Electroretinogram of Human, Monkey and Mouse

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Introduction

The electroretinogram (ERG) is a useful tool for objective, non-invasive assessment of retinal function both in the clinic and the laboratory. It is a mass electrical potential that represents the summed response of all the cells in the retina to a change in illumination. Recordings can be made in vivo under physiological or nearly physiological conditions using electrodes placed on the corneal surface. For standard recordings in the clinic, ERGs such as those illustrated in Figure 24.1 are recorded from alert subjects who are asked not to blink or to move their eyes. Anesthesia, selected for having minimal effects on retinal function, may be required for recordings from some very young human subjects, and is generally used for recordings in animals. The positive and negative waves of the ERG reflect the summed activity of overlapping positive and negative component potentials that originate from different stages of retinal processing. The choice of stimulus conditions and method of analysis will determine which of the various retinal cells and circuits are generating the response. Information about retinal function provided by the ERG is useful for diagnosing and characterizing retinal diseases, as well as for monitoring disease progression, and evaluating the effectiveness of therapeutic interventions.

Much of the basic research on the origins, pathophysiology and treatment of retinal diseases that occur in humans is carried out in animal models. The ERG provides a simple objective approach for assessing retinal function in animals. In recent years it has been of particular benefit in studies of mouse (rat, and other species) models of retinal disease, and it has been valuable for evaluating the retinal drug toxicity. The ERG also has been useful for characterizing changes in retinal function that occur as a consequence of genetic alterations in mice and other models, which affect the transmission and processing of visual signals in the retina.

This chapter provides information on retinal origins and interpretation of the ERG, with emphasis on advances in our understanding of the ERG that have occurred through pharmacological dissection studies in macaque monkeys whose retinas are similar to those of humans. The similarity of the waveforms of flash ERGs of humans and macaques can be seen in Figure 24.2. The chapter also will examine origins of the mouse ERG, and consider similarities and differences between mouse and primate ERG. Although the focus of this chapter is on primate and rodent retinas, it is important to note that the ERG is a valuable tool for assessing retinal function of all classes of vertebrates. This includes amphibians and fish in which classical studies of the retina were carried out (for a review, see Dowling) and in which current work continues to improve our understanding of retinal function under normal, genetically altered and pathological conditions.

This chapter provides useful background information for interpreting the ERG, but does not provide a comprehensive review of the characteristics of ERGs associated with retinal disorders encountered in the clinic. Recent texts by Fishman et al., Heckenlively & Arden and Lam are recommended resources for learning more about clinical applications.

Generation of the ERG

Radial current flow

The ERG is an extracellular potential that arises from currents that flow through the retina as a result of neuronal signaling, and for some slower ERG waves, potassium (K+) currents in glial cells. Local changes in the membrane conductance of activated cells give rise to inward or outward ion currents, and cause currents to flow in the extracellular space (ECS) around the cells, creating extracellular potentials. Although all retinal cell types can contribute to ERGs recorded at the cornea, the contribution of a particular type may be large, or hardly noticeable in the recorded waveform, depending upon several factors detailed below.

The orientation of a cell type in the retina is a major factor in determining the extent to which its activity will contribute to the ERG. A schematic drawing of the mammalian retina, with the various cell types labeled, can be found in Chapter 21 (Figs 21.1 & 21.2). When activated synchronously by a change in illumination, retinal neurons that are radially oriented with respect to the cornea, i.e. the photoreceptors, and bipolar cells, make larger contributions to the major waves of the ERG than the more laterally oriented cells and their processes, i.e. the horizontal and amacrine cells. The major waves at light onset are, as marked in Figures 24.1 and 24.2, an initial negative-going a-wave (mainly from photoreceptors),
which is truncated by a positive-going b-wave, mainly generated by ON (depolarizing) bipolar cells. For longer duration flashes (Fig. 24.2, bottom row), the light-adapted ERG includes another positive-going wave, the d-wave, at light offset, with major contribution from OFF (hyperpolarizing) bipolar cells. Currents that leave retinal cells and enter the ECS at one retinal depth (the current source), will leave the ECS to re-enter the cells at another (the current sink), creating a current dipole. These retinal currents also travel through the vitreous humor to the cornea, where the ERG can be recorded non-invasively, as well as through the extraocular tissue, sclera, choroid and high resistance of the retinal pigment epithelium (RPE) before returning to the retina. Local ERGs can be recorded near the retinal generators using...
intraretinal microelectrodes in animals, while simultaneously recording the global ERG elsewhere in the current path, e.g. from the corneal surface, or using an electrode in the vitreous humor, with a reference electrode behind the eye. Such recordings have provided useful information about the origins of the various waves of the ERG.

**Gliarial currents**

The ERG waveform also will be affected by gliarial currents. Retinal gliarial cells include Müller cells, RPE cells, and radial astrocytes in the optic nerve head. One crucial function of gliarial cells is to regulate extracellular $K^+$ concentration, $[K^+]_o$, to maintain the electrochemical gradients across cell membranes that are necessary for normal neuronal function. Membrane depolarization and spiking in retinal neurons that occur in response to changes in illumination lead to leak of $K^+$ from the neurons and to $K^+$ accumulation in the ECS. Membrane hyperpolarization, in contrast, leads to lower $[K^+]_i$, as the membrane leak conductance is reduced, but the Na$^+$/$K^+$ ATPase in the membrane continues to transport $K^+$ into the cell.

$K^+$ currents in Müller cells move excess $K^+$ from areas of high $[K^+]_i$ to areas of lower $[K^+]_i$, by a process called spatial buffering. Return currents in the retina are formed by Na$^+$ and Cl$^-$. The regional distribution and electrical properties of inward rectifying $K^+$ (Kir) channels in Müller cells (see Fig. 24.3C) are critical for the spatial buffering capacity of the cells. Studies of Kofuji and co-workers have shown that strongly rectifying Kir2.1 channels are distributed in the Müller cell membrane. $K^+$ in synaptic regions enters Müller cells through Kir2.1 channels, and exits through weakly rectifying Kir4.1 channels concentrated in the ILM, OPL and Müller cell processes around blood vessels. (Modified from Kofuji et al 2002, used with permission.)

**Figure 24.3 Distal retinal components of the direct current (dc)-ERG, and distribution of inward rectifying potassium channels, Kir2.1 and Kir4.1 in Müller cells.**

(A) Simultaneous intraretinal (local) and vitreal (global) ERG recordings from the intact eye of the cat. Top: Trans-retinal, and trans-epithelial (TEP) potentials were recorded in response to a 5-minute period of illumination. The a- and b-waves cannot be seen due to the compressed time scale. The intra- and intraretinal recordings show the two (sub) components of the c-wave: (1) the retinal pigment epithelium (RPE) c-wave, which is the TEP recorded between a microelectrode in the subretinal space (SRS) and a retrobulbar reference, and (2) slow PIII, the cornea-negative trans-retinal component generated by Müller cell currents (see text), recorded between the same microelectrode and the vitreal electrode. The vitreal ERG (bottom), recorded between the vitreal electrode and retrobulbar reference, is the sum of the RPE c-wave, and slow PIII. The c-wave is followed by the fast oscillation trough (FOT) and then the light peak (LP), both of which are exclusively RPE responses. (From Steinberg et al 1985, used with permission.)

