Metabolic profiling of activated retinal glia

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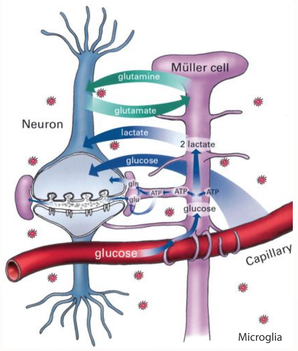
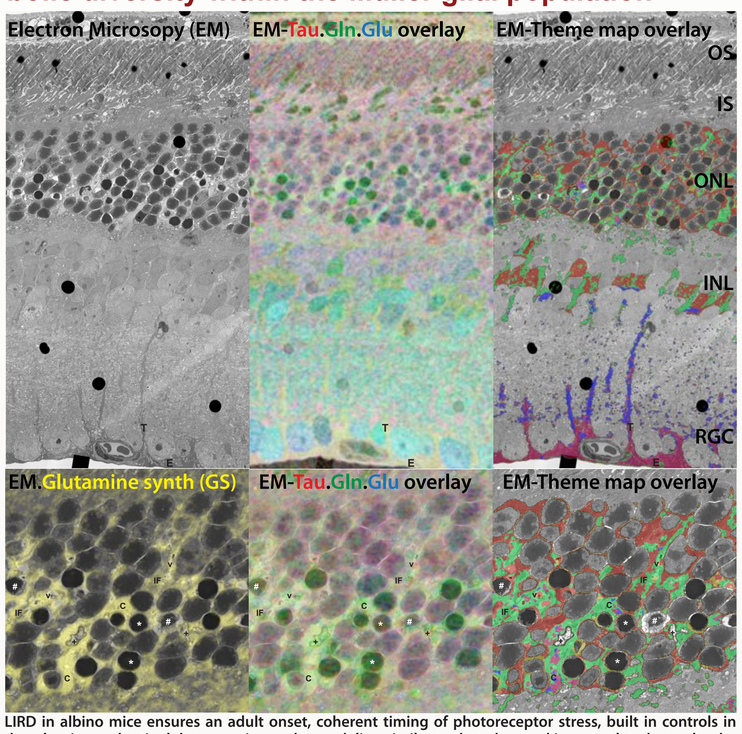
Glia can decrease their metabolic support during degeneration. Thus our long-term goal is to enhance neuronal survival by prolonging the ability of Müller glia to support metabolism. Our first aim is to visualize and quantify the metabolic states of activated glia during various forms and stages of retinal degeneration.



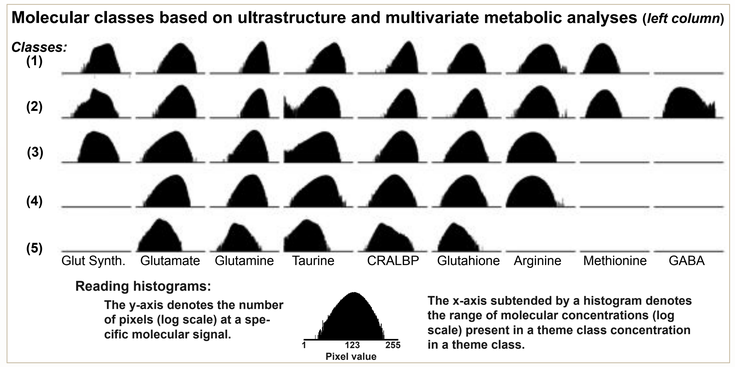
Metabolic neuron-glia interactions.
Adapted from Antonetti et al., *Diabetes*, 2006

Light-induced retinal damage (LIRD) induces metabolic diversity within the Müller glial population



LIRD in albino mice ensures an adult onset, coherent timing of photoreceptor stress, built in controls in dorsal retina and retinal degeneration and remodeling similar to that observed in age-related macular degeneration. Ultrastructure and metabolic analyses after 24 h light exposure (LX). EM mosaic represents nearly 200 tiles at 5000x. Adjacent ultrathin sections (90 nm) were probed with specific anti-hapten IgGs and visualized with silver-intensification of 1.4 nm gold granules coupled to specific secondary IgGs. Taurine.Glutamine.Glutamate (RGB) composite overlaid on EM has an opacity of 60%. Theme map of Müller glial metabolic profiling using CMP, see next section.

