Roles Of Retinoic Acid Signaling In Neuritogenesis During Light-Induced Retinal Degeneration

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Purpose: Work in our laboratory and others has revealed striking neuritogenesis in the neural retina subsequent to photoreceptor stress and degeneration. While the initiators of this process remain unknown, our data indicate that major changes in retinoid processing occur prior to neuritogenesis. We hypothesize that alterations in retinoic acid (RA) signaling may influence the evolution of neuritogenesis and subsequent retinal remodeling.

Methods: Adult balb/c albino mice were exposed to constant intense light (24 h) by excluding one normal night cycle (12 h) to establish the light-induced retinal degeneration (LIRD) animal model. Retinas were harvested at various post-light exposure day (pLX) 0, 1, 7, 30, 60, 90 and 120 for RA signaling analysis by morphological, metabolic and protein profiling.

Results: Cellular retinoic acid-binding protein II (CRABP II, a transcriptional regulator), RA receptors α, β (RARα, β), and retinoid X receptors α β, γ (RXRα, β, γ) were expressed in inner nuclear layer (INL) and ganglion cell layer (GCL). RARγ was not detected in control retina. The protein levels of CRABP II, RARβ, RXRα, RXRβ, and RXRγ were fluctuated in the neural retina after light stress, while RARα levels had no alteration. These changes were followed by bipolar cell neuritogenesis revealed by PKCα staining in the survivor zone where the gross histology of the neural retina seemed normal. However, signals of the examined RA signaling components were reduced in INL of the light-damage (LD) zone, where photoreceptor loss and early dendritic remodeling occurred. Nevertheless, we observed neuritogenesis revealed by SV2 staining in INL of the LD zone, where ganglion cells showed strong signals of RA signaling.

Conclusions: RA signaling displays large alterations early in retinal degeneration, suggesting RA signaling pathways may be responsible for survival-related neuritogenesis and reactive neuronal plasticity. Deficiencies in RA signaling may contribute to neurite degeneration.

Background Retinoic acid (RA), a biologically active metabolite of vitamin A (retinol), is one of the most ubiquitous inductive signals, and is involved in retinal and eye development. RA controls the transcription of its target genes by binding to and activating two gene superfamilies. One superfamily encodes cellular RA-binding proteins (CRABP I, II). The other superfamily encodes RA receptors (RARα, β, and γ) and retinoid X receptors (RXRα, β, and γ), which themselves heterodimerize and bind to a sequence of DNA known as the RA-response element (RARE). This binding activates the transcription of target genes and influences adult phenotypic attributes.

In the adult brain, all-trans RA increases neurite outgrowth and restores neurogenesis in vitamin A-deprived animals. RA modulates neurite outgrowth in dissociated embryonic brain cultures, retinal explants, and olfactory receptor neurons. Neuritogenesis, a striking feature of retinal degeneration, is a pathologic form of neuroplasticity and a barrier to therapeutic strategies.

Pathways for the synthesis and mechanism of action of RA.

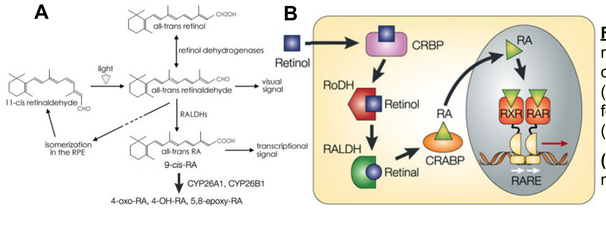

Figure 1. **(A)** The metabolic pathway that converts vitamin A (retinol) into the various forms of retinoic acid (RA).

(B) The cellular mechanism of RA action.

References:

1. Marc RE, Jones BW, Watt CB, Vazquez-Chona F, Vaughan DK, Organisciak DT 2008 Extreme retinal remodeling triggered by light damage: Implications for AMD. Mol Vis 14:782-806.
2. Jones BW and RE Marc 2005 Retinal remodeling during retinal degeneration. Exp Eye Res 81: 121-244.
3. Maden M 2002 Retinoid signalling in the development of the central nervous system. Nat Rev Neurosci 3: 843-853.
4. Luo T, Sakai Y, Wagner E, Dräger UC 2006 Retinoids, eye development, and maturation of visual function. J Neurobiol 66: 677-686.
5. Fujii H, Sato T, Kaneko S, Gotoh O, Fujii-Kuriyama Y, Osawa K, Kato S, Hamada H 1997 Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. EMBO J 16: 4163–4173.
6. Dmetrichuk JM, Carlone RL, Spencer GE 2006 Retinoic acid induces neurite outgrowth and growth cone turning in invertebrate neurons. Dev Biol 294: 39-49.
7. Bonnet E, Touyarot K, Alfos S, Pallet V, Higueret P, Abrous DN 2008 Retinoic acid restores adult hippocampal neurogenesis and reverses spatial memory deficit in vitamin A deprived rats. PLoS One 3: e3487.

