Central Hypothesis

We will test the central hypothesis that the mechanisms and outcomes of IL-6 signaling in RGCs are directly dependent upon the structural and functional state of their axons.

Specific Aim #1

Preliminary finding #1: IL-6 deficiency during development results in RGCs with slower rates of axon transport and lethargic transmission of light-induced stimulation.

In Specific Aim 1, we will test the working hypothesis that IL-6 is not only beneficial, but required for proper formation of RGC axons during retinal development. Using conditional IL-6Ra mice, we will:

1) Define the temporal period for IL-6 signaling during RGC development
2) Determine sufficiency and/or necessity of IL-6 signaling for the proper development and function of RGC axons
3) Identify the contributions of classical and trans-signaling pathways of IL-6 to axon formation and function

Experimental Design: Propose all of the above plus patch clamp in IL-6R conditional knockout (global and RGC-specific) and both models with sIL-6R induction only

Specific Aim 1 Prelim Data:

CTB and WGA transport WT vs IL-6 KO

Caveolin-1 and GM1 expression WT vs IL-6 KO

Brn3a+ counts in WT vs IL-6 KO – development series

OKT WT vs IL-6 KO

Gross eye and retina development WT vs IL-6 KO – development series

F-VEP data WT vs IL-6 KO

Nodes of Ranvier WT vs IL-6 KO (?)

IL-6Ra global conditional recombination feasibility

IL-6Ra conditional KO CTB transport (?)

CaMKIIa specific conditional cre genotyping

CaMKIIa specific conditional recombination feasibility (?)

