Abstract 833 B806

Excitatory Self-signaling In Retinal Remodeling

RE Marc, BW Jones • Moran Eye Center • University of Utah

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RH Lucas • School of Biological Sciences • University of Manchester

Purpose: Our goal was to assess the excitatory behavior of remodeling neuronal arrays in retinal degenerations, after loss of all photoreceptor cells.

Methods: Glutamate-gated channel activation was visualized as *in vivo* and *in vitro* AGB permeation and combined with computational molecular phenotyping (CMP, Marc and Jones 2002 J Neurosci 22: 413) in rodent models of inherited retinal degenerations (transgenic *rdcl* and natural *rd* mice, > PND 60-100) after complete loss of photoreceptors. Datasets were visualized as *rgb* and theme maps of classified cell types, thus reporting the excitation states of identified neuronal populations.

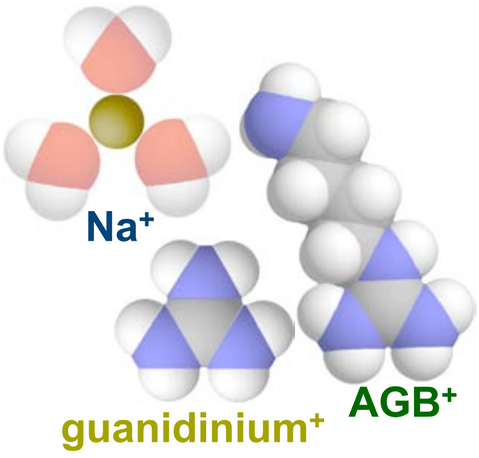
Results: The mid-stage remodeling mouse retina is extremely active in the absence of photoreceptors, indicating that light-independent events generate endogenous glutamatergic signaling. There are remarkable deviations from the normal retina: Rod bipolar cells (BCs) are inactive, as are all rod pathway neurons. Since rod BCs in the *rdcl* mouse lack a glutamate signal, cation channels gated by the mGluR6 pathway should be open. They are not, consistent with loss of the channel activation path (Strettoi *et al*. 2003 and Varela *et al*. 2003 Vision Res). Some OFF cone BCs are moderately active, implying re-entrant signaling. Some ON cone BCs are extremely active, as are their target amacrine (AC) and ganglion (GC) cells. The mechanism of high ON cone BC activity is unknown. In early remodeling, most cone-pathway ACs and GCs are active, without any BC activity, implying a cryptic source of glutamate receptor activation. We have new evidence that dopaminergic ACs have a glutamatergic rather than a GABAergic signature, and they may provide an excitatory drive. Advanced remodeling also triggers migration of neurons. Migrating GCs are quiescent and suppression of glutamate receptor expression may be a prerequisite or consequence of migration.

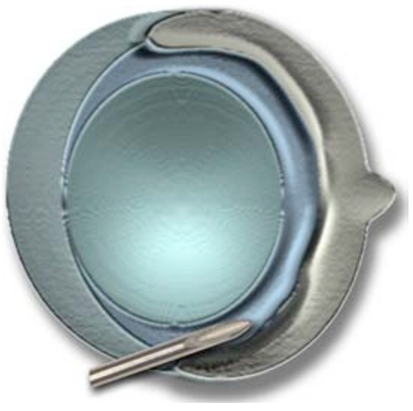
Conclusions: Light-independent glutamate-drive persists in the retinas of rodents after total photoreceptor loss. Initially limited to ACs and GCs, the source of endogenous glutamatergic-drive is unknown but may arise from dopaminergic / glutamatergic ACs. Remodeling recruits some BCs to rejoin excitatory networks, probably as re-entrant loops. Advanced remodeling events such as migration seems to induce loss of glutamate receptor expression.