Light stress induced photoreceptor death and retinal degeneration in adult mice

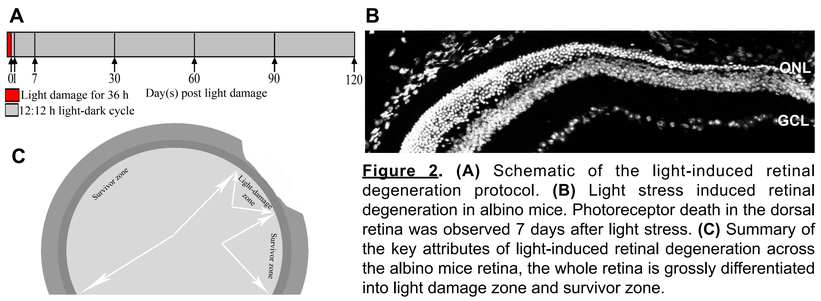

Figure 2. **(A)** Schematic of the light-induced retinal degeneration protocol. **(B)** Light stress induced retinal degeneration in albino mice. Photoreceptor death in the dorsal retina was observed 7 days after light stress. **(C)** Summary of the key attributes of light-induced retinal degeneration across the albino mice retina, the whole retina is grossly differentiated into light damage zone and survivor zone.

Neuritogenesis and anomalous fascicles occurred in the survivor zone during retinal degeneration in mice

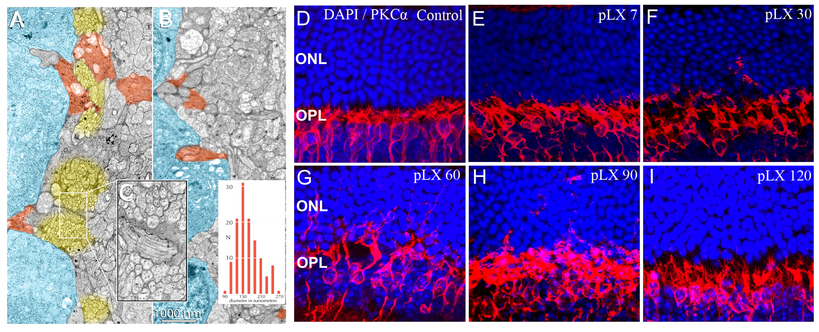

Figure 3. Neuritogenesis in light-induced retinal degeneration mice. Comparison of the distal inner plexiform layer (IPL) neuropil of **(A)** pLX22 and **(B)** normal cyclic lighting mouse retinas. At the IPL-ACL margin, anomalous fascicles (yellow) of 30-50 neurites ≈ 160 ± 47 (mean ± 1sd) nm in diameter (histogram inset) run beneath the AC somas (blue) **(C)** Each neurite contains 2-10 critically spaced microtubules. Such bundles are absent from wt retina (sampled over 1.5 mm of retina in 200+ micrographs). **(D-I)** Vertical cryostat sections of rod bipolar cells during LIRD probed with PKCα (red) antibody and DAPI and visualized by confocal imaging. In control retina, rod bipolar cell dendrites are confined to the outer plexiform layer, but by pLX7 rod bipolar cells in the survivor zone start to extend dendrites into the outer nuclear layer, implying that remodeling is beginning.

CRABP II was expressed in adult mice retina and protein levels of CRABP II were increased after light stress

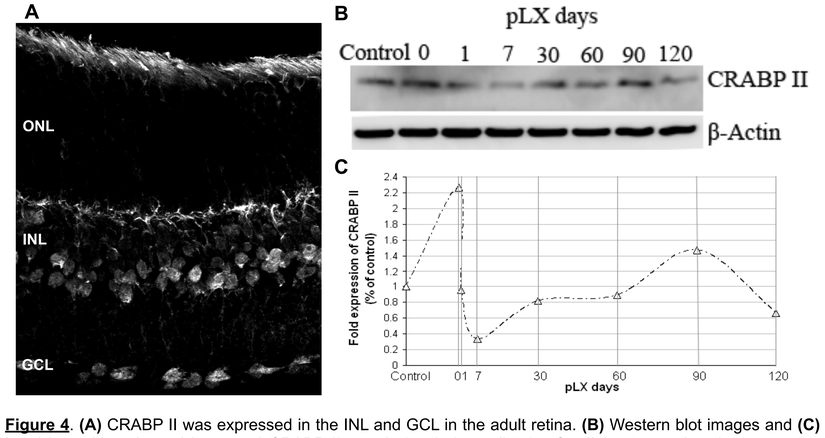

Figure 4. **(A)** CRABP II was expressed in the INL and GCL in the adult retina. **(B)** Western blot images and **(C)** band intensities showed increased CRABP II protein levels immediately after light stress, then its expression fluctuated. INL, inner nuclear layer; GCL, ganglion cell layer.