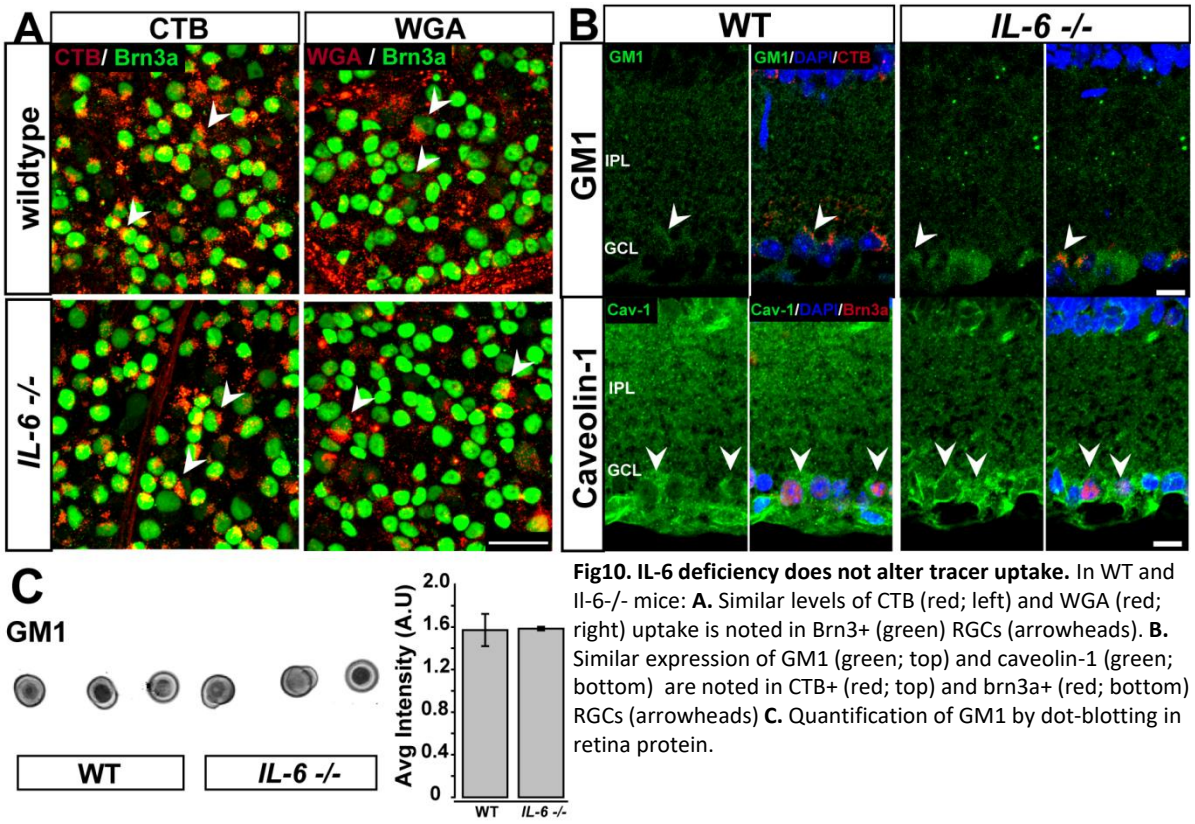

Fig10. IL-6 deficiency does not alter tracer uptake. In WT and Il-6-/- mice: **A.** Similar levels of CTB (red; left) and WGA (red; right) uptake is noted in Brn3+ (green) RGCs (arrowheads). **B.** Similar expression of GM1 (green; top) and caveolin-1 (green; bottom) are noted in CTB+ (red; top) and brn3a+ (red; bottom) RGCs (arrowheads) **C.** Quantification of GM1 by dot-blotting in retina protein.

