Published in final edited form as:

Vis Neurosci. 2009 ; 26(4): 349–363. doi:10.1017/S0952523809990113.

A spectral model for signal elements isolated from zebrafish photopic electroretinogram

Ralph Nelson¹ and Nirmish Singla^{1,*}

¹Basic Neurosciences Program, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Rockville MD 20892

Abstract

The zebrafish photopic ERG sums isolatable elements. In each element red, blue, green and UV (r, g, b, u) cone signals combine in a way that reflects retinal organization. ERG responses to monochromatic stimuli of different wavelengths and irradiances were recorded on a white, rod suppressing background using superfused eyecups. Onset elements were isolated with glutamatergic blockers and response subtractions. CNQX blocked ionotropic (AMPA/kainate) glutamate receptors; L-AP4 or CPPG blocked metabotropic (mGluR6) glutamate receptors; TBOA blocked glutamate transporters; and L-Aspartate inactivated all glutamatergic mechanisms. Seven elements emerged: photopic **PIII**, the L-Aspartate-isolated cone response; **b1**, a CNQX-sensitive early b-wave element of inner retinal origin; **PII**, a photopic, CNQX-insensitive, composite b-wave element from ON bipolar cells; **PIIm**, an L-AP4/CPPG-sensitive, CNQX-insensitive metabotropic sub-element of PII; **PIInm**, an L-AP4/CPPG/CNQX-insensitive, non-metabotropic sub-element of PII; **a1nm**, a TBOA-sensitive, CNQX/L-AP4/CPPG-insensitive, non-metabotropic, post-photoreceptor a-wave element; and **a2**, a CNQX-sensitive a-wave element linked to OFF bipolar cells. The first five elements were fit with a spectral model that demonstrates independence of cone color pathways. From this $V_{\max}$ and half-saturation values (k) for the contributing r- g- b- and u-cone signals were calculated. Two signal patterns emerged. For PIII or PIInm the $V_{\max}$ order was $V_r > V_g \gg V_b \approx V_u$. For b1, PII, and PIIm the $V_{\max}$ order was $V_r \approx V_b > V_g > V_u$. In either pattern u-cone amplitude (V_u) was smallest, but u-cone sensitivity (k_{u362}) was greatest, some 10-30 times greater than r-cone (k_{r570}). The spectra of b1/PII/PIIm elements peaked near b-cone and u-cone absorbance maxima regardless of criteria, but the spectra of PIII/PIInm elements shifted from b- towards r-cone absorbance maxima as criterion levels increased. The greatest gains in $V_{\max}$ relative to PIII occurred for the b- and u-cone signals in the b1/PII/PIIm b-wave elements. This suggests a high-gain, prolific metabotropic circuitry for b- and u-cone bipolar cells.

Keywords

zebrafish; spectral sensitivity; b-wave; PIII; ON cone bipolar cells

Corresponding author: Ralph F. Nelson, Ph. D., Basic Neurosciences Program, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 5625 Fisher's Lane, Room TS-09, Rockville, Maryland 20892-9406, TEL (301) 496-8133, FAX (301) 480-3734, nelsonr@ninds.nih.gov.

Send reprints to corresponding author.

*Current Address: University of Michigan, Ann Arbor, MI.

Introduction

The need for component analysis of zebrafish electroretinogram

Zebrafish is a genetic model for visual system studies (Fadool & Dowling, 2008). More visually alert than mouse, in zebrafish, as in primates, color perception is very well developed. Forward-directed genetic research on zebrafish suggests that there are many important genes with undiscovered function for visual-system organization. These genes are revealed as developmental, neuroanatomical, behavioral, or electrophysiological deficits induced by random mutations. The zebrafish electroretinogram (ERG) is often screened in such studies, but is as yet a blunt tool, as there is no ERG component analysis for zebrafish as there is for cats (Granit, 1933; Sieving *et al.*, 1986; Robson & Frishman, 1995), mice (Robson *et al.*, 2004) and primates (Bush & Sieving, 1994; Sieving *et al.*, 1994; Robson & Frishman, 1998).

The ERG b-wave: a measure of ON bipolar-cell function

The ERG is the light evoked field potential generated by retina. It sums extracellular limbs of current loops set in motion by light stimulation of all retinal cells, producing net, light-evoked voltage changes at the retinal surface (Newman, 1980), culminating in a series of named ERG peaks and troughs. The challenge is to disentangle the various summations and cancellations among these current loops that produce the characteristic waveform so as to understand the physiology and circuitry of the various cell types of origin. The ERG b-wave, a vitreal positive going peak at light onset, is thought to be generated in large measure by currents circulating around ON-type retinal bipolar cells (Stockton & Slaughter, 1989). This is in part because of the disproportionately large bipolar-cell representation in the inner nuclear layer of the retina (Strettoi & Masland, 1995; Jeon *et al.*, 1998; Connaughton *et al.*, 2004), but also because of the especially long ON-bipolar-cell axon (Famiglietti *et al.*, 1977; Nelson, 1977) that provides a large radial dipole for extracellular currents circulating around these cells.

Bipolar cell pathways express unique glutamate-receptor types

ON bipolar cells employ unique synaptic glutamate receptors in contacts with rods and cones. These receptors are insensitive to the AMPA/kainate antagonists that inactivate most excitatory synapses in the nervous system. Because of this, synaptic currents of ON bipolar cells, and the b-wave responses they generate, can be isolated. Conversely the synapses between OFF type bipolar cells and photoreceptors employ AMPA/kainate type glutamate receptors common to retina and brain (DeVries, 2000). Light responses of OFF-type bipolar cells, horizontal cells, amacrine cells, and ganglion cells, are all blocked by AMPA/kainate antagonists, leaving photocurrents only from photoreceptors and ON-type bipolar cells. In many species ON bipolar cells express only mGluR6 in synapses with photoreceptors. mGluR6 is a type III metabotropic glutamate receptor, that is insensitive to AMPA/kainate antagonists, (Masu *et al.*, 1995), and continues to function even in the presence of such antagonists. In these species the light responses of ON-bipolar cells are completely blocked by agonists and antagonists of type III metabotropic receptors (Slaughter & Miller, 1981), as are ERG b-waves. In zebrafish and other cyprinids, however, both metabotropic and non-metabotropic, glutamate currents combine in ON-bipolar-cell function (Grant & Dowling, 1995, 1996; Connaughton & Nelson, 2000; Wong *et al.*, 2005b). This dual glutamate pathway is reflected in zebrafish b-wave responses (Saszik *et al.*, 2002a; Wong, 2004; Wong *et al.*, 2005a) which persist even in the presence of the metabotropic agonists and antagonists. Nonetheless, the remaining non-metabotropic b-wave, like the metabotropic b-wave, is insensitive to AMPA/kainate antagonists (Wong *et al.*, 2005a).

Combining wavelengths and drugs to dissect circuits

In zebrafish Saszik *et al* (2002a) found that L-AP4 (a type III metabotropic agonist) blocked the b-wave in wavelength-dependent fashion. Both modeling and raw data indicated ERG b-waves were greatly suppressed for blue and UV stimulation but less so for red and green, suggesting a u- and b-cone ON-bipolar circuitry biased towards metabotropic synapses. This opened the possibility that, through a combination of selective glutamate agonists and antagonists, and selective wavelength of stimulation, individual, cone-selective, ON bipolar pathways could be isolated in zebrafish ERG responses. In effect the 4 endogenous 'channel rhodopsins' of zebrafish retina, the r- g- b- and u-cone pigments (Robinson *et al.*, 1993), could be combined with selective glutamate blockers to probe circuitry. Dissection of the ON pathway was further aided by the existence of at least two different glutamate mechanisms in ON-bipolar-cells: a metabotropic mechanism and a non-metabotropic one, the latter based in a glutamate transporter-gated chloride current (Connaughton & Nelson, 2000) or I_{glu} (Grant & Dowling, 1995, 1996). There is a happy confluence of 6 endogenous handles that can be combined in various fashions to study circuitry using an evoked potential approach.

The zebrafish ERG revisited

The Bilotta laboratory measured classic spectral sensitivity functions for the native zebrafish b-wave and developed linear models based on pigment absorbance to analyze them (Hughes *et al.*, 1998). Perturbations in b-wave spectral properties by individual drugs such as L-AP4 were also investigated (Saszik *et al.*, 2002a), but no attempt was made to isolate the ERG elements arising from individual synaptic mechanisms, such as cone synapses with ON bipolar cells. In the present study we use non-linear models to characterize the spectral summation within isolated ERG elements. The membrane signals from cones, and ON-bipolar-cell contacts with cones, are identified through pharmacological blockade and response subtraction. The spectra of these ERG elements are modeled with sums of saturable Hill functions, one for each cone pigment mechanism. In Hill-type models of ERG-elements, the fit parameters V_{max} , the cone-color saturation amplitude, and k , the half-saturation amplitude are closely analogous to similar measures that might be made on cones and bipolar cells. V_{max} provides an index of maximal membrane current contributed by signals from each cone color type. In this way synaptic gains are estimated. K compares intrinsic sensitivity among signals arising from different cone pathways.

To isolate synaptic actions, we developed an in vitro, perfused eyecup preparation for adult zebrafish (Wesolowska *et al.*, 2002) that conveniently allows a layering of multiple drugs to isolate ERG elements. Through response subtractions among the waveforms generated by the layered drug treatments, isolated ERG elements generated by photoreceptors, ON bipolar cells, and inner retinal neurons can be calculated and modeled. Further both metabotropic and non-metabotropic contributions to the ON bipolar response are distinguished. The proposal for a selective synaptic circuitry and chemistry for different cone pathways is further tested, and some emergent properties of the zebrafish ERG are defined.