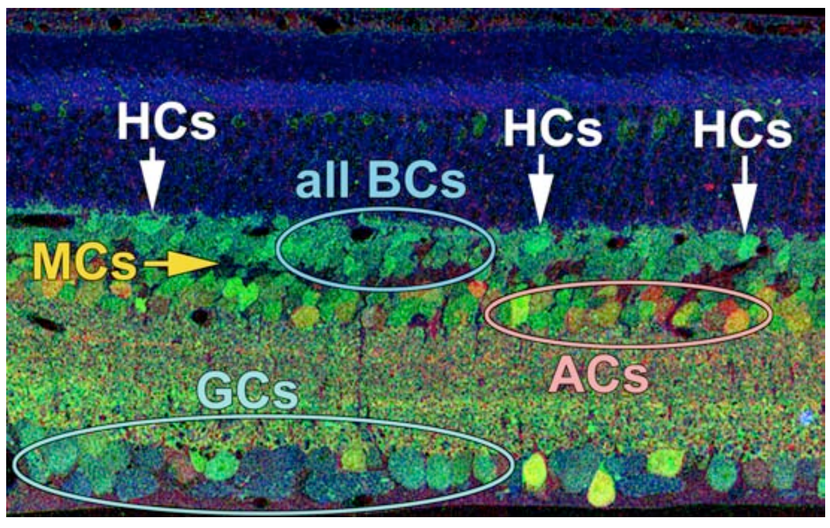
Commercial Relationship: Jones, Lucas,- none; Marc - Signature Immunologics, F,E

A. Retinal excitation mapping *in vivo*

The physiological behavior of neurons in retinas devoid of photoreceptors due to inherited degenerations is *terra incognita* since no easily controlled stimulus can be applied, especially in view the emergence of a confluent distal glial seal. Electrophysiology is a poor tool because of its weak sampling and short epochs. Excitation mapping with the glutamate-gated channel permeant probe AGB (1-amino-4-guanidobutane) enables concurrent sampling of the integrated excitation histories of all retinal neurons *in vitro* (Marc JCN 1999, Marc and Jones J Neurosci 2002). It also possible to map excitation *in vivo* by intravitreal injections yielding 5mM intraocular AGB, followed by a 60 min response epoch and harvest for CMP analysis. All neurons can be visualized and analyzed for their intrinsic excitation histories.





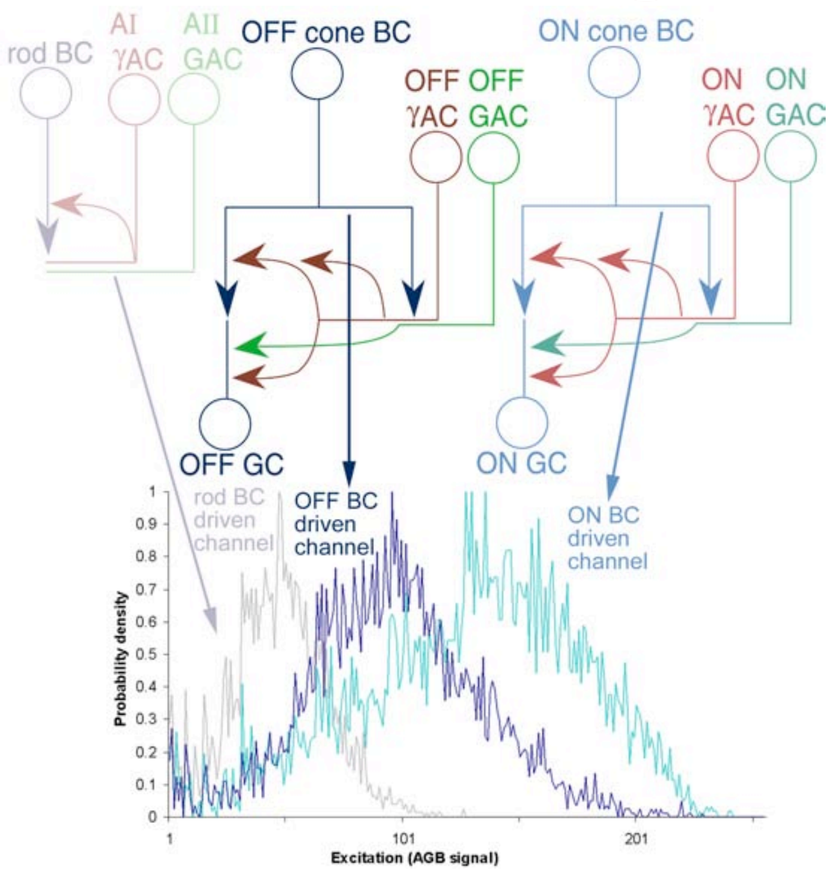


Na^+, guanidinium$^+$ and AGB^+ all permeate ligand-gated iGluR channels effectively.

