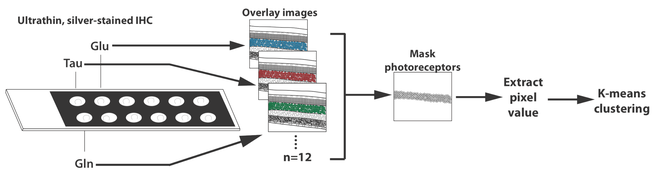
Metabolic mapping of photoreceptor degeneration

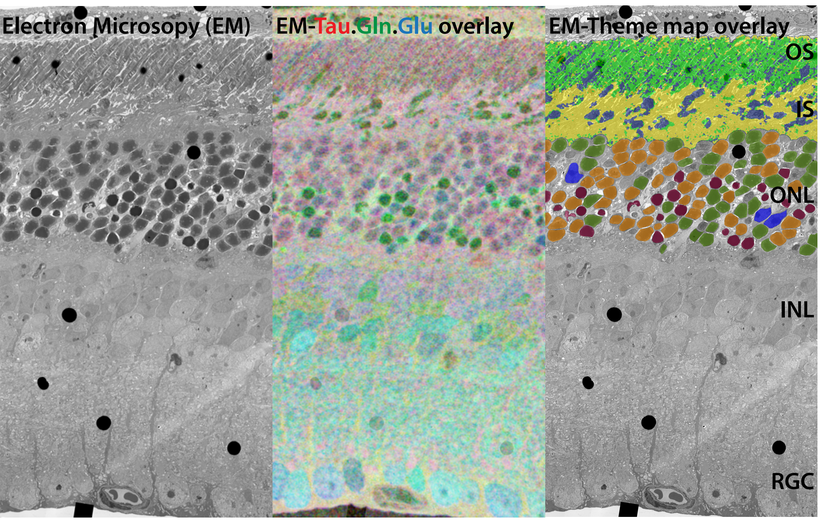
Ferrell WD, Vázquez-Chona FR, Jones BW & Marc RE
Moran Eye Center University of Utah

Photoreceptors function in an oxidatively challenging environment and are vulnerable to metabolic and energetic imbalances. Yet, in-depth profiling of photoreceptor metabolism during the degenerative process remains incomplete. We relate the classic sequelae of photoreceptor degeneration and glial activation to small molecular metabolic signals using computational molecular profiling (CMP).

CMP explores metabolite concentration with high spatial resolution and high dimentionality



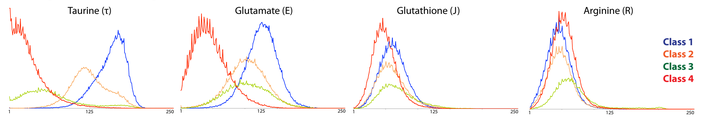
Light-induced oxidative damage induces metabolic diversity within the photoreceptor population



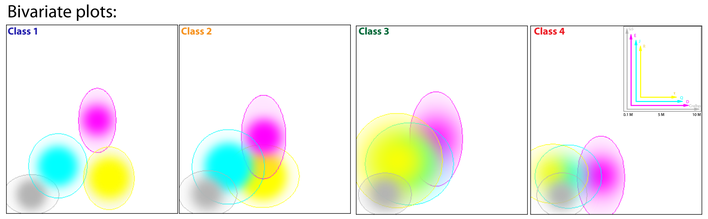
Ultrastructure and metabolic analyses of albino BALB/c retina after 24 h light damage. EM mosaic represents nearly 200 tiles at 5000x. Taurine.Glutamine.Glutamate (RGB) composite overlaid on EM has an opacity of 60%. Theme map of photoreceptor metabolic profiling using CMP, see next section.

Photoreceptor degeneration sequela is parsable into quantifiable metabolic profiles

Histograms showing number of pixels vs pixel intensity:

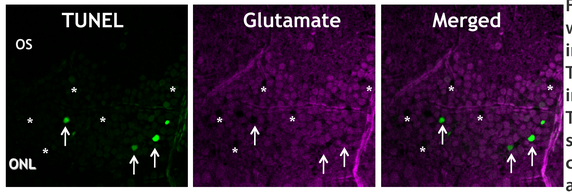


Bivariate plots:



Molecular profiles were derived using k-means algorithm. Each profile can produce a histogram for their respective metabolite or proteomic signal. Stacked bivariate histograms describing metabolic diversity within the photoreceptor cohort. Each plot represent eight signals mapped on x-y concentration coordinates. Each plot spans estimated concentration range 0.1-10 mM.

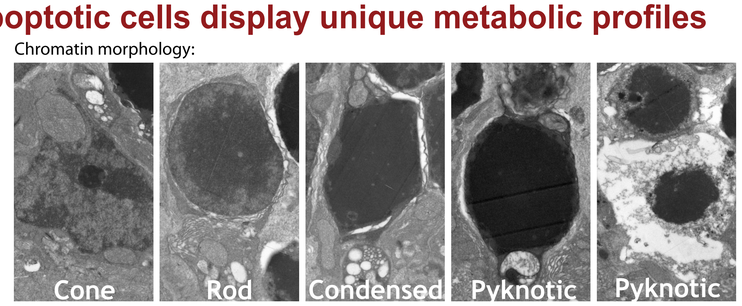
Glutamate depletion correlate with DNA fragmentation



Fluorescent TUNEL labeling in green with Glutamate staining in magenta in LR white resin. Arrows indicate TUNEL$^+$/Glutamate$^-$ cells. Asterics indicate Tunel$^-$/Glutamate$^-$ cells. These findings suggest that TUNEL staining is a late DNA indication of cell death events that are more acutly represented in the metabalome.

