Cellular Basis of the Electroretinogram

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Cellular origins and mechanisms of generation of the various waves of the ERG

Sherry, 2002

Modified from Dowling, 1987
The ERG – a non-invasive tool for assaying retinal function and integrity

- The ERG can inform us about the functional status of retinal cells and circuits
- The ERG is useful for detecting and monitoring the progression of diseases that affect the retina
- The ERG can be used to monitor effects of therapeutic interventions

Non-invasive recording of the ERG in man, monkey, mouse etc.

Electrodes: Conductive materials in contact with the cornea
Example: Contact lens electrodes and DTL fiber electrodes

- Pupils – fully dilated for full field flash ERG
- Corneas – covered with moistening/ionic conducting solution
- Fixation (spot or cross)
- Responses – repeated trials averaged when signals are small
- Animals and sometimes young children: generally anesthetized using drugs with minimal effects on retinal function
ERG Stimulus - The diffuse flash full-field or “ganzfeld” ERG

Current LED-based ganzfeld stimulators

Traditional white light (xenon flash tube is common) within a Ganzfeld globe

Retinal cells and circuits

Rods

Cones

Müller cells

ON and OFF Bipolar Cells

Amacrine cells

ON and OFF Ganglion cells
Rod- and cone-driven ERGs

Dark-adapted (Scotopic)

Light-adapted (Photopic)

Modified from Dowling, 1987

ISCEV “standard” clinical testing

McCulloch et al., 2015
Origins of the ERG waves

- Intraretinal localization
- Pharmacological dissection
- Modeling
- Site-specific lesions/pathology
- Targeted mutations
- New stimulation/analysis techniques

Animal model for necessarily invasive experiments: macaque monkey (primate) retina and ERG are very similar to those of humans
Intraretinal localization: Radial Currents
Generation of the a-wave (Plll)
Circulating current and resulting a-wave from the rods

A-wave

After Penn & Hagins, 1972; Pugh et al., 1998

Intraretinal localization
Current source density analysis in monkey retina localizing a-waves to photoreceptors, b-waves to bipolar cells
Pharmacological dissection: Origins of the dark-adapted ERG: Ragnar Granit, 1933

Component processes:
Disappearing waves – during ether induction:
P1- RPE: c
P2- Bipolar cells: b
P3- Photoreceptors: a

First pharmacological dissection of the ERG

Derived photoreceptor component, PIII: paired flash approach

• Modeled rod photoreceptor response (PIII, kinetics determined via paired-flash technique) shows the negative a-wave and the slow time course of the rod response

PIII revealed by removing the b-wave
• Pharmacologic blockade
• Anoxia – ischemia – occlusion of inner retinal circulation
• cCSNB in human
The isolated a-wave, recovers faster than the outer segment photo-current

P3 revealed by removing the b-wave
- Pharmacologic blockade
- Anoxia – ischemia – occlusion of inner retinal circulation
- cCSNB in human

Modeling – transduction cascade* & the a-wave

Mouse rod OS photocurrent recording (Field & Rieke, 2002)

Lamb & Pugh, 1992; Pugh et al., 1998
Hood and Birch, 1990
*a-wave generated by OS current

R&F, 2014
Modeling – OS current, membrane resistance (R) and capacitance (C) & the a-wave

Photocurrent

Robson & Frishman, 2014

Other photoreceptor-dependent events:
The c-wave and slow P3: photoreceptor response-dependent $[K^+]_0$ changes in subretinal space between photoreceptors and RPE affect RPE cells (TEP c-wave) and Müller cells (slow P3)

Waves in the DC ERG with origins in the retinal pigment epithelium (RPE)

Cat: Steinberg et al (1985)
Spatial buffer currents in Müller cells move $K^+$ from areas of high extracellular to areas of low extracellular concentration

**Slow P3**
- A corneal negative subcomponent of the C-wave
- A major photoreceptor-dependent component generated by Müller cell currents
- The $K^+$ channel blocker, barium ($Ba^{2+}$) eliminates slow P3
- Knocking out Kir4.1 channels in mice eliminates slow P3 (the b-wave remains intact)

*Kofuji et al (2000)*

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**Postreceptoral contributions to the ERG**

*Pharmacological dissection studies*
Dark-adapted ERG from postreceptoral retina

The rod pathway
- Rods
- Rod bipolar cells
- All amacrine cells
- Cone bipolar cells
- Ganglion cells

Dark-adapted ERGs are similar in primates and rodents
Removal of amacrine and ganglion cell contributions to the ERG to isolate the RBC component (PII)

