Rapid ionotropic glutamate receptor plasticity in light-induced retinal stress

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Introduction Remodeling research has uncovered extensive plasticity in the neural retina ensuant to compromised photoreceptor function. The rodent light-induced retinal degeneration (LIRD) model evokes a coherent deafferentation of the retina mimicking central nervous system (CNS) traumas. Our hypothesis is that retinal glutamate receptor trafficking/ regulation is modulated after LIRD.

Methods Adult Balb/C albino mice were used as our LIRD cohort. LIRD mice entrained on a 12:12 h light-dark cycle then received constant light by excluding one normal night cycle. They were harvested at the end of the following day, defined as post-light exposure day zero (pLX) 0. The total constant light exposure was 34 h. Retinas were harvested at pLX 0, 1, 7, 30 and 60 for metabolic profiling, immunostaining, and Western blotting (WB) analysis in triplicate. Ionotropic glutamate receptors (iGluRs: GluR1, GluR2) and metabotropic glutamate receptor type 6 (mGluR6) levels were assessed by Western Blotting and compared to a range of neuronal tracking proteins (PKCα, PSD95, β-actin).

Results

Figure 1. WB analysis of synaptic signaling markers

GluR2 subunits showed the largest, fastest response to LIRD. GluR2 protein levels increased immediately after LIRD (pLX0), recovering to control levels by pLX30, and increasing again by pLX60 when new neurite growth begins. GluR5 and mGluR6 levels decreased firstly then recovered. Conversely, GluR1 levels changed little. Similarly, the post-synaptic marker protein PSD-95 and the ON bipolar cell marker PKCα did not alter measurably. As in brain, stress appears to regulate GluR2 protein expression.

