# NEURAL PLASTICITY REVEALED BY LIGHT-INDUCED PHOTORECEPTOR LESIONS

B.W. Jones, R.E. Marc, C.B. Watt, D.K. Vaughan, D.T. Organisciak. *

## 1. INTRODUCTION

The retina has long been assumed to remain in stasis after photoreceptor degeneration effectively deafferents the neural retina (Zrenner, 2002). However, a growing literature reveals the more insidious details of retinal degeneration and evidence of early plasticity. Retinal degenerations typically undergo three phases. Early changes observed in phase one are triggered by photoreceptor stress and include misrouting of rhodopsin to the inner segments of photoreceptors (Milam et al., 1998) followed by rhodopsin delocalization to processes extending down in fascicles projecting into the inner nuclear and ganglion cell layers (Li et al., 1995; Milam et al., 1996). Phase two is characterized by active photoreceptor cell death eventually deafferenting bipolar cell populations and eliminating light mediated signaling to the neural retina. Also observed in phase two is the formation of the Müller cell (MC) seal, entombing or walling off the remnant neural retina from what is left of the retinal pigment epithelium and vascular choroid (Jones et al., 2001; Jones et al., 2003; Marc et al., 2003). Formation of the Müller cell seal is likely due to collapse of distal elements of Müller cells, but is also possibly due to hypertrophic processes. Before completion of phase two, all dendritic elements of bipolar cells have retracted and horizontal cells typically have sent axonal processes into the inner plexiform layer (IPL). (Strettoi and Pignatelli, 2000; Park et al., 2001; Strettoi et al., 2002; Strettoi et al., 2003). The final stage of remodeling, phase three, was originally described in the GHL mouse (Jones et al., 2001), however at the time the extent of remodeling across models and the implications for vision rescue was not appreciated. Subsequent work in naturally occurring and genetic models (Jones et al., 2003) revealed extensive remodeling in response to photoreceptor degeneration. This remodeling involves the evolution of processes from all classes of neurons into fascicles that may run for >100 microns in addition to elaboration of new "tufts" of IPL (microneuromas) that form outside the boundaries of the normal stratification of the IPL. These microneuromas are populated with synaptic contacts corruptive of normal visual

* B.W. Jones, R.E. Marc, C.B. Watt, Ophthalmology, Univ Utah/Moran Eye Center, Salt Lake City, UT; D.T. Organisciak, Biology, Univ of Wisconsin Oshkosh, Oshkosh, WI; D.K. Vaughan, Biochemistry and Molecular Biology, Wright State Univ, Dayton, OH.

processing (Marc et al., 2003). Finally, migration of adult neuronal phenotypes throughout the vertical axis of the retina is observed with all cell classes participating. It is believed that in order to maintain normal gene expression, neurons will sprout processes to seek lost glutamatergic signaling. Failing to achieve synaptic contact may result either in cell death or cellular soma migration to other regions of the retina. Amacrine cells are commonly observed translocating to the ganglion cell layer with ganglion cells also migrating up into the inner nuclear layer.

The work with naturally occurring human, and natural and genetically engineered animal models has revealed plasticity and retinal neural remodeling as the typical response to apparent sensory deafferentation. Therefore, while light damage has long been recognized as a way to "kill" photoreceptors, this study was designed to use the light damage methodology (LD) to assess the nature and scope of plasticity in the neural retina in response to deafferentation in an environmental rather than a genetic model of deafferentation..

## 2. Methods

Over 90 albino Sprague-Dawley rat retinas were exposed to light (Organisciak et al., 1998) of varying durations, pre-adaption states, circadian phases and survival times. Post-euthanasia, enucleated eyes were rapidly fixed in glutaraldehyde, resin-embedded and thin sections (250nm) were serially probed with IgGs generated against aspartate, glutathione, glutamate, glutamine, glycine, GABA, and taurine, key retinal metabolites and cell specific markers. Primary immunohistochemical labeling was followed by silver intensification with a secondary goat anti-rabbit IgG adsorbed to 1nm gold particles and visualized with silver intensification (Kalloniatis and Fletcher, 1993). All images of immunoreactivity were captured as 8-bit greyscale images and registered to < 250 nm root-mean-square error. Computational molecular phenotyping (Marc and Jones, 2002) was then employed to identify neurons. EM overlay was employed to identify signatures at the ultrastructural level (Marc and Liu, 2000).

