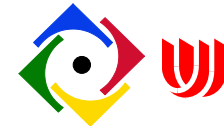


THE MOLECULAR SIGNATURES OF RETINAL GANGLION CELLS

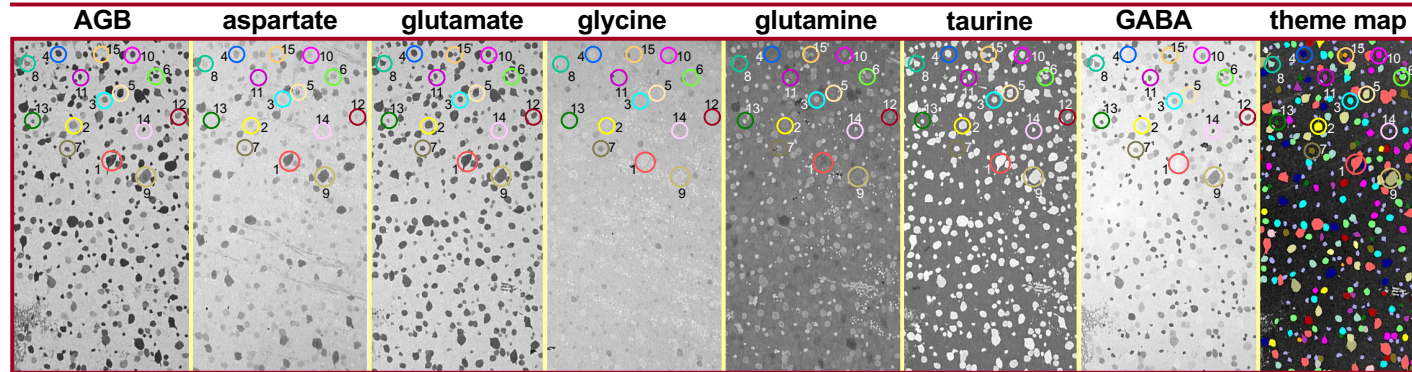
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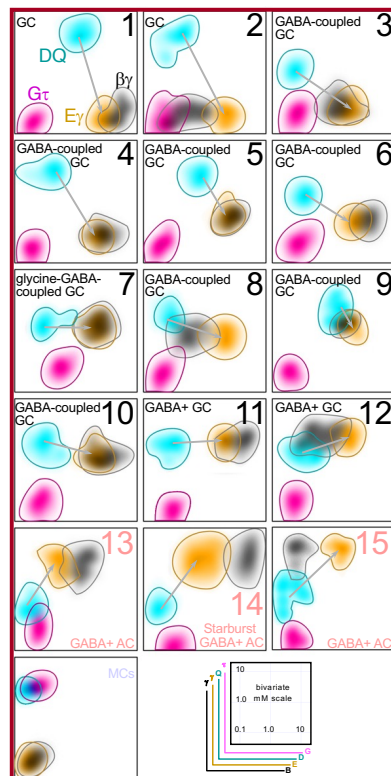


Purpose: The goal of this work was to define the molecular signatures (phenotypes) of all cells in the ganglion cell layer of the rabbit retina.

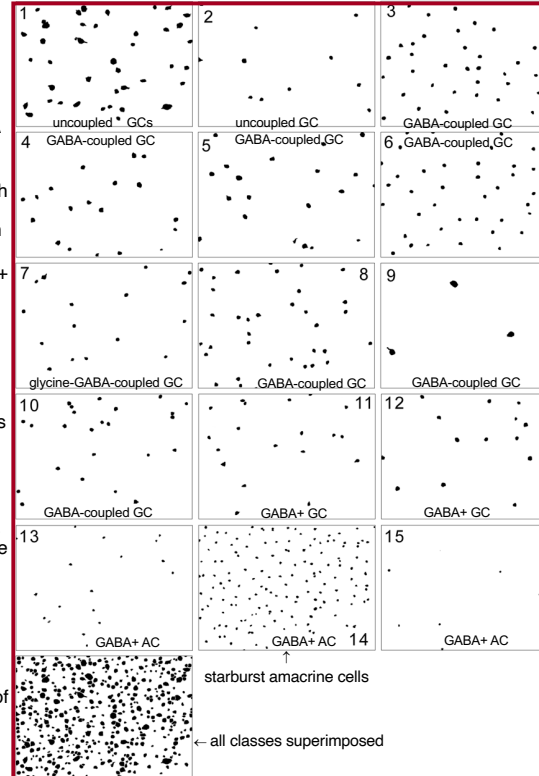
Methods: Pieces of rabbit retina incubated in Ames medium + AGB (1-amino-4-guanidobutane, a marker of ionotropic glutamate channel activation) were activated with glutamate agonists followed by glutaraldehyde quenching. Molecular signatures were extracted for every cell in serial thin horizontal sections by probing with IgGs targeting AGB, GABA, aspartate, glutamate, glutamine, glycine and taurine, followed by registration and classification using the ISOCCLUS algorithm.



