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Sparse network principles of GABAergic amacrine cell heterocellular coupling

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Purpose: Heterocellular coupling between specific classes of ganglion cells (GCs) and GABA (γ) containing amacrine cells (γACs) in the mammalian retina is well established, but the actual gap junctions supporting these connections have never been visualized. Given the abundance of γ+ GCs in the mammalian retina, we sought to comprehensively map the spatial distributions and sizes of gap junctions associated with γACs and their presynaptic bipolar cell (BC) inputs or postsynaptic BC, AC and GC targets.

Methods: γAC networks in the ultrastructural rabbit retinal connectome RC1 were annotated with the Viking viewer, and explored by 3D rendering and graph visualization of connectivity (Anderson et al., 2011 J Microscopy). Small molecule signals embedded in RC1 combined with morphological reconstruction and connectivity analysis allow robust cell classification. Gap junctions were validated by TEM re-imaging with tilt at 0.3 nm resolution.

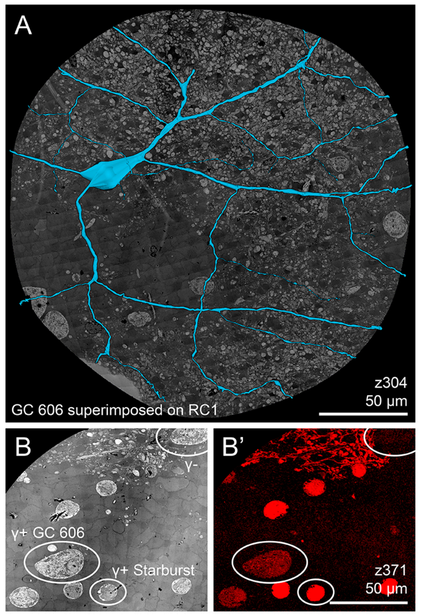


Figure 1. GC 606 is a candidate coupling partner for γACs.
(A) GC 606 is a candidate ON alpha ganglion cell. GC 606 has a large 30 µm diameter soma and a dendritic arbor spanning beyond the 0.25 mm diameter of RC1. (B,B') GC 606 exhibits a strong γ signal.

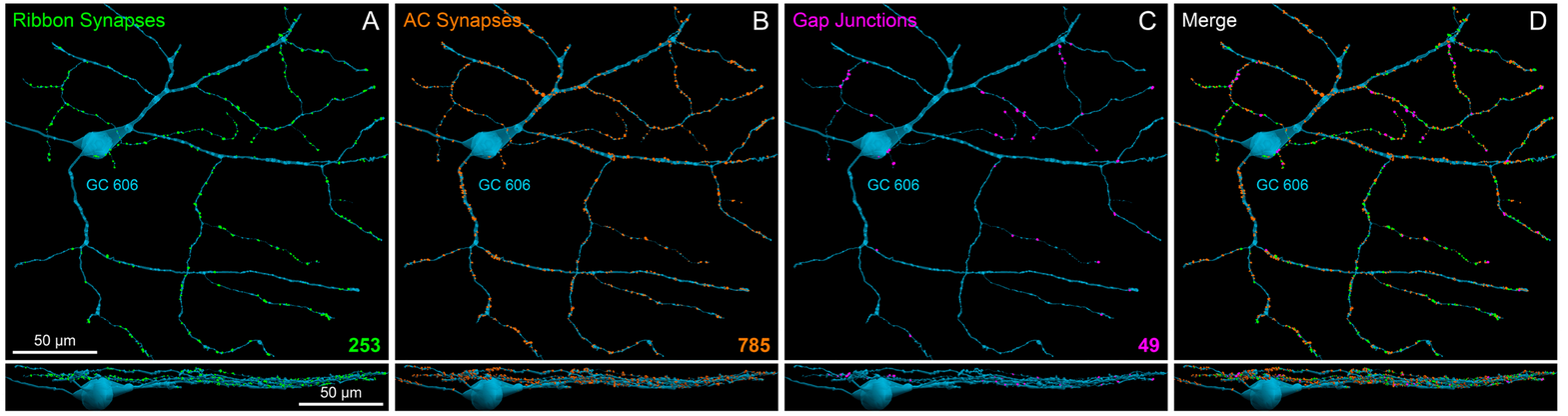


Figure 2. GC 606 participates in sparse gap junctional coupling.
(A) GC 606 is driven by at least 52 ON cone (CBb) BCs, each contributing 1-20 ribbon synapses (green dots). Multi-ribbon synapses from CBbs onto GC 606 are rare, but both di- and tri-ribbon synapses are present. (B) GC 606 also receives over 700 AC synapses (orange dots), most from γACs. (C) Within the RC1 volume, GC 606 makes 49 identified gap junctions (pink dots). These gap junctions range in size from 60-360 µm and are widely dispersed along the major GC dendrites. (D) Spatial relationships of all identified chemical and electrical synapses mapped onto GC 606. For ease of visibility, all synapses are plotted and scaled by a factor of 3, with ribbon synapses and gap junctions size normalized.

