IONOTROPIC GLUTAMATERGIC DRIVE HISTORIES OF AMACRINE CELL LAYER NEURONS REPORTED BY 1-AMINO-4-GUANIDOBUTANE (AGB) *IN VIVO*

B.W JONES, J. HOWARD, A. BEG, C.B. WATT and R.E. MARC. MORAN EYE CENTER, UNIVERSITY OF UTAH

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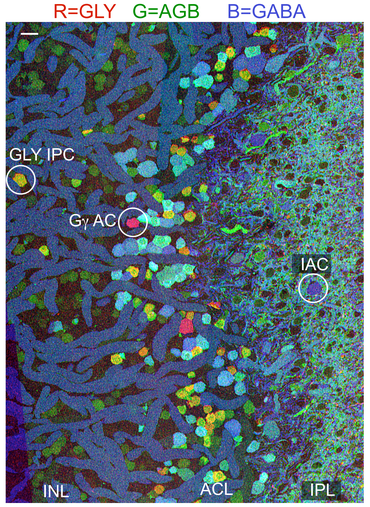
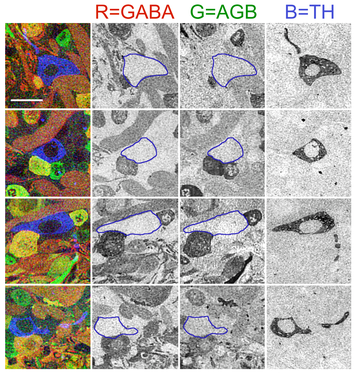
Purpose: The goal of this work was to classify cells in the amacrine cell layer (ACL) by their neurochemical signatures and their *in vivo* ionotropic glutamate receptor (iGluR) function as measured by AGB permeation. Over 70 cyprinid amacrine cell (AC) / interplexiform cell (IPC) morphs are known. In spite of three decades of physiological analysis, few descriptions of synaptic drive exist for them. Most ACs are posited to be driven by bipolar cell (BC) inputs, but evidence for this is limited, as is any description of the natures of the iGluRs they bear. The organic cation AGB permeates most mammalian iGluR-gated channels and can be applied *in vivo* to assay the excitation history of all retinal cells, while preserving characteristic neurochemical / metabolic signatures.

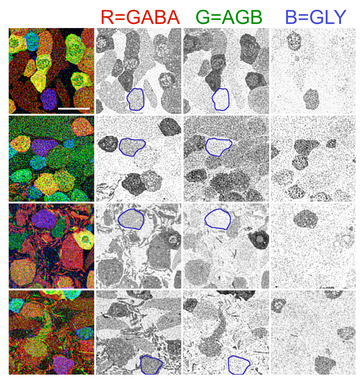
Methods: Adult, light-adapted goldfishes (*Carassius auratus*) and koi (*Cyprinus carpio*) were anesthetized briefly with MS-222 until respiratory movements ceased and then injected intravitreally with 7-10 μl of 130 mM AGB, yielding an estimated 5-10 mM intraocular AGB. The animals recovered within 5 minutes in normal aquarium water and were permitted to swim for 60 minutes in ambient room light (fluorescent source ≈ 2.5×10^4 photons/s/μm², 400-700 nm). The animals were anesthetized, sacrificed by cervical transection, eyes removed, and eyecups fixed in 2.5% glutaraldehyde, 1% paraformaldehyde, 3% sucrose in 0.08 M phosphate buffer. The tissues were processed for conventional epoxy resin embedding, serially sectioned at 250 nm in vertical and horizontal planes and serially probed with IgGs selective for AGB, γ-aminobutyrate (GABA or γ), glycine (G), glutamate (E), and tyrosine hydroxylase (TH). Signals were visualized with silver-intensified gold reagents (1nm gold streptavidin after biotinylated 2° IgG or 1 nm gold 2° IgG) or Cy3™ 2° IgG fluorescence. Signals were captured with a calibrated CCD camera at resolutions of 2.08-10.4 pixels/μm, digitally mosaicked/registered with GCPWorks (PCI Remote Sensing, Arlington VA), signals analyzed with Image Pro Plus (Media Cybernetics, Silver Spring, MD), and images assembled in PhotoShop® 5.0 (Adobe Systems Inc., San Jose, CA). Scale bars on all images = 10 μm.