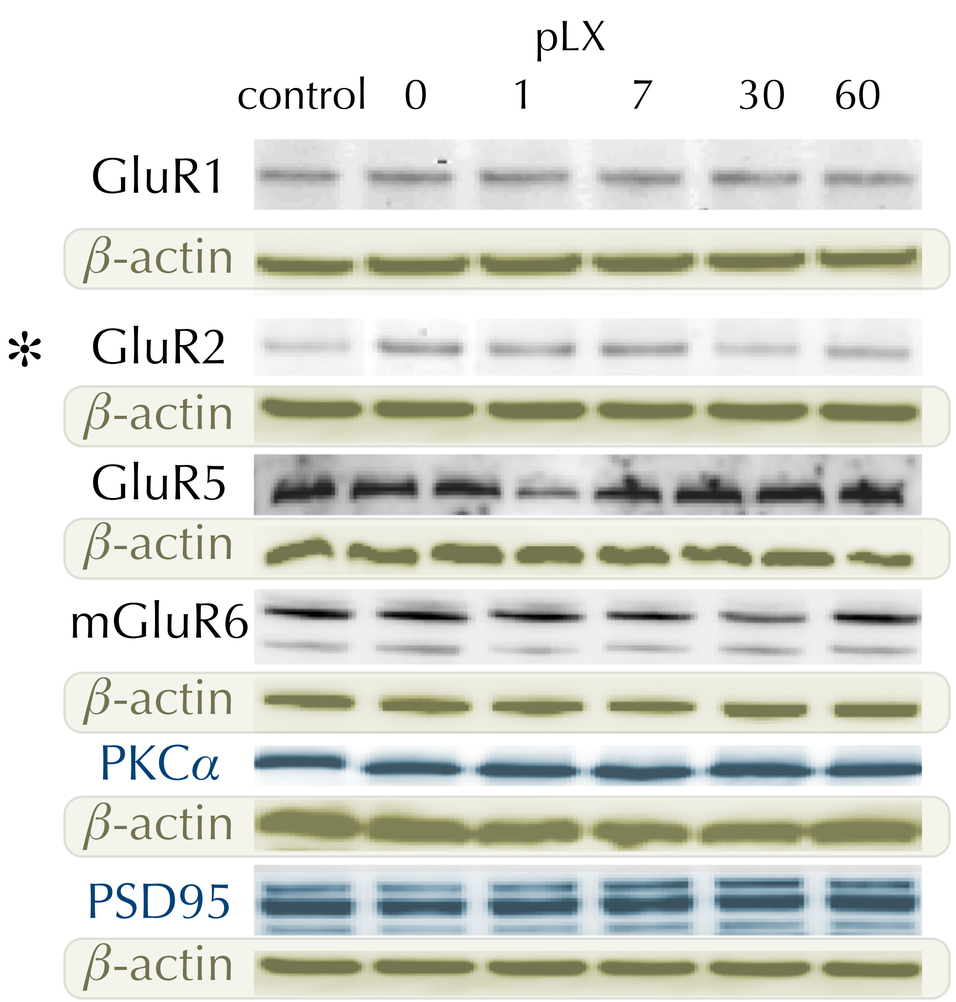
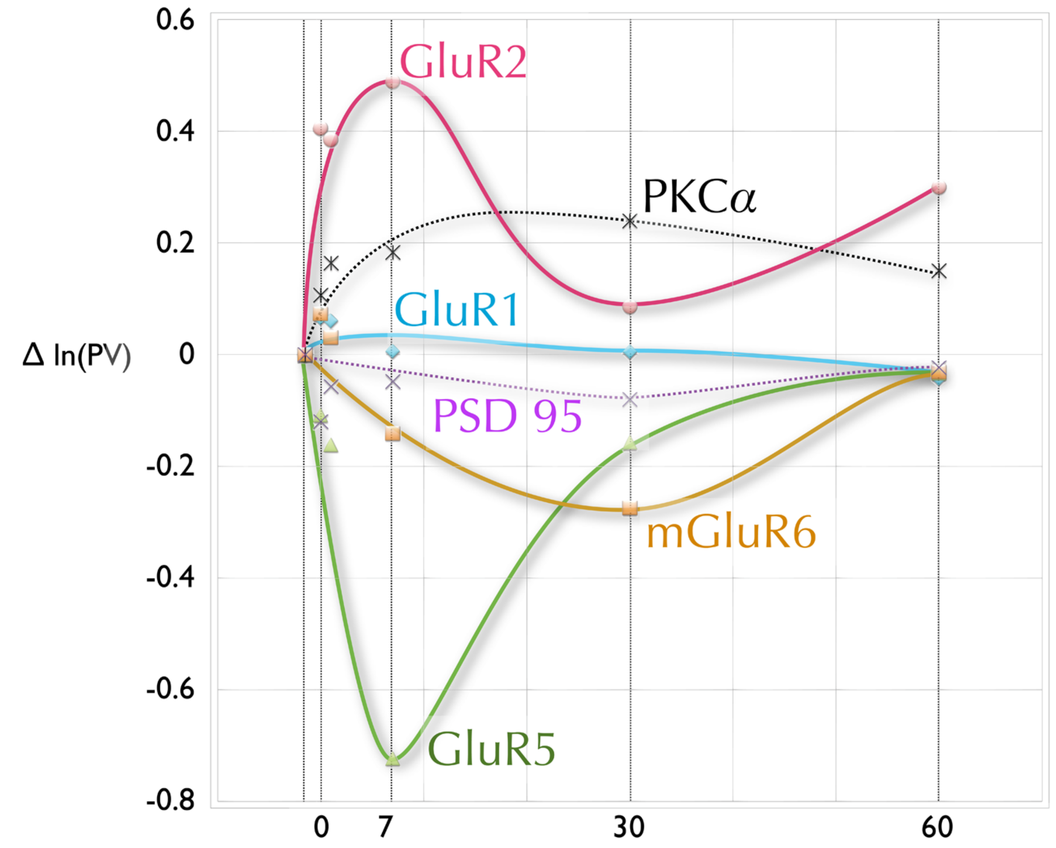


Figure 2. Kinetics of synaptic signaling markers in LIRD

Certain markers showed fast responses to LIRD. Low conductance AMPA receptor GluR2 subunits showed at 0.5 Δ ln increase (65%) in protein level while high conductance KA receptor GluR5 subunits showed a 0.7 Δ ln decrease (50%). AMPA receptor GluR1 subunits showed no significant change in expression. Each point represents the summed protein signal of three pooled retinas at each time.