(B) dc-ERG recorded from a C57BL/6 mouse in response to a 7-minute period of illumination. FO, LP, and OFF-response amplitudes are marked by arrows. Upper record shows the early portion of the response on an expanded (×5) time scale. Amplitude calibration: 0.5 mV. (From Wu et al 2004, used with permission.)

(C) Strongly rectifying Kir2.1 channels are widely distributed in the Müller cell membrane. $K^+$ is synaptically released into the extracellular ‘sink’ areas with low $[K^+]_o$, to maintain the vitreous humor, subretinal space (SRS), and blood vessels. (Modified from Kofuji et al 2002, used with permission.)
other retina cells with glial function, such as the RPE, include the c-wave and slow PIII (Fig. 24.3A,B), both related to the reduction in $[K^+]_o$ in the SRS that occurs when photoreceptors hyperpolarize in response to a strong flash of light.\textsuperscript{15,17–21} The negative scotopic threshold response (nSTR) and photopic negative response (PhNR), both originating from inner retinal activity, are also thought to be mediated by glial $K^+$ currents in retina or optic nerve head.\textsuperscript{22–29}

**Stimulus conditions**

Aside from structural and functional aspects of the retina, stimulus conditions are of great importance in determining the extent to which particular retinal cell or circuits contribute to the ERG. Signals will be generated in rod pathways, in cone pathways or both depending upon the stimulus energy, wavelength and temporal characteristics, as well as upon the extent of background illumination, with rods responding to, and being desensitized by lower light levels than cones. Fully dark-adapted, ERGs driven by rods only (i.e. scotopic ERGs) are thus useful for assessing rod pathway function (Figs 24.1 and 24.2, top), and light-adapted ERGs driven only by cones (i.e. photopic ERGs), for assessing cone pathway function (Figs 24.1 and 24.2, top). Light-adapted ERGs are then utilized to the ERG. Signals will be generated in rod pathways, in cone pathways or both depending upon the stimulus energy, wavelength and temporal characteristics, as well as upon the extent of background illumination, with rods responding to, and being desensitized by lower light levels than cones. Fully dark-adapted, ERGs driven by rods only (i.e. scotopic ERGs) are thus useful for assessing rod pathway function (Figs 24.1 and 24.2, top), and light-adapted ERGs driven only by cones (i.e. photopic ERGs), for assessing cone pathway function (Figs 24.1 and 24.2, bottom). Figure 24.1, bottom right, also shows responses to 30 Hz flicker which isolates cone-driven responses because rod circuits do not resolve well such high frequencies. Very bright light flashes elicit small wavelets superimposed on the b-wave called oscillatory potentials (OPs) that are generated by circuits proximal to bipolar cells.\textsuperscript{30,31} OPs can be isolated by bandpass filtering: 75–300 Hz in Figure 24.1, top right.

The spatial extent of the stimulus is an important factor in ERG testing. For standard clinical tests, as illustrated in Figure 24.1, and listed in Box 24.1A, as well as for most testing of animal models, a full-field (Ganzfeld) flash of light is used (Fig. 24.2B). A full-field stimulus generally elicits the largest responses because more retinal cells are activated and the extracellular current is larger than for focal stimuli. It also has the advantage that all regions of retina are evenly illuminated, and with respect to background illumination, evenly adapted. Pupils are generally dilated for full-field testing of animal models, a full-field (Ganzfeld) flash of light is used (Fig. 24.2B). A full-field stimulus generally elicits the largest responses because more retinal cells are activated and the extracellular current is larger than for focal stimuli. It also has the advantage that all regions of retina are evenly illuminated, and with respect to background illumination, evenly adapted. Pupils are generally dilated for full-field stimulation. More spatially localized (focal) stimuli are useful for analysis of function of particular retinal regions, e.g. foveal vs peripheral regions in primates. Multifocal stimulation allows assessment of many small regions simultaneously.

ERG responses illustrated in Figure 24.1 are to a minimum set of stimuli selected by the International Society for the Clinical Electrophysiology of Vision (ISCEV) to efficiently acquire standard, comparable data on rod and cone pathway function from clinics and laboratories around the world.\textsuperscript{1} Names of the standard tests are listed in Box 24.1A. Tests using stimuli presented over a fuller range of stimulus conditions to allow more complete or specific evaluation of retinal function, are listed in Box 24.1B, and a few of these tests will be described later in this chapter.

**Non-invasive recording of the ERG**

ERGs can be recorded from the corneal surface using various types of electrodes. A commonly used electrode, with good signal-to-noise characteristics, is a contact lens with a conductive metal electrode set into it (Burian Allen electrode). It has a lid speculum to reduce effects of blinking and eye closure. In the bipolar form of the electrode, the outer surface of the lid speculum is coated with conductive material that serves as the reference. This type of electrode is best tolerated (in alert subjects) when a topical anesthetic is used. Other types of contact lens electrodes have been used as well, e.g. the jet electrode which is disposable. Some clinicians and researchers use thin mylar fibers impregnated with silver particles, called DTL electrodes,\textsuperscript{32} as illustrated in Figure 24.2B, or gold foil, or wire loop electrodes (H-K loop) that hook over the lower eyelid. For rodents, metal wires, in loops or other configurations placed in contact with the corneal surface are often used. Some labs use cotton wick electrodes and some use DTL fibers under contact lenses, or another form of contact lens electrode.\textsuperscript{26,33} Corneas are kept hydrated with a lubricating conductive solution in all cases. The reference electrode can be placed under the ey lid, e.g. the speculum of a contact lens electrode, as described above, or remotely, for example on the temple, the forehead, or the cornea of the fellow eye. ERG signals ranging from > microvolt to a millivolt or more, peak to peak, for responses to strong stimuli, are amplified, and digitized for computer averaging and analysis. Filtering is done to remove signals outside the frequency range of retinal responses to stimulation (<1 and >300 Hz), and to remove line frequency noise (e.g. 50 or 60 Hz).

**Box 24.1 Standard and More Specialized ERG Tests**

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<thead>
<tr>
<th>A. Standard ERG tests</th>
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<tr>
<td>Described by ISCEV Standard for full-field clinical Electroretinography (2008 update).\textsuperscript{1} All numbers are stimulus calibrations in cd.s.m–2</td>
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<tr>
<td>• Dark-adapted 0.01 ERG (“rod response”)</td>
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<tr>
<td>• Dark-adapted 3.0 ERG (“maximal or standard combined rod–cone response”)</td>
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<td>• Dark-adapted 3.0 oscillatory potentials (“oscillatory potentials”)</td>
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<td>• Light-adapted 3.0 ERG (“single-flash cone response”)</td>
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<td>• Light-adapted 3.0 flicker ERG (“30 Hz flicker”)</td>
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<td>• Recommended additional response: either dark-adapted 10.0 ERG or dark-adapted 30.0 ERG</td>
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<tr>
<th>B. Specialized types of ERG and recording procedures</th>
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<tr>
<td>• Macular or focal ERG</td>
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<tr>
<td>• Multifocal ERG (see published guidelines\textsuperscript{162})</td>
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<tr>
<td>• Pattern ERG (see published standard\textsuperscript{39})</td>
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<tr>
<td>• Early receptor potential (ERP)</td>
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<td>• Scotopic threshold response (STR), negative and positive</td>
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<td>• Photopic negative response (PhNR)</td>
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<td>• Direct-current (dc) ERG</td>
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<tr>
<td>• Electro-oculogram (see published standard\textsuperscript{39})</td>
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<tr>
<td>• Long-duration light-adapted ERG (ON–OFF responses)</td>
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<td>• Paired-flash ERG</td>
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<td>• Chromatic stimulus ERG (including S-cone ERG)</td>
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<td>• Dark and light adaptation of the ERG</td>
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<td>• Dark-adapted and light-adapted luminance-response analyses</td>
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<tr>
<td>• Saturated a-wave slope analysis</td>
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<tr>
<td>• Specialized procedures for young and premature infants\textsuperscript{163}</td>
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related responses that remained the longest. PII and PIII are generated predominantly by the RPE; PII, the b-wave, generated (P)I, the slow c-wave response that follows the b-wave; generation of ether anesthesia. (Modified from Granit 1933, used with permission.)

