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Noninvasive Electoretinographic Procedures for the Study of the Mouse Retina

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Abstract

Overall retinal function can be monitored by recording the light-evoked response of the eye at the corneal surface. The major components of the electroretinogram (ERG) provide important information regarding the functional status of many retinal cell types including rod photoreceptors, cone photoreceptors, bipolar cells and the retinal pigment epithelium (RPE). The ERG can be readily recorded from mice, and this unit describes procedures for mouse anesthesia, and the use of stimulation and recording procedures for measuring ERGs that reflect the response properties of different retinal cell types. Through these, the mouse ERG provides a non-invasive approach to measure multiple aspects of outer retinal function, including the status of the initial rod and cone pathway, rod photoreceptor deactivation, rod dark adaptation, the photoreceptor-to-bipolar cell synapse, and the RPE.

Keywords

mouse; electrophysiology; retina; photoreceptor; bipolar cell; retinal pigment epithelium; electroretinogram

INTRODUCTION

In response to light, the retina generates a field potential referred to as the electroretinogram (ERG). ERGs have been studied for more than 150 years, and are known to reflect the combined response of multiple cell types (Perlman, 2017). For several ERG components, the underlying cellular generators have been established (Frishman, 2006). The ability to link a change in an ERG component to altered function of retinal cells, coupled with the fact that ERGs can be recorded noninvasively using a corneal electrode, makes the ERG a powerful functional measure for application to many vision research applications. In human subjects, ERGs are recorded to address basic research questions and are used clinically for patient

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Conflicts of Interest

The authors have no conflicts of interest to declare.

diagnosis and to define the natural history of disease progression, among other applications (Heckenlively and Arden, 2006). Animal subjects can be examined under anesthesia, and ERGs have been reported for many species to address a wide range of experimental questions. Over the last ~25 years, the laboratory mouse has become a primary model for retinal research, as naturally occurring mutants were discovered (Baehr and Frederick, 2009; Veleri et al., 2015), and other models were generated by mutagenesis (Krebs et al., 2017) and through genetic engineering based on the identification of human disease genes or genes expressed in retinal cells (Veleri et al., 2015). In the characterization of these models, the ERG has provided a key measure of overall retinal function. This article describes basic protocols for noninvasive recording of the light-evoked response of the initial stages of the visual system (flash ERG), and of the slower electrophysiological response evoked by the retinal pigment epithelium (RPE) when photoreceptors respond to light (dc-ERG).

Basic Protocol 1 describes methods to record the flash ERG from anesthetized mice. Alternate Protocol 1 describes a variation on Basic Protocol 1 that can be used to monitor deactivation of the phototransduction process. Alternate Protocol 2 describes a variation on Basic Protocol 1 that can be used to measure dark adaptation recovery following a photobleach. Alternate Protocol 3 describes a variation on Basic Protocol 1 that can be used to measure a response component that reflects activity of the RPE. Basic Protocol 2 describes methods to record the direct current-coupled ERG (dc-ERG).

NOTE

All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or must conform to governmental regulations regarding the care and use of laboratory animals.

Basic Protocol 1

Flash Electroretinogram Analysis of Photoreceptor and Bipolar Cell Function

The flash ERG reflects the mass response of the outer retina to light. Based on the contribution of many laboratories using a variety of experimental techniques, the Flash ERG can be used to monitor the functional status of several, but not all, of the major retinal cell types. In this protocol, we focus on three cell types: rod photoreceptors, rod bipolar cells (BCs), and cone depolarizing BCs. Rod-driven signals are obtained by presenting flash stimuli to the dark-adapted eye. The rod pathway can be selectively monitored using low luminance stimuli that are too dim to stimulate the less sensitive cone photoreceptors. Under these conditions, the dark-adapted ERG is dominated by the b-wave component, which reflects the response of rod BCs. Note that older literature links the b-wave to Müller cell activity (e.g., Newman and Odette, 1984). But this link was disproven when Kofuji et al. (2000) reported ERGs from mice lacking Kir4.1. In response to light flashes, these mice lacked the Müller cell response, but retained the ERG b-wave. Further work has solidified the link between the ERG b-wave and BC activity (Robson and Frishman, 1995; Hood and Birch, 1996; Sharma et al., 2005). The ERG b-wave is valuable in the study of mouse mutants that have altered photoreceptor-to-BC signaling due to mutations in genes encoding proteins that (a) control glutamate release at photoreceptor terminals, (b) are involved in the

depolarizing BC signal transduction cascade or its regulation, (c) are instrumental in formation of the ribbon synapse between photoreceptors and depolarizing BCs, or play other roles in BC survival or physiology (reviewed in Pardue and Peachey, 2014).