Statistics: Heteroscedastic one-tailed t-tests were performed for each marker pooled from all LIRD time points (5 measures) against all eighteen LIRD β-actin measures as a global housekeeping protein and ten LIRD GluR1+PSD-95 measures as synaptic housekeeping proteins.

Protein	P-value (vs β-actin)	P-value vs (GluR1+PSD-95)
GluR1	NS	NS
GluR2	1.1×10^{-3}	2.6×10^{-3}
GluR5	7.1×10^{-2}	5.1×10^{-2}
mGluR6	NS	NS
PKCα	1.1×10^{-4}	3.1×10^{-5}
PSD-95	NS	NS

Figure 4. Rapid degeneration in outer but not inner retinal layers early in LIRD visualized with anti-CRALBP IgGs

The normal retinal displays CRALBP signals throughout the retina predominantly in Müller cells, photoreceptor outer segments and the RPE. Signals are intensified by pLX0 and pLX1 as light-stress photoreceptors degenerate. However there are no detectable signs of inner retinal layer alteration at this time either in the outer nuclear layer (ONL) or inner plexiform layer (IPL), even though GluR2 and GluR5 expressions are changing rapidly. By pLX45 some areas if the retina are devoid of photoreceptors or limited to a single layer of cones. Outside the LIRD region, the survivor zone is appears normal, though the outer segment layer (OSL) may be slightly shorter. However, we do see evidence bipolar cell neuritogenesis in the survivor zone (Fig. 4).

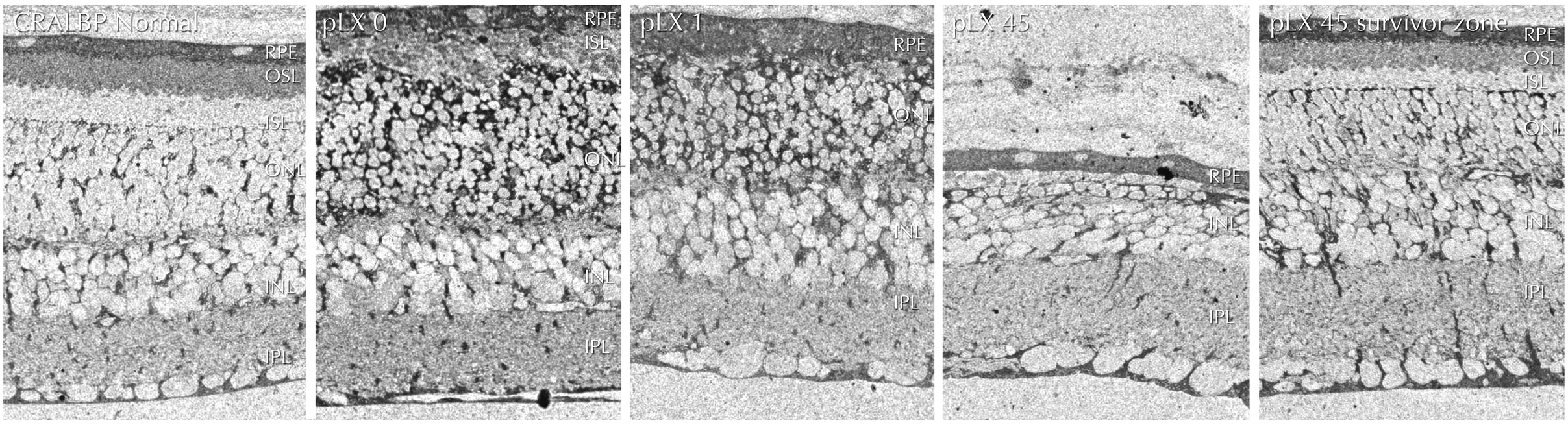
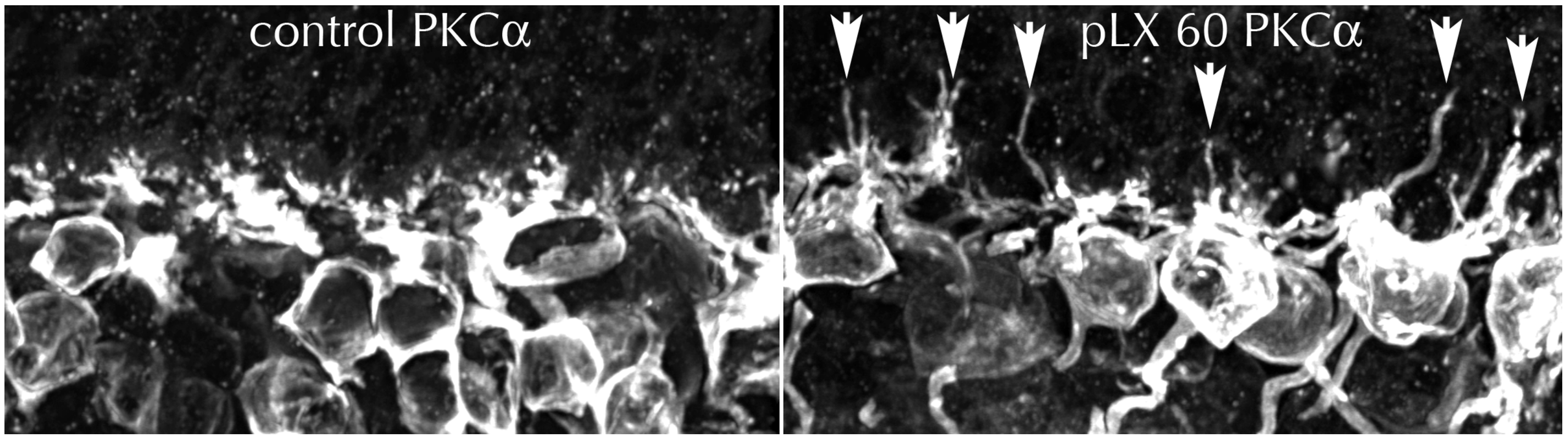


