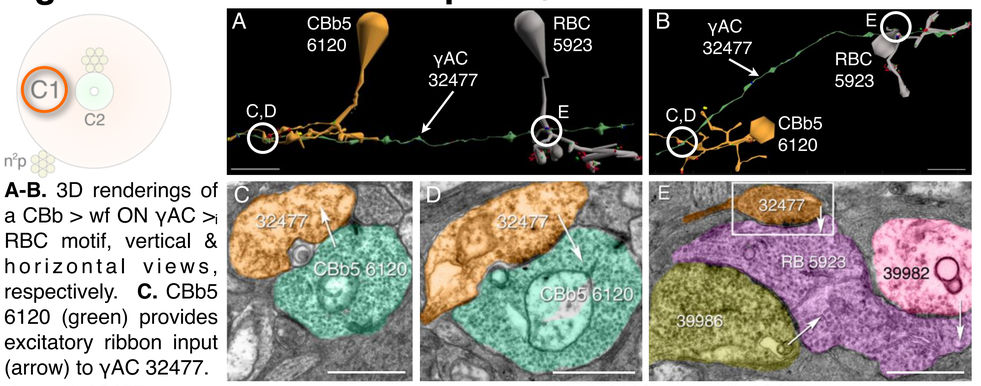


B

Motif	Gain	Chain
C1	n^2p	C >m CBb > wf ON γAC >i RBC
C2	n^2p	C >m CBb > nf ON GAC >i RBC
R1	n^3pc	R >m RBC > A_{II} :: CBb > wf ON γAC >i CBb
R2	n^3p^2	R >m RBC > A_{II} >i CBa > wf OFF γAC >i CBa

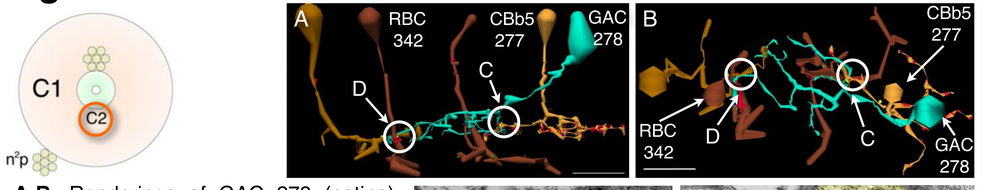
A. Graphical representation of four rod-cone inhibitory motifs. **B.** Gains and synaptic chains associated with each motif. C cone, R rod, CBb ON cone BC, CBa OFF cone BC, RBC ON rod BC, γAC GABAergic AC, GAC glycinergic AC, wf wide-field, nf narrow field, > sign-conserving high gain (3,4) ionotropic glutamate synapses with gain n, >m sign-inverting high gain ionotropic glutamate synapses with gain n, >i sign-inverting low gain (5,6) iono-tropic GABAergic or glycinergic synapses with gain p, ::, sign-conserving gap junction coupling with gain c.

Figure 4. C1 Motif: CBb > γAC >i RBC



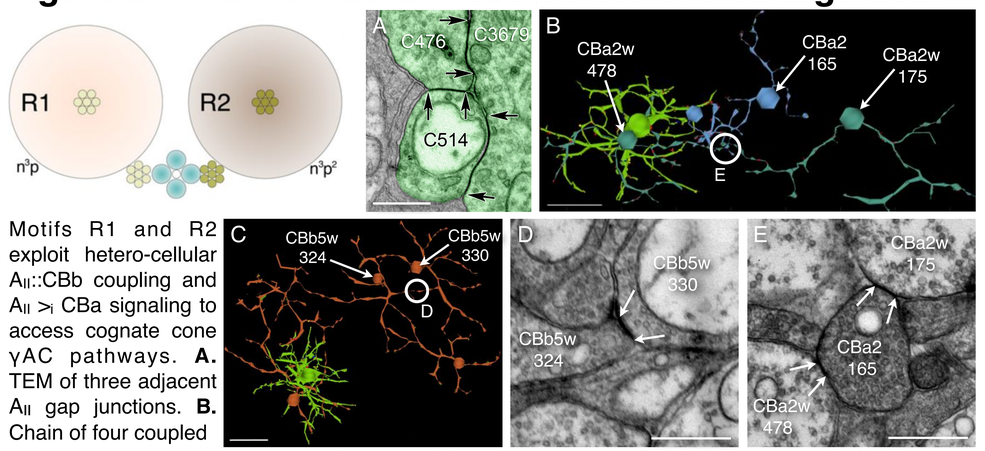
A-B. 3D renderings of a CBb > wf ON γAC >i RBC motif, vertical & horizontal views, respectively. **C.** CBb5 6120 (green) provides excitatory ribbon input (arrow) to γAC 32477. **D.** γAC 32477 provides reciprocal inhibition (arrow) to CBb5 6120. **E.** γAC 32477 (orange) is pre-synaptic (arrow) to rod BC 5293 (blue) which receives input from another motif C1 cell (γAC 39982 pink) and a classical A_I AC (γAC 39986). Scale 10 µm for A-B, 1.0 µm for E.

Figure 5. C2 Motif: CBb > GAC >i RBC



A-B. Renderings of GAC 278 (patina) forming a C2 motif. **C.** CBb5 277 forms a ribbon synapse onto GAC 278. **D.** RBC 342 receives convergent conventional synapses from from GAC 278 and γAC 39998 (γAC not shown in renderings for clarity), in the same plane of section as it drives A_I AC 31700. Scale 10 µm for A-B, 1.0 µm for C-D.

Figure 6. R1 & R2 Motifs Preserve ON-OFF Antagonism



Motifs R1 and R2 exploit hetero-cellular A_{II}::CBb coupling and A_{II} >i CBa signaling to access cognate cone γAC pathways. **A.** TEM of three adjacent A_{II} gap junctions. **B.** Chain of four coupled CBas and a chemically synaptic A_{II} involved in an R2 motif. **C.** Chain of five coupled CBbs and a coupled A_{II} involved an R1 motif. **D.** Exemplary gap junction between CBbs. **E.** Exemplary gap junction between CBas. Arrows delineate gap junctions. Scale 0.5 µm for A, 20 µm for B-C, 0.5 µm for D-E.