## 3. Results

In the LD model, Müller cells undergo dramatic transformations in areas with complete rod and cone loss. In these regions, plasticity ensues as evidenced by neuronal migration via formation of hypertrophic MC columns throughout the axis of the retina, with some neurons migrating from the retina into the remnant choroid. Synaptic remodeling is demonstrated with neurites engaging in novel, corrupt circuitry via GABAergic, glycinergic and glutamatergic synapses. Within 14 days of even a brief, 3 hr LD treatment, focal photoreceptor loss was accompanied by irregular 2-4 fold increases in RPE glutamine and rod aspartate levels, perhaps presaging cell death. The onset of MC remodeling (formation of a fibrotic glial seal in regions of extensive rod/cone cell death) is accompanied by a dramatic >10-fold increase in MC glutamine. This occurs only in MCs engaged in seal formation; MCs a mere 0.1 mm away are normal (Figure 1). By 60 days post exposure, most photoreceptors have died and the glial seal has become complete in those areas with complete photoreceptor loss. Small microneuromas have begun to form, originating from sprouting bipolar cells and amacrine cells. Aditionally, fluid channels begin to form, likely originating from the Müller cell seal walling off of

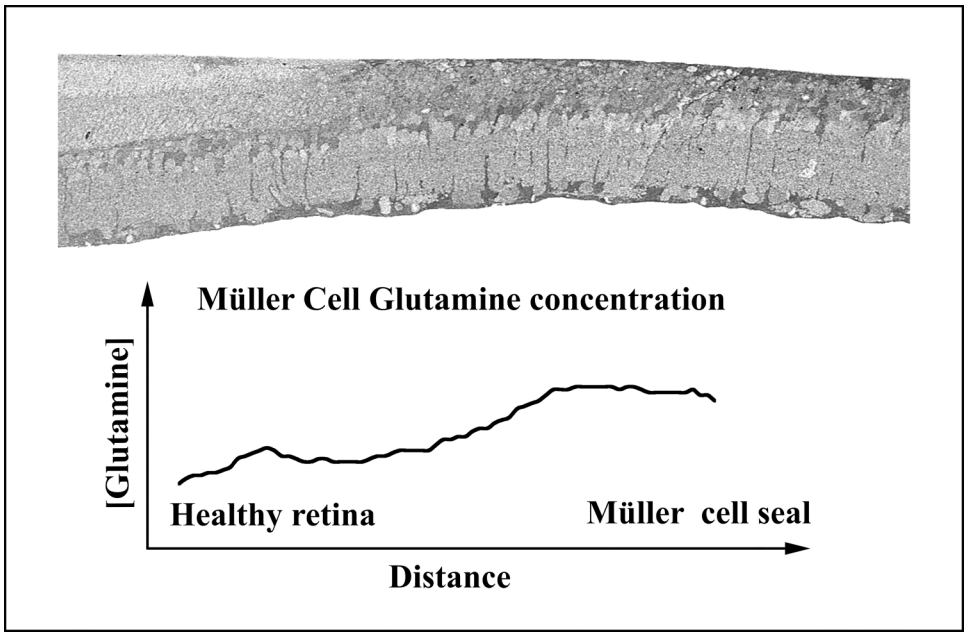


**Figure 1.** Early in the degenerative process, Müller cells exhibit large increases in glutamine concentration (often >10-fold increases) in areas where photoreceptor death is complete allowing Müller cells to begin seal formation.

the neural retina from the vascular choroid. This seal likely impairs transretinal water flow (Bringmann et al., 2004), resulting in the formation of fluid channels or cysts. Those bipolar cells that have not sprouted and found targets to contact have begun the process of dying. For the most part however, at 60 days post exposure the normal lamination of the IPL is intact and most populations of cells other than the photoreceptors appear in approximately normal numbers. At approximately 120-240 days post-LD, when both neuronal migration on hypertrophic MC columns and synaptic remodeling are initiated, other more dramatic changes ensue. Synaptic remodeling is evinced by neuropil arising from new neurites in the remnant distal retina containing GABAergic, glycinergic, and glutamatergic synapses in novel circuits (Figure 2). Distal migration of MC nuclei, MC hypertrophy and disorganization of the inner nuclear layer, including cell loss, match remodeling processes in advanced genetic forms of retinal degeneration, including human retinitis pigmentosa. Neuronal migration throughout the axis of the retina is common. All classes of neurons participate including glycinergic amacrine cells migrating into the ganglion cell layer and ganglion cells can be observed migrating into the inner nuclear layer. By 240 days post-LD, the RPE has been obliterated, the vascular choroid has been compromised and there is extensive emigration of MCs and neurons from the neural retina proper into the remnant choroid, similar to that described by Sullivan et al. (Sullivan et al., 2003) for the aged ambient-LD rat. These neurons posess signatures unchanged from their signatures in the retina proper.