Müller glia displayed enhanced metabolic signals for glutamate metabolism, osmoregulation, retinoid metabolism, and anti-oxidation after LIRD



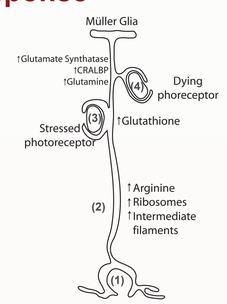
Müller glia were classified using the glutamine synthetase and glutamate signal. Cell classes were derived from computational molecular phenotyping (CMP) utilizing the k-means algorithm. Each class is segmented by its N-dimensional metabolite/protein histogram. Signals not statistically different from background were omitted, including rhodpsin (1D4), LWS opsin and glycine.

Müller glia are highly sensitive to neuronal stress and compartmentalize their metabolic response

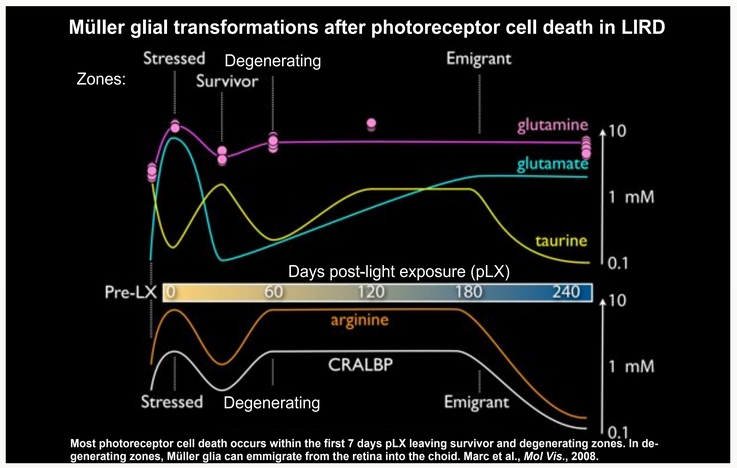
Correlation of metabolic class with ultrastructure features

Class	Region	Ultrastructure	Signature
(1) pink	Endfeet	Ribosomes Intermediate filament	High: GS, E, Q, TT, CRALBP Moderate: J, R
	Near engulfed material	vacuoles	Low: Methionine (M)
(2) blue	IPL Trunk	Ribosomes Intermediate filament	High: J, E, Q, CRALBP Moderate: GS, TT, R Low: M, GABA,
(3) green	near dying cells	Cytoplasm + vacuoles	High: Q, CRALBP Moderate: GS, E, TT, J
	around MG nuclei	Intermediate filament	Low: R
(4) red	around stressed cells	Thick interm. filament Bundles	High: Q Moderate: E, TT, J, CRALBP
	nucleus	Chromatin	Low: GS, R