Figure 4. Slow neuritogenesis by rod bipolar cells after LIRD

Vertical cryostat sections probed with PKCα antibody and visualized by confocal imaging (Olympus FV1000) to reveal rod bipolar cell dendrites in survivor retina. In control retina, rod bipolar cell dendrites are confined to the outer plexiform layer but by pLX60 rod bipolar cells even in the survivor zone start to extend dendrites into the outer nuclear layer, implying that even there remodeling is beginning. The immunochemistry is consistent with the increased mass of rod bipolar cell neurites.



Conclusions

Though the overall anatomy of the neural retina, and especially the inner plexiform layer seems normal early in LIRD, key synaptic marker show that the inner retina can sense photoreceptor stress. Further, GluR2 subunits of AMPA receptors predominantly associated with inner retinal processing displayed rapid and highly significant increases in protein level, although these increases are too small to be detected by either immunocytochemistry or gene expression arrays.

A 65% increase in GluR2 subunit availability is sufficient to add one subunit to every AMPA receptor that does not already express one. Moreover, a single GluR2 subunit has the potential to perform three protective actions:
… it decreases the Ca^{2+} permeability of the entire channel unitary conductance by 10x (nearly abolishes it)
… it decreases the conductance of the entire channel by over 50%
… it prevents GluR1 phosphorylation-dependent increases in channel conductance
Thus even this small change has a powerful role in neuroprotection by decreasing Ca^{2+} loads in neurons. The fact that GluR1 subunits do not change expression is consistent with the dominant role of GluR2 in brain as a of AMPA receptor expressing cells during glutamate stress.

A 50% decrease in GluR5 KA receptor subunit suggests that OFF BCs expressing Kainate receptors also have a protective response. As GluR5 can form homomers or heteromers with GluR6 and/or GluR7, this could reflect a total decrease in receptor availability by up to 50%.

The LIRD model demonstrates that photoreceptor-specific stress signals rapidly propagate through the neural retina via the same pathways used in brain for neuroprotection.