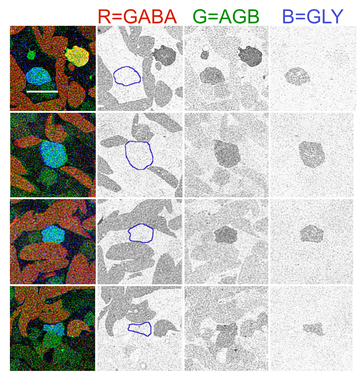
Results [1]: ACs / IPCs exhibit diverse iGluR-mediated excitation histories
This image is a registered triplet of three serial 250 nm sections, viewed at 4.2 pixels/μm, with glycine / AGB / GABA → *rgb* mapping. The frame is a subset of a larger sample encompassing 1183 ACs. The green AGB signal represents the *in vivo* excitation history of varied cell types. The intercellular variation in AGB signal strength arises from differences across classes of cells, rather than extreme variation within a class, each class member possessing a signature characteristic of its class. In this image, one can observe glycinergic IPCs, Gγ ACs, interstitial ACs, and a broad range of glycine+ and GABA+ ACs. Some neurons possess strong excitation signals, while others clearly possess very weak signals.



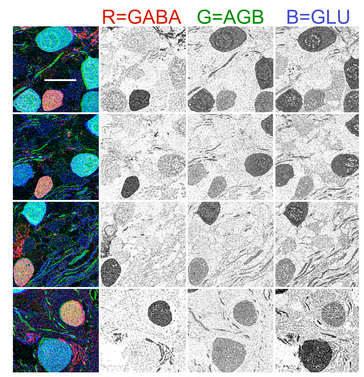
Results [2]: Dopaminergic IPCs lack iGluR-mediated excitatory drive
Cyprinid dopaminergic IPCs, identified as TH+ profiles in the AC layer (ACL) are sparse, with densities of 50-90 mm². The panel above shows four instances from a collection of >20 DA IPCs. In each horizontal strip, the left tile is intensity-scaled, GABA / AGB / TH → *rgb* mapped and the following tiles are the corresponding monochrome, density-scaled images of GABA, AGB and TH signals. DA IPCs remain unlabeled under normal photopic drive *in vivo* even though neighboring ACs are strongly activated. Do DA IPCs lack iGluRs altogether or simply lack AGB-permeant channels? Previous studies of [³H] DA release in cyprinid and reptilian retinas indicate that iGluR agonists are ineffective at stimulating DA release. We conclude that iGluR-mediated transmission is not part of the signaling received by dopaminergic IPCs.



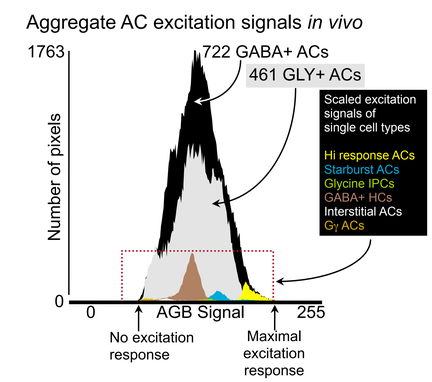
Results [3]: Gγ ACs lack iGluR-mediated excitatory drive
Cyprinid Gγ ACs are a sparse population of strongly glycine+, moderately GABA+ ACs that comprise 1.5-2% of all glycinergic neurons. Their connections and light responses are unknown. The panel above shows four instances from a collection of 10 Gγ ACs. In each horizontal strip, the left tile is intensity-scaled, GABA / AGB / GLY → *rgb* mapped and the following tiles are the corresponding monochrome, density-scaled images of GABA, AGB and GLY signals. No AGB permeation is activated by photopic drive *in vivo*. Do Gγ ACs lack iGluRs altogether or simply lack AGB-permeant channels? This can be assessed by using 1 mM glutamate or 10-100 μM kainate depolarization activate transport-mediated efflux from retinal neurons *in vitro*. After such stimulation, Gγ ACs are still present, while almost all other glycine+ cells are absent. Thus, Gγ cells lack any iGluR-driven excitation.



