CaMKII Signaling Is Contributive To Neuritogenesis In Light-Induced Retinal Degeneration

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Purpose: Retinal degenerations (RD) such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) lead to primary loss of photoreceptors and secondary remodeling of the surviving retina. A striking feature of remodeling is neuritogenesis, while the initiators of this process remain unknown. We hypothesize that Ca2+/calmodulin-dependent protein kinase II (CaMKII) signaling may influence the evolution of neuritogenesis and subsequent retinal remodeling.

Methods: Adult 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 post-light exposure day (pLX) for CaMKII signaling analysis with morphological, metabolic profiling and biochemical parameters.

Results: αCaMKII and βCaMKII were expressed in the neural retina. Low intracellular Ca2+ is known to favor expression of βCaMKII over αCaMKII, and our results showed that light stress immediately increased the protein levels of βCaMKII, but αCaMKII levels showed no change. Increase in βCaMKII/αCaMKII protein ratio correlated with elevated levels of low Ca2+-permeable GluA2 AMPAR subunit and with no change in highly Ca2+-permeable GluA1 AMPAR subunit expression. These changes were followed by bipolar cell neuritogenesis revealed by PKCα staining in the survivor zone. Inhibitors of CaMKII kinase activity (KN-62) accelerated neuritogenesis compared with LIRD retina.

Conclusions: Even though the gross histology of the neural retina in the survivor zone seems normal early in LIRD, alterations to the fine dendritic circuitry in fact is underway. CaMKII signaling displays large alterations, suggesting potential CaMKII signaling and post-synaptic Ca2+ are responsible for neuritogenesis and reactive neuronal plasticity.

Background Neuritogenesis, a striking feature of retinal degeneration, is a pathologic form of neuroplasticity and a barrier to therapeutic strategies. Therapeutic windows for genetic, optogenetic, molecular, cellular and bionic rescues are severely limited by bipolar cell dendrite truncation, glutamate receptor reprogramming, atypic de novo neuritogenesis, and rewiring.

CaMKII plays a central role in synaptic plasticity and senses cytosolic Ca2+ fluxes, largely mediated by glutamate-activated AMPA or NMDA receptors in CNS neurons. αCaMKII and βCaMKII are neuron-specific. While αCaMKII is activated by high Ca2+ levels, βCaMKII is more sensitive to lower levels, and heteromeric CaMKII Ca2+ responsivity and activity depends on the α/β subunit ratio.

References:

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Light stress induced photoreceptor death and retinal degeneration in adult mice

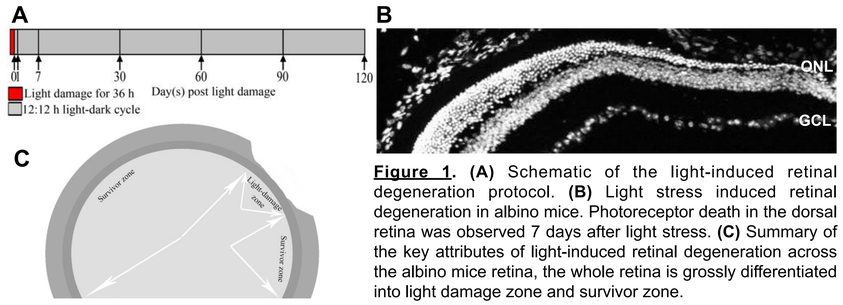

Figure 1. **(A)** Schematic of the light-induced retinal degeneration protocol. **(B)** Light stress induced retinal degeneration in albino mice. Photoreceptor death in the dorsal retina was observed 7 days after light stress. **(C)** Summary of the key attributes of light-induced retinal degeneration across the albino mice retina, the whole retina is grossly differentiated into light damage zone and survivor zone.

Neuritogenesis occurred in the survivor zone during light-induced retinal degeneration

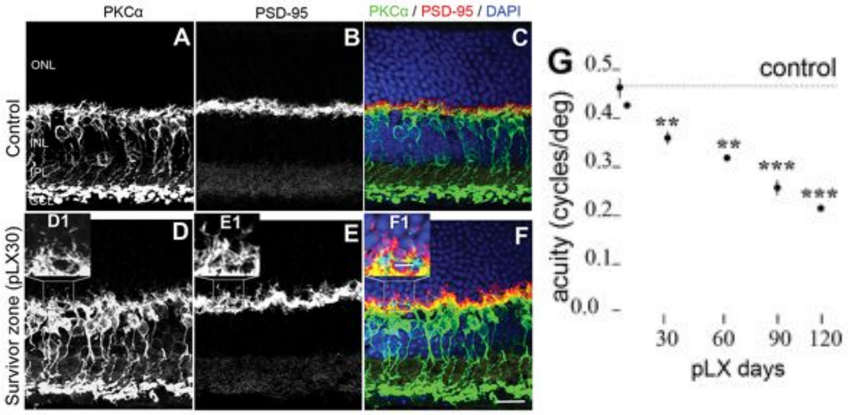

Figure 2. Neuritogenesis occurred in light-induced retinal degeneration retinas. **(A-F)** Vertical cryostat sections of LIRD retina were probed with antibodies against PKC and Postsynaptic density-95 (PSD-95) and visualized by confocal imaging. PKCαand PSD-95 immunoreactivity mapping showed that in control retina, rod bipolar cell dendrites (PKCα) and photoreceptor terminals (PSD-95) were confined to the outer plexiform layer. While obvious rod bipolar cell neuritogenesis happened in the survivor zone by pLX30, the rod bipolar cell dendrites extended far past the outer plexiform layer into the outer nuclear layer, rod spherules and cone pedicles were retracted into the survivor zone ONL. Scale bar = 20 μm. **(G)** LIRD decreased vision acuity measured by Optometry, n = 5, **P < 0.01, ***P < 0.001 vs control.