PIII of the ERG can be separated into a fast and a slow portion: fast PIII is the a-wave, which reflects photoreceptor current (see below), and slow PIII results from Müller cell activities induced by photoreceptor-dependent reduction in \([K^+]_o\), in the SRS (Fig. 24.3C). Negative-going slow PIII and the positive-going pigment epithelial response to the same reduction in subretinal \([K^+]_o\), add together to form the c-wave, which, as shown in Figures 24.3 and 24.4, is positive-going in the dark-adapted ERG of the cat. This is because, as illustrated by the intraretinal recordings from intact cat eye in Figure 24.3A, the positive-going RPE contribution, is larger than the negative-going Müller cell contribution. In mice, the c-wave also is positive-going, as shown in Figure 24.3B. In humans and monkeys, slow PIII and the RPE c-wave are more equal in amplitude, and the corneal c-wave is less positive. Two slower potentials that arise from the RPE, the fast oscillation potential (FO) and light peak (LP), are also present in cat and mouse dc-ERG recordings (Fig. 24.3). The mouse LP is much smaller in amplitude than that in the cat. The cellular mechanisms that generate these slow waves were reviewed in more detail in previous reviews of ERG origins.

In alert human subjects it is not possible to obtain stable dc-ERG recordings necessary for recording slow events, e.g. those arising from the RPE, that occur over seconds or minutes, because the eyes move too frequently. Therefore, to measure slow potentials, the electro-oculogram (EOG), an eye-movement-dependent voltage, is recorded. The EOG is a corneo-fundal potential that originates largely from the RPE, its amplitude changes with illumination, being maximal at the peak of the light peak. Use of EOGs to evaluate retinal/RPE function is described in an ISCEV Standard publication on clinical EOG.

**Classical definition of components of the ERG**

The origins of the various waves of the ERG have been of longstanding interest to clinicians and researchers. Our current understanding of the cellular origins of the ERG profits from extensive knowledge, as described in previous chapters, of the functional microcircuitry of the retina, and particularly of the physiology and cell biology of the retinal cell types, and the identity and action of retinal neurotransmitters, their receptors, transporters and release mechanisms. However, a classical study, using ether anesthesia, provided the first pharmacological separation of ERG components.

Granit’s classical pharmacological dissection of the ERG, illustrated in Figure 24.4, provided valuable insights on origins of ERG waves as well as a nomenclature for waves based on their distinct retinal origins. Component “processes” were found to disappear from the ERG during the induction of ether anesthesia in the following order: process (P)I, the slow c-wave response that follows the b-wave; generated predominantly by the RPE; PII, the b-wave, generated by bipolar cells; and eventually PIII, the photoreceptor-related responses that remained the longest. PII and PIII are still commonly used terms for ERG components generated by ON bipolar cells and photoreceptors respectively.

**Slow PII, the c-wave and other slow components of the direct current (dc)-ERG**

Figure 24.5 shows full-field dark-adapted ERG responses to a range of stimulus strengths for a human subject (left), a macaque monkey, whose retina and ERG are similar to that of human (middle), and a C57BL/6 mouse (right). The mouse ERG is similar to the primate ERG, but larger in amplitude (see calibrations). For higher stimulus strengths than shown in the figure, the mouse ERG develops larger oscillatory potentials than those generally seen in the primate ERGs (e.g. Fig. 24.6). The ERGs in Figure 24.5 were generated almost entirely, except for responses to the strongest stimuli, by the most sensitive, primary rod circuit. This circuit is described more fully in previous chapters. For all three subjects, the strongest stimuli evoked an a-wave, followed by a b-wave. For stimuli more than two log units weaker than the strongest one, b-waves were still present, but a-waves were no longer visible. B-waves can be seen in responses to weaker stimuli than a-waves partly because of the convergence of many rods (20–40) onto the rod bipolar cells generating the response, which increases their sensitivity, and partly because of the large radial extent of ON bipolar cells in the retina. The slow negative wave in the ERGs of the three subjects in response to the weakest stimuli, called the (negative) scotopic threshold response (nSTR), and the equally sensitive positive (p)STR are related to amacrine and/or ganglion cell activity, as described more fully in a later section.

The high sensitivity of the STRs relative to b-waves (and a-waves) reflects the additional convergence of rod signals in the primary rod circuit in the inner retina proximal to the rod bipolar cells.
Full-field dark-adapted (Ganzfeld) flash ERG

Negative ERGs

The receptor origin of the a-wave also was demonstrated in early studies in amphibians using compounds that blocked synaptic transmission, Mg\(^{2+}\), Co\(^{2+}\), and Na\(^{+}\)-aspartate, and isolated photoreceptor signals in the ERG, while abolishing responses of postreceptoral neurons.\(^{49,50}\) These manipulations also caused the b-wave to disappear, indicating its postreceptoral origin. As our understanding of synaptic pharmacology has improved, it has become more common to use glutamate agonists and antagonists to block transfer of signals from photoreceptors to specific second-order neurons. For example, blocking metabotropic transmission to depolarizing (ON) bipolar cells, with

**Dark-adapted a-wave**

It has long been appreciated that the dark-adapted a-wave primarily reflects the rod receptor photocurrent. The a-wave generator was localized to photoreceptors in classical intraretinal recording studies in mammalian retinas, some of which included current source density (CSD), or source-sink, analyses.\(^{30,11,46–48}\) The most direct demonstration of the a-wave’s cellular origin was provided in such experiments by Penn and Hagins\(^{47,48}\) in isolated rat retina. These experiments produced evidence that light suppressed the circulating (dark) current of the photoreceptors, and the investigators proposed that this suppression is seen in the ERG as the a-wave.

**Negative ERGs**

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An essentially identical negative ERG to that after APB occurs in mice in which the mGluR6 receptor is genetically deleted,56 or when there are mutations in the mGluR6 receptor or other proteins whose function is necessary for normal signal transduction in ON bipolar cells.57 For example, Figure 24.6 shows the typical negative ERG of the dark-adapted Nob1, i.e., no b-wave, mouse.58,59 The Nob1 mouse has a mutation in the Nyx gene that encodes nystagmin, a protein found in ON bipolar cell dendrites.60,61 Mutation of this protein produces a negative ERG in human patients who are diagnosed with X-linked complete congenital stationary night blindness (CNSB-1).56,61 Negative ERGs also occur for other forms of CNB caused by mutations in mGluR6 receptors, and in Nob3 and Nob4 mice with such mutations.70,71 as well as in Nob2 mice in which glutamatergic transmission from photoreceptors is compromised.72 Although the ERG of the Nob1, and other mice lacking b-waves, is almost entirely negative-going, it rises from its trough at the timecourse of the c-wave. Photoreceptor-dependent slow responses such as c-wave and slow PIII are not affected by blockade of postreceptor responses in neural retina.