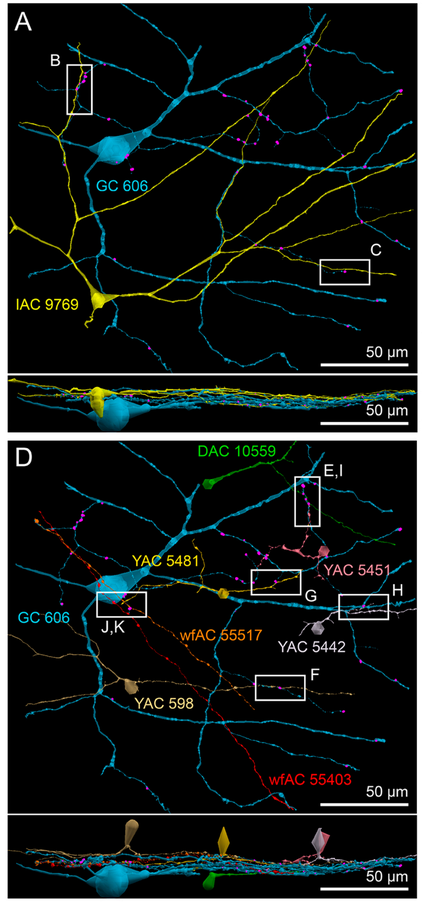
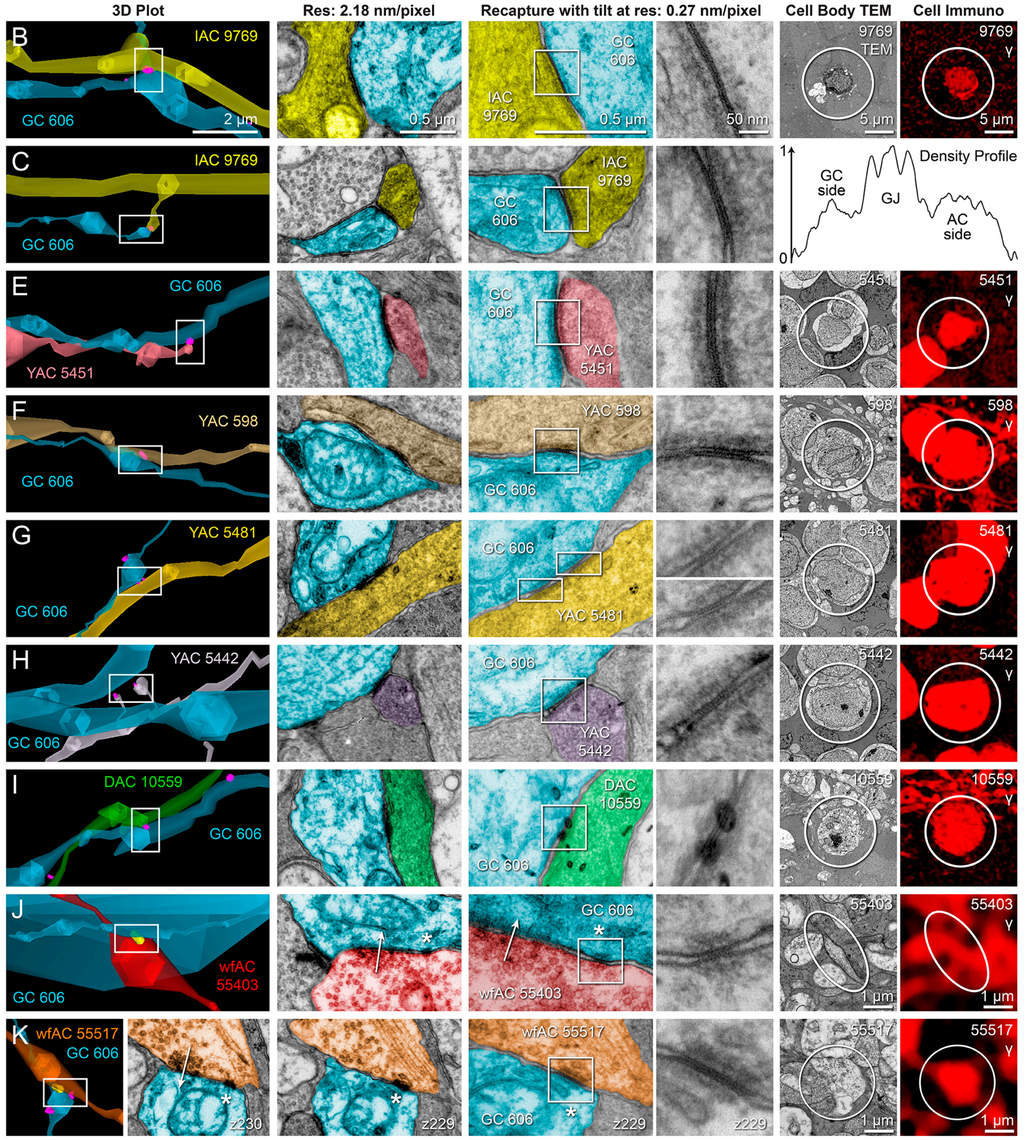