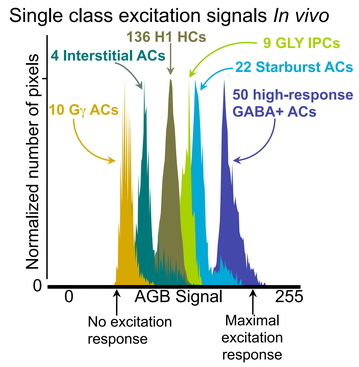
Results [4]: Glycinergic IPCs possess iGluR-mediated excitatory drive
Glycinergic IPCs are glycine+ neurons with large somas located in mid-INL at very low densities (20-50 mm²). The natures of their connections in the IPL are largely unknown and there have been no known recordings from them. The panel at left shows four instances from a collection of 9 glycinergic IPCs. In each horizontal strip, the left tile is intensity-scaled, GABA / AGB / GLY → *rgb* mapped and the following tiles are the corresponding monochrome, density-scaled images of GABA, AGB and GLY signals. Every cell has moderate AGB signals indicating that part of the input to glycinergic IPCs must arise from BCs and iGluR-gated channels. These cells also receive extensive somato-dendritic input from GABAergic H1 HCs. The natures of their light responses remain unknown and cannot be inferred from current data.



Results [5]: Cyprinid Starburst ACs possess iGluR-mediated drive
Many recordings have been made from mammalian starburst ACs and ACh release in the rabbit retina is clearly driven by iGluRs. There are no known recordings from cyprinid starburst ACs. However, displaced starburst ACs are the only GABA+ neurons in the cyprinid GC layer. The panel at left shows four instances from a collection of 22 starburst ACs. In each horizontal strip, the left tile is intensity-scaled, GABA / AGB / GLU → *rgb* mapped and the following tiles are the corresponding monochrome, density-scaled images of GABA, AGB and GLU signals. A consistent, AGB permeation is activated by photopic drive *in vivo*. All starburst ACs show similar iGluR-mediated signals, though other nearby GCs and other GABA+ ACs in the ACL have quite different signal strengths.



Results [6]: The *in vivo* photopic excitation patterns of AC populations are diverse.
The response histograms for the entire GABA+ and glycine+ cohorts are clearly aggregates of responses from many small AC populations with distinct, restricted response ranges (see below).



Results [7]: The *in vivo* photopic excitation patterns of single neuronal classes are precise.
Sampled individually, each distinct neuronal class exhibits a characteristic excitation history. For example, Gγ ACs show no evidence of excitatory drive through AGB-permeant channels gated by iGluRs. A well-known class, starburst ACs exhibit strong endogenous signals. A distinct cohort of GABA+ ACs possesses the highest response strengths in the ACL, but their morphological and physiological identities are yet unknown.

Conclusions: The "AGB response" of an individual neuron *in vivo* is determined by (1) the number and unitary conductances of iGluR-gated, AGB-permeant channels, (2) the strength and temporal properties of endogenous glutamate drive. It is not possible to untangle these two features for all cells, but some conclusions can be drawn for some cell types and explicit hypotheses generated for others. For example, if two cell types can be demonstrated to have similar iGluRs but differ in their AGB responses *in vivo*, one could reasonably posit that their excitatory drive histories were different - more explicitly, sustained cells may have stronger AGB responses than transient cells. Taken together, these individual examples show that identified neurons have consistent and homogenous AGB signals under photopic drive *in vivo*. By further considering glutamate-agonist activated release experiments, we make the following assessments of specific cell groups in the cyprinid retina:

[1] Dopaminergic IPCs lack iGluR-mediated drive

[2] Gγ ACs lack iGluR-mediated drive

[3] Glycinergic IPCs possess iGluR-mediated drive

[4] Starburst ACs possess iGluR-mediated drive

[5] Interstitial ACs have very weak AGB permeation and possess either iGluR2(R)-like channels or extremely transient excitation histories.

[6] A new type of GABA+ neuron in the INL possesses iGluR-mediated drive

[7] A small cohort of GABA+ ACs possess the strongest iGluR-mediated drive in the ACL

[8] Most ACs appear to possess some iGluR-mediated drive, but some GABA+ ACs display extremely weak AGB permeation, and they may either lack iGluRs or possess AGB-impermeant iGluR-gated channels.