Orange (5) class overlapped with red (4) class or green (3) class



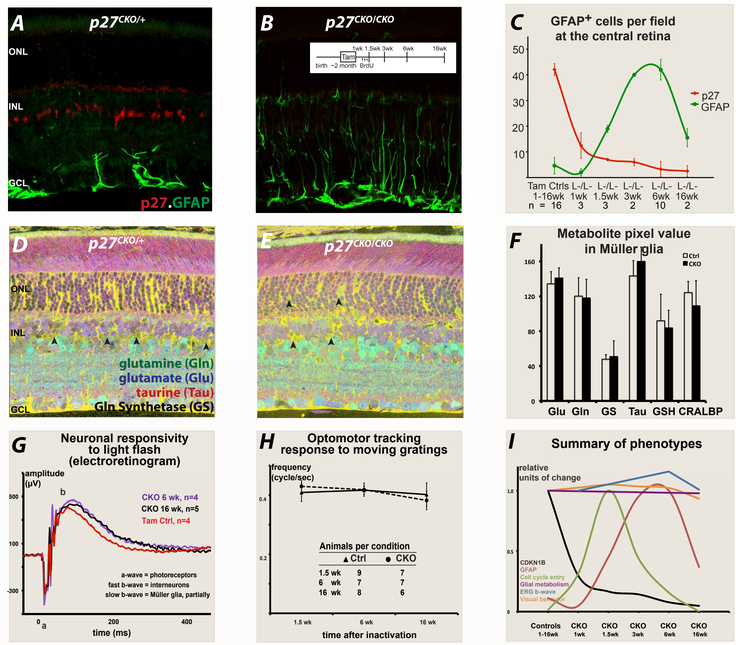
Decrease in glial metabolic support correlates with loss of photoreceptors



Most photoreceptor cell death occurs within the first 7 days pLX leaving survivor and degenerating zones. In degenerating zones, Müller glia can emmigrate from the retina into the choid. Marc et al., *Mol Vis.*, 2008.

Proliferative reactive gliosis is compatible with glial metabolic support and neuronal function

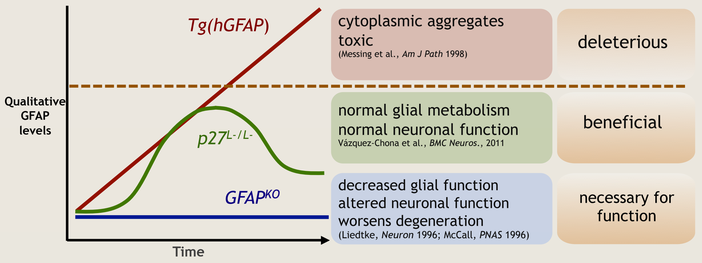
We tested the hypothesis that reactive gliosis is incompatible with neuronal survivial and function by developing an inducible model of proliferative reactive gliosis in the absence of degenerative stimuli [Vázquez-Chona et al., *BMC Neuroscience*, 2011]. The genetic inactivation of the cyclin-dependent kinase inhibitor p27 in adult mouse (*p27^{Lox};CreER^{TM}*) yielded surprising results:
(*A-C*) Conditional inactivation of p27 results in glial proliferation and GFAP upregulation.
(*D-F*) Reactive p27$^{CKO/CKO}$ Müller glia provide homeostatic metabolic support.
(*G-I*) Normal electrophysiology and visual acuity in mice with reactive p27$^{CKO/CKO}$ Müller glia.



Methods: (*A-C*) We conditionally targeted the p27 coding region in mice harboring *LoxP* sites at the *p27* locus (*p27^{L+}*), and expressing a tamoxifen-regulated Cre recombinase under the control of the chimeric chicken beta-actin promoter, CAGG::CreER^{T2}. (*D-F*) We visualized and quantified metabolite distribution using CMP. The yellow background is the distinctive taurine-glutamine signature of Müller glia. The pink and red compartments in the photoreceptor outer segments and inner segments contain distinct taurine-glutamate-glutamine mixtures. While various blue-to-azure cells in the interneuron layer and ganglion cell layer are neurons containing distinctive glutamate-glutamine mixtures. Metabolite pixel value was extracted using the GLUL signal. (*G* and *H*) Neuronal responsivity and visual acuity were measured using an electroretinogram system and a virutal optomotor system. (*I*) Data values were standaridzed for cross comparisons [Vázquez-Chona et al., *BMC Neurosc.*, 2011].

Moderate levels of reactive gliosis are neuroprotective but prolonged levels are detrimental

Genetic models of GFAP expression and its effect on neuronal survival and function:



Preconditioning models can upregulate GFAP and neuroprotect
(Casson et al., *IOVS* 2003; Garnier, *Brain R*, 2001)

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