Excitatory-Inhibitory balance in glaucoma

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Introduction

We aimed to assess alterations to excitatory and inhibitory (E/I) circuits in the IPL of mice following to IOP elevation to uncover sites of adult retinal plasticity in response to RGC loss.

Design & Methods

Induction of RGC loss in adult mice:
Adult CD1 mice underwent unilateral photocoagulation of episcleral and limbal vessels, with the controlateral eye serving as control. IOP was followed for 7 days via rebound tonometry until back to baseline values. Mice were eithanized either 7, 14 or 30 days from laser and retinas

Labeling of E/I synapses in the IPL :
Retinas were immunolabeled for pre- and post-synaptic components of excitatory synapses (RibeyeA and PSD95 antibody, respectively) as well as inhibitory synapses (VGAT and Gephyrin, respectively). Individual synapses were automatically recognized using ObjectFinder 10.

Labeling of E/I synapses on individual RGCs :
Retinas were biolistically labeled with cmv-psd95-YFP or cmv-gephyrin-yfp plasmids to label either excitatory or inhibitory postsynaptic; and co-transfected with cmv-cfp to hightlight the dendritic morphology. Dendrites were skeletonized with Imaris and synaptic density along dendrites calculated with ObjectFinder 10.

Results

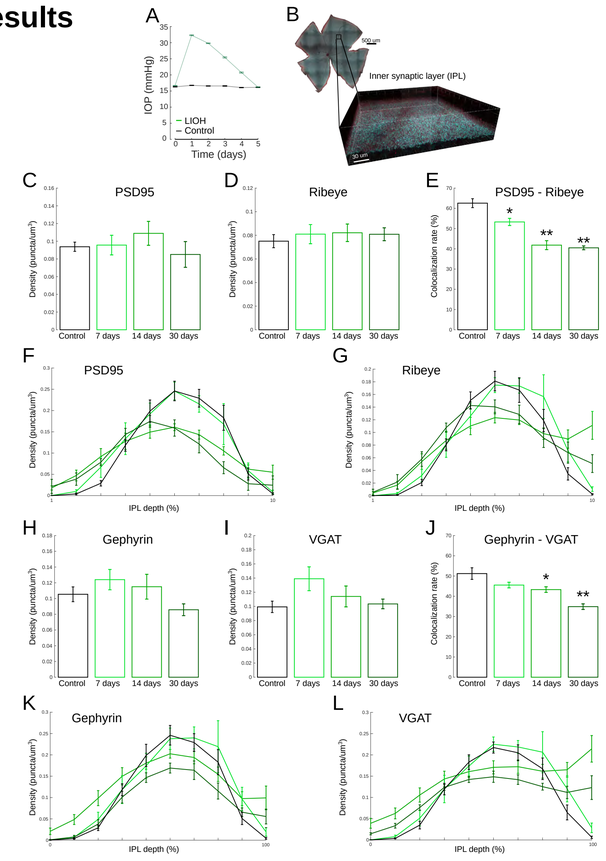

Fig.1: Excitatory and Inhibitory synaptic proteins are lost in the IPL

A) Acute intraocular pressure elevation profile following to laser photocoagulation of the episcleral and limbal vessels (LIOH). B. Example labeling of excitatory and inhibitory synaptic proteins. C,D) Quantification of excitatory synaptic protein density (*: p<0.05, **: p<0.01, rank-sum test) E) Colocalization rate of pre- and post-synaptic proteins. F,G) Spatial distribution of excitatory synaptic proteins as a function of IPL depth. H,I) Quantification of inhibitory synaptic proteins (*: p<0.05, **: p<0.01, rank-sum test). J) Colocalization rate of pre- and post-synaptic inhibitory proteins. K,L) Spatial distribution of inhibitory synaptic proteins as a function of IPL depth. N = 6 animals per time point.

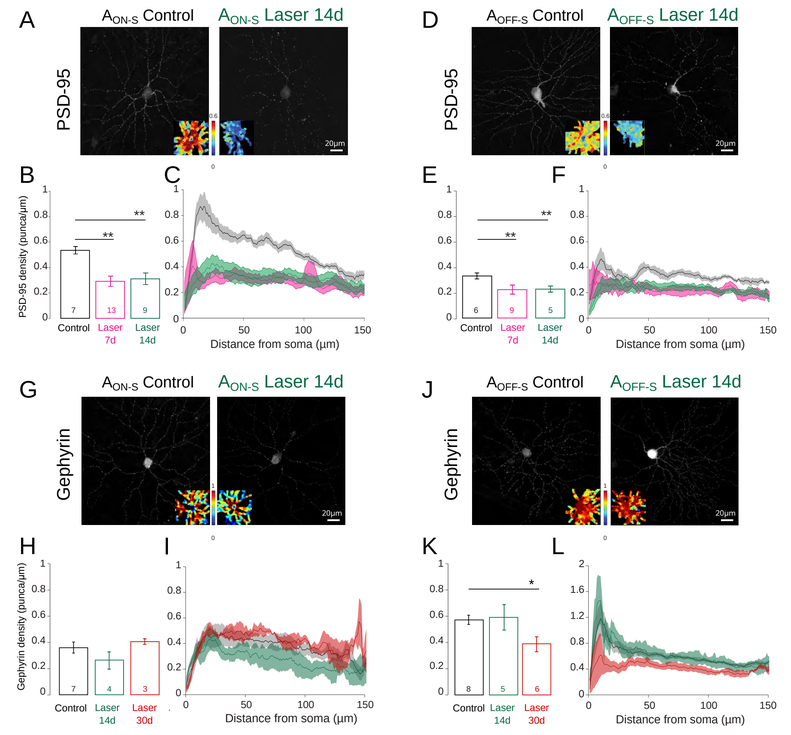

Fig.2 : Loss of excitatory synapses precedes inhibitory in A_{RGCs}

A,G) Example of an individual $A_{ON\text{-}S}$ RGC biolostically transfected to express PSD-95 and Gephyrin tagged with YFP, respectively. Inset heatmaps represents the local density of postsynaptic proteins along the dendrites of the transfected cell D,J) Example of an individual $A_{OFF\text{-}S}$ RGCs.
B,E,H,K) Average density of postsynaptic protein along dendrites. C,F,I,L) Linear density as a function of distance from the some of the labeled neurons. Histograms express average value ± SEM. *p<0.05 **P<0.01 Rank-sum test. n = number of cells at base of each bar plot from N=6 animals per time point.

Conclusions:

Loss of both excitatory and inhibitory synapses occurrs in the IPL, but follows different spatial, temporal and cell-type specificity patterns. These differences can be potentially leveraged to detect early functional alterations in glaucoma.