GABA  NMDA

TTX (Na⁺-dependent spikes)

Remove scotopic threshold response (STR) pharmacologically

Scotopic b-wave originates from rod-driven bipolar cells

Rod bipolar cell current from patch recordings in mouse retinal slice (Field & Rieke, 2002) compared with isolated human (by weak light adaptation) and mouse scotopic b-waves (ERG RBC response, PII)

The scotopic b-wave (PII): mainly from radial currents around bipolar cells - late Müller cell contribution

Barium (BaCl₂) an inward rectifying K⁺ channel blocker removes a slow component of the b-wave by blocking Kir channels in Müller cell membranes

Origins of the light-adapted waves of the ERG from postreceptoral retina – using glutamate pharmacology to block ON or OFF pathways

- **APB**: elimination of ON bipolar cell activity (b-wave) via agonist effect on metabotropic GluRs
- **PDA**: Blockade of ionotropic GluRs on OFF bipolar (& Hz cells), amacrine and ganglion cells
Origins of waves in the light-adapted ERG of the primate

- **b-wave**: ON cone bipolar cells – removed by APB.
- **d-wave**: OFF bipolar cells + cone offset – reduced by PDA
- push-pull or inhibition makes b-wave small
- **a-wave**: partially OFF bipolar cell response

Monkey: Origins of a-waves in the light-adapted ERG

- **a-wave**: OFF cone bipolar cell contribution removed by PDA
Light adapted ERGs are different in primates and rodents

Long duration rodent ERGs do not have a d-wave or negative plateau.

Brief flash ERG b-waves are slower in rodents than in primates

Postreceptoral origins of “30 Hz” fast flicker ERG in nonhuman primates

Bush & Sieving, 1996
Oscillatory potentials (OPs)

OPs: wavelets superimposed on the b-wave. occur in scotopic and photopic ERG

Involvement of amacrine cells is well established; mechanisms of generation are not understood

OPs are reduced in eyes with diabetic retinopathy

High frequency OPs (peak at 150 Hz) are related to ganglion cell function.

Retinal ganglion cells – contributions to flash ERG?
The scotopic threshold response (STR) originates from amacrine cells and retinal ganglion cells
The PhNR (and PERG) in primate photopic ERG: selectively and similarly reduced by experimental glaucoma which causes retinal ganglion cell death

PhNR amplitude is reduced in humans and animals with optic neuropathies

- Open angle glaucoma
- Optic atrophy
- NAION
- Optic neuritis (MS)
Multifocal ERG*
(many small focal “ERGs” recorded simultaneously)

Hood (2000)

*Developed in late 20th century by Dr. Erich Sutter

Multifocal ERG – cellular origins in primates

A

APB
Positive potential:
ON bipolar cells

APB + PDA
Initial negative potential
OFF bipolar cells and cone photoreceptors

TTX+NMDA
Oscillatory activity from amacrine and ganglion cells removed

Hood et al (2002)
ISCEV “Standard” Clinical Testing

Retinal cells contributing to waves of the flash and flicker ERG

ISCEV “Standard” clinical testing

**a-wave**
- Photoreceptors (rods and cones) and late postreceptoral OFF bipolar, and ON pathway contributions

**b-wave / (d-wave)**
- Bipolar cells
  - Scotopic **b-wave**: rod bipolar cells (RBC), slow Müller cell;
  - Photopic **b-wave**: ON cone bipolar (DCB) and OFF cone bipolar (HCB) bipolar cells, horizontal (Hz) cell feedback;
  - Photopic **d-wave**: OFF bipolar and offset of cone photoreceptor response

**Oscillatory potentials (OPs)**
- Amacrine and ganglion cells (membrane oscillations, feedback loops)

“30 Hz” fast flicker
- ON and OFF cone bipolar cells, small cone photoreceptor contribution (small contribution from spiking activity)
Retinal cells contributing to waves of the ERG

More specific tests

PhNR, STR, PERG
- Ganglion cells and their axons; amacrine cells as well for STR; glial currents (astrocytes?)

C-wave
- Retinal pigment epithelial (PPE) cell slow potentials; photoreceptor-dependent changes in \([K_+]^c\). Corneal signal has slow PIII subtracted from the RPE signal, which is generally larger.

Slow P3
- Müller (glial) cell currents across the neural retina; light-evoked photoreceptor-dependent changes in \([K_+]^c\)

The end

References:
Useful website for knowledge about the visual system, includes retinal structure and function.
Webvision: http://webvision.med.utah.edu/

Review chapters with reference list for papers on ERG origins:
