BIOCHEMICAL SIGNATURE ANALYSIS OF THE HUMAN GANGLION CELL LAYER

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Purpose: The goal of this work was to extend previous signature analyses of fish and rabbit ganglion cell (GC) populations to human retinas, defining in greater detail the small molecule GC signatures in humans.

Methods: Glutaraldehyde/formaldehyde fixed human retinas were resin embedded and serial horizontal thin sections quantitatively probed with IgGs for glutamate, aspartate, alanine, glutathione, glutamine, taurine, GABA, and glycine, followed by registration and pattern recognition analysis.

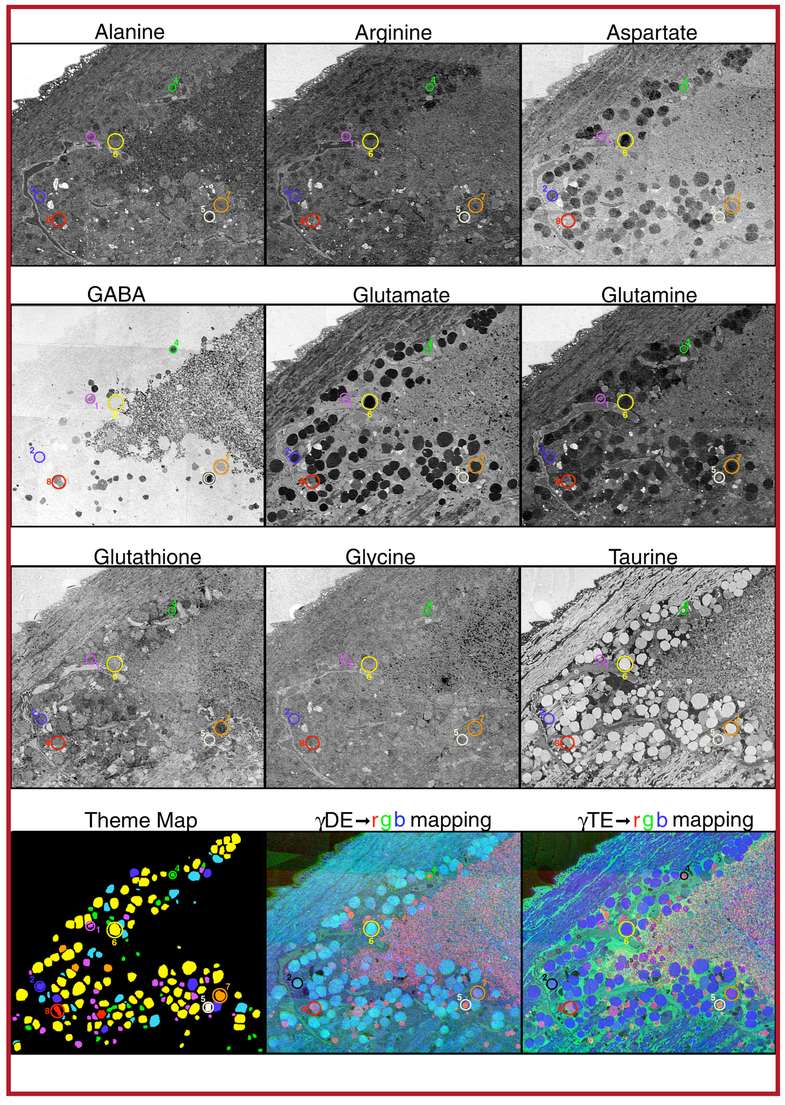


Fig1: Cells are classified according to their amino acid content revealing distinct metabolic functional groupings. This frame is a subset of a larger sample encompassing 441 cells in the GC layer. Peripheral retina was taken post mortem, glutaraldehyde fixed, and resin embedded. Serial 200 nm sections were quantitatively probed for 9 selected amino acids, visualized with silver intensification, and digitally captured. Images were then registered and classified. Nine classes were identified with five belonging to ganglion cells, three classes belonging to amacrine cells and one class structurally present, but metabolically empty, therefore appearing to be pathologic. Ganglion cell classes separate according to their (1) degree of amacrine cell coupling; (2) intrinsic GABA content; (3) overall metabolic phenotypes. Amacrine cell classes separate according to taurine content, the levels of GABA and glutamate, and their overall metabolic phenotype.

