IONOTROPIC GLUTAMATERIC 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

Abstract 2321 Support: NIH EY02576 and Research to Prevent Blindness

Purpose: The goal of this work was to classify cells in the amacrine cell layer (ACL) by their neurochemical signatures and their in vivo ionotrophic glutamate-gated (iGluR) function as measured by AGB permeation. Over 70 cyprinid amacrine cell (AC) or 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 poised to be driven by bipolar cell (BC) inputs, but evidence for this is limited, as is any description of the nature 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 = 2.5×10^6 photons/µm², 400-700 nm). The animals were anesthetized, sacrificed by cervical transection, eyes removed, and eyeballs 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 µm in vertical and horizontal planes and serially probed with IgGs selective for AGB, glycin (GABA or G), glycine (G), glutamate (E), and taurine dihydroxylase (TH). Signals were visualized with silver-intensified gold reagents (110 µg/ml tetrathionate in 2% IgG or 1 ml 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: [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 TH+ DA release in cyprinid and reptilian retinas indicate that GABA 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] G+ ACs lack iGluR-mediated excitatory drive
Cyprinid G+ ACs are a sparse population of strongly glycine+, moderately GABA+ ACs that comprise 1-5% of all glycine+ neurons. Their connections and light responses are unknown. The panel above shows four instances from a collection of 10 G+ 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 G+ ACs lack iGluRs altogether or simply lack AGB-permeant channels? This can be assessed by using 1 µm glutamate or 10-100 µM kainate depolarization activate transport-mediated efflux from retinal neurons in vitro. After such stimulation, G+ ACs are still present, while almost all other glycine+ cells are absent. Thus, G+ cells lack any iGluR-driven excitatory response.

Results: [4] Glycinergic IPCs possess iGluR-mediated excitatory drive
Glycinergic IPCs are glycine+ neurons with large somas located in mid- and lower densities. These neurons 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 glycineergic IPCs must arise from BCs and GABA-gated channels. These cells also receive extensive somato-dendritic input from GABAergic H1 HCs. The nature of the 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 AGB release in the rabbit retina is clearly driven by iGluRs. There are also known recordings from cyprinid starburst ACs. However, displaced starburst ACs are the only iGluR-gated 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 / GLY → rgb mapped and the following tiles are the corresponding monochrome, density-scaled images of GABA, AGB, and GLY 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 consistent phototransduction history. For example, G+ 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, while their morphological and physiological identities are yet unknown.