Materials and methods

Preparation of zebrafish retina-eyecups

Light-adapted adult zebrafish (*Danio rerio*, EK strain) were decapitated according to an Animal Study Protocol approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke, NIH, in accordance with National Research Council Guidelines and Public Health Service Policy on Humane Care and Use of Laboratory Animals. Fish were kindly donated by The Laboratory of Molecular Genetics, NICHD, NIH. Eyes were removed and mounted upright on squares of black nitrocellulose filter paper (Millipore, Billerica MA). Under a dissecting microscope (Leica MZ12-5, Bannockburn IL)

corneas were punctured with sharp iridectomy scissors; then cornea, iris and lens were cut away. Eyes, rinsed for 10 min in bubbled (95% O₂, 5% CO₂) minimum essential medium (MEM, Invitrogen, Carlsbad, CA), were transferred to a stimulation, recording, and perfusion chamber mounted on a microscope stage (Olympus BX51, Olympus America, Center Valley PA). Retina-eyecups were perfused at room temperature (22° C) from the vitreal side (0.3 ml·min⁻¹) through a 3.25 inch gel-loader pipetter tip (Thomas, Swedesboro NJ), leaving a semi-submerged preparation. Excess media was pumped out of the chamber at a rate of 2 ml·min⁻¹ (Pre-Set Volume Pumps Series I, The Lee Company, Westbrook, CT).

Optical Source and Calibration

The vitreal sides of retina-eyecups were illuminated through a 4× UV plan-apo objective and microscope epifluorescence illumination system. A Xenon source focused onto a liquid light guide through intervening filter wheels and shutter (Uniblitz, Vincent Associates, Rochester NY) provided the stimulation. Interference filters mounted on one wheel (Lambda 10-2, Sutter Instruments, Novato CA) provided wavelengths from 330-650nm at 40nm intervals (20nm half-band, Chroma Technology Corporation, Brattleboro VT). Stimulus attenuations from 0.0 to 4.5 log units (metallic ND, Andover Corporation, Salem NH) were provided by another filter wheel, with further attenuation of 1-3 log units attained by manually inserting filters in the path. Monochromatic illumination was quantified with a calibrated silicon detector (Newport Corporation, Irvine CA). Maximum irradiance in the plane of the retina ranged from 6.3 log hv·μm⁻²·s⁻¹ at 330nm to 8.0 log hv·μm⁻²·s⁻¹ at 490nm, though stimuli this bright were not delivered in the physiological protocol. Except for 330nm, quantal output was relatively flat.

A second liquid light guide, illuminated through a second port on the Xenon source, provided background stimulation. (3.9 ND filters, 0.05 μW·cm⁻², J16 Digital Photometer, Tektronix, Beaverton OR). The background was white, and adjusted to evoke a low-level ERG signal with photopic waveform. Xenon 'white' delivers approximately equal quanta across r- g- b- and u-cone absorbance peaks, but the optical elements in the background pathway were partially UV absorbent. The background was found sufficient to prevent dark-adaptation of rods, without significant adaptation of cones.

Stimulus protocol

The stimulus protocol consisted of 280 flashes of different wavelengths and irradiances delivered in a fixed sequence over a period of 15.8 min. At each wavelength and irradiance, 4 stimuli were averaged. An irradiance-response series of 7 or 14 points, covering a 3.0 log unit range in 0.5 log unit steps was acquired at each wavelength. In total the spectrum was covered with 70 wavelength-irradiance mean responses. Irradiance ranges were pre-adjusted to correspond to the dynamic range of the ERG at each wavelength. Peak irradiances ranged from 5.4 to 6.0 log hv·μm⁻²·s⁻¹. Flash duration was 300 msec and the repetition rate was 3.0 s. A 12 second pause between each wavelength's irradiance series allowed for recovery. Wavelengths were staggered in the order 650, 570, 490, 410, 330, 650, 610, 530, 450, 370nm.

Evoked potential (ERG) recording

The reference electrode was an 18 gauge chlorided silver wire placed in the chamber outflow pool. The recording electrode was a patch pipette, filled with 0.9% NaCl, filed to a ~100 μm tip, and mounted on the head stage of a DAM 80 AC differential amplifier (World Precision Instruments, Sarasota FL), set for 0.1 Hz (τ = 1.6 s) to 1 kHz bandpass, and for a gain of 10⁴. Amplifier output was digitized with a Digidata 1200 series interface and Clampex 8.2 software (Axon Instruments/Molecular Devices, Sunnyvale CA). Digitization was at 0.5 msec intervals for 2 s starting 400 msec before stimulus onset. The coefficient of variation in b-wave amplitudes among different preparations was wide (~0.4), nonetheless drug-evoked wave-shapes and preparation stability were similar regardless of preparation amplitude.

The spectral protocol was initiated through Clampex sequencing keys. A second DOS computer programmed in Quick Basic 7.0 (Microsoft, Redmond WA) was used to respond to Clampex TTL requests to trigger acquisition, and to send protocol commands to filter wheels and Uniblitz reflective shutters (Vincent Associates, Rochester NY). Data collection including all drug treatments required 3-4 hrs.

Drugs

Concentrated stock solutions of the drugs CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, Tocris Cookson Inc, Ellisville, MO), L-AP4 (L-(+) Amino-4-phosphonobutyric acid, Tocris Cookson Inc, Ellisville, MO), CPPG ((*RS*)- α -Cyclopropyl-4-phosphonophenylglycine, Tocris Cookson Inc, Ellisville, MO), TBOA (DL-*threo*- β -Benzyloxyaspartic acid, Tocris Cookson Inc, Ellisville, MO) and L-aspartic acid (RBI/Sigma, St. Louis, MO) were diluted by controlled injection into the main perfusion line and applied to the vitreal surface of the retina. Stock solutions of CNQX were 5 mM (dissolved in H₂O) and were diluted to 30-50 μ M at the vitreal surface. Stock solutions of L-AP4 were 5 mM, those of CPPG were 5-20 mM (both dissolved in 1N NaOH, then neutralized to pH 7). L-AP4 was diluted to 20-100 μ M at the vitreal surface; CPPG, to 120-180 μ M. Stock solutions of TBOA were 2.5-10 mM (dissolved in H₂O) and diluted to 20-40 μ M at the vitreal surface. Stock solutions of L-Aspartic acid were 500 mM (dissolved in H₂O and titrated to pH 7.0) and diluted to 3000-7000 μ M at the retinal surface.

Dilution of drugs

Dilution was accomplished by adjusting flows from syringe pumps. These were 1 cc disposable syringes with plungers advanced by motorized micrometers (Motor Mike Actuators, Newport/Oriel, Irvine CA). Actuators were driven in pulsed mode (12V, 5 msec) allowing for very slow speeds with delivery nearly proportional to pulse rate. Infusion of stock solutions, and resultant concentrations on the retina, could be varied in 8 steps by switch, each differing by a factor of approximately 2. The main perfusion flow of 0.3 ml/min was measured by drop counting, and pressure on the main perfusion reservoir adjusted by computer to maintain the drop rate constant. Delivered drug concentrations were calculated from the ratio of drug infusion rate to the main flow rate of the media. Between 10 and 30 minutes were allowed for equilibration of drugs with the retina, as monitored by ERG waveform changes.

Data analysis

Clampex protocol files containing averaged responses for each drug treatment were imported into Origin (v. 7.5, OriginLab, Northhampton MA) making use of the Origin Script language LabTalk. The slower processes of response subtractions and nonlinear-least-squares fitting (NLSF) were written in Origin C language. After importing, records were 'box-car' smoothed (33 point running average, covering one cycle of 60Hz line noise) and graphed. Peak amplitude of PIII (baseline to trough) and of b-wave elements (trough to peak) were measured and fit with the spectral model (Eq. 1). Both spectral-sensitivity and irradiance-response curves were generated from the model. Results from multiple experiments were compiled in a Filemaker database (Filemaker Inc, Santa Clara CA).

The spectral model

The zebrafish photopic ERG provides the unique challenge of modeling the circuitry integration of 4 vertebrate cone signals, r, g, b and u. As drug treatments used in this study heavily suppress the synaptic interactions through retinal interneurons, including those that might introduce non-linear interactions between cone signals, a simple summation model seemed worth testing and, in fact, fit the response-wavelength-irradiance datasets well.

We have chosen to model each isolated zebrafish ERG element as a sum of 4 Hill functions each with an exponent of one, and each with separate saturation nonlinearities. A similar model was used to quantify rod and r-cone summation in cat horizontal cells (Nelson, 1977).

$$V = \frac{V_r I}{I + k_{r570}/A_r(\lambda)} + \frac{V_g I}{I + k_{g480}/A_g(\lambda)} + \frac{V_b I}{I + k_{b415}/A_b(\lambda)} + \frac{V_u I}{I + k_{u362}/A_u(\lambda)} \quad (1)$$

Amplitudes (V) for the complete sequence of 70 stimuli of different wavelengths (λ) and intensities (I) were fit by this 8 parameter 3-dimensional model. The model in turn generated both irradiance-response curves (holding λ constant) and spectral-sensitivity curves (holding V , the criterion response level, constant). The parameters V_r , V_g , V_b and V_u are the saturated amplitudes for r- g- b- and u-cone signals. The parameters k_{r570} , k_{g480} , k_{b415} and k_{u362} are the half-saturating values of irradiance for each cone signal, as measured at the wavelength of maximal absorption as given by Robinson *et al* (1993). These values are sometimes referred to as 'sensitivities'. The absorbance functions of the r- g- b- and u-cones, $A_r(\lambda)$, $A_g(\lambda)$, $A_b(\lambda)$ and $A_u(\lambda)$, are each normalized to the peak wavelength for that cone. In equation 1 dividing each 'k' value by absorbance at a particular wavelength in effect adjusts the effective k at that wavelength for reduced off-peak absorbance efficiency.