Retinal ischemia due to compromised inner retina circulation also isolates the a-wave and eliminates postreceptor ERG components. This was demonstrated in early experiments in monkeys by occlusion of the central retinal artery.66,67 A “negative ERG” in which b-waves are reduced or missing is a common clinical readout of central retinal artery and vein occlusions, as well as other disorders affecting postreceptor retina such as melanoma-associated retinopathy, X-linked retinoschisis, muscular dystrophy or toxic conditions.73

Modeling

The utility of the a-wave in studies of normal and abnormal photoreceptor function was advanced by the development of quantitative models based on single cell physiology that could predict the behavior both of the isolated photoreceptor cells and ERG a-wave in the same or similar species. Hood and Birch49,69 demonstrated that the behavior of the leading edge of the dark-adapted a-wave in the human ERG can be predicted by a model of photoreceptor function derived to describe in vitro suction electrode recordings of currents around the outer segments of single primate rod photoreceptors.70 Lamb and Pugh71,72 followed a simplified kinetic model of the leading edge of the photoreceptor response (in vitro current recordings initially in amphibians) that took account of the stages of the biochemical phototransduction cascade in vertebrate rods (see Chapter 21). This model was subsequently shown to predict the leading edge of the human dark-adapted a-wave generated by strong stimuli,73 and has been used extensively in clinical studies of retinal disease, and in analyses of photoreceptor function in animal models. A simplified formulation presented by Hood and Birch49,73 often is used (see legend to Fig. 24.7), and can be adjusted to application to cone signals as well. Figure 24.7 shows fits of Hood and Birch’s model to the dark-adapted a-wave of a normal human subject and a patient with retinitis pigmentosa (RP).

Photoreceptor models of Hood and Birch,74–76 Lamb and Pugh,72 and more recent ones, with improved fits,55 all provide parameters to represent the maximum amplitude of the a-wave, which is Rmax, in equations of Hood and Birch, and the sensitivity (S) of the response. Rmax and S vary depending upon the pathology and stimulus conditions (e.g., adaptation level). For example, S is thought to be more affected than Rmax in eyes whose photoreceptors are hypoxic and more generally for abnormalities in the transduction cascade or increases in retinal illumination, whereas Rmax is more affected by photoreceptor loss. Both parameters may be affected in RP, but in the case illustrated in Figure 24.7, Rmax was affected more than S.77

Although models of the leading edge of the a-wave yield useful parameters for describing the health of the photoreceptors, simpler approaches, using stronger flashes than those advised by the ISCEV Standard (Fig. 24.1 and Box 24.1)4 are helpful. Hood and Birch,77,78 and other investigators,79 have noted that a pair of strong flashes, or even a single flash that nearly or just saturates the rod response can be analyzed without fitting a model, to make a rough estimate of Rmax, and to measure a peak time that is related to S. Such measurements for a single flash77 are illustrated in the bottom row of Figure 24.7. The flash strength used was 4.0 log sc td.s which is 63 times (1.8 log units) higher than the current ISCEV standard flash1 for mixed rod-cone ERG (assuming an 8 mm pupil).

Mixed rod-cone a-wave

For weak to moderate stimuli flashed from darkness, a-waves are dominated by rod signals, but stronger flashes such as those used for responses in the top row of Figure 24.5 elicit mixed rod–cone ERGs. To investigate relative contributions of rod and cone-driven responses to the ERG, it is necessary to separate them. Figure 24.8 shows the ERG (open circles) of a dark-adapted macaque in response to two different flash strengths, and the individual rod- and cone-driven contributions.

Rod-driven responses (filled circles) in Figure 24.8 were extracted by subtracting the isolated cone-driven response to the same stimulus from the full mixed rod–cone response. Isolated cone-driven responses (triangles) were obtained by briefly (1 s) suppressing, rod-driven responses with an adapting flash, and then measuring the response to the original test stimulus, presented 300 ms after offset of the rod-suppressing flash. Cone-driven responses in primates recover to full amplitude within about 300 ms, whereas rod-driven responses take at least a second, making it possible to isolate the cone-driven responses. The cone photoreceptor-driven portion of the leading edge of the a-wave represented about 20 percent of the saturated response in Figure 24.8. Model lines for the rod (purple) and cone (blue) photoreceptor responses are modifications of Lamb and Pugh’s model.75,72 The entire cone-driven a-wave (cyan line) is larger than the modeled photoreceptor contribution (blue line) because it includes additional negative-going signals from postreceptor OFF pathway that can be eliminated with ionotropic glutamate receptor antagonists, as described in a later section on light-adapted ERG.
Full-field dark-adapted (Ganzfeld) flash ERG

Cones. Note that the (derived) photoreceptor response is quite large at its peak time for the ERG responses to weaker stimuli, when the leading edge of the a-wave is too small to be seen. The nicely illustrates the overlapping nature of ERG components arising from different stages of retinal processing.

**Dark-adapted b-wave (PII)**

It is well accepted that the dark-adapted b-wave arises primarily from ON bipolar cells. Results of intraretinal recording and CSD analyses were consistent with its originating from bipolar (or Müller cells), and as described above, pharmacologic blockade of postreceptoral responses and specifically those of ON bipolar cells was found to eliminate the b-wave, as did mutations that prevent signaling by ON bipolar cells (Fig. 24.6).

There also is good evidence that for most of its dynamic range, the dark-adapted b-wave is generated by rod bipolar
cells in the primary rod circuit. Only for very strong stimuli, will rod signals pass via gap junctions to cones and then to cone bipolar cells, which also will then contribute to the dark-adapted flash response.41,44,81,82 For the dark-adapted, scotopic ERGs it has been possible to isolate Granit’s PII from other ERG components and to compare its characteristics with those of rod bipolar cells recorded in retinal slices. One approach is to pharmacologically isolate PII.

Figure 24.10A shows the dark-adapted ERG of a C57BL/6 mouse before and after intravitreal injection of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) to suppress inner retinal activity. GABA receptors are present in bipolar cell terminals, as well as in amacrine and ganglion cells of inner retina, as described in Chapter 23. The figure shows that the most sensitive positive and negative going waves (p- and n-STR) were removed by GABA, leaving a pharmacologically isolated PII, likely generated by rod bipolar cells. Loss of the sensitive STRs was not due to a general loss of retinal sensitivity; a-wave amplitude and b-waves, Müller cells also contribute. The PII component, pharmacologically isolated from macaque ERG (Fig. 24.11B), is similar in its rising phase to PII of the other species illustrated in Figure 24.10 legend. These findings are consistent with a single mechanism, i.e. one retinal cell type, producing the isolated PII component of the ERG.

Although bipolar cells play a major role in generating b-waves, Müller cells also contribute. The PII component, pharmacologically isolated from macaque ERG (Fig. 24.11B), is similar in its rising phase to PII of the other species illustrated in Figure 24.11A. However PII in macaque (and cat) recovers more slowly to baseline. Macaque PII in Figure 24.11B has been analyzed into a fast component, and a slow component that is a low-pass filtered version of the fast component. In cat, intravitreal injection of Ca²⁺ to block inward rectifying K⁺ channels in Müller cells did not eliminate the b-wave,55,87 but did remove a slow portion of isolated PII that is similar in timecourse to a low pass filtered version of the response.68 Although the slow component (in response to a brief flash) is lower in amplitude than the fast component, the area under the two curves is similar in both cat and the macaque. With longer-duration stimuli such as those used in early studies in amphibians,66 contributions of neuronal and glial generators of the b-wave could be equal.

