Neural Activity in the Inner Retina After Photocoagulation

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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 effects. Our goal was to assess the inner retina activity after photocoagulation with different levels of severity.

Methods: Laser exposures of "barely visible" and "moderate" grades were applied to rabbit retina (20ms, 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 (iGluR, mGlurR6). The eyes were then aldehyde fixed in-situ, embedded in 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 anatomic 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.

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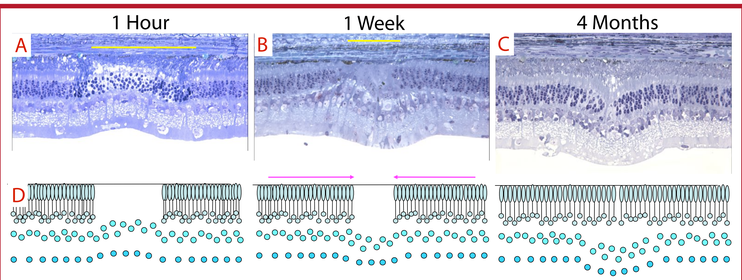
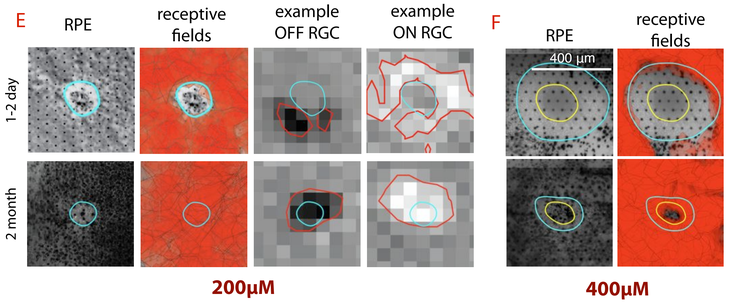
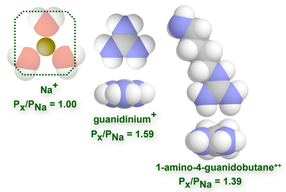


By standard toluidine blue stains, nuclei of photoreceptors in the photocoagulation site appear pyknotic at 1 hour A, and disappear within 2 days. Inner nuclear layer and ganglion cell layers appear intact but are deformed due to the changes in the photoreceptor and RPE layers. By one week B, Müller glia fill in the photoreceptor decimated region. The RPE layer appears restored, though hypo-pigmented in the center of the lesion and contracted to 50% of its original size. At 4 months C, photoreceptor organization in the center of the lesion continues to improve and is distinguished from adjacent normal retina only by a narrow column of Müller glia, and a slight elevation on the vitreal side of the retina. Photoreceptor morphology within lesions is otherwise indistinguishable from that in the untreated retina and appear to shift from the adjacent areas into the lesion, filling it over time, as schematically shown in D.