Light-induced retinal degeneration decreased the ratio of α/β CaMKII proteins by upregulating transcription of βCaMKII

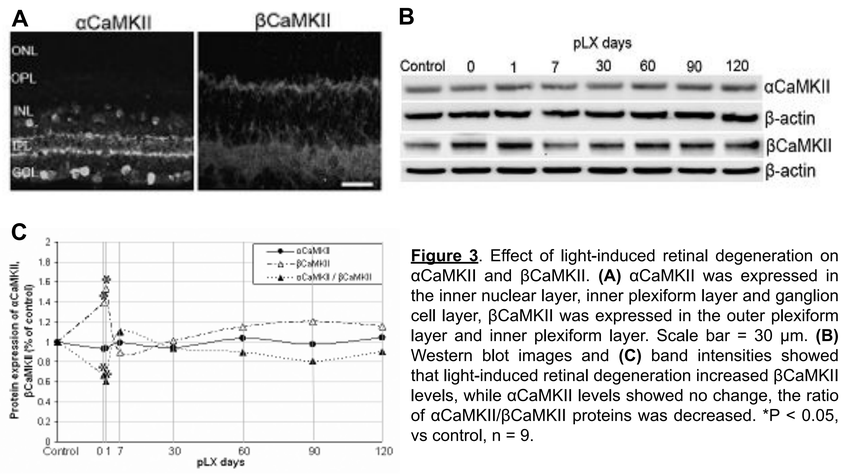

Figure 3. Effect of light-induced retinal degeneration on αCaMKII and βCaMKII. **(A)** αCaMKII was expressed in the inner nuclear layer, inner plexiform layer and ganglion cell layer, βCaMKII was expressed in the outer plexiform layer and inner plexiform layer. Scale bar = 30 μm. **(B)** Western blot images and **(C)** band intensities showed that light-induced retinal degeneration increased βCaMKII levels, while αCaMKII levels showed no change, the ratio of αCaMKII/βCaMKII proteins was decreased. *P < 0.05, vs control, n = 9.

Light-induced retinal degeneration increased the protein levels of GluA2

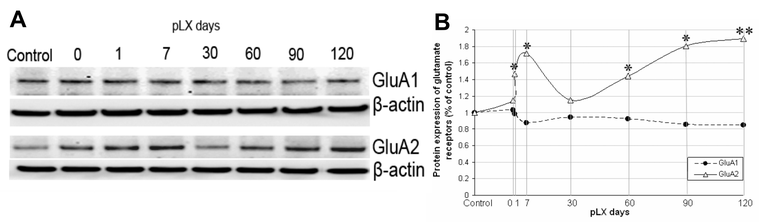

Figure 4. Effect of light-induced retinal degeneration on glutamate receptors protein expression. **(A)** Western blot images and **(B)** band intensities showed that GluA2 levels were immediately increased by pLX0, but recovered to baseline by pLX30, then continued increasing. However, GluA1 levels showed no change until pLX120. *P < 0.05, **P < 0.01 *vs* control, n = 9.

CaMKII signaling inhibition enhanced neuritogenesis

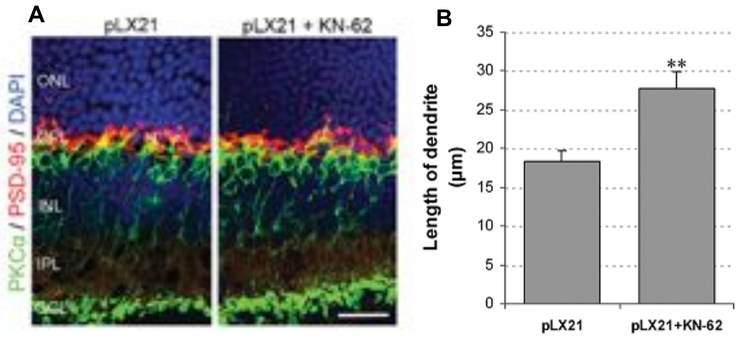

Figure 5. Effect of CaMKII inhibitor KN-62 on neuritogenesis. **(A)** KN-62 accelerated neuritogenesis by rod bipolar cells, terminals of photoreceptor were retracted to the outer nuclear layer. Scale bar = 20 μm. **(B)** Summary data (mean ± s.e.m.) for the length of rod bipolar cell dendrites, **P < 0.01 vs pLX21, n = 3.

Effect of CaMKII inhibitor KN-62 on α/β CaMKII proteins expression

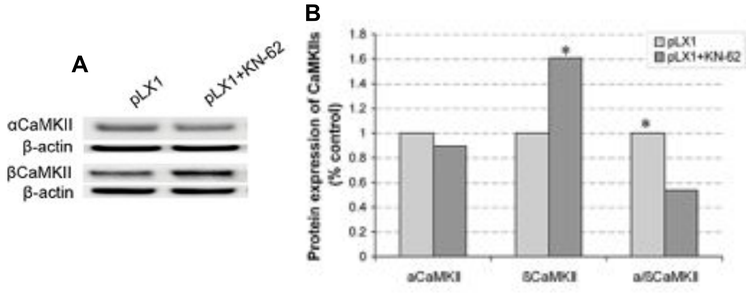

Figure 6. CaMKII inhibitor KN-62 decreased the ratio of α/β CaMKII proteins by upregulating transcription of βCaMKII. **(A)** Western blot images and **(B)** band intensities showed that subretinal injection of KN-62 increased βCaMKII levels by pLX1. However, αCaMKII levels showed no change. The ratio of αCaMKII/βCaMKII protein levels was decreased, *P < 0.05 *vs* pLX21, n = 5.

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