Scotopic threshold response (STR)

As illustrated in Figures 24.5 and 24.10, for very weak flashes from darkness, near psychophysical threshold in humans,27,89...
The nSTR and pSTR dominate the ERG of most mammals studied. The nSTR was shown in intraretinal analyses in cat to be generated more proximally in the retina than PII.27 In studied. The nSTR was shown in intraretinal analyses in cat (NMDA) receptors present on inner retinal neurons, also in Figs 24.10 and 24.11, or glycine).90 Blockade of non-invasive recordings, the p and nSTR can be suppressed, or unable to respond due to characteristics not only from PII, but also from the pSTR, nSTR (and PII) components of the mouse ERG. These components add together to form the ERG response (colored lines) at a particular time after the stimulus flash. The model assumes that each ERG component (colored lines) initially rises in proportion to stimulus strength, and then saturates in a characteristic manner, as has been demonstrated in single cell recordings in mammalian retinas92,93 as well as for a- and b-waves in numerous other studies. To isolate PII at higher stimulus strengths when the photoreceptor contribution to the ERG is significant (see Fig. 24.9), the PIII (photoreceptor) component must be removed, A model line for PII is shown in Figure 24.10B and D for the higher stimulus strengths.26

The neuronal origins of the nSTR and pSTR are species-dependent. In macaques, the nSTR may originate predominantly from ganglion cells; it was eliminated in severe experimental glaucoma with selective ganglion cell death, whereas the pSTR remained.43 In contrast, in rodents the pSTR requires the healthy function of ganglion cells. The pSTR was eliminated in mice and rats by removing ganglion cells (optic nerve crush (ONC) or transection and subsequent degeneration) and in mice in which ganglion cells were deleted genetically, whereas the nSTR was only partially removed in rat, and of normal amplitude in mice.22,24-95 In cat (and human) the nSTR also survives ganglion cell loss,96 and in cat, it has been demonstrated that nSTR generation involves K+ currents in Müller cells.24

Mice lacking connexin 36 gap junction proteins (Cx36) provide additional insights to the neuronal origins of the STR. Cx36 is expressed in the electrical synapses (gap junctions) between AII amacrine cells, AII amacrine cells and ON cone bipolar cells as well as between the rod and cone terminals.82,97 In the scotopic ERG of mice with Cx36 genetically deleted, pSTR remains, whereas the nSTR is absent.44 Because Cx36 is essential for transmission of ON pathway signals to ganglion cells in the primary rod pathway,82 preservation of the pSTR in Cx36−/− mice suggests that the pSTR, shown to be ganglion cell related in rodents, must depend upon activity of OFF, rather than ON ganglion cells. The nSTR, in contrast, depends on the syncytium of AII amacrine cells and their electrical synapses with ON cone bipolar cells, no longer functional in Cx36-deficient mice. Figure 24.11A includes an example of PII isolated from the scotopic ERG of a Cx36−/− mouse (already lacking the nSTR) but also lacking the pSTR, due to a ganglion cell lesion subsequent to ONC. This isolated PII is very similar to PII isolated pharmacologically or by light adaptation, as well as to the rod bipolar cell recordings from the retinal slice.

Because of their specific inner retinal origins, the n- and pSTRs are of particular interest in animal models of diseases that affect the inner retina, such as glaucoma. They provide non-invasive readouts of pathologic changes in function, and can be used to document effects of neuroprotective agents.22,43,98

**Light-adapted, cone-driven ERGs**

**Isolating cone-driven responses**

To study the cone-driven (photopic) flash ERG, rods must be suppressed, or unable to respond due to characteristics of the stimuli selected. Typically for recording photopic ERGs, rods are rendered unresponsive by imposing a steady background light sufficient to saturate their responses (i.e. >25 cd.m–2). Another approach is to briefly saturate rod responses, and to wait for cones to recover. Cones recover more quickly than rods: in primates, in about 300 ms.
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Spectral sensitivity of their medium wave-length M-cones peaks near 515 and 510 nm respectively and overlaps with that of rods, which peaks around 500 nm. Scotopic stimulus calibrations are thus adequate, and more appropriate than photopic calibrations (based on human spectral sensitivity) for the rodent M-cone responses. However, for any subject, spectral separation of responses of individual cone types is possible using ERG flicker photometry. In mice and rats, the recovery time of cone signals is longer, about a second.

Rod and cone signals also can be separated by selection of appropriate stimulus wavelength. In trichromatic primates, L-cones can be isolated using red light, at wavelengths greater than 630 nm, and excluding wavelengths much lower. However mice and rats do not have L-cones, and the spectral sensitivity of their medium wave-length M-cones peaks near 515 and 510 nm respectively and overlaps with that of rods, which peaks around 500 nm. Scotopic stimulus calibrations are thus adequate, and more appropriate than photopic calibrations (based on human spectral sensitivity) for the rodent M-cone responses. However, for any subject, spectral separation of responses of individual cone types is possible using ERG flicker photometry.

Figure 24.10 Dark-adapted mouse ERG before and after intravitreal injection of GABA: analysis of ERG components. (A) ERG of a C57BL/6 mouse in response to brief flashes of increasing stimulus strength recorded before and after intravitreal injection of GABA (30 mM intravitreal concentration assuming for this and subsequent panels, a 20 µl vitreal volume in mouse). (B) ERGs of three mice measured at 110 ms (mean ± SEM) after a brief flash, at the peak of the pSTR and b-wave. The modeled full ERG (blue line) includes four components: PII, pSTR, nSTR and PIII. All components are assumed to increase in proportion to stimulus strength before saturating. Explicitly, the exponential saturation of the pSTR, nSTR and PIII is defined by:

\[ V = V_{\text{max}}(1 - \exp(-I/I_o)) \]

where \( V_{\text{max}} \) is the maximum saturated amplitude, and \( I_o \) the stimulus strength for an amplitude of \((1 - 1/e)V_{\text{max}}\).

The hyperbolic relation used for PII is described by the function:

\[ V = V_{\text{sat}}/I(I + I_o) \]

where \( V_{\text{sat}} \) has the same meaning as for the exponential function but \( I_o \) is the stimulus strength at which the amplitude is \( V_{\text{sat}}/2 \).

(C) ERGs measured at 6 ms after a brief flash, to measure a-wave amplitude on its leading edge. (D) ERGs measured at 200 ms to measure nSTR amplitude. Model lines for the full response and for component parts are as described for panel B.
Figure 24.11 Rod bipolar cell component, PII, of the dark-adapted ERG of a human subject, macaque monkey and mouse.

(A) Comparison of rod bipolar cell current from patch recordings in mouse retinal slice (Field & Fieke, 2002) with isolated PII (by weak light adaptation) from humans, isolated PII from ERGs of six C57Bl/6 mice by intravitreal injection of GABA (32–46 mM) and from a Cx36(-/-) mouse lacking ganglion cells. Contributions to ERGs from melanopsin-driven responses are thought to be minimal.107

(B) PII of macaque, isolated by pharmacologic blockade [6,7-dinitroquinoxaline-2,3-dione (DNQX), 0.1 mM; N-methyl-D-aspartic acid (NMDA, 3 mM)] of inner retinal responses. PII has been analyzed into a fast component, proposed to be a direct reflection of the post-synaptic current, and a slow component that is a low-pass filtered version of the faster component, believed to be the Müller-cell contribution. (From Robson & Fishman 1998, unpublished observations.)