When flash strength is increased, the b-wave is preceded by a cornea-negative component, the ERG a-wave. The a-wave reflects the light-induced closure of cyclic-nucleotide gated channels along the rod outer segment (Pugh and Lamb, 2000). The a-wave is of value when evaluating mouse mutants involving proteins that are expressed in photoreceptors including those that (a) play a role in phototransduction or its regulation, (b) are used in the visual cycle whereby light-sensitivity is restored to photoreceptor outer segments, (c) are required for outer segment formation or the trafficking of outer segment proteins from the inner to the outer segment. Many of these models share a common phenotype of photoreceptor apoptosis, and are thus reflective of the genetically heterogeneous human condition retinitis pigmentosa (retinet.org). In others, photoreceptors are retained, and the ERG can be used to monitor the impact of the mutation on photoreceptor physiology.

The vast majority of photoreceptors in the mouse retina are rods, but some 2–3% are cones (Carter-Dawson and LaVail, 1979). Although the ERG recorded in response to stimuli presented in the dark is dominated by rod-driven activity, it is possible to isolate cone-driven ERGs by superimposing stimuli upon a steady adapting field which desensitizes the rods.

Mouse models have been identified with selective or predominant loss of rod function (e.g., Calvert et al., 2000) or cone function (e.g., Chang et al., 2006). Basic Protocol 1 describes an approach to measure rod- and cone-mediated function using, respectively, dark-adapted and light-adapted mouse ERGs.

Materials

Mouse subject

Anesthesia

ketamine HCl (100 mg/ml)

xylazine (100 mg/ml)

0.9% saline

0.3 cc syringe

Eye drops

1% tropicamide

2.5% phenylephrine HCl

1% cyclopentolate HCl

1% proparacaine HCl

1% carboxymethylcellulose

Needle electrodes

Corneal electrode

X-Y-Z positioner, or alternative electrode position

Long-wavelength darkroom light

Ganzfeld visual stimulator

Signal averaging system

Heating pad

Initial preparation

- 1 Pre-test dark adaptation. Dark adapt for at least two hours. Overnight dark adaptation is preferred. This is because the visual system requires time to reach maximum sensitivity in the dark and dark adaptation can be substantially delayed in mouse models that impact the processes that are involved in dark adaptation (e.g. Saari et al., 2001). Overnight dark adaptation is preferable because it ensures that dark adaptation will be complete, and allows testing to begin in the morning. *The use of a temperature- and humidity-regulated space is usually acceptable to IACUCs from the standpoints of housing outside of the animal vivarium.*
- 2 Once dark adaptation has been initiated, perform all animal procedures under dim long wavelength illumination.
- 3 Anesthetize mouse with intraperitoneal ketamine (80 mg/kg) and xylazine (16 mg/kg). For accuracy, it is helpful to dilute these agents with saline (0.5 ml of 100 mg/ml ketamine; 0.1 ml of 100 mg/ml xylazine; 4.4 ml of saline). Alternative anesthetics can be used (cf., Nair et al., 2011).
- 4 Dilate pupils by applying mydriatic eye drops (tropicamide, phenylephrine HCl, cyclopentolate HCl). Atropine is an alternative mydriatic. Anesthetize corneal surface with proparacaine HCl eye drops. One eye drop per eye is sufficient. Some labs use a micropipette to administer eye drop solutions.
- 5 Place mouse on temperature-regulated heating pad, set at 37 deg C (mouse body temperature).
- 6 Place needle electrode in the tail and connect to ground. Place a second needle electrode in the cheek and connect to reference.
- 7 Wet the active electrode with carboxymethylcellulose and place in contact with the center of the cornea of the test eye. We use a home-made electrode (Goto, 1995) constructed from thin stainless steel wire and curled into a loop at the contact. The electrode is mounted on a X-Y-Z positioner with a magnetic base, that allows the loop to be carefully positioned in the center of the cornea of the test eye. Note that other types of corneal electrodes are possible (e.g., Sagdullaev et al., 2005).