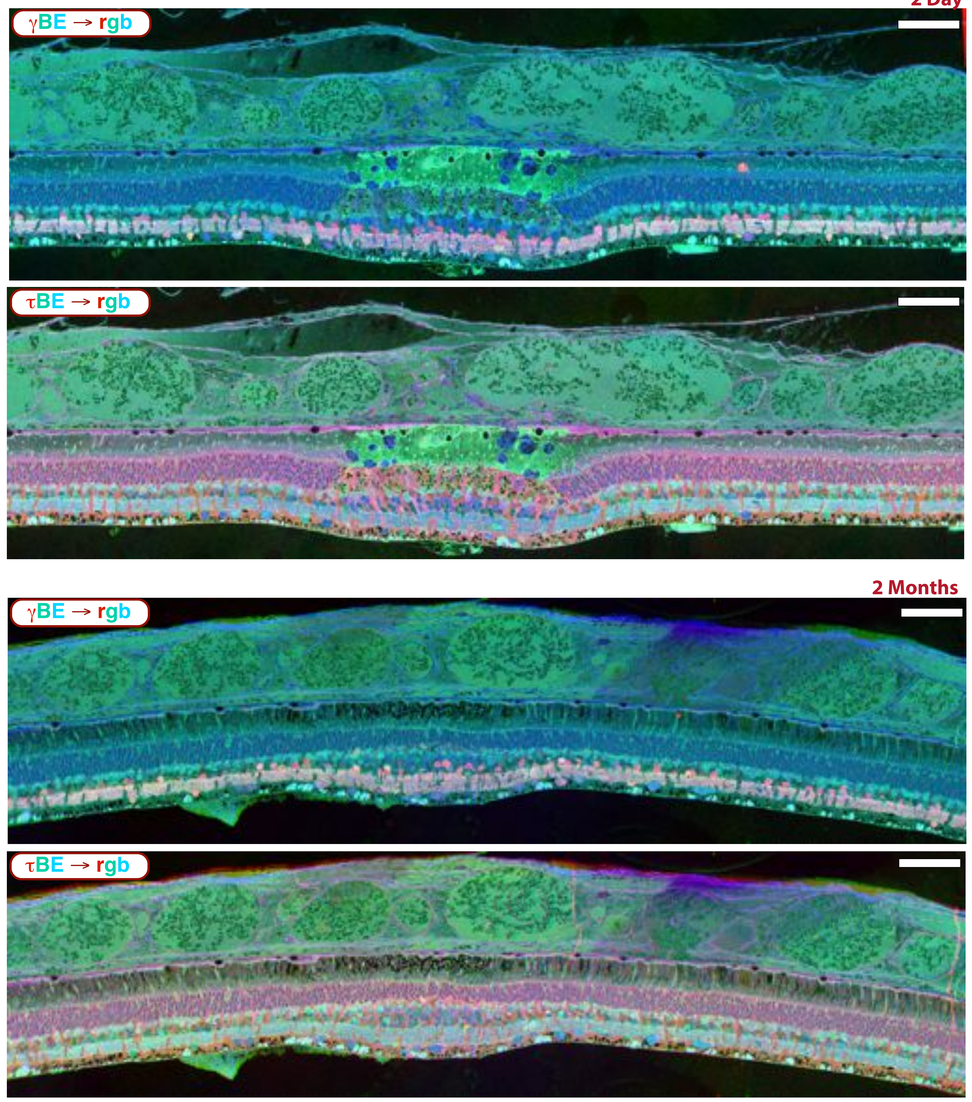
Retinal function was characterized through measurement of spike triggered average responses of retinal ganglion cells to spatio-temporal white noise visual stimulus. For this purpose, several hundred cells were recorded simultaneously on 512-electrode array with 60 micron interelectrode spacing. Acute (1 to 2 day) and 2 month old lesions made with 200µM and 400µM diameter laser beams E and F. Images of retina on the electrode array identify the location of the lesion relative to the electrode array. As expected, the receptive fields of the recorded ganglion cells show no response over the acute lesion sites of both 200µM diameter burns E, and the 400µM diameter burns F, with the sensitivity drop being larger for 400µM lesions. In contrast, the blind spot resolved in the 2 month old 200µM lesion E. Examples of receptive fields of individual ganglion cells show that the sensitivity restoration occurred for both ON and OFF ganglion cell populations (data not shown). These results demonstrate that previously reported anatomical data showing restoration of retinal continuity after barely visible laser lesions corresponds to the restoration of retinal sensitivity at the coagulation sites. Furthermore, this restoration takes place in the two major visual pathways sensitive to the ON- and OFF-pathways in the retina. Incomplete restoration of sensitivity in the larger size lesions corresponds to anatomical observations of the limited healing in the experimental time frame, but might also be associated with the maximum distance the photoreceptors can travel before the Müller cells inside the lesion deactivate.



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 intravitreal injections/ vitrectomies yielding 5-10mM intraocular [AGB], followed by a 30-60 minute response epoch. 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 γBE :: rgb and τBE :: rgb mapping employed, assigning GABA, AGB and L-glutamate and taurine, AGB, L-glutamate to red, green and blue color channels respectively.

Barely Visible Clinical Grade Burn



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 Müller cells appear to have become activated, altering their taurine and glutamate metabolism. However, by 2 months in the barely visible clinical grade burn, Müller 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, Müller 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, regions adjacent to the burn and glial scar recover substantially, though neuronal signaling indicated by AGB permeation does not quite normalize with some populations (horizontal cells) showing substantially increased AGB signals underneath ablated photoreceptors. Scale Bar = 120µM.