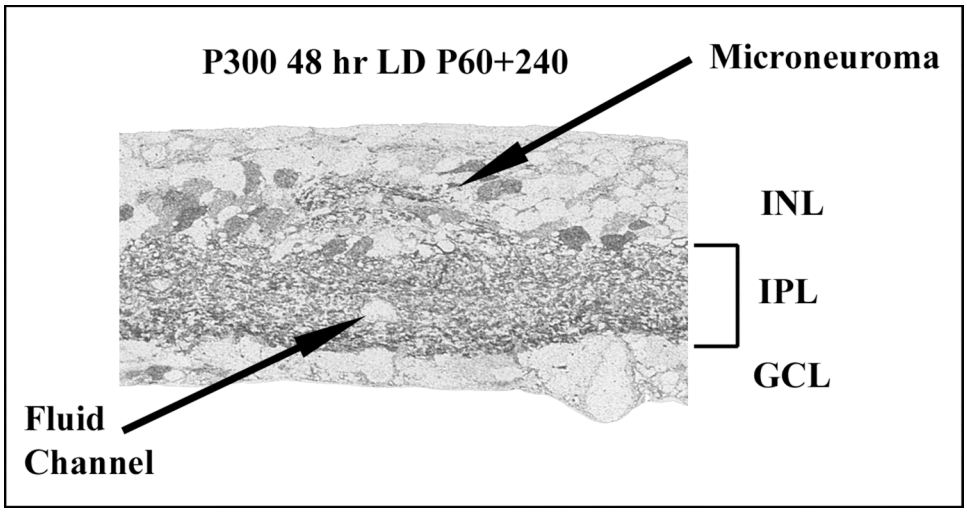


**Figure 2.** A 300 day old rat retina harvested 240 days after light damage at 60 days of age immunohistochemically labeled for GABA. Elaboration of microneuromas outside the normal lamination of the IPL are seen composed of tangles processes labeling for GABA, glutamate and glycine from amacrine cells, horizontal cells, bipolar cells and ganglion cells (Jones et al., 2003). Also observed in this image is the beginning formation of an aqueous fluid channel.

Confirming the exit from the neural retina proper required ultrastructural analysis. Therefore, electron microscopy (EM) with light microscopy overlay (Marc and Liu, 2000) was employed to demonstrate emigration of cells with mature neuronal phenotypes through Brüch's membrane. Figure 3 shows one such neuron: a GABAergic neuron that has completely passed through a small hole in Brüch's membrane demonstrating adult neuronal phenotypes remain stable when migrating through the retina and into the remnant choroid

## 4. Conclusions

All insults that kill photoreceptors represent sensory deafferentations that trigger retinal remodeling akin to CNS plasticities, including neuronal loss, growth of new neurites, formation of new synapses, and reorganization of the neuronal and glial somatic positions. These data show LD that models exhibit plasticity that mirrors pathology observed in other models of retinal degeneration. LD is a fast, effective trigger of large-scale remodeling (perhaps due to the high temporal coherence of the insult) and enables study of circuitry defects emergent from remodeling.

Sparing of the ventral retina allows for a "built in" control, allowing us to compare within the same preparation both normal and remodeled portions of tissue. Furthermore, the LD model, with the possible exception of the conditional genetic knockouts, is the only model in which we know there are no developmental abnormalities with respect to circuitry and genetics throughout development making the LD model possibly the best model available for Age Related Macular Degeneration (AMD) and AMD like disorders.

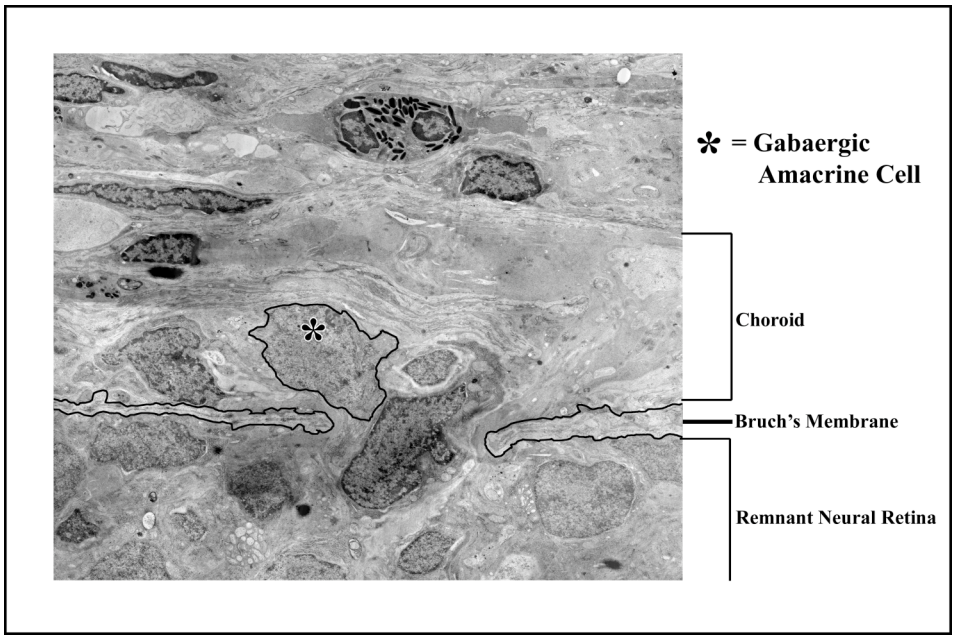


**Figure 3.** An electron micrograph of a GABAergic amacrine cell (confirmed by CMP EMoverlay (Marc and Liu, 2000)) . Bruch's Membrane has been breached allowing neurons to escape the remnant neural retina into the choroid.