Immunolocalization of retinoic acid receptors and retinoid X receptors in adult mice retina

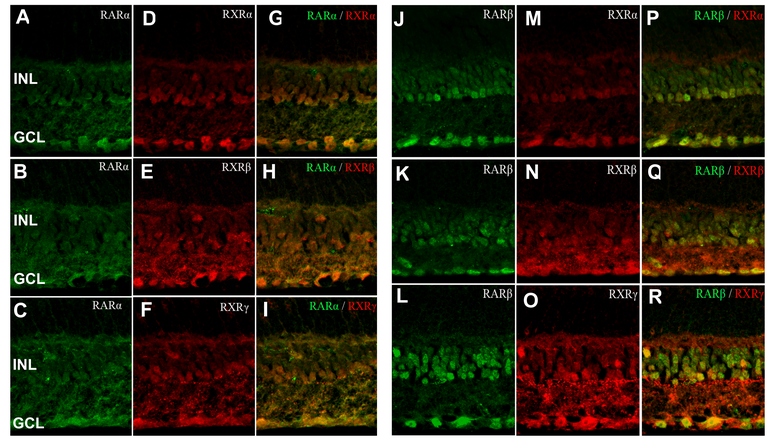

Figure 5. Immunolocalization of retinoid receptors in the adult mouse retina. RARα**(A, B, C)**, RARβ**(J, K, L)**, RXRα**(D, M)**, RXRβ**(E, N)**, and RXRγ**(F, O)** were expressed in INL and GCL, while RARγ was not found in adult albino mice. Colocalization of RARα/RXRs **(G, H, I)** and RARβ/RXRs **(P, Q, R)** was observed both in INL and GCL. INL, inner nuclear layer; GCL, ganglion cell layer.

Alterations in protein levels of retinoic acid receptors and retinoid X receptors in adult mice retina after light stress

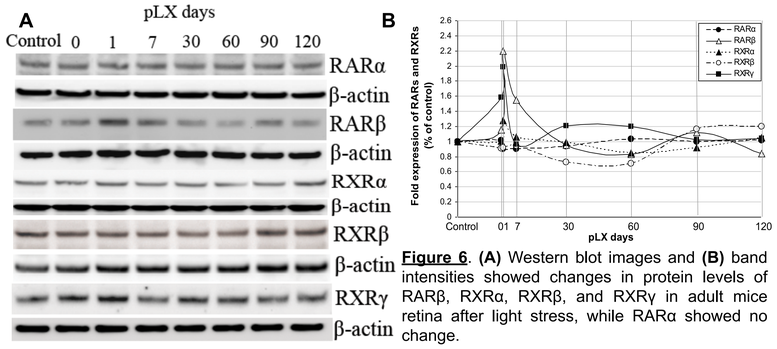

Figure 6. **(A)** Western blot images and **(B)** band intensities showed changes in protein levels of RARβ, RXRα, RXRβ, and RXRγ in adult mice retina after light stress, while RARα showed no change.

Neuritogenesis occurred in inner nuclear layer of the light-damage zone

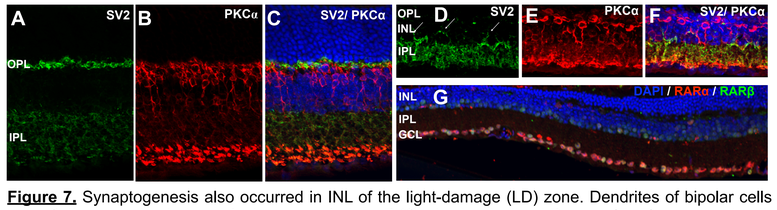

Figure 7. Synaptogenesis also occurred in INL of the light-damage (LD) zone. Dendrites of bipolar cells were flattened, retracted and lost as revealed by PKCαstaining in the LD zone **(E, F)** compared with control retina **(B)**. SV2 was expressed in IPL and OPL in control retina **(A, C)**, but its signals disappeared in OPL and were detected in INL of LD zone **(D,F)**, where signals of CRABP II, RARs and RXRs (for example, RARα and RARβ) remained strong in GCL **(G)**, although their signals were reduced in INL. Outer plexiform layer: OPL; inner plexiform layer: IPL; inner nuclear layer: INL; ganglion cell layer: GCL.

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