The absorbance functions for r, g, b and u cones were calculated from absorption nomograms (Eq. 2).

$$\log[A(\lambda)] = \sum_0^n C_n \left(\frac{\lambda_{\max}}{\lambda} \right)^n \quad (2)$$

The coefficients for r-, g-, b-, and u-cone nomograms appear in Table 1. One nomogram is used for r, g and b cones (Hughes *et al.*, 1998). Palacios *et al* (1996) conclude that the u-cone nomogram in giant danio, a species related to zebrafish, is actually narrower than the nomogram for the other pigments. For this reason the Palacios u-cone data is used for the 362nm zebrafish u-cone pigment.

Response-wavelength-irradiance data were fit with the spectral model (Eq.1) using NLSF (Levenberg-Marquardt). There are the constraints on the stimulus ranges to which this model can be applied. We choose colors that included both peak and flanking wavelengths for each of the 4 zebrafish cones. Irradiances included response threshold and extended to 6.0 log $\text{hv} \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1}$ at r- and g-cone spectral peaks, and 5.4 log $\text{hv} \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1}$ at b- and u-cone spectral peaks. These maximal stimuli were from 1.6 to 2.3 log units greater than the semi-saturation values for the respective cone mechanisms as seen below, or from 96% to 99% saturating. Considering the whole stimulus protocol, at some point, each cone type is saturated. No stimulus saturated all cone mechanisms simultaneously, however. The model (Eq. 1) does not detect very small cone color signals, and sometimes fails to converge in the search for them. In the case of such small signals, the cone color was eliminated from the NLSF.

Results

Nomenclature for ERG elements and choice of spectral model

Among the ERG response waveforms evoked in this study with 5 drug treatments, and further in examination of the subtracted differences between them, 10 peaks or troughs were named. Seven of these are interpreted in terms of the isolated functions of cones, ON bipolar cells,

OFF bipolar cells, or inner retinal neurons, and five of these isolated functional elements were spectrally studied. For ease of reference, the colloquial names given these elements, their physiological interpretation, and a short summary of the way in which each is calculated appear in Table 2. The PII, PIII nomenclature is adapted from Granit (1933) who noted functional waveform changes similar to those seen below using progressive ether anesthesia.

The spectral model (Eq.1) is an independent summation model for cone signals within each functional element. It takes into account that most wavelengths, except for 650nm, stimulate multiple cone types. It assigns saturated amplitudes and sensitivities to each cone signal, and sums them so as best to fit all the 70 response-wavelength-irradiance data points. Irradiance-response curves presented below are based on this full, modeled dataset, rather than being based just on the 7 or 14 data points acquired for a particular wavelength. These irradiance-response curves are model predictions for a particular wavelength based mainly on the more extensive data acquired at other wavelengths. One of the model successes is fitting saturated-response values in the various ERG onset elements. These progressively increase as wavelength becomes shorter, as more saturable cone signals are recruited. This experimental fact in itself precludes other models, such as those with joint saturation for multiple cone signals. The resulting irradiance-response curves provide an accounting by visual inspection of the appropriateness of the model to the datasets. Another assessment of model performance is provided by R^2 .

The model (Eq. 1) can be plotted as spectral sensitivity. Cord sensitivity was chosen as an index. This is the criterion voltage divided by the irradiance required to meet this criterion at each wavelength. Cord sensitivity is a nanovolts per photon measure, with units of $\text{nV} \cdot \text{h}\nu^{-1} \cdot \mu\text{m}^2 \cdot \text{s}$. V_r was chosen to define the 4 criterion levels ($0.2V_r$, $0.4V_r$, $0.6V_r$, $0.8V_r$) so as to guarantee that sensitivity points could be calculated for all wavelengths.

PIII, the contribution of cones to the ERG

To determine the spectral sensitivity and the intensity response functions for zebrafish cones without interference from the retinal network we blocked all glutamatergic transmission with L-Aspartic acid (Sillman *et al.*, 1969b, a), herein supplemented with additional blockers. Sample responses at 450nm appear in Fig. 1A. These waveforms are representative of any of the 9 wavelengths tested. Photopic PIII responses so isolated are smoothly varying functions of irradiance. Maximum PIII negativity is plotted against quantal irradiance for 370nm, 450nm and 650nm (Fig. 1B), 3 of the 9 wavelengths tested. The continuous curves are model predictions (Eq.1) based on the 70 irradiance-response points garnered from all 9 wavelengths.

At low criterion voltages ($0.2V_r$, $0.4V_r$, and $0.6V_r$), spectral sensitivity (Fig. 1C) is maximal near u-cone peak absorbance, but flattens to a broad maximum near the g-cone absorbance peak for the highest criterion ($0.8V_r$). In 17 such spectra, maxima shifted from $440 \pm 13\text{nm}$ (mean and SE) at $0.2V_r$ to $509 \pm 10\text{nm}$ at $0.8V_r$. PIII parameter fits for this eyecup (Fig. 1D) reveal that the largest signal component arises from r cones (V_r). Note that the $0.8V_r$ criteria ($-10\mu\text{V}$), while near 650nm saturation for r cones, was not particularly large for PIII as a whole, as net saturation, the sum of V_{max} 's from all cone types, was $-24\mu\text{V}$ (Fig. 1D). The success of the sum-of-Hill-functions model (Eq.1) can be judged by the 0.99 R^2 value.

R-, b-, g- and u-cone signals within zebrafish PIII differ in half-saturation irradiances and in amplitude contributions to PIII (Table 3). Combining results from 17 preparations, g-cone saturated amplitudes were 40-50% that of r cones, while b- and u-cone saturated amplitudes were 10-20% that of r cones (Table 3, col. 2). The median g-cone signal was about 3 times more sensitive than for r cones. B- and u-cone signals were 20-30 times more sensitive (Table 3, col. 4). All the cone-signal V_{max} ratios (Table 3, col. 2) differ significantly ($P < 0.01$, t-test), except for b and u cones ($P > 0.05$, t-test). The half-saturating irradiances (Table 3, col. 3)

differed significantly among cone types ($P < 0.01$, t-test), except for b and u cones ($P > 0.05$, t-test). G, b, and u cones were significantly more sensitive than r cones (Table 3, col. 4).

AMPA/kainate block reveals b1 and b2, fast and slow b-wave elements

CNQX is an AMPA-kainate antagonist. Most glutamatergic synapses in the retina, except between cones and ON-type bipolar cells, the principal contributors to the ERG b-wave, were blocked by this antagonist. The blockade limits GABAergic and glycinergic synaptic activity as well, by blocking excitatory input to horizontal cells and amacrine cells. The use of CNQX at the outset limits ERG currents to just those contributed by ON bipolar cells and photoreceptors.

In the untreated ERG (Fig. 2A) there is a b-wave (b) at stimulus onset and a d-wave (d) at stimulus offset. CNQX had 4 effects: increasing the b-wave amplitude, delaying the b-wave peak (b2, Fig. 2B), inducing onset negativity (a1, Fig. 2B), and eliminating the d-wave. The increase in b-wave amplitude is thought to reflect suppression of subtractive OFF-bipolar cell signals, and suppression of inhibitory circuits involving horizontal and amacrine cells. Similar amplitude increases were previously noted in zebrafish (Wong, 2004) and originally reported in monkey (Bush & Sieving, 1994; Sieving *et al.*, 1994). The unmasking of an a-wave element (a1) and the delay in b-wave peak (b2) merit further study.

The AMPA/kainate driven ERG elements blocked by CNQX can be calculated by arithmetic subtraction of the traces in Fig. 2B from those of Fig. 2A. This gives the traces of Fig. 2C. It came as some surprise that, by this analysis, part of the b-wave rising phase (b1, Fig. 2C) involves AMPA/kainate receptors. The loss of this element accounts for the slower peak time in the remaining b-wave (b2, Fig. 2B). The next element blocked is a2, a slowly decaying negativity maintained throughout the stimulus ON phase. At stimulus offset, the d-wave is also blocked. The CNQX blockade of a2 and d (Fig. 2C) is interpreted as the expected suppression of AMPA/kainate-mediated light responses from OFF bipolar cells.

The CNQX-isolated b-wave (b2, Figs. 2B, 3A) is not a purely ON-bipolar-driven element, as cone PIII field potentials also actively summate within this response. In Fig. 3C this PIII cone component has been removed from b2 by subtracting PIII (Fig. 3B), recorded a little later in the drug treatment protocol for this eyecup. This leaves the isolated ON-bipolar signals (PII, Fig. 3C). Interestingly, the subtraction does not fully eliminate the a1 a-wave (Fig. 3A), at least half of which remains (Fig. 3C). This remaining a-wave element appears to be post-photoreceptor, and associated with ON-bipolar-cell activity.

Cone contributions to b-wave elements

The b1 and PII b-wave elements differ in time course, but are similar in response-irradiance and spectral characteristics. In figures 4A and 5A irradiance-response plots for 450nm or 370nm stimuli yield near double the saturated amplitudes seen at 650nm. Spectral sensitivities (Figs. 4B and 5B) are b-cone and u-cone dominated, with peaks shifting little with changes in criterion. R and g cones contribute a low lying plateau at longer wavelengths. In 13 such spectral sets for b1, peaks followed the b-cone absorbance maximum shifting from $416 \pm 16\text{nm}$ at $0.2V_r$ to $421 \pm 15\text{nm}$ at $0.8V_r$ (means and SE's). Similarly in 15 such spectral sets for PII, peaks were also stable near the b-cone absorbance maximum shifting from $409 \pm 15\text{nm}$ at $0.2V_r$ to $425 \pm 13\text{nm}$ at $0.8V_r$. In accordance with the b-cone-tracking spectral peaks for both b1 and PII, the fit parameters for these elements (Figs. 4C and 5C) show $V_b > V_g$, and both $\log(k_{b415})$ and $\log(k_{u362})$ less than either $\log(k_{g480})$ or $\log(k_{r570})$.