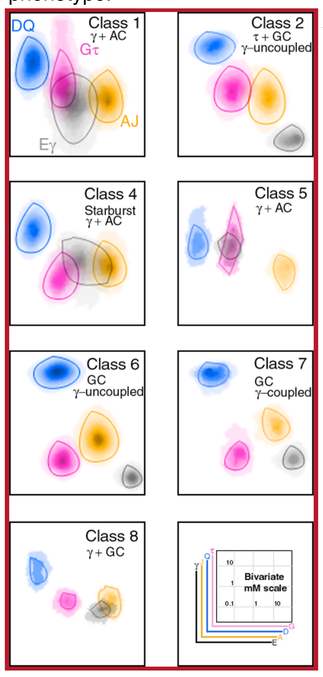
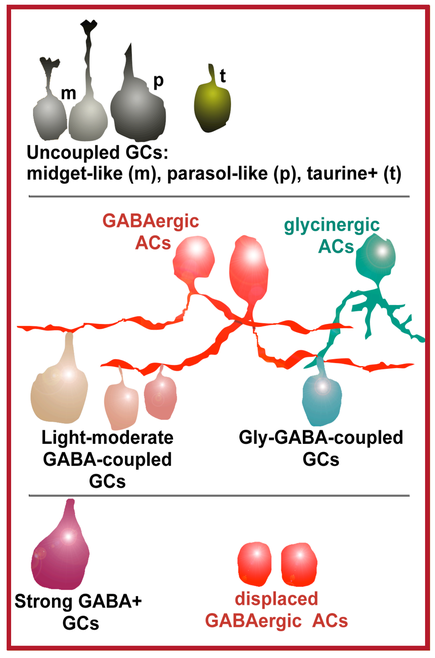


Fig. 2. Each class displays a unique molecular phenotype visualized as a hyper-dimensional metabolic phenotype map. Each class is represented by 4 superimposed bivariate density plots. Each two dimensional data cloud is enclosed by 2 SD borders. The x,y axis pairs are glutamate,GABA (Eγ); alanine, glutathione (AJ); aspartate, glutamine (DQ) ; glycine, taurine (Gτ); spanning 0.1 - 10 mM. Classes one, four and five correspond to amacrine cells with Class four comprising the starburst amacrine cells population. Class five contains the highest level of GABA of any cell in this analysis. Class two and class six are GABA uncoupled ganglion cells with class two distinguished by having a higher taurine content than class six. Class seven comprises the ganglion cell cohort that is GABA coupled, while class eight contains those ganglion cells that contain the highest levels of GABA present in ganglion cells. This concentration of GABA approaches levels found in GAD+ starburst amacrine cells indicating the possible presence of GAD in this cohort. Class three appeared to be pathological and therefore was not displayed in this figure. Class nine (Not Shown) comprises rare GABA-glycine coupled ganglion cells present at perhaps 1-2% of the total ganglion cell population.



Results: At least four superclasses of ganglion cells in human retina can be parsed according to their amino acid contents visualized with N-dimensional signatures. One large polymorphic superclass is distinguished by absence of GABA/glycine signals. A second polymorphic superclass possesses weak GABA signals that indicated heterologous coupling with GABAergic amacrine cells (ACs). A small third GC superclass has relatively high levels of GABA (but with a signature distinct from GABAergic ACs) suggesting that these cells may actually synthesize GABA. A final GC class has a distinctive glycine signal suggesting unique coupling with a glycinergic AC population. In addition, three classes of amacrine cells can be distinguished. Starburst amacrine cells are easily distinguished by their metabolic phenotype in addition to being separable according to their number, size and distribution. The second class of amacrine cells is separated according to their taurine content, while the third AC class separates from starburst according to their taurine content and their high amounts of GABA.

Conclusion: Metabolic phenotyping of cells in the ganglion cell layer of the human retina can reveal distinct metabolic classes based on probes generated towards amino acids. Human ganglion cell cohorts possess diverse signature classes indicative of functional grouping of cells. Some of these signatures, such as glycine AC-coupled GCs, are shared with species as diverse as cyprinids. Further refinement of classes may be obtainable through the use of AGB mapping.