Light-adapted a-wave

The a-waves in the photopic ERG are smaller than those in the scotopic ERG, reflecting the large difference in retinal densities of cones vs rods. In human and macaque about 5 percent of the photoreceptors are cones, and only up to 3 percent in mice, and fewer in rats are cones. In addition, use of glutamate analogs has shown that much of cone-driven a-wave is postreceptoral in origin. Results of using glutamate analogs in macaque109,110 are illustrated in Figure 24.13. The a-wave was reduced in amplitude by the ionotropic glutamate receptor antagonists PDA (cis-2,3-piperidinedicarboxylic acid) and kynurenic acid, KYN, not shown) that block kainate and AMPA receptors of OFF bipolar and horizontal cells as well as cells of inner retina. In contrast, APB, which eliminates the b-wave, did not reduce the amplitude of the leading edge of the a-wave. Figure 24.13 also shows that PDA had a similar effect to that of aspartate, used to block all glutamatergic transmission, or to cobalt (Co2+), used to block voltage-gated Ca2+ channels that are essential for vesicular release of the neurotransmitter glutamate, for signal transmission to postreceptor neurons.

PDA-sensitive postreceptor neurons, rather than cones, or APB-sensitive ON bipolar cells (and more proximal cells receiving ON signals), were found to generate the leading edge of the a-wave for the first 1.5 log units of increasing flash strength that elicited a cone-driven a-wave in macaque, reaching an amplitude of 10–15 µV in experiments of the studies illustrated in Figure 24.13. Postreceptoral cells continued to contribute 25–50 percent of the leading edge of the a-wave for higher stimulus strengths when cone photoreceptor contributions also were present. The relative sizes of the postreceptor and receptor-driven portions of the photopic a-wave were modeled in Figure 24.8 for cone-isolated responses in the macaque dark-adapted ERG-based results after PDA. Responses up to ~5 ms after the flash in Figure 24.8 were cone photoreceptor responses, as indicated by the blue model line.35

Experiments in mouse to investigate the origins of the photopic a-wave yielded similar results to those in macaque. PDA-sensitive responses dominated the leading edge of the a-wave for the initial 1.5 log units, and at least half of the response to stronger stimuli. Photoreceptor

mice and rats such studies have isolated short-wavelength UV (S-) cones that peak around 358 or 359 nm.100,102

Separation of rod and cone signals in mice can also be achieved using genetically manipulated models in which either rods or cones have been inactivated. Examples of such models include: Rho(rod opsin knockout) mice which have cone function only, but also eventual cone degeneration108, Tra(−/−) (transducin α knockout) with cone function only109, Cnga3(−/−) (cone cyclic nucleotide channel deficient) mice or GNAT2(−/−) (Gq protein) mutant mice with rod function only.100 Mice with inactivated or degenerated rods and cones, i.e. rodless, coneless mice, can be used to study function of a more recently identified photoreceptor pigment, melanopsin, found in a small population of retinal ganglion cells. Contributions to ERGs from melanopsin-driven responses are thought to be minimal.107

Figure 24.12 shows photopic ERG responses to brief stimulus flashes on a rod-saturating background for human, macaque monkey, and mouse. As shown above for the dark-adapted ERG, the responses to brief flashes are similar in man and macaque. In mice, however, the b-wave rises more slowly to its peak, does not return to baseline as early, and OPs are more prominent. Very strong stimuli produce more prolongation of responses in mice, and produce larger OPs (inset on the right of Fig. 24.12) than in primates. For macaques, a second peak after the b-wave, probably an OFF pathway response (i-wave), emerges, and in some cases (not shown) more of the response is below the baseline level.
signals were present in response to strong flashes up to 8 ms after the flash. A significant portion of the a-wave also was removed by NMDA, which suppresses responses of inner retinal neurons, indicating contributions to the leading edge of the a-wave from more proximal neurons in the OFF pathway (unpublished observations, Maeda, Kaneko, and Frishman).

**Light-adapted b-wave**

Intraretinal recording studies described above for studying origins of the dark-adapted ERG, indicated a bipolar cell origin for light-adapted b-waves, but ON cone bipolar cells, rather than rod bipolar cells were implicated. Origins of the photopic b-wave also were studied in the same series of experiments in macaques as those illustrated in Figure 24.13. Figure 24.14A, top right, shows the macaque photopic ERG response to long-duration flashes, before and after intravitreal injection of APB (eye 1, left). APB removed the transient b-wave, supporting a role for ON bipolar cells in generating the response (although the possibility of glial mediation was not eliminated in these studies). In contrast, when PDA was injected first (Eye 2, right), to remove OFF pathway and inner retinal influences (and horizontal cell inhibitory feedback), the b-wave was larger in amplitude and the maximum amplitude was sustained for the duration of the stimulus. These findings indicate that cone-driven b-waves in primates are normally transient because they are truncated by (PDA-sensitive) OFF pathway contribution of opposite polarity to the ON bipolar cell contribution (a push–pull effect), or by inhibitory feedback via cones from horizontal cells. Only an isolated photoreceptor response of slow onset, but more rapid offset, remained after all postreceptoral activity was eliminated, either by APB followed by PDA, or PDA followed by APB.

The outcome in mouse of parallel experiment to that in Figure 24.14A to determine origins of the photopic b-wave is shown in Figure 24.16B. The figure shows that the control response to a 200 ms stimulus in a C57BL/6 mouse is different from that in primates, and in fact, the b-wave looks similar to the response in macaque after PDA. APB removed the b-wave in the mouse, but did not reveal a d-wave, only a tiny positive intrusion was present. Similar results have been observed in rat. PDA injection in mouse produced only a small increase in b-wave amplitude; but it removed OPs, and the small positive intrusion. APB combined with PDA left a negative ERG of slower onset than in the normal ERG due to removal of the leading edge of the a-wave. The slow recovery of the isolated photoreceptor-related response, compared to that of the macaque, was due to the presence, in mouse, of slow PIII in the ERG.
Light-adapted d-wave

The d-wave is a positive-going wave at light offset that is a characteristic of the photopic ERG. The d-wave is prominent in all-cone retinas, but also can be seen in the photopic ERG of the mixed rod–cone retina of man and macaque monkey for stimulus durations of 150 or 200 ms, as shown in Figures 24.2A and 24.14A. As described above, the mouse (and rat) photopic ERG does not include a d-wave in response to offset of light flashes of only a few hundred ms in duration.114–116

Intraretinal analysis in monkey retina initially indicated that d-waves represent a combination of a rapid positive-going offset of cone receptors followed by the negative-going offset of the b-wave.66,67,118 However intravitreal injection of glutamate analogs in macaques revealed a prominent role, at light offset, for OFF cone bipolar cells as well, in generating d-waves (see Fig. 24.14).105,115 The relatively slower offset of cone photoreceptor responses in rodents may explain, in part, the lack of d-waves for stimuli that elicit them in primates. The lack of d-waves in rodents is not due to differences in relative numbers of ON and OFF cone bipolar cells in rodents and primates, the proportions are similar.120

Figure 24.13 Postreceptoral contributions to the a-wave of the macaque monkey ERG. Comparison of effects of intravitreally injected L-2-amino-4-phosphonobutyric acid (APB, 1 mM) and APB+PDA (5 mM) with effects of aspartate (ASP, 0.05 M) and cobalt (Co²⁺, 1 mM) on photopic a-waves of three different eyes of two macaques. The inset at the top shows the control response of eye #1 to the 200 ms stimulus of 3.76 log td (100 cd m⁻²) on a steady background of 3.3 log td (35.5 cd m⁻²). The a-waves are shown below on an expanded time scale. For the stimulus used, the a-wave leading edge (10 µV) was mostly postreceptoral in origin.