Comparison of b1 and PII to PIII

The modeled cone signal composition within the b1 and PII b-wave elements is summarized and compared to PIII in Tables 4 and 5. As a group, modeled cone color signals within b1 and PII are 2-13 times larger in saturated amplitude than for PIII (col. 2, Tables 4 and 5). Sensitivity, as measured by the ratios of half-saturation irradiances (col. 4, Tables 4 and 5) was significantly increased over PIII for r and g cones. B-cone saturated amplitudes are 10-13 times larger than seen in PIII (col. 2, Tables 4 and 5). By comparison $V_{\max}$ gains for r-cone and g-cone signals are in the range 2-4. U-cone signals are intermediate with $V_{\max}$ gains of 6-7. Clearly ON pathways, as represented by b1 and PII, create b-cone signals of disproportionately large amplitude. This contributes to b-cone-tracking of spectral peaks for both b1 and PII, while relegating r-cone and g-cone contributions to shoulders on the spectral profile.

Metabotropic and non-metabotropic components within PII

In order to identify ON-bipolar sub-elements within b2 that might be mediated by the metabotropic receptor mechanism, and further, to identify non-metabotropic signals that might remain, L-AP4, a metabotropic glutamate agonist, was added to the perfusion mixture, while continuing to treat retinas with the AMPA/kainate blocker CNQX. A CNQX treated a1 and b2 response appears in figure 6A, similar to figures 2B and 3A. With the addition of L-AP4, a smaller biphasic waveform, composed of a-wave and a diminished b-wave, persisted (Fig. 6B). To view the L-AP4-blocked metabotropic elements in isolation, the non-metabotropic response (Fig. 6B) is subtracted from the CNQX isolated response (Fig. 6A). PII_m, an isolated b-wave element results (Fig. 6C). PII_m is the metabotropic b-wave, a sub-element of b2 that is blocked by L-AP4. No a-wave is associated with this element.

Not all the zebrafish b-wave is blocked by L-AP4 (Fig. 6B). A non-metabotropic, non-AMPA/kainate sub-element remains. This is disentangled from PIII in Fig. 7 by subtracting PIII, isolated by L-Aspartate a little later in the same preparation (Fig. 7B), from the response set seen in the presence of CNQX and L-AP4 (Fig. 7A). The result of this subtraction (Fig. 7C) is biphasic in waveform, composed of a1_{nm} and PII_{nm}. PII_{nm} is a non-metabotropic, non-AMPA/kainate, post-photoreceptor, b-wave element. Further, a1_{nm} is a non-metabotropic, non-AMPA/kainate, post-photoreceptor, a-wave element.

Spectral properties of PII_m

When PII_m irradiance-response functions for red, blue and UV stimuli are examined (Fig. 8A), saturated amplitudes in the blue (450nm) and UV (370nm) were found nearly triple those in the red (650nm). Correspondingly spectral sensitivity (Fig. 8B) is dominated by b- and u-cone absorbance, and peaks at 365nm for the 0.2V_r criterion, with red shift only as far as 390nm for the 0.8V_r criterion. Spectral contributions from r-cones and g-cones form only a low shoulder on the spectral profile. Similar results were obtained when CPPG, an mGluR6 antagonist (120-180 μM) was used in place of L-AP4, an mGluR6 agonist. In 6 such spectral sets for PII_m, peaks were intermediate between b-cone and u-cone absorbance maxima shifting from 375 ± 20nm at 0.2V_r to 390 ± 13nm at 0.8V_r (means and SE's). The model parameters (Fig. 8C) explain this with two factors, first the dominant b-cone saturation voltage (V_b), and second, the high sensitivity ($\log(k_{u362})$) for the u cone signal.

Comparison of PII_m with PII

As a sub-element of PII, it is not surprising that PII_m cone signal amplitudes are smaller than found for PII. The loss is selective, however, and limited to r-cone and g-cone signals, while b-cone and u-cone amplitudes remain about the same (col. 1, Tables 5 and 6). Correspondingly the r-cone and g-cone $V_{\max}$ gains relative to PIII are reduced to 1-2 for the PII_m element, as compared to 2-4 for PII (col. 2, Tables 5 and 6). The b-cone and u-cone signal gains of 10 and

7, respectively, are about the same in PII and PII_m. The changes in sensitivity for cone signals relative to PIII (col. 4, Tables 5 and 6) do not appear to differ among PII and PII_m. Owing to the relative rejection of r-cone and g-cone signals, the metabotropic PII_m sub-element represents a more cleanly b- and u-cone pathway than any of the other zebrafish ERG elements.

Spectral properties of the non-metabotropic PII_{nm} b-wave element

PII_{nm} response-irradiance functions (Fig. 9A) are similar to PIII. While the saturated amplitudes for blue (450nm) and UV (370nm) are larger than red (650nm), the difference appears less than double. The very large b cone and u cone signals seen in the metabotropic pathway are lacking. Spectral sensitivity (Fig. 9B) is more evenly distributed among cone signals. The PII_{nm} spectral profile (Fig. 9B) shifts in peak from 380nm for the 0.2V_r criterion, to 530nm for the 0.8V_r criterion. Similar waveforms and spectra for PII_{nm} were seen when CPPG, an mGluR6 antagonist (120-180 μM) was used in place of L-AP4, an mGluR6 agonist, to block metabotropic receptors. In 5 such spectral sets for PII_{nm}, peaks were intermediate between b-cone and r-cone absorbance maxima shifting from 459 ± 20nm at 0.2V_r to 527 ± 8nm at 0.8V_r (means and SE's). Contributions from r-cones and g-cones are very much in evidence. Modeled sensitivities (Fig. 9C, col. 3) for b- and u-cone signals are higher than for r- or g-cone signals, but V_{max} gains for b- and u-cone signals are low, so that the V_{max} distribution among cone types more resembles the PIII distribution ratio than the PII distribution ratio.

Comparison of PII_{nm} with PII and PIII

Cone V_{max} amplitudes within PII_{nm} are smaller than PII, consistent with PII_{nm} as a sub-element of PII (col.1, Tables 5 and 7). While r-cone and g-cone V_{max}'s were slightly reduced with respect to PII, b-cone and u-cone V_{max}'s were greatly reduced. The distribution of cone V_{max}'s (col. 1, Tables 3 and 7) is quite similar to PIII. Correspondingly V_{max} gains compared to PIII are in the range 2-4 for signals from r, g, b and u cones (col. 2, Table 7). As with other post-photoreceptor onset elements, sensitivities (col. 4, Table 7) are significantly increased compared to PIII only for r and g cones, but these sensitivity gains are smaller than seen in PII_m (col. 4, Table 6). For the PII_{nm} sub-element the processing of signals appears relatively uniform for all cone color signals.

Actions of TBOA, a glutamate uptake antagonist thought to block the non-metabotropic b-wave

Wong et al (2005b) suggest that zebrafish non-metabotropic b-waves are mediated through glutamate transporters and can be blocked by transporter antagonists. The actions of TBOA, a broad-spectrum, non transported antagonist appear in Fig. 10. A CNQX-isolated a1 and b2 response appears in figure 10A. A diminished and slowed b-wave (delayed b2) appears on addition of TBOA (Fig. 10B). The a1 component (Fig. 10A) is reduced 85% by TBOA treatment (Fig. 10B). This a1 blockade was seen in 4 of 5 treatments (one had no a1 element). Remaining a-wave elements are accounted for by PIII. The b2 amplitude is reduced by more than a factor of 2 (delayed b2, Fig. 10B). The time-to-peak for this delayed b2 is greatly increased. This was seen in all TBOA treatments (N = 5). Higher doses of TBOA further reduce or eliminate even the delayed b2 responses (not shown). Because of major alteration in response kinetics, the tool of response subtractions is not useful. It appears likely that TBOA diminishes and/or delays both PII_m and PII_{nm} elements, and can progressively and non-selectively alter many post-photoreceptor ERG elements.

Discussion

Zebrafish ERG elements and the implications of a spectral model

The existence of separable signals within the photopic ERG of zebrafish that can be attributed to isolated cellular mechanisms is confirmed. Seven onset elements are discerned in this study. Traditional elements are PII and PIII (Granit, 1933). Photopic PIII is an isolated, composite cone signal. Photopic PII is a non AMPA/kainate, composite b-wave element arising from ON bipolar cells. This can be separated into PIIm, a metabotropic signal element, and PIInm, a non-metabotropic signal element, by agonists or antagonists of metabotropic glutamate receptors. AMPA/kainate antagonists blocked two elements traditionally associated with OFF bipolar cells (Sieving *et al.*, 1994). These are a2, a maintained vitreal hyperpolarization, and the stimulus offset d-wave. Two novel onset elements emerged from this study. The first, b1, is a rising-phase b-wave element that is blocked by AMPA/kainate antagonists. The second is a1nm, an early a-wave element. The a1nm element arises neither from AMPA/kainate glutamate receptors nor from metabotropic glutamate receptors, but is blocked by antagonists of glutamate transporters.

An ERG spectral model based on summation of independent color processing circuits fits the pharmacologically and arithmetically isolated ERG elements well, and provides a cone signal component analysis from response-wavelength-irradiance data. The model shows no single photopic spectral sensitivity function in zebrafish ERG, even on neutral backgrounds, or for isolated ERG elements; rather, sets of nested spectral curves are generated with shapes and peaks that depend on criterion levels chosen. The pattern of nested spectral curves differ among the elements. The PIII cone-signal profile produces broad, flat spectral curves with peaks ranging from UV to green, as does PIInm. By contrast for PIIm, the metabotropic ON-bipolar cell signal, spectra are greatly enriched in b-cone and u-cone signals and in large part reject r-cone and g-cone signals, as implied by the work of Saszik *et al* (2002a). Spectral curves are sharply peaked between b-cone and u-cone absorbance maxima.