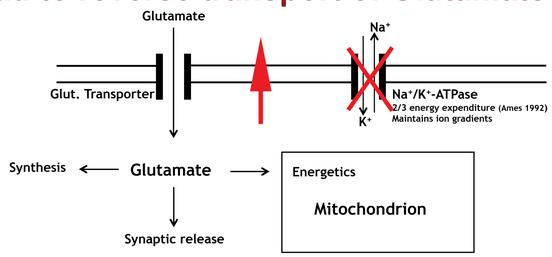
Surviving cones & rods as well as degenerating & apoptotic cells display unique metabolic profiles

Chromatin morphology:



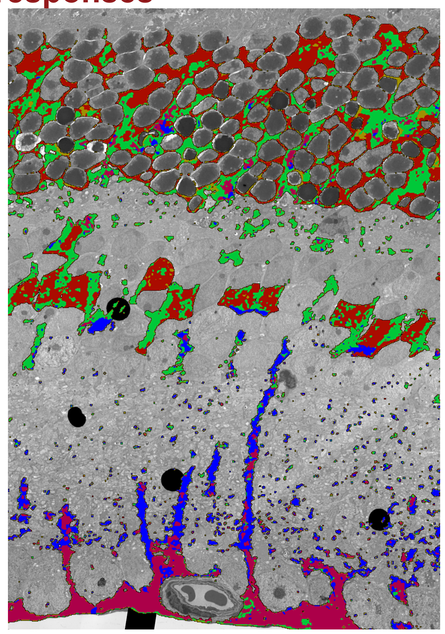
metabolic profile	N	cone chromatin	rod chromatin	condensed chromatin	pyknotic nucleus
τ, E, J	38	38	0	0	0
↓τ, J	150	3	137	11	1
↓↓ τ, E, J	133	3	64	66	8
↓↓↓τ, E, J	369	1	35	333	305

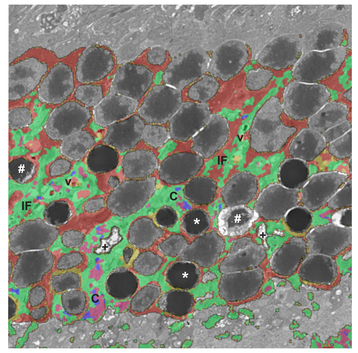
Na+/K+ ATPase collapse may decrease ion gradients and lead to reverse transport of Glutamate & Taurine

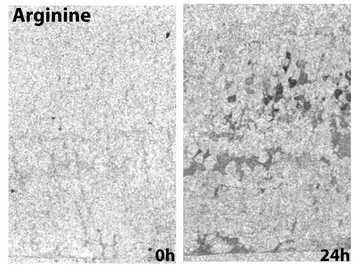


Corroborating evidence:
Sodium channel blockers reduce glutamate release (Torp et al., 1993)
Increasing extracellular K+ concentration increases glutamate release (Szatkowski et al., 1990)

Müller glia are highly sensitive to neuronal stress and respond to stress events with focal small molecular responses



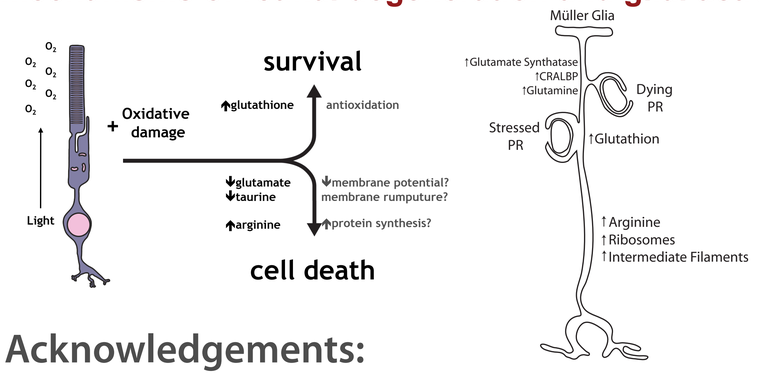




Class	Region	ultrastructure	signature
(1) pink	Endfeet	Ribosomes Intermediate filament	High: GS, E, Q, TT, CRALBP Moderate: J, R
	Near engulfed material	vacuoles	Low: Methionine (M)
(2) blue	IPL Trunk	Ribosomes Intermediate filament	High: J, E, Q, CRALBP Moderate: GS, TT, R Low: M, GABA,
(3) green	near 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

Metabolic theme map of responsive Müller glia. The theme map displays the results from clustering to extract molecular phenotypes. Pink areas represent rich expression of glutamate synthantase (GS) and CRALBP and rich levels of glutamate, glutamine, and taurine; moderate levels of glutathione and arginine; and low levels of methionine. Green areas displayed similar distributions but at lower levels. Blue areas represent high glutathione and low GABA concentration. Red areas have low glutamate sythantase expression.

Visualization of metabolic diversity can help predict mechanisms of retinal degeneration and glial activation



Acknowledgements:

Funding from: NIH Grants EY02576 (RM), EY015128 (RM), EY014800 Vision Core (RM), Research to Prevent Blindness Career Development Award (BWJ), Edward N. & Della L. Thome Memorial Foundation, Bank of America, N.A. Trustee (BWJ), NIH Dev Bio training grant HD07491 (FVC), International Retinal Research Foundation (FVC), Knights Templar Eye Foundation (FVC), Fight for Sight (FVC & WDF), Undergraduate Research Opportunities Program (WDF). Special thanks to Jai-Hui Yang, Carl Watt, James Anderson, and Maggie Shaw for their technical support.