Neural Activity in the Inner Retina After Photocoagulation

Bryan William Jones1, Phil Huie2, Haimei Wang1, Alexander Sher3, Robert E. Marc1, Daniel Palanker2, Moran Eye Center, University of Utah, Salt Lake City, UT1, Stanford University2, University of California, Santa Cruz.2. Support: RPB CDA (BWJ), Thome Foundation (BWJ), BWF CASI (AS), NIH EY02576, EY015128, EY014800 Vision Core, NSF 0941717 (RM), Research Prevent Blindness (Moran Eye Center), Research to Prevent Blindness CDA (BWJ)

Purpose: Retinal photocoagulation is a common clinical intervention in many retinopathies. Though clinically effective, current laser therapies result in scotomas and scarring. We have demonstrated that during healing of small and light photocoagulation lesions, photoreceptors from adjacent areas migrate into the coagulated zone, restoring retinal continuity. This approach could allow for retinal laser therapy without the common detrimental size effect.

Methods: Laser exposures of "barely visible" and "moderate" grades were applied to rabbit retina (20μm, 200μm). 2 days and 2 months after photocoagulation the eyes were vitrectomized, retina was incubated for 30 minutes in vivo with 10mM 1-amino-4-guanidobutane (AGB) while exposed to flickering light, allowing AGB to permeate activated cation channels (GluR, GABA). The eyes were then embedded, plastic, sectioned and processed for computational molecular phenotyping (CMP).

Results: In the burns of moderate grade the two-month-old lesions were only partially filled with migrated photoreceptors, leaving scotomas. In the barely visible lesions, retinal pigment epithelium and photoreceptors were selectively ablated, but amacrine and metabolic signatures revealed robust bipolar, amacrine, horizontal and ganglion cell populations. These lesions filled in with photoreceptors after 2 months. Light evoked activity of the inner retina (horizontal, bipolar and amacrine cells) as measured through AGB probing, was reduced 2 days after photocoagulation but was restored to almost normal levels after 2 months.

Conclusions: Optimizing the laser spot size, radiant exposure and pulse duration to target photoreceptors, while preserving inner retina allows the adjacent photoreceptors to shift and rewire to the local inner neurons. This procedure, while achieving its therapeutic goal of reducing metabolic load through reduction in the number of photoreceptors, may help avoid scarring, vision loss and other associated side effects of current photocoagulation protocols. Additionally, targeted coagulation of photoreceptors may represent an adjustable and reversible model of retinal degeneration and neural plasticity.

Commercial Relationship: BW Jones, None; P Huie, None; H.Wang, None; A. Sher, None; RE Marc, Signature Immunodiagnostics; D. Palanker, Patent - Topcon Medical Laser Systems.

Barely Visible Clinical Grade Burn

AGB+ is injected in vivo to yield vitreal levels of 5 mM, ~3% of permeant cations. 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 becomes possible to map excitation in vivo by intrastrial injections/ vitrectomies yielding 5-10μM intraretinal AGB, focal 5-60 minute response epochs. Tissues are then harvested for CMP analysis (below and right).

Computational Molecular Phenotyping (CMP) of retinal lesions was performed after AGB perfusion via-vitrectomy in the rabbit eye. Combining AGB labeling with CMP reveals all neuronal excitation histories. AGB labeling in the 2-day post moderate burn permeates all neuronal and glial populations in the retina.

Analysis of moderate and light lesions 2 day, and 2 months post-burn are shown on above with - rgb and BE: rgb mapping employed, assigning GABA, AGB and L-glutamate and taurine, AGB, L-glutamate to red, green and blue color channels respectively.

The data reveal that laser burns at barely visible clinical grade (images above) result in complete ablation of retinal pigment epithelium and photoreceptors, yet with preservation of more inner retinal neurons than deeper or higher energy burns. Coherent excitatory signaling in the region of the burn appears to have been attenuated at 2 days post burn and Muller cells appear to have become activated, altering their taurine and glutamate metabolism. However, by 2 months in the barely visible clinical grade burn, Muller cells appear to normalize and photoreceptors appear to have migrated into the space vacated by the burn. Most importantly, vertical channel excitatory responses in bipolar, some amacrine and ganglion cells in the lesion also appear to be recovering, though at reduced levels compared to normal adjacent retina. "Moderate" clinical grade burns (data not shown) result in substantial impact to the retina ablating essentially all retinal cell populations and leaving a glial scar. At 2 days post moderate clinical burn, Muller glia are no longer performing osmoregulation as demonstrated by the taurine signal and all neuronal populations are assumed to be compromised or stressed, if not destroyed. GABAergic processes from amacrine cells appear to be present, but all amacrine cell bodies in the region of the burn have been ablated. The remaining GABA labeling is likely from GABAergic amacrine cells adjacent to the burn. By 2 months post moderate burn, retinal laser therapy could allow for retinal photocoagulation without the common detrimental size effect. AGB perfusion via-vitrectomy in the rabbit eye. Combining AGB labeling with CMP reveals all neuronal excitation histories. AGB labeling in the 2-day post moderate burn permeates all neuronal and glial populations in the retina.

Analysis of moderate and light lesions 2 day, and 2 months post-burn are shown on above with - rgb and BE: rgb mapping employed, assigning GABA, AGB and L-glutamate and taurine, AGB, L-glutamate to red, green and blue color channels respectively.