Recording

- 8** Dark-adapted stimulus series. During this phase of the recording session, present stimuli in order of increasing flash strength within a ganzfeld, a circular integrating sphere which results in a homogeneous stimulus flash. We use a series of 10 flash levels that range from $-3.6 \log \text{cd s/m}^2$ to $2.1 \log \text{cd s/m}^2$. Use signal averaging to improve signal-to-noise ratio. Increase the interstimulus interval between flashes as flash luminance increases. We average 20 successive responses at our lowest flash level, and use a 4 sec interstimulus interval. As flash luminance increases, we decrease the number of responses averaged and increase the interstimulus interval.

In wild type mice, signal averaging is particularly important when recording low-amplitude responses. In disease models with low amplitude ERGs, signal averaging may be of value for all stimulus conditions, and it may be helpful to increase the number of successive responses that are averaged together to improve the signal-to-noise ratio. With increasing flash strength, increase the inter-flash interval to avoid light adaptation. Light adaptation will be seen as a reduction in response amplitude with successive flash presentations. If this is noted, then increase the inter-flash interval.

Figure 1A presents responses obtained from a wild-type adult mouse to a series of 10 flash levels presented to the dark-adapted eye. The total acquisition time used here is 512 ms, with a 20 msec pre-stimulus baseline (to establish that the recording preparation was stable). The amplifier band-pass was set to 0.3 – 1,000 Hz and amplifier gain was varied according to overall response amplitude.

- 9** Light-adapted stimulus series. After completion of the dark-adapted stimulus series, present a steady field of light within the ganzfeld and wait 7 minutes. The purpose of the adapting field is to desensitize rods so that flash stimuli presented against the adapting field evoke cone-mediated responses. We use a 20 cd/m^2 adapting field. The 7-minute adapting period reflects that the amplitude and timing of the cone ERG changes during the first ~7 minutes of light adaptation (Peachey et al., 1993). To ensure stable responses, we recommend that responses be obtained only after waiting 7 minutes. We use a series of 7 flash levels that range from $0.8 \log \text{cd s/m}^2$ to $2.1 \log \text{cd s/m}^2$. These are presented in order of increasing luminance and we average 25 successive responses to stimuli presented at 2.1 Hz.

This 7-min period provides a good opportunity to verify that the active electrode has not moved and, if needed, to administer a supplemental anesthetic (~25% of initial dose).

Figure 2A presents responses obtained from a wild-type mouse to a series of 7 flash levels superimposed upon a steady 20 cd/m^2 adapting field. Amplifier and time base settings are the same as used for dark-adapted recordings.

Analysis

- 10 Dark-adapted ERGs have two major components. ERG waveforms are plotted so that the b-wave component is up (cornea positive) and the a-wave is down (cornea negative). In response to the lower stimulus range used here, there is a single positive component, the ERG b-wave. Two components appear at the upper stimulus range, and the slower b-wave is preceded by the cornea-negative a-wave.
- 11 The amplitude of the b-wave is measured to the major positive peak, as diagrammed in Figure 1B for three flash conditions that cover the stimulus range. For flash stimuli where there is no initial a-wave, this measurement is made from the pre-stimulus baseline. For flash stimuli, which evoke an initial negative potential, this measurement is made from the lowest point or trough of that potential. This analysis can be done using cursors provided on most commercial ERG systems. Alternatively, ERG measurement can be accomplished off-line after importing the waveforms into Excel or similar program.
- 12 The amplitude of the a-wave is measured from the pre-stimulus baseline. As diagrammed in Figure 1C for three flash conditions, this measurement is made to a specific time point after the flash (8 msec in this example). Historically, the a-wave has been measured to the negative trough. But this convention is complicated by several factors. One is the presence of negative polarity components generated by the inner retina that contribute to the ERG, which do not directly reflect the photoreceptor response. Another is that this convention relies on the initiation of the b-wave to interrupt the a-wave, and thus the measure would be affected by the many conditions that impact the b-wave (Pardue and Peachey, 2014). Bearing in mind that the main purpose of a-wave amplitude measurement is to document rod photoreceptor activity, the convention developed by Robson and Frishman (1998–1999) is recommended, in which the a-wave is measured to a fixed point in time that precedes the actual trough. While the ERG reflects many generators, in non-human primates, Robson et al. (2003) have demonstrated that this procedure has less contamination by responses from the inner retina or cone photoreceptors.