Figure 24.14 Effects of APB and PDA on the light-adapted ERG of macaque monkey and mouse. (A) Macaque: Intravitreal injections were given in two different eyes sequentially: APB followed by PDA for eye 1, and PDA followed by APB for eye 2. The vertical line shows the time of the a-wave trough in the control response. The 200 ms stimulus and drug concentrations were the same as reported for Fig. 24.15. (From Bush & Sieving 1994,109 used with permission from Association for Research in Vision and Ophthalmology.) (B) Mouse: Control ERG and response after intravitreal injection of APB (1 mM) and PDA (0.5 mM)+ APB in a C57BL/6 mouse. The 200 ms stimulus was 4.6 log sc td (3.9 log cd m⁻²) on a steady background of 2.6 log sc td (63 cd m⁻²). (From Shiota & Finshman, unpublished.)
The interactions of ON and OFF pathways in flicker ERGs have been examined in macaque over a wide range of temporal frequencies. The typical temporal frequency response function of the photopic ERG of the macaque, illustrated in Figure 24.15A, is similar to that seen in humans. Response amplitude dips at frequencies around 10–12 Hz before rising to a maximum amplitude around 50 Hz. Injections of APB and PDA revealed that the dip is due to cancellation of contributions to the response at different phases from ON and OFF pathways. Other experiments demonstrated small more proximal retinal contributions to the flicker responses, at the fundamental frequency of stimulation, and prominent TTX-sensitive contributions to the second harmonic component of the response that peaked around 8 Hz.
Flicker ERGs in mice differ from those in primates in several ways. The frequency response for the primary rod pathway in mice extends up to 30 Hz and secondary rod pathway responses up to 50 Hz. These ranges overlap substantially with the range for cone pathway responses shown in Figure 24.15B for a control C57BL/6 mouse. The overlap of rod- and cone-driven frequency responses means that flicker cannot easily be used in rodents to selectively stimulate cone-driven responses. The lower photopic frequency response of mice relative to primates is due at least in part to the slower recovery of cone photoreceptors in mice. Figure 24.17B also shows the frequency response of Nob1 mice that lack ON pathway responses. The low amplitude of responses in Nob1 mice indicates that OFF pathway contributions to the light-adapted frequency response are smaller in mice than in primates. The figure also shows that ON and OFF pathway responses do not interact to cancel responses at midrange frequencies in mice. Similar to macaques, however, the photoreceptors, whose contribution was isolated by injection of PDA, contributed only a small signal. Second harmonic responses in C57BL/6 mice, that peak around 17 Hz, were found to be greatly reduced either by loss of ON pathway responses or injection of TTX.

### Oscillatory potentials

Oscillatory potentials (OPs) in flash ERG responses to strong stimuli consist of a series of high-frequency, low-amplitude wavelets superimposed on the b-wave. OPs are present under dark- and light-adapted conditions. The mixed rod–cone flash ERG of a human (Fig. 24.1 top): includes at least four OPs that can be extracted by filtering the response to remove frequencies lower than ~75 Hz signals. The number of wavelets varies between four and ten depending upon stimulus conditions; the temporal characteristics of OPs vary as well. As an example, a recent study described dark-adapted OPs in humans as ranging in frequency from 100 Hz or less to more than 200 Hz, with a peak around 150 Hz for moderate stimulus strengths, but closer to 100 Hz for the strongest stimuli. Light-adapted OPs also can include a high-frequency band peaking around 150 Hz, but have a lower-frequency band peaking that peaks around 75 Hz and extends to frequencies of about 50 Hz, below the low-frequency cutoff (75 Hz) for isolating OPs recommended by ISCEV.

There is consensus, from experiments in amphibians and mammals, that OPs are generated by inner retinal neurons, making them useful for evaluating inner retinal function. However the discrete origins of different OPs, numbered in order from the first to occur, and mechanisms of their generation, are not well understood. Intraretinal studies using stimuli that elicited responses from both rod and cone systems in macaques localized OPs, as a group, to inner retina. For a brief light flash, the major OPs in the photopic ERG were found to be APB-sensitive, indicating an origin in the ON pathway, but later OPs in the brief flash response, and at light offset for longer duration stimuli were found to originate from OFF pathway. TTX reduced amplitudes of later OPs more than early ones in rabbits, but this finding has not been consistent across species. Inhibitory neurotransmitters glycine and GABA suppress OPs in monkeys and other mammals, as do ionotropic glutamate receptor antagonists that block transmission of signals from bipolar cells to amacrine and ganglion cells. Consistent with this, PDA removed OPs from the mouse light-adapted ERG illustrated in Figure 24.16B. GABAergic involvement is limited to specific receptor types; genetic deletion of GABA receptor D in mice enhanced amplitudes of the OPs, whereas blockade of GABA receptors removed OPs in C57BL/6 mice (unpublished observations).

Although the observations described above indicate involvement of amacrine or retinal ganglion cells in generating OPs, the role of ganglion cells has been controversial. Loss of OPs after ganglion cell death has not been observed consistently across species, making amacrine cells more likely generators. Reductions in OPs have been reported in conditions such as diabetic retinopathy that compromise inner retinal circulation, but not ganglion cell function selectively. However, in Ogden’s studies in macaques, optic nerve section and subsequent ganglion cell degeneration lead to disappearance of the OPs. Further, in the macaque photopic “flash” ERG studies of macular regions (using a multifocal stimulus), both severe experimental glaucoma and TTX removed a high-frequency band of OPs (centered at 143 Hz), while a lower-frequency band (at 77 Hz) remained intact. In contrast, OPs were still present in full-field dark-adapted ERGs. The mechanisms for generating OPs are likely to involve both intrinsic membrane properties of cells and neuronal interactions/feedback circuits. Oscillatory activity has been observed most frequently in amacrine cells (ACs). For example, GABAergic wide-field ACs (WFAC) isolated from white bass retina generated high-frequency (~100 Hz) oscillatory membrane potentials (OMPs) in response to extrinsic depolarization. These OMPs were shown to arise from “a complex interplay between voltage-dependent Ca 2+ currents and voltage- and Ca 2+-dependent K+ currents.” A feedback mechanism is consistent with effects on OPs of GABA and glycine, both of which participate widely in feedback circuits in inner retina, e.g. between amacrine cells and bipolar cells or other amacrine cells (see Chapter 23). A feedback model has been proposed to account for high-frequency oscillatory (or “rhythmic”) activity seen in some mammalian ganglion cell recordings from whole retina that involves electrical synapses indicated by tracer coupling patterns, as well as inhibitory feedback circuits between amacrine and ganglion cells.