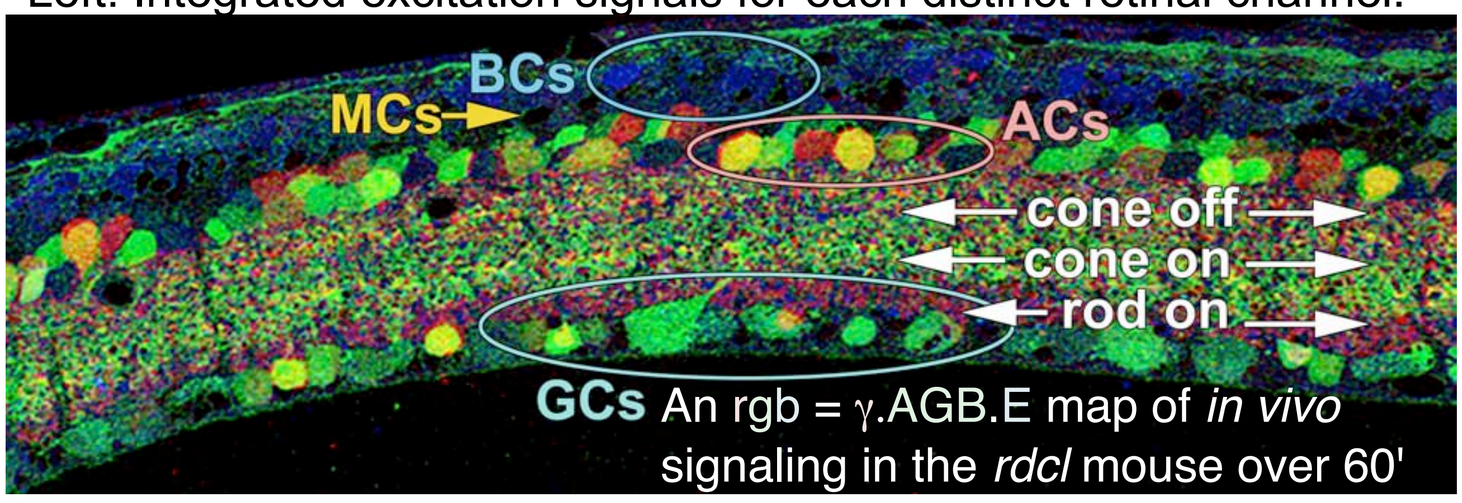
AGB^+ can be injected *in vivo* to yield vitreal levels of 5 mM, ≈ 3% of permeant cations.

An rgb = γ.AGB.E map of *in vivo* signaling in the normal *wt* mouse over a 60 min epoch. Virtually all BCs, HCs and ACs are active. GC signaling varies and MCs are quiet.

B. Self-signaling in the *rdcl* mouse retina

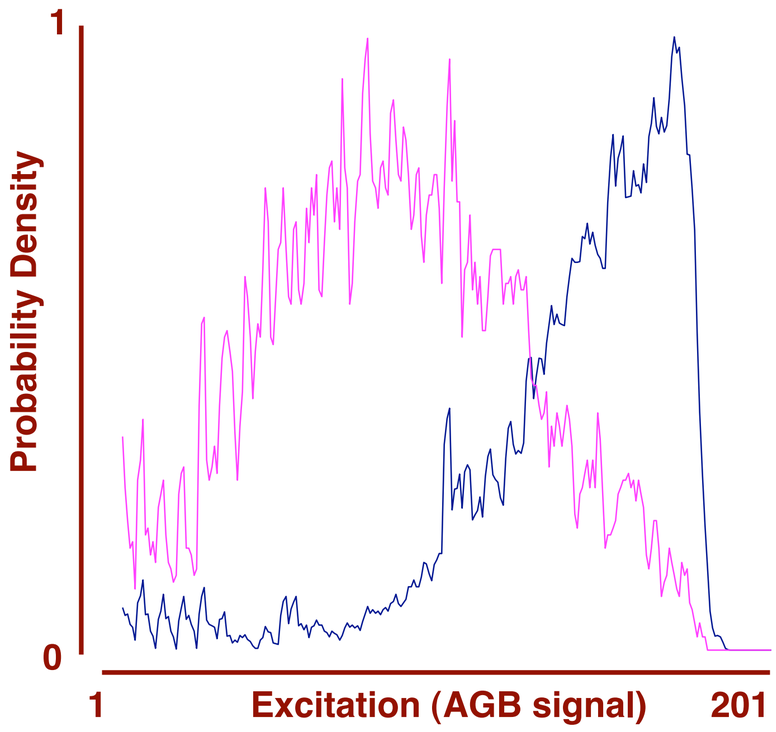


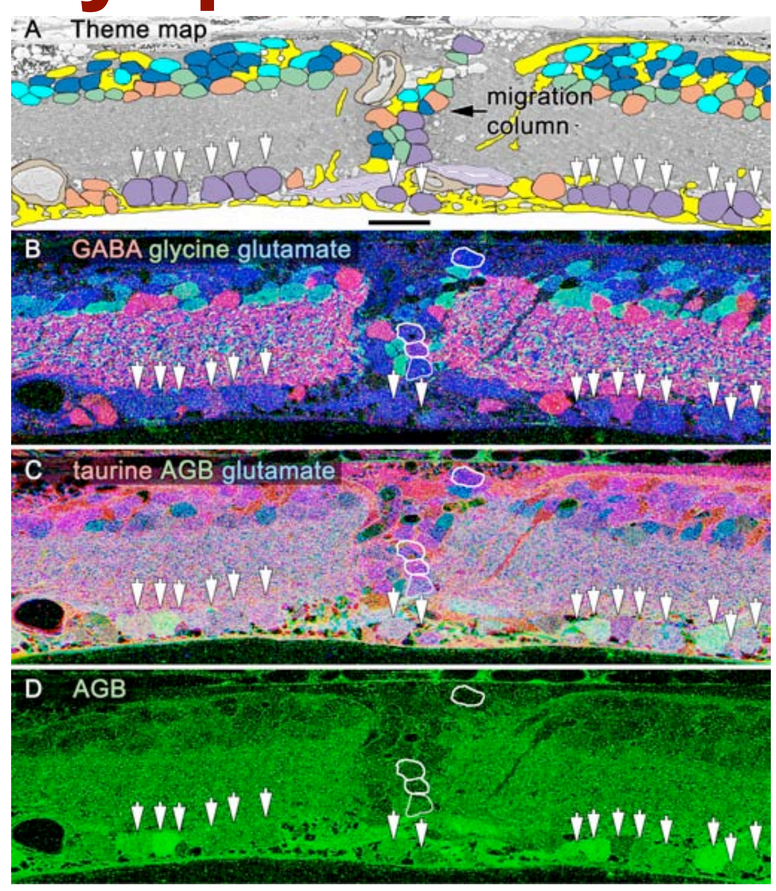
Despite complete loss of rods and cones in the *rdcl* mouse, the retina continues signal, **but only in cone pathways**. The rod pathway is quiescent, though the absence of rod glutamate input should depolarize the entire path. This is consistent with a shutdown of rod BC mGluR6 transduction. Most but not all BCs are quiescent, and the source of excitation for cone pathways remains an enigma. Left: Integrated excitation signals for each distinct retinal channel.



C. Migrating GCs are synaptically quiescent

Neuronal migration on glial columns to ectopic sites is a key defect in retinal degenerations. Ultrastructural data suggest that neurons retract many of their dendrites in remodeling. AGB mapping shows that **GCs in migration columns lack excitation signals.** Thus, glutamate signaling elements (pre- and/or postsynaptic) are absent during migration. The lack of IPL banding and weak AC signaling near columns implies neurite pruning and scrambling during remodeling.





D. Potential sources of endogenous excitation.

There are five possible sources of endogenous excitation in the remnant retina:

- re-entrant BC circuits: this occurs in advanced remodeling, but cannot account for early self-signaling
- cholinergic ACs: would have to invoke a glutamate source and be spontaneously active
- vGlut3 ACs (Haverkamp & Wässle): a possible glutamate source, but would have to be spontaneously active
- GC ↔ AC coupling: would have to invoke a glutamate source and be spontaneously active
- **Dopaminergic ACs:** These cells are potentially spontaneously active and have a glutamate signature (see Jones et al., ARVO 2004, Poster B759, Thurs AM).

We theorize that self-signaling emerges from pre-existing mechanisms in the inner retina; that self-signaling provides essential excitation for survivor neurons; that rewiring, migration and microneuromas (Watt et al., ARVO 2004, Poster B750, Sun AM) are devices that remnant neurons exploit to enhance survival.

A. An rgb = γ.G.E map of the mammalian AC layer.

B. The same map + tyrosine hydroxylase [TH] signals. TH+ ACs have a glutamatergic signature.