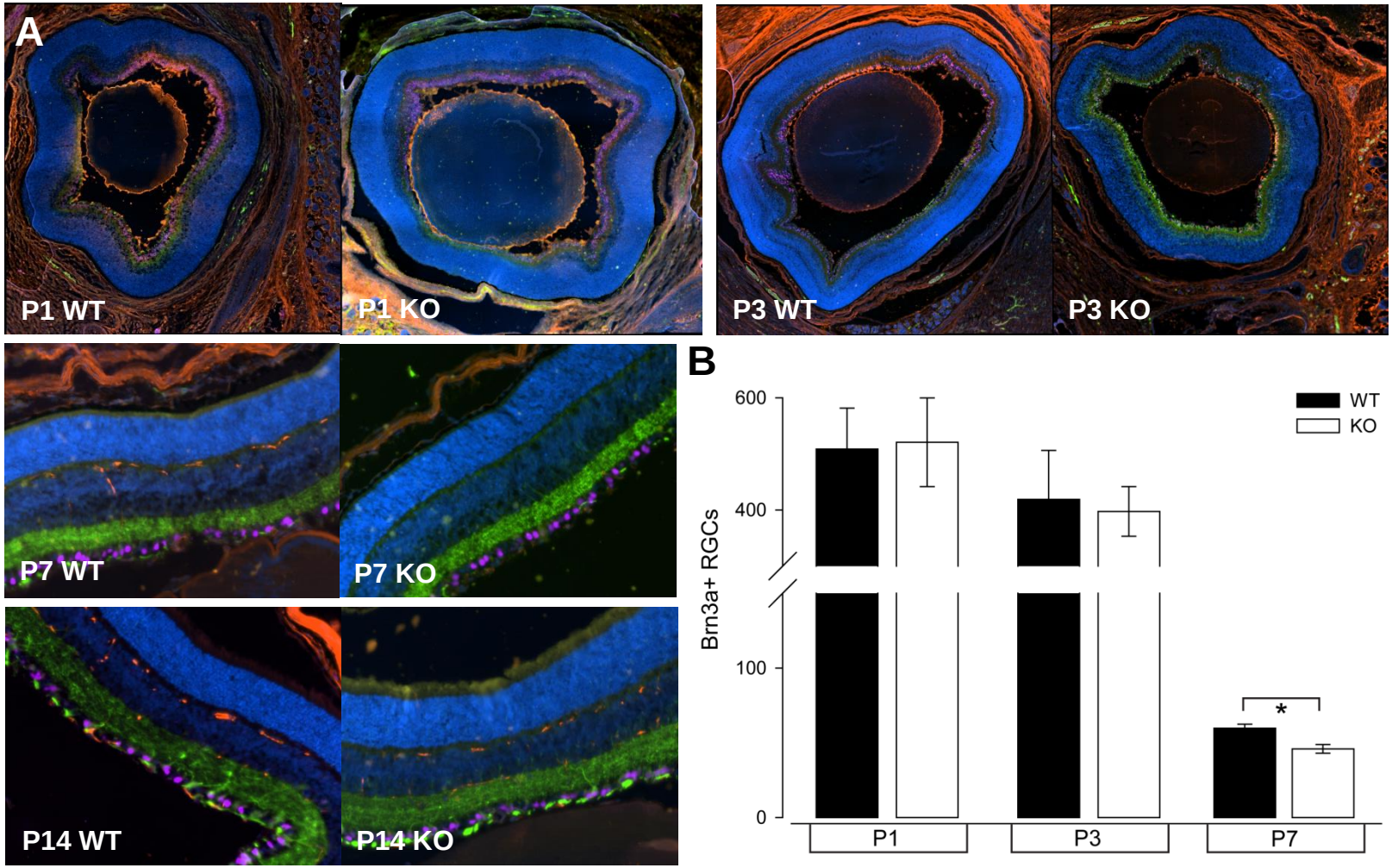

Fig12. IL-6 deficiency reduces RGC number by postnatal day 7. A. Representative micrographs of Brn3a (purple), beta-tubulin (green) and GFP (red) co-immunolabeling with DAPI counterstain (blue) in whole eye sections of eyes from WT and Il-6-/- mice 1 day (P1), 3 days (P3), 7 days (P7) and 14 days (P14) after birth. **B.** Quantification of the number of beta-tubulin+/brn3a+ RGCs per whole eye section. Asterisks indicates $p<0.01$.

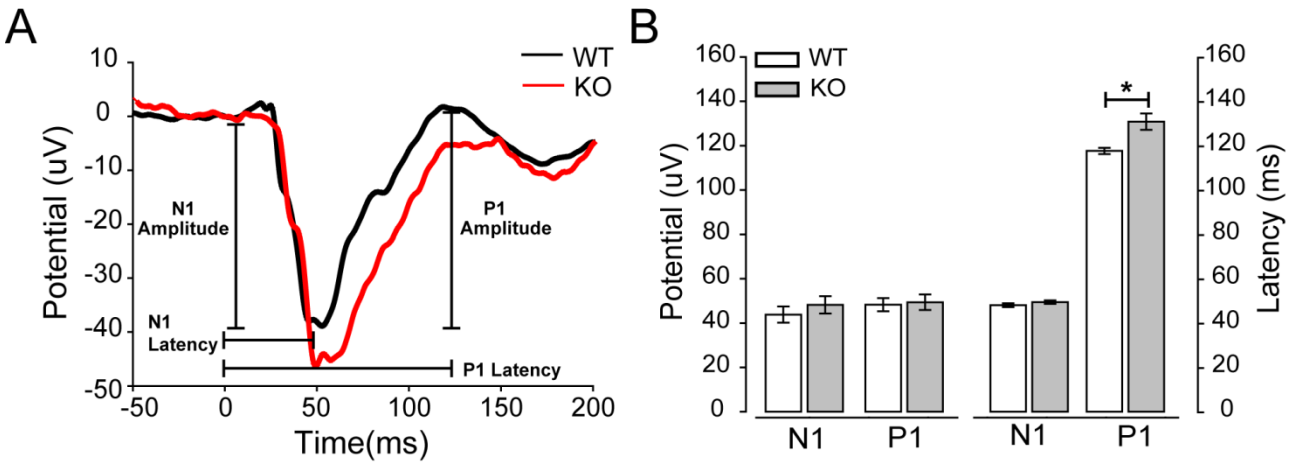

Fig13. IL-6 deficiency increases P1 latency of F-VEP waveform in naïve mice. A. Mean F-VEP trace from WT and IL-6-/- mice at 3 months of age. **B.** Quantification of N1 and P1 components of the waveform reveals no change in amplitudes (uV), but an increase in latency (ms) of P1 component. Asterisks indicates p<0.01.