PIII, the cone field potential

PIII is decomposed into signals originating with r, g, b, or u cones. A ready hypothesis to predict saturated cone-signal amplitudes within PIII is that they reflect the densities of cone types. In zebrafish the cone mosaic is ordered. There are 6 cones in each repeat: 2 unequal double cones, a short single cone and a long single cone (Engstrom, 1960). The double cones are made up of a principal r-cone and an accessory g-cone. The long and short singles are b-cones and u-cones, respectively. This gives a ratio of 2:2:1:1 for r:g:b:u (Robinson *et al.*, 1993). On this stoichiometry, saturated r-cone and g-cone signal amplitudes within PIII should be equal, as should b-cone and u-cone amplitudes. The amplitude of the latter pair should be half that of the former. Though this idea accurately predicts greater amplitudes for r-cone and g-cone signals than for b-cone or u-cone signals, and the equality b-cone and u-cone signals, it does not predict that r-cone amplitudes are double those of g-cones, or 7 times those of b-cones or u-cones. Evidently intrinsic properties of each cone, not just their numbers, must also come into play.

The PIII decomposition also suggests that cones differ in intrinsic half-saturation. On this measure b- and u-cones are more sensitive than r-cones by about a factor of 20-30. G-cones appear 3 times more sensitive than r cones. Terrestrial solar irradiance at the u-cone peak is half that at the r- and g-cone peaks (Bird & Riorden, 1986), and even less on the short wavelength limb. Sensitive b- and u-cones might be an adaptation. The greater sensitivity of b- and u-cones is carried forward to all postsynaptic elements, giving blue and UV stimulation the threshold sensitivity edge in all ERG onset elements.

b1, an AMPA/kainate-mediated b-wave element

The earliest phase of the zebrafish b-wave is CNQX sensitive. This is b1. The origin might be inner retina. Horizontal cells utilize AMPA/kainate receptors as do OFF bipolar cells. Both cell types are hyperpolarized by AMPA/kainate antagonists, and the light responses are blocked (Hankins & Ikeda, 1991; Hensley *et al.*, 1993; Krizaj *et al.*, 1994; Nelson *et al.*, 2003). In experiments with horizontal cells hyperpolarized by adapting backgrounds, outer retina, post-synaptic cone signals increase in amplitude, but peak times shorten (Chappell *et al.*, 1985; Pflug *et al.*, 1990). On this argument the delayed peak-time of the CNQX-treated b-wave, which leads to the b1/b2 distinction, is not likely caused by CNQX-hyperpolarized horizontal cells, though the b2 amplitude increase might be. CNQX blocks amacrine responses with attendant loss of GABAergic and glycinergic inhibition of bipolar cells (Connaughton *et al.*, 2008). These actions are not expected to be fast enough to alter the rising phase of the ON-bipolar-cell response. We speculate that b1 results from synaptic currents in the inner retina of zebrafish, perhaps synapses onto amacrine and ganglion cells. In the mammalian rod system, AII amacrine cells are quicker in rise-time than rod-bipolar-cell inputs (Nelson, 1982; Dacheux & Raviola, 1986), and inner retina ERG responses are prominent for very dim stimuli (Sieving *et al.*, 1986). The zebrafish b1 element might be similar to positive scotopic threshold responses (pSTR) in mammals (Saszik *et al.*, 2002b; Bui & Fortune, 2004).

PII, a composite, non AMPA/kainate, ON-bipolar-cell response

Isolation of photopic PII, the ON-cone-bipolar contribution to the light adapted ERG, was pioneered in monkey retina by Sieving *et al* (1994). This group combined pharmacological blockades with response subtractions. We have used a similar approach to isolate zebrafish PII. The tetrachromatic cone signals that combine in zebrafish photopic PII are of particular interest for studies of color vision. Therefore we additionally studied the spectral properties of isolated PII signals.

The zebrafish PII element is composed of 2 sub-elements: one (PIIm) is blocked by the metabotropic glutamate ligands L-AP4 or CPPG, and the other (PIInm), a non metabotropic sub-element, is not. In the cyprinid *giant danio* TBOA, a non-transported, broad spectrum, glutamate transporter antagonist (Shimamoto *et al.*, 1998), partially blocked the ERG b-wave. This suggested that the non-metabotropic element originated with glutamate-transporter-associated chloride channels (Wong *et al.*, 2005a), agreeing with findings of similar glutamate-gated currents by patch physiology on the membranes of ON bipolar cells of zebrafish (Connaughton & Nelson, 2000), *giant danio* (Wong *et al.*, 2005b), and hybrid bass (Grant & Dowling, 1995, 1996). The hypothesis then is that the PIIm sub-element is metabotropic, and the PIInm sub-element is transporter mediated. TBOA affects all retinal glutamate transporters producing a variety of actions. Several authors have noted that without care in dosing, delay in b-wave peak-time and total b-wave loss can result (Nelson & Connaughton, 2004; Wong *et al.*, 2005a). For this reason PIInm is most reliably revealed by response subtraction after L-AP4 or CPPG treatments.

PIIm cone signals

The PIIm irradiance response function has triple the amplitude for blue or UV stimulation as for red and the highest b- and u-cone signal composition of any onset element. Peak amplitude gains for b- and u-cone signals (relative to PIII) are in the range of 7-10, as compared to 1-2 for g-cone and r-cone signals. Spectral peaks for this element lie between b- and u-cone absorbance maxima for all criteria tested. Despite b- and u-cones being 1/3rd of the cone population, 80% of the PIIm saturated amplitude arises from b- and u-cone signals. While the PIIm signal represents all cones, the weighting suggests that subsynaptic contacts between b cones, u-cones and bipolar cells are special, driving more light-evoked current through the ON-bipolar-cell pathway. Saszik *et al* (2002a) were the first to note the enrichment of b- and u-

cone signals in the zebrafish L-AP4-sensitive b-wave. The present study confirms this, but adds that all cone types make metabotropic glutamate synapses with ON-type bipolar cells.

PIInm cone signals

PIInm spectral sensitivities are oriented towards longer wavelengths. Spectral profiles are broad. Peaks shift gradually from the long-wavelength limb of b-cone absorbance to the short-wavelength limb of r-cone absorbance as criterion levels increase. The distribution of cone color signal amplitudes resembles that found in cone PIII, suggesting that the PIInm, EAAT5 chloride-channel mechanism (Arriza *et al.*, 1997) is uniformly shared among post-synaptic contacts between different cone types and ON bipolar cells. The mechanism does not particularly redistribute cone-color amplitudes or sensitivities. The gains in maximal signal amplitudes (relative to PIII) are in the range 2-4 for all cone spectral signals.

a1nm and a2, post-photoreceptor a-waves

In the photopic ERG of monkeys, both fast and slow a-wave components sensitive to AMPA/kainate blockers can be isolated (Bush & Sieving, 1994). These components are post-receptor and appear to originate with hyperpolarizing cone bipolar cells. The analogous ERG element in zebrafish is a2, a maintained, cornea hyperpolarizing, CNQX-sensitive element. There is a further post-photoreceptor, a-wave element in zebrafish, a1nm. This element is CNQX insensitive, and not driven by AMPA/kainate receptors. As AMPA-kainate glutamate synapses provide excitation to amacrine and ganglion cells, an inner retina origin for a1nm is unlikely. So a1nm may not be analogous to the post-photoreceptor scotopic threshold response (STR) of mammals (Sieving *et al.*, 1986) or to corneal negative OFF cone bipolar signals identified in primate ERG (Sieving *et al.*, 1994). The a1nm element is also insensitive to blockers of metabotropic glutamate receptors, and is therefore non-metabotropic. In *giant danio* TBOA abolished this 'a-wave' (Wong *et al.*, 2005a), similar to present results for zebrafish. We propose that zebrafish a1nm is intrinsic to PIInm, and that the non-metabotropic ON-bipolar mechanism generates biphasic current flow. Perhaps a loss of inward sodium current as light stops PIInm glutamate transport is followed by loss of an outward chloride current, as transporter chloride channels later disassociate, generating a biphasic waveform. PIIm, by contrast, generates no associated a-wave.

Spectra and circuitry

The zebrafish photopic ERG presents a study in cone system integration for an animal model with rich genetics, and equally rich color capabilities. Seven functionally distinguishable elements, PIII, a1nm, a2, b1, PII, PIIm and PIInm contribute to responses at light onset. All of the elements studied increase in saturated amplitude as stimuli move towards shorter wavelengths that progressively recruit more cone color types. This result is successfully represented by a model that sums independently saturable sources for each cone color signal. The result is not unexpected for the composite PIII signal arising from independent r, g, b and u cones. For the b-wave elements the implication is similar: separate ON-cone bipolar pathways for each cone color type. A single cone bipolar pathway with pooled cone input sharing a common saturation level would not produce the observed and modeled outcome. The model is likely most applicable to the pharmacologically isolated b-wave elements, as these are studied under conditions that suppress cone-signal color interactions through interneurons. Hughes *et al* (1998) show opponent cone color signals in the native b-wave. This suggests the underlying independent pathways studied here are entangled through interneuron circuitry.