Cells outside the boundaries of the neural retina have escaped. Furthermore, they appear to have normal amacrine and bipolar cell signatures indicating their metabolic status appears to be stable. Many of these neurons have rewired and apparently have established some form of connectivity keeping them alive. Furthermore, the glial cells are also emigrating, leaving an abandoned retina which, for all intents and purposes may be dead. Rescue at this point is impossible.

These data show light-damaged models exhibit plasticity that mirrors pathology observed in other models of retinal degeneration. We suggest that all insults resulting in loss of photoreceptors represent sensory deafferentations, triggering retinal remodeling akin to CNS plasticities, including neuronal loss, growth of new neurites, formation of new synapses, and reorganization of neuronal and glial somatic positions and finally, adult neurons can migrate without first de-differentiating.

**References**

Bringmann A, Reichenbach A, Wiedemann P. 2004. Pathomechanisms of cystoid macular edema. Ophthalmic Res 36:241-249.

Jones BW, Baehr W, Frederick JM, Marc RE. 2001. Aberrant remodeling of the neural retina in the GHL transgenic mouse. In: Invest Ophthalmol Vis Sci.

Jones BW, Watt CB, Frederick JM, Baehr W, Chen CK, Levine EM, Milam AH, LaVail MM, Marc RE. 2003. Retinal remodeling triggered by photoreceptor degenerations. Journal of Comparative Neurology 464:1-16.

Kalloniatis M, Fletcher EL. 1993. Immunocytochemical localization of the amino acid neurotransmitters in the chicken retina. J Comp Neurol 336:174-193.

Li ZY, Kljavin IJ, Milam AH. 1995. Rod photoreceptor neurite sprouting in retinitis pigmentosa. Journal of Neuroscience 15:5429-5438.

Marc RE, Jones BW. 2002. Molecular phenotyping of retinal ganglion cells. Journal of Neuroscience 22:412-427.

Marc RE, Jones BW, Watt CB, Strettoi E. 2003. Neural Remodeling in Retinal Degeneration. Prog Ret Eye Res 22:607-655.

Marc RE, Liu W. 2000. Fundamental GABAergic amacrine cell circuitries in the retina: nested feedback, concatenated inhibition, and axosomatic synapses. Journal of Comparative Neurology 425:560-582.

Milam AH, Li ZY, Cideciyan AV, Jacobson SG. 1996. Clinicopathologic effects of the Q64ter rhodopsin mutation in retinitis pigmentosa. Invest Ophthalmol Vis Sci 37:753-765.

Milam AH, Li ZY, Fariss RN. 1998. Histopathology of the human retina in retinitis pigmentosa. Prog Ret Eye Res 17.

Organisciak DT, Darrow RM, Barsalou L, Darrow RA, Kutty RK, Kutty G, Wiggert B. 1998. Light history and age-related changes in retinal light damage. Invest Ophthalmol Vis Sci 39:1107-1116.

Park SJ, Kim IB, Choi KR, Moon JI, Oh SJ, Chung JW, Chun MH. 2001. Reorganization of horizontal cell processes in the developing FVB/N mouse retina. Cell Tissue Res 306:341-346.

Strettoi E, Pignatelli V. 2000. Modifications of retinal neurons in a mouse model of retinitis pigmentosa. Proc. Natl. Acad. Sci. 97:11020-11025.

Strettoi E, Pignatelli V, Rossi C, Porciatti V, Falsini B. 2003. Remodeling of second-order neurons in the retina of rd/rd mutant mice. Vision Res 43:867-877.

Strettoi E, Porciatti V, Falsini B, Pignatelli V, Rossi C. 2002. Morphological and functional abnormalities in the inner retina of the rd/rd mouse. Journal of Neuroscience 22:5492–5504.

Sullivan R, Penfold P, Pow DV. 2003. Neuronal migration and glial remodeling in degenerating retinas of aged rats and in nonneovascular AMD. Invest Ophthalmol Vis Sci 44:856-865.

Zrenner E. 2002. Will Retinal Implants Restore Vision? Science 295:1022-1025.