Figure 3. Infrequent, yet routine gap junctions exist between multiple classes of γACs and GC 606.
(A-C) Of the 49 identified gap junctions for GC 606 in RC1, 11 are with interstitial γAC (IAC) 9769. Gap junctions with IAC 9769 occur in situations of tangency (B) and through small extensions from IAC 9769 (C). (D-K) GC 606 also possesses gap junctions with several additional classes of γACs, including wide field γACs (wfAC) and a displaced γAC (DAC). Two wfACs (J-K) make mixed synapses (both chemical and electrical within the same varicosity). Gap junctions (pink dots) enlarged for visualization for A,D; shown with true scale in B-K. γ signature of ACs confirmed by immunolabeling on adjacent sections.



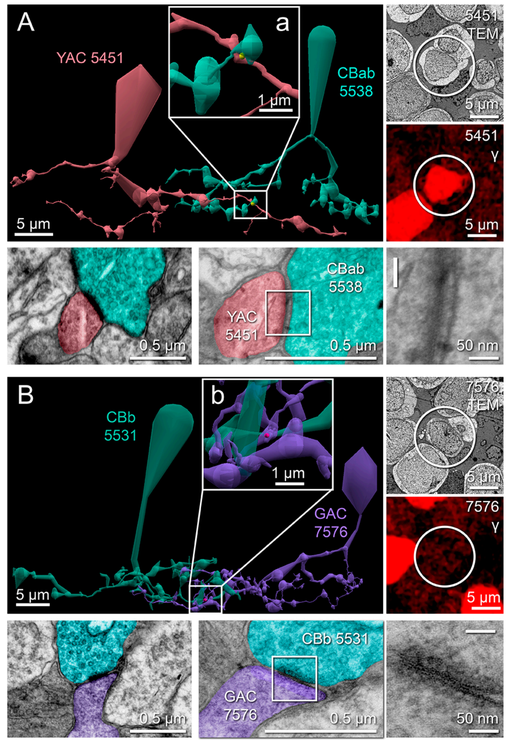


Figure 4. Sparse heterocellular coupling events between non-AII ACs and BCs.
(A) Candidate heterocellular gap junction (yellow dot) between a γAC and a CB cell identified as a bipolar cell conventional synapse (BCS) upon recapture at 0.27 nm resolution and goniometric tilt. Note synaptic cleft and absence of cytoplasmic density on the CB cell side. (B) Confirmed CB cell gap junction (pink dot) involves a GAC (non-AII) AC. Gap junction and BCS enlarged for visualization for A,B; shown with true scale in a,b. γ signature of ACs confirmed by immunolabeling on adjacent sections.

Conclusions: Coupling instances (::) in heterocellular γAC::GC and networks are sparse. We calculate that a single gap junction serves an equivalent GC receptive field area of ≈ 1000 µm^2: a patch ≈ 30 µm in diameter, at least 2 times larger than the area served by a single ribbon. GC 606 is large and one of the most strongly coupled cells based on γ signal strength, so GCs with weaker γ signals or smaller fields may display markedly fewer gap junctions. Assuming these gap junctions are electrophysiologically active, there may not be a 1:1 correlation between γACs (or GACs) and their coupling targets, as a single gap junction appears to control two highly coupled CBb cells.

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Poster available online:
http://webvision.med.utah.edu/2013/05/sparse-network-principles-of-gabaergic-amacrine-cell-heterocellular-coupling/