Among ERG onset elements there are two distinct spectral mixing patterns. The PIII mixture is spectrally broad, and is shared by PIInm. The PIIm mixture is b- and u-cone focused. This focus is accompanied by increased voltage gain in the metabotropic b- and u-cone signals. The gain might be accounted for in two ways. The first is higher current densities through synaptic

and non-synaptic ion channels. The second is increased membrane area, as would result from a greater proliferation of cells. As ON-type bipolar cells of zebrafish do not differ greatly in dimensions (Connaughton *et al.*, 2004), simply larger cells seems unlikely. This suggests specialized b-cone and u-cone bipolar cells, in addition to being metabotropic, may exist in a relatively high density as compared to other ON-type bipolar cells.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, NINDS. We express admiration for, and take inspiration from, the pioneering research of the late Joe Bilotta and his students and colleagues. We thank Anna Wesolowska, Risa Gordon, and Victoria Connaughton for critical contributions to the development of evoked potential recordings in zebrafish eyecup.

References

- Arriza JL, Eliasof S, Kavanaugh MP, Amara SG. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc Natl Acad Sci U S A* 1997;94:4155–4160. [PubMed: 9108121]
- Bird R, Riorden C. Simple solar spectral model for direct and diffuse irradiance on horizontal and tilted planes at the Earth's surface for cloudless atmospheres. *Journal of Applied Meteorology* 1986;25:87–97.
- Bui BV, Fortune B. Ganglion cell contributions to the rat full-field electroretinogram. *J Physiol* 2004;555:153–173. [PubMed: 14578484]
- Bush RA, Sieving PA. A proximal retinal component in the primate photopic ERG a-wave. *Invest Ophthalmol Vis Sci* 1994;35:635–645. [PubMed: 8113014]
- Chappell RL, Naka K, Sakuranaga M. Dynamics of turtle horizontal cell response. *J Gen Physiol* 1985;86:423–453. [PubMed: 4056733]
- Connaughton VP, Graham D, Nelson R. Identification and morphological classification of horizontal, bipolar, and amacrine cells within the zebrafish retina. *Journal of Comparative Neurology* 2004;477:371–385. [PubMed: 15329887]
- Connaughton VP, Nelson R. Axonal stratification patterns and glutamate-gated conductance mechanisms in zebrafish retinal bipolar cells. *J Physiol* 2000;524:135–146. [PubMed: 10747188]
- Connaughton VP, Nelson R, Bender AM. Electrophysiological evidence of GABA_A and GABA_C receptors on zebrafish retinal bipolar cells. *Vis Neurosci* 2008;25:139–153. [PubMed: 18442437]
- Dacheux RF, Raviola E. The rod pathway in the rabbit retina: a depolarizing bipolar and amacrine cell. *J Neurosci* 1986;6:331–345. [PubMed: 3950701]
- DeVries SH. Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. *Neuron* 2000;28:847–856. [PubMed: 11163271]
- Engstrom K. Cone types and cone arrangement in the retina of some cyprinids. *Acta Zool* 1960;41:277–295.
- Fadool JM, Dowling JE. Zebrafish: a model system for the study of eye genetics. *Prog Retin Eye Res* 2008;27:89–110. [PubMed: 17962065]
- Famiglietti EV Jr, Kaneko A, Tachibana M. Neuronal architecture of on and off pathways to ganglion cells in carp retina. *Science* 1977;198:1267–1269. [PubMed: 73223]
- Granit R. The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. *J Physiol* 1933;77:207–239. [PubMed: 16994385]
- Grant GB, Dowling JE. A glutamate-activated chloride current in cone-driven ON bipolar cells of the white perch retina. *J Neurosci* 1995;15:3852–3862. [PubMed: 7538566]
- Grant GB, Dowling JE. On bipolar cell responses in the teleost retina are generated by two distinct mechanisms. *J Neurophysiol* 1996;76:3842–3849. [PubMed: 8985882]
- Hankins MW, Ikeda H. Non-NMDA type excitatory amino acid receptors mediate rod input to horizontal cells in the isolated rat retina. *Vision Res* 1991;31:609–617. [PubMed: 1688218]

- Hensley SH, Yang XL, Wu SM. Identification of glutamate receptor subtypes mediating inputs to bipolar cells and ganglion cells in the tiger salamander retina. *J Neurophysiol* 1993;69:2099–2107. [PubMed: 7688801]
- Hughes A, Saszik S, Bilotta J, Demarco PJ Jr, Patterson WF 2nd. Cone contributions to the photopic spectral sensitivity of the zebrafish ERG. *Vis Neurosci* 1998;15:1029–1037. [PubMed: 9839967]
- Jeon CJ, Strettoi E, Masland RH. The major cell populations of the mouse retina. *J Neurosci* 1998;18:8936–8946. [PubMed: 9786999]
- Krizaj D, Akopian A, Witkovsky P. The effects of L-glutamate, AMPA, quisqualate, and kainate on retinal horizontal cells depend on adaptational state: implications for rod-cone interactions. *J Neurosci* 1994;14:5661–5671. [PubMed: 7521912]
- Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita M, Fukuda Y, Sasaki H, Hiroi K, Nakamura Y, Shigemoto R, Takada M, Nakamura K, Nakao K, Katsuki M, Nakanishi S. Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. *Cell* 1995;80:757–765. [PubMed: 7889569]
- Nelson R. Cat cones have rod input: a comparison of the response properties of cones and horizontal cell bodies in the retina of the cat. *J Comp Neurol* 1977;172:109–135. [PubMed: 838876]
- Nelson R. All amacrine cells quicken time course of rod signals in the cat retina. *J Neurophysiol* 1982;47:928–947. [PubMed: 6177841]
- Nelson R, Bender AM, Connaughton VP. Stimulation of sodium pump restores membrane potential to neurons excited by glutamate in zebrafish distal retina. *J Physiol* 2003;549:787–800. [PubMed: 12730339]
- Nelson R, Connaughton VP. Glutamate transporter drives the b-wave in zebrafish retina. *Investigative Ophthalmology and Visual Science* 2004;45:815.E-abstract
- Newman EA. Current source-density analysis of the b-wave of frog retina. *J Neurophysiol* 1980;43:1355–1366. [PubMed: 6246222]
- Palacios AG, Goldsmith TH, Bernard GD. Sensitivity of cones from a cyprinid fish (*Danio aequipinnatus*) to ultraviolet and visible light. *Vis Neurosci* 1996;13:411–421. [PubMed: 8782369]
- Pflug R, Nelson R, Ahnelt PK. Background-induced flicker enhancement in cat retinal horizontal cells. I. Temporal and spectral properties. *J Neurophysiol* 1990;64:313–325. [PubMed: 2213120]
- Robinson J, Schmitt EA, Harosi FI, Reece RJ, Dowling JE. Zebrafish ultraviolet visual pigment: absorption spectrum, sequence, and localization. *Proc Natl Acad Sci U S A* 1993;90:6009–6012. [PubMed: 8327475]
- Robson JG, Frishman LJ. Response linearity and kinetics of the cat retina: the bipolar cell component of the dark-adapted electroretinogram. *Vis Neurosci* 1995;12:837–850. [PubMed: 8924408]
- Robson JG, Frishman LJ. Dissecting the dark-adapted electroretinogram. *Doc Ophthalmol* 1998;95:187–215. [PubMed: 10532405]
- Robson JG, Maeda H, Saszik SM, Frishman LJ. In vivo studies of signaling in rod pathways of the mouse using the electroretinogram. *Vision Res* 2004;44:3253–3268. [PubMed: 15535993]
- Saszik S, Alexander A, Lawrence T, Bilotta J. APB differentially affects the cone contributions to the zebrafish ERG. *Vis Neurosci* 2002a;19:521–529. [PubMed: 12511084]
- Saszik SM, Robson JG, Frishman LJ. The scotopic threshold response of the dark-adapted electroretinogram of the mouse. *J Physiol* 2002b;543:899–916. [PubMed: 12231647]
- Shimamoto K, Lebrun B, Yasuda-Kamatani Y, Sakaitani M, Shigeri Y, Yumoto N, Nakajima T. DL-threo-beta-benzoyloxyaspartate, a potent blocker of excitatory amino acid transporters. *Mol Pharmacol* 1998;53:195–201. [PubMed: 9463476]
- Sieving PA, Frishman LJ, Steinberg RH. Scotopic threshold response of proximal retina in cat. *J Neurophysiol* 1986;56:1049–1061. [PubMed: 3783228]
- Sieving PA, Murayama K, Naarendorp F. Push-pull model of the primate photopic electroretinogram: a role for hyperpolarizing neurons in shaping the b-wave. *Vis Neurosci* 1994;11:519–532. [PubMed: 8038126]
- Sillman AJ, Ito H, Tomita T. Studies on the mass receptor potential of the isolated frog retina. I. General properties of the response. *Vision Res* 1969a;9:1435–1442. [PubMed: 5367433]

- Sillman AJ, Ito H, Tomita T. Studies on the mass receptor potential of the isolated frog retina. II. On the basis of the ionic mechanism. *Vision Res* 1969b;9:1443–1451. [PubMed: 5367434]
- Slaughter MM, Miller RF. 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science* 1981;211:182–185. [PubMed: 6255566]
- Stockton RA, Slaughter MM. B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *J Gen Physiol* 1989;93:101–122. [PubMed: 2915211]
- Strettoi E, Masland RH. The organization of the inner nuclear layer of the rabbit retina. *J Neurosci* 1995;15:875–888. [PubMed: 7823187]
- Wesolowska A, Nelson R, Connaughton V. Glutamate Mechanisms Involved in the OFF Pathway of Zebrafish Retina. *Invest Ophthalmol Vis Sci* 2002;43:1826.E-abstract
- Wong KY. Glutamatergic Mechanisms in the Outer Retina of Larval Zebrafish: Analysis of Electroretinogram b- and d-Waves Using a Novel Preparation. *Zebrafish* 2004;1:121–131. [PubMed: 18248224]
- Wong KY, Adolph AR, Dowling JE. Retinal bipolar cell input mechanisms in giant danio. I. Electroretinographic analysis. *J Neurophysiol* 2005a;93:84–93. [PubMed: 15229213]
- Wong KY, Cohen ED, Dowling JE. Retinal bipolar cell input mechanisms in giant danio. II. Patch-clamp analysis of on bipolar cells. *J Neurophysiol* 2005b;93:94–107. [PubMed: 15229214]