OPs are more prominent in mice than in primates or rats. Peak temporal frequencies of OPs, in dark-adapted C57BL/6 mice range from 100 to 120 Hz or higher, as in humans, whereas they are about 70–85 Hz in light-adapted mice. In rats where OPs are much smaller overall, two frequency bands of OPs have been reported for dark-adapted ERG, one peaking around 70 Hz and the other, more like other species, around 120–130 Hz. In the light-adapted ERG, only the lower-frequency band is present, similar to the findings in mice. In mice and rats, OPs under dark- and light-adapted conditions are present at frequencies lower than common in primates, i.e. down to 50 Hz, and in mice small OPs can occur at frequencies as low as 30–40 Hz.

### Photopic negative response

A slow negative wave after the b-wave, called the photopic negative response (PhNR) can be seen in the light-adapted
ERG, more prominently in primates than in mice and rats. In humans and monkeys the PhNR is thought to reflect spiking activity of retinal ganglion cells. As shown in Figure 24.16, the PhNR is reduced in macaque eyes with experimental glaucoma (or after TTX injection) and in humans with primary open angle glaucoma (POAG). It also is reduced in several other disorders affecting the optic nerve head and inner retina. The slow timecourse of the PhNR suggests glial involvement, perhaps via K+ currents in astrocytes in the optic nerve head set up by increased \([K^+]_o\) due to spiking of ganglion cells. PhNRs can be evoked using white flashes on a white background, if the flash is very bright. However a red LED flash on a blue background, as was used for responses in Figure 24.16, elicits PhNRs over a wider range of stimulus strengths. The red flash may minimize spectral opponency that would reduce ganglion cell responses, and use of a blue background suppresses rods while minimizing light adaptation of L-cone signals. The PhNR can be exaggerated relative to other major ERG components using slowed focal stimuli confined to the macula.

In rodents, with relatively fewer ganglion cells than in primates, the PhNR is small, and probably originates mainly from amacrine cells. It is reduced in amplitude by TTX, PDA blockade of block transmission to inner retina in rats and mice, NMDA to suppress inner retinal activity, but not by loss of ganglion cells in mice, as shown in the inset to Figure 24.17. Glial involvement in generation of the PhNR in rodents is likely. In Royal College of Surgeons rats, a large photopic ERG negative response develops in the degenerating retina, due at least in part to increased density of Kir4.1 channels in Müller cell endfeet. These channels are critical for producing glial currents that contribute to the ERG.

**Pattern ERG**

A common technique for assessing ganglion cell function is to record a pattern ERG (PERG). The stimulus is usually a contrast-reversing checkerboard or grating pattern for which changes in local luminance occur with each reversal, but the mean luminance remains constant. This causes the linear signals that produce a- and b-waves to cancel, leaving only the non-linear signals in the ERG. Non-linear signals that compose the PERG are known to depend upon...
Figure 24.17 Transient pattern electroretinogram (PERG) of human, macaque monkey and mouse. (A) Representative transient PERG of a normal human subject, elicited with a contrast-reversing checkerboard pattern (0.8 deg checks), mean luminance > 80 cd m\(^{-2}\), modulated at 2 Hz, 100 percent contrast. Amplitude for normal humans ranges from 2 to 8 μV, according to the "ISCEV standard for clinical pattern Electroretinography 2007 update" from which the figure was adapted (from Holder et al 2007, used with permission). (B) Transient PERG of a macaque, elicited with a checkerboard (0.5 deg checks), mean luminance of 55 cd m\(^{-2}\) modulated at 2 Hz; 84 percent contrast. (Unpublished, Luo & Frishman). (C) PERG of a C57BL/6 mouse before (red line) and 40 days after (red line) unilateral optic nerve crush (ONC) when ganglion cells had degenerated. The pattern was a 0.05 c/deg horizontal bar grating, mean luminance 45 sc cd m\(^{-2}\); contrast-reversed at 1 Hz; 90 percent contrast. Inset shows brief flash ERGs for the same eye: 57 cd s m\(^{-2}\) flash on a 63 sc cd m\(^{-2}\) background before (black line) and 40 days after (red line) ONC. (From Miura et al 2009, used with permission.)

The PERG is also useful for study of rodent models of glaucoma. The DBA/2J mouse model of inherited glaucoma has normal ganglion cell numbers at 2 months of age, and progresses to massive retinal ganglion cell degeneration by 12–14 months. This is reflected in the PERG which is of normal amplitude in young DBA/2J mice, whereas it is practically eliminated in older mice. Because the PERG is a non-invasive measure it can be used in these mice and other rodent models of glaucoma to track progression of ganglion cell loss as well as to document effects of therapies to slow progression and protect neurons. PERGs can be recorded as transient responses to low reversal frequencies, 1–2 Hz, or as steady state responses to higher frequencies, e.g. 8 Hz. The transient PERG has prominent early positive and later negative waves, named respectively, P50 and N95 (see Fig. 24.17) in humans, denoting the timing of the peak and trough following each pattern reversal. Both waves reflect ganglion cell activity, although P50 may include some non-spiking input. The early positive (P1) and later negative (N2) component (Fig. 24.17) in the mouse transient PERG, different in exact timing, but similar general appearance to P50 and N95 in primates.

**Multifocal ERG**

The multifocal ERG (mERG) provides a technique for simultaneously recording local ERG responses from many small retinal regions, typically 60 or 103, over 35–40 degrees of the visual field. Focal ERGs from individual regions are recorded in some clinics and labs, but sampling more than a couple of regions is time consuming. Figure 24.18A illustrates the stimulus and mERG records from a normal human subject. The individual hexagons of the stimulus were reversed in contrast following a pseudorandom (m-) sequence which is shifted in time uniquely for each sector to enable extraction via correlation of individual responses. The reversal rate was locked to the frame rate of the visual stimulator, 75 Hz for CRTs, 60 Hz for LCD. Each hexagon had a 50 percent chance of reversing contrast on each frame change. The test is usually done under light-adapted conditions, for which the foveal response is large, allowing changes in function, such as occur in Stargardt’s disease, or other macular dystrophies to be easily seen. The scotopic mERG, rarely attempted, was found to be more susceptible than light-adapted recordings, to effects of scattered light. Dark-adapted recordings in rodents required use of large focal regions, and although difficult, some light-adapted recordings in rodents have been possible.

Studies using glutamate analogs have shown that despite different methods of generation and response timing of the mERG, the cellular origins of the major positive and negative waves are essentially the same as the light-adapted flash ERG; see Figure 24.18B. This was the case for the standard fast mERG, as well as when the stimulus presentation was slowed by interleaving blank frames between m-steps, to allow the full ERG to form. A small optic nerve head component from axons of retinal ganglion cells may also be observed in the mERG, especially when stimuli are arranged to optimize the response.
The optic nerve head component is the likely generator of high-frequency oscillatory potentials in the slow sequence mfERG that are reduced in experimental glaucoma.

**Closing comments**

The ERG provides a means for non-invasive evaluation of normal and abnormal retinal function in humans and in animal models. Its value as a test has increased as we have better understood the retinal circuits involved and mechanisms of generation for each wave. Our current understanding of the origins of the ERGs recording in standard testing, and in more specialized testing, as reviewed in this chapter, are summarized in Boxes 24.2 and 24.3 respectively.
References


SECTION 7 Visual processing in the retina

Chapter 24 Electroretinogram of Human, Monkey and Mouse