← Fig. 1. Each molecular signal displays a unique pattern; classification algorithms reveals the underlying statistical classes of cells. A patch of retina on the midline ≈ 3 mm below the visual streak was activated with 25 μ M AMPA to initiate AGB permeation. After glutaraldehyde quenching and resin processing, serial 200 nm sections were quantitatively probed for 7 molecular species, visualized as density-scaled silver images, registered and classified. Fifteen major cell classes accounting for $>98\%$ of all cells in the ganglion cell layer were identified. Twelve classes were identified as ganglion cells and three as amacrine cells based on their characteristic metabolic phenotypes. Ganglion cell classes differ in (1) the strengths of their AMPA responses; (2) their degree of amacrine cell coupling; (3) their intrinsic metabolic profiles.



← Fig. 2. Each class displays a unique molecular phenotype visualized as an N-dimensional phenotype map or "pawprint". Each class is represented by 4 superimposed bivariate density plots. Each blob is delimited by 2 SD borders. The x,y axis pairs are AGB,GABA ($\beta\gamma$); glutamate,GABA ($E\gamma$); aspartate, glutamine (DQ); glycine, taurine ($G\gamma$); spanning 0.1 - 10 mM. Presumed GCs are characterized by a high glutamate signal (right displacement of the $E\gamma$ blob) and a glutamine content similar to or much higher than the glutamate content (upward displacement of the DQ blob). Presumed GABA+ amacrine cells have a lower glutamate level and a much lower glutamine level than ganglion cells. Thus most $DQ \rightarrow E\gamma$ vectors are downward-to-level in ganglion cells and all $DQ \rightarrow E\gamma$ vectors are upward in GABA+ amacrine cells. Class 1 and 2 ganglion cells show no coupling to GABA+ amacrine cells, while classes 3-10 show progressively increased coupling strengths. Class 7 is notable for its coupling to both GABA+ and glycine+ amacrine cells. Class 11 and 12 ganglion cells have high GABA levels and may correspond to the GAD+ ganglion cells described by Yu *et al* (1985). Class 14 cells are starburst amacrine cells, while classes 13 and 15 are two sparse amacrine cell populations. Each class is also characterized by the strength of its responsivity to AMPA. For example, starburst amacrine class 14 cells and class 1 ganglion cells are the most responsive of all cells. Classes 2 and 8 are the least responsive of ganglion cells. Class 15 GABA+ presumed amacrine cells are unique in having no measurable response to AMPA.



← Fig. 3. Parsing the theme map into component classes reveals the unique size and pattern groupings of each class. Though it is not possible to deconvolve the total size histogram of the ganglion cell layer to discover all the cell classes, classification algorithms achieve this by discovering cell classes through their molecular phenotypes and then displaying their size classes.

Class	%	μ M	Diam	1 SD	cell type	size not different from class (p<0.01)
1	8.8	18.2	3.6	uncoupled GCs	5	
2	2.6	13.4	1.5	uncoupled GCs	3,6,7,8,10,11,12	
3	7.5	13.2	1.3	GABA-coupled GCs	2,7,8,10,12	
4	4.1	15.4	2.1	GABA-coupled GCs	5,12	
5	4.3	16.2	2.5	GABA-coupled GCs	1,4	
6	9.2	12.4	1.3	GABA-coupled GCs	2,7,10,11,12	
7	3.4	13.0	1.8	gly-GABA-coupled GCs	2,3,6,8,10,11,12	
8	7.5	13.4	1.1	GABA-coupled GCs	2,3,7,10,12	
9	0.9	22.8	2.1	GABA-coupled GCs	-	
10	5.2	13.0	1.2	GABA-coupled GCs	2,3,6,7,8,11,12	
11	4.1	12.3	1.1	GABA+ GCs	2,6,7,10	
12	3.2	13.9	1.9	GABA+ GCs	2,3,4,6,7,8,10	
13	3.9	8.3	1.1	GABA+ ACs	14,15	
14	32.7	8.0	1.0	GABA+ ACs	13,15	
15	1.7	6.9	2.3	GABA+ ACs	13,14	
total	99.1%					
465 cells						

Results: Molecular phenotyping demonstrates that $>98\%$ of the cells in the ganglion cell layer derive from 12 ganglion cell and 3 amacrine cell classes. Each emerges as a distinct size group with differential AMPA responses and spatial patterns. Four superclasses of ganglion cells can be parsed:

- Classes 1 and 2 are uncoupled.
- Classes 3-10 are pure GABA-coupled.
- Class 7 is mixed glycine-GABA-coupled.
- Classes 11,12 are strongly GABA+ and may be GABAergic.

Conclusion: Molecular phenotyping of ganglion cells reflects underlying functional classes. Of the 12 signature classes, at least one and perhaps three contain more than one functional class. This argues for no less than 13-15 functional types of rabbit retinal ganglion cells.