Fig. 1. Spectral shape of photopic PIII. **A.** The nested irradiance-response series was evoked with 450nm stimuli from a light-adapted zebrafish eyecup treated with the synaptic blocking agents listed in the figure (L-Asp, L-Aspartate). The 7 irradiance levels are 2.7, 3.3, 3.7, 4.2, 4.7, 5.2 and 5.7 $\log(h\nu \cdot \mu m^{-2} \cdot s^{-1})$. **B.** Peak negativities are plotted vs. stimulus irradiance at 370, 450 and 650nm. Curves are model predictions (Eq.1) based on the entire spectral dataset. Horizontal lines are spectral sensitivity criterion levels. **C.** PIII spectral sensitivity plots vary with criterion levels. Stars mark spectral maxima. Other points mark the test wavelengths. Spectral peaks range from the UV (370nm at the 0.2Vr criteria) to the blue-green (500nm at the 0.8Vr criteria). Both points and curves are calculated from the model fits (Eq. 1). **D.** The fit parameters (Eq. 1) show saturation amplitudes from r cones (V_r) are greatest, while those from b and u cones (V_b , V_u) are least. Cone sensitivity is given as the log of half-saturation irradiance. Sensitivities of b and u cones, $\log(k_{b415})$ and $\log(k_{u362})$, are greater than those of r and g cones, $\log(k_{r570})$ and $\log(k_{g480})$. Spectral peaks are dominated by b and u cones at low criteria. In this and the following figures the rectangular stimulus trace (A) is adjusted in height and level to equal the maximum and minimum response voltage excursions during the entire 70-record, 9-

wavelength dataset. Irradiance-response curves (B) are predictions based on NLSF fits to Eq. 1 to the whole dataset, not just the points for individual wavelengths. The 650nm points (B) are doubled because this wavelength is repeated as a check for preparation stability. Cord sensitivity (C) is the irradiance required to reach criterion voltage divided by the criterion voltage. Spectral voltage criteria are set to 0.2, 0.4, 0.6, and 0.8 of V_r (Eq. 1).

Fig. 2.

ERG elements blocked by AMPA/kainate antagonists. *A*. 450nm stimulation evokes both onset b-wave and offset d-wave responses in the untreated eyecup. *B*. CNQX, an AMPA/kainate antagonist, induces onset negativity (a1), doubles b-wave amplitude, delays the b-wave peak time (b2), and abolishes d-wave responses (d). *C*. Arithmetic subtraction of CNQX-treated responses (*B*) from untreated responses (*A*) reveals three AMPA/kainate generated elements sensitive to CNQX: a fast b-wave element (b1), a maintained ON negativity (a2) and the d-wave (d). Irradiances of stimulation are the same as Fig. 1. Responses in *A* and *B* are recorded consecutively in the same eyecup.

Fig. 3.

PII, the non-AMPA/kainate, b-wave element. A. Non-AMPA/kainate ERG elements a1 and b2 remain after treating an eyecup with CNQX (same as Fig. 2B). B. PIII cone signals are revealed after complete synaptic blockade (same as Fig. 1A). C. The photoreceptor-generated PIII signal within A is removed by subtracting B from A, revealing PII, a post-photoreceptor, non-AMPA/kainate element that originates selectively with ON-type bipolar cells. An a-wave element remains even after subtracting cone-generated PIII. Responses are recorded consecutively in the same eyecup in A to B order. All stimuli are 450nm with the same irradiances as in Fig. 1.

Fig. 4.

Spectral properties of b1, a b-wave element generated by AMPA/kainate receptors. **A.** Peak responses vs. stimulus irradiance at 370, 450, and 650nm are fit by curves predicted from the spectral model (Eq. 1). Maximal amplitudes are larger for simulation with UV (370nm) or blue (450nm) than red (650nm). Horizontal lines are criteria for spectral sensitivity. **B.** Model-generated spectral curves for b1 vary with criterion. Stars indicate spectral peaks, which remain in the UV or blue (380-405nm) for all criteria tested. Other points mark test wavelengths. **C.** The table lists model (Eq.1) fit parameters for this b1 dataset. These parameters generate the curves in **A**, and both curves and points in **B**. While V_r and V_g are in similar ratio as found for PIII (Fig. 1), V_b is much increased. Other conventions are the same as Fig. 1

Fig. 5. Spectral shape for PII, a b-wave element insensitive to AMPA/kainate blockade. **A.** PII irradiance-response data are fit by predicted model curves (Eq. 1) at three wavelengths. Maximal responses are much larger for UV (370nm) or blue (450nm) stimulation than for red (650nm). Horizontal lines are criterion levels for spectral sensitivity. **B.** Model-generated PII spectral sensitivity curves vary with criterion level. Stars mark spectral peaks, ranging from 380nm (0.2V_r criterion) to 405nm (0.8V_r criterion). Other points mark test wavelengths. **C.** The table lists model (Eq.1) fit parameters for PII. These parameters generate the curves in A and both curves and points in B. While the ratio of V_r to V_g is similar to PIII (Fig. 1), V_b is much increased. Other conventions are the same as Fig. 1.

Fig. 6. Extraction of PIIIm, the metabotropic b-wave. *A.* Non-AMPA/kainate ERG elements a1 and b2 remain after treating an eyecup with CNQX (similar to Fig. 2B). *B.* Treatment with L-AP4, a blocker for type III metabotropic glutamate receptors, in addition to the AMPA/kainate blocker CNQX used in *A*, leaves a1 little affected, but reduces the b2 element. *C.* Arithmetic subtraction of responses treated in both CNQX and L-AP4 (*B*) from responses treated only with CNQX (*A*) reveals PIIIm, the metabotropic b-wave. PIIIm arises from ON-bipolar-cell metabotropic glutamate receptors post-synaptic to zebrafish cones. The subtraction (*C*) reveals little a-wave element for PIIIm. Stimuli are all 450nm with irradiances of 1.7, 2.6, 3.2, 3.7, 4.2,

4.7 and 5.2 $\log(h\nu \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1})$. Responses in *A* and *B* are recorded consecutively in the same eyecup. Other conventions are the same as Fig.1.

Fig. 7.

Extraction of PIIm, a non-metabotropic, non AMPA/kainate b-wave element. *A*. An eyecup treated with the AMPA/kainate antagonist CNQX and the metabotropic glutamate agonist L-AP4 responds with a-wave and b-wave, (same records as figure 6B). *B*. Addition of L-Aspartate (L-Asp), a non-specific blocker of glutamatergic transmission, and TBOA, a glutamate uptake blocker, eliminates post-photoreceptor responses leaving PIII, the cone signal. *C*. Arithmetic subtraction of PIII (*B*) from the responses in *A* reveals a post-photoreceptor, non-metabotropic, non-AMPA/kainate a-wave (a1nm) and b-wave (PIIm). Both elements originate with ON bipolar cells. Responses were recorded consecutively in the same eyecup in *A* to *B* order.

Irradiances of 450nm stimuli are the same as in Fig. 6. Other conventions are the same as Fig. 1.

Fig. 8.

Spectral characteristics of PIIm, the metabotropic b-wave. **A.** The PIIm peak responses are compared to predicted model curves at 3 wavelengths. Saturated amplitudes in the UV (370nm) and blue (450nm) are nearly triple those in the red (650nm). Horizontal lines mark criteria for spectral sensitivity. **B.** Spectral sensitivity curves for PIIm vary with criterion level. Stars mark spectral peaks. These range from 365nm at the 0.2V_r criteria to 390nm at the 0.8V_r criteria. Other points show test wavelengths. **C.** The table lists model (Eq.1) fit parameters for this dataset. These parameters generate the curves in **A** and both curves and points in **B**. For PIIm, V_b is the largest signal element. Other conventions are the same as Fig. 1.

Fig. 9. Spectral shape for PIInm, the non-metabotropic, non-AMPA/kainate b-wave element. **A.** Plots of PIInm peak responses vs. stimulus irradiance at 3 wavelengths reveal saturated amplitudes in the UV (370nm) and blue (450nm) are somewhat larger than in the red (650nm), but not to the extent seen for the PIIm (Fig. 8). Horizontal lines mark criterion levels for spectral sensitivity. Curves are model predictions (Eq. 1). **B.** Spectral sensitivity curves for PIInm shift with criterion level. Stars mark peaks in the modeled broad spectral functions. These range from the UV (380nm) at the 0.2V_r criteria to the green (520nm) at the 0.8V_r criteria. **C.** The table lists model (Eq.1) fit parameters for this data set. These parameters generate the curves

in A and both curves and points in B . Except for scale factors, $PIInm$ resembles $PIII$ in spectral properties. Other conventions are the same as given for Fig 1.

Fig. 10.

TBOA, a glutamate uptake antagonist, blocks a1 and slows b2. A. a-wave (a1) and b-wave (b2) remaining after AMPA/kainate responses are blocked in an eyecup treated with CNQX (same as Fig. 2B). The combined waveform is composed of metabotropic and non-metabotropic glutamate signals, as well as cone signals. B. Addition of the glutamate uptake blocker TBOA to CNQX abolishes the a1 component, delays b2 peak time (delayed b2) and reduces b2 amplitude. Results suggest that a1, and its major component a1nm, are mainly a transporter phenomenon. Actions on b2 are more complex. Because TBOA causes major changes in b2 response kinetics, the method of waveform subtraction for showing TBOA actions is difficult to interpret, and is not shown.

Table 1

Polynomial coefficients of normalized absorption nomograms for zebrafish cones. When entered into (Eq.2) these coefficients generate pigment absorbance functions $A_r(\lambda)$, $A_g(\lambda)$, $A_b(\lambda)$ and $A_u(\lambda)$. The nomogram for r, g and b cones is from Hughes et al (1998). The nomogram for the u cone is from Palacios et al (1996).

coefficient	r, g & b cones	u cone
$c0$	-93.262	-48139.554
$c1$	617.458	309007.504
$c2$	-2815.735	-770875.542
$c3$	7339.277	834365.023
$c4$	-10714.545	-57657.482
$c5$	9048.982	-815689.388
$c6$	-4410.667	871189.984
$c7$	1154.235	-389189.665
$c8$	-125.738	66989.122

Table 2

Onset elements in zebrafish photopic ERG. Colloquial names, physiological interpretations, and brief description of the method for extracting the element are given. All elements are photopic, isolated on a rod-saturating, weak photopic background.

Element	Interpretation	Method
b1	An early AMPA/kainate b-wave element.	CNQX & subtraction.
b2	A late, AMPA/kainate insensitive b-wave peak.	CNQX
delayed b2	glutamate uptake blocker slows b2	CNQX, TBOA
PII	ON bipolar cell signals.	PIII subtracted from b2.
PIIm	Metabotropic ON bipolar cell signals.	L-AP4/CPPG & b2 subtraction.
PIInm	<i>Non</i> metabotropic ON bipolar cell signals.	L-AP4/CPPG & PIII subtraction.
PIII	The summed signals from r, g, b & u cones.	L-Aspartate
a1	An early a-wave element.	CNQX
a1nm	EAAT-linked, post-photoreceptor a-wave.	L-AP4, CPPG, CNQX & PIII subtraction.
a2	AMPA/kainate OFF bipolar cell signals.	CNQX & subtraction.

Table 3

Cone color signals within photopic PIII, isolated by L-Aspartate. Saturated amplitudes ($V_{\max \text{ PIII}}$, col. 1) and half-saturation irradiances (k_{PIII} , col. 3) for r-, g-, b- and u-cone signal elements are extracted by the spectral model (Eq. 1). $V_{\text{PIII}}/V_{\text{r PIII}}$ (col. 2) gives $V_{\max}$ relative to r cones. $k_{\text{r PIII}}/k_{\text{x PIII}}$ (col. 4) gives median half-saturation irradiances relative to r cones, so that values larger than 1 indicate greater sensitivity. Values significantly greater than 1 are marked by asterisks ($P < 0.05$, t-test). Response-wavelength-irradiance datasets from 17 preparations were fit by NLSF to the spectral model (Eq. 1) with a median R^2 of 0.98. R cones contribute the largest signal amplitude to PIII, but at a lower sensitivity than g, b, or u cone types. In this and the following tables, $V_{\max}$ refers to V_{r} , V_{g} , V_{b} and V_{u} for the cone signal within the ERG element. The k values are in $\text{h}\nu \cdot \mu\text{m}^{-2} \cdot \text{sec}^{-1}$. Values are means $\pm$ SE, except for the k ratio. This follows a non-Gaussian distribution best characterized by the median. All ratios are computed from paired values within each preparation.

Cone	$V_{\max \text{ PIII}} \mu\text{V} \pm \text{SE}$	$V_{\text{x PIII}}/V_{\text{r PIII}} \pm \text{SE}$	$\log(k_{\text{PIII}}) \pm \text{SE}$	$k_{\text{r PIII}}/k_{\text{x PIII}}$ median
red	-12.26 ± 1.02	$1.00 \pm \text{n/a}$	4.57 ± 0.06	1.0
green	-5.40 ± 0.65	0.44 ± 0.05	4.05 ± 0.09	3.4*
blue	-1.74 ± 0.14	0.15 ± 0.01	3.27 ± 0.08	25.9*
UV	-1.69 ± 0.42	0.13 ± 0.03	3.10 ± 0.24	21.2*

Table 4

Cone color signals in b1, the AMPA/kainate-sensitive b-wave element. Saturated amplitudes ($V_{\max b1}$, col. 1) and half-saturation irradiances (k_{b1} , col. 3) for r, g, b and u cone signals are extracted from 13 preparations by the spectral model (Eq. 1). The median R^2 was 0.98. $|V_{mib1}/V_{m PIII}|$ (col. 2) gives the absolute gain in $V_{\max}$ relative to PIII. In column 4, k_{PIII}/k_{b1} is an index of change in sensitivity (half-saturation irradiance) relative to PIII. Positive values with asterisks indicate significant sensitivity increases ($P < 0.05$, t-test). R cones and b cones contribute the greatest signal amplitudes. $V_{\max}$ signal gains relative to PIII are largest for b and u cones, while sensitivity gains are seen for r and g cones. Other conventions are the same as Table 3.

Cone	$V_{\max b1} \mu V \pm SE$	$ V_{m b1}/V_{m PIII} \pm SE$	$\log(k_{b1}) \pm SE$	k_{PIII}/k_{b1} median
red	27.37 ± 4.42	2.42 ± 0.37	3.74 ± 0.21	6.0*
green	14.59 ± 1.56	3.13 ± 0.26	3.46 ± 0.16	4.4*
blue	21.20 ± 3.00	13.04 ± 1.95	3.49 ± 0.15	0.5
UV	7.25 ± 1.13	6.74 ± 0.93	2.51 ± 0.28	4.2

Table 5

Cone color signals for PII, the AMPA/kainate-insensitive b-wave element. Saturated amplitudes ($V_{\max \text{ PII}}$, col. 1) and half-saturation irradiances (k_{PII} , col. 3) for r, g, b and u cone signals are extracted by the spectral model (Eq. 1) from 16 preparations. The median R^2 was 0.99. While r and g cones contribute the largest signal amplitudes, $V_{\max}$ gains relative to PIII (col. 2) are largest for b- and u-cone signals. Sensitivity gains (col. 4) are significant for r and g cones ($P < 0.05$, t-test). Conventions are the same as Table 4.

Cone	$V_{\max \text{ PII}} \mu\text{V} \pm \text{SE}$	$ V_{\text{m PII}}/V_{\text{m PIII}} \pm \text{SE}$	$\log(k_{\text{PII}}) \pm \text{SE}$	$k_{\text{PIII}}/k_{\text{PII}}$ median
red	30.79 ± 4.16	2.82 ± 0.36	3.87 ± 0.11	5.2*
green	17.80 ± 1.92	3.74 ± 0.53	3.45 ± 0.10	3.7*
blue	16.98 ± 2.91	10.70 ± 1.59	3.29 ± 0.17	0.8
UV	6.64 ± 0.95	5.68 ± 0.65	2.77 ± 0.15	2.9

Table 6

Cone color signals for PII_m, the metabotropic b-wave element. Saturated amplitudes ($V_{\max \text{ PII}_m}$, col. 1) and half-saturation irradiances (k_{PII_m} , col. 3) for r- g- b- and u-cone signals are extracted by a spectral model (Eq. 1) for 6 preparations, 4 treated with L-AP4, and 2 with CPPG. The median R^2 (all fits) was 0.99. The saturated amplitude of b-cone signals is greatest, but similar to r-cone signals. $V_{\max}$ gains relative to PIII (col. 2) are greatest for b- and u-cone signals. Sensitivity gains (col. 4) are significant for r- and g-cone signals ($p < 0.05$, t-test). Other conventions are the same as Table 4.

Cone	$V_{\max \text{ PII}_m} \mu\text{V} \pm \text{SE}$	$ V_{\text{m PII}_m}/V_{\text{m PIII}} \pm \text{SE}$	$\log(k_{\text{PII}_m}) \pm \text{SE}$	$k_{\text{PIII}}/k_{\text{PII}_m}$ median
red	16.18 ± 2.53	1.58 ± 0.23	3.75 ± 0.32	7.2*
green	10.35 ± 0.96	2.42 ± 0.45	3.57 ± 0.11	4.1*
blue	17.04 ± 3.23	10.51 ± 0.93	3.53 ± 0.17	0.6
UV	6.21 ± 1.34	5.92 ± 0.53	2.63 ± 0.23	5.1

Table 7

Cone color signals for PII_{nm}, the non-metabotropic b-wave element. Saturated amplitudes ($V_{\max \text{ PII}_{nm}}$, col. 1) and half-saturation irradiances ($k_{\text{PII}_{nm}}$, col. 3) for r- g- b- and u-cone signals were extracted using a spectral model (Eq. 1) for 5 preparations, 3 treated with L-AP4, an mGluR6 agonist, and 2 with CPPG, an mGluR6 antagonist. The median R^2 (all fits) was 0.99. The distribution of $V_{\max}$ values is similar to PIII, and $V_{\max}$ gains relative to PIII are all in the range 2-4 (col. 2). Sensitivity gains (col. 4) were small, but significant for r and g cones ($P < 0.05$, t-test). Conventions are similar to Table 4.

Cone	$V_{\max \text{ PII}_{nm}} \mu V \pm SE$	$V_{m \text{ PII}_{nm}}/V_{m \text{ PIII}} \pm SE$	$\log(k_{\text{PII}_{nm}}) \pm SE$	$k_{\text{PII}}/k_{\text{PII}_{nm}}$ median
red	21.99 ± 3.95	2.20 ± 0.39	4.17 ± 0.23	1.9*
green	11.00 ± 2.58	2.83 ± 0.63	3.39 ± 0.24	2.7*
blue	5.85 ± 2.00	3.88 ± 0.06	3.03 ± 0.56	0.5
UV	2.74 ± 1.18	3.42 ± 1.52	2.99 ± 0.20	0.5