Alternate Protocol 1

ERG-BASED ANALYSIS OF ROD PHOTORECEPTOR DEACTIVATION—The absorption of light by a rhodopsin molecule causes the isomerization of 11-*cis* retinal to all-trans retinal and the formation of activated rhodopsin (R*) which drives the phototransduction process (Lamb and Pugh, 2006). R* is deactivated in a series of steps, involving phosphorylation of C-terminal serine residues by rhodopsin kinase (Mendez et al., 2000) and arrestin binding (Xu et al., 1997). At the single-cell level, deactivation is accompanied by restoration of the photoreceptor dark current and recovery of photoreceptor response amplitude (Chen et al., 1995; Xu et al., 1997; Mendez et al., 2000). This recovery can be monitored using a two-flash protocol (Pepperberg et al., 1996), at the single-cell level and by ERG analysis. Alternate Protocol 1 can be used to monitor photoreceptor

deactivation in mice (Chen et al., 1995; Kim et al., 2005; Budzynski et al., 2010; Shin et al., 2017).

Materials / Initial Preparation

- 1 Overnight dark adaptation, pupil dilatation, and other aspects of animal preparation as described under Basic Protocol 1.

Recording

- 2 This protocol consists of a series of two-flash trials using high luminance stimuli. The duration between Flash 1 and Flash 2 (interstimulus interval, ISI) is varied across the different trials. Trials are presented in order of decreasing ISI. Wait at least 2 minutes between each trial. The interval is sufficient if the response to the Flash 1 does not change across trials. We use ISI values of 64 sec, 32 sec, 16 sec, 8 sec, 4 sec and 2 sec. In this example (Figure 3A), the luminance of the second flash (Flash 2) was chosen to evoke a large amplitude a-wave, which measures the extent of recovery of the rod photoresponse. The luminance of the first flash (Flash 1) can vary, but in the example shown here matches that of Flash 2. As the ISI becomes shorter, the a-wave evoked by Flash 2 will decrease, reflecting incomplete recovery of the photoresponse during a given ISI.

Analysis

- 3 Measure a-wave amplitude as described under Basic Protocol 1. As shown in Figure 3B, the data are summarized by expressing the ratio of the amplitude of the a-wave obtained to Flash 2 (R2) to that obtained to Flash 1 (R1), as a function of ISI.

Alternate Protocol 2

ERG-BASED ANALYSIS OF ROD DARK ADAPTATION—Dark adaptation refers to the processes by which the visual system recovers sensitivity after exposure to a bright light. While dark adaptation involves non-retinal factors such as pupil dilation, dark adaptation is governed in large part by the visual cycle and the restoration of light-sensitive rhodopsin in rod photoreceptor outer segments. In humans, dark adaptation is typically measured by monitoring the time course over which the threshold to detect a light stimulus declines (Lamb and Pugh, 2004). Collectively, dark adaptation reflects the ability of rods to eliminate all-*trans* retinol, of the RPE to recycle all-*trans* retinol to 11-*cis* retinal and of rods to use 11-*cis* retinal to regenerate light-sensitive rhodopsin. Many proteins are involved, and mutations in the genes that encode these proteins have been linked to human retinal disease (Lamb and Pugh, 2004) and these discoveries have also been used to guide the development of relevant mouse models. In these models, dark adaptation recovery is monitored in terms of the growth of the amplitude of the ERG as a function of time following exposure to a bright light that causes a substantial fraction of the rhodopsin molecules to be bleached (e.g., Saari et al., 2001; Kim et al., 2005; Budzynski et al., 2010; Shin et al., 2017). Alternative Protocol

2 can be used to monitor the recovery of the ERG to a previously determined dark-adapted baseline level.

Materials / Initial Preparation

- 1 Overnight dark adaptation, pupil dilatation, and other aspects of animal preparation as described under Basic Protocol 1.

Recording

- 2 Record dark-adapted baseline ERG using a flash stimulus that evokes a large amplitude a-wave.
- 3 Expose the eye to a bright light for 2 minutes. The adapting light used for Basic Protocol 1 can be used for this purpose. Other light exposure regimens can also be used.
- 4 Monitor ERG amplitude at 5-minute intervals for up to 60 minutes of dark adaptation. Figure 4A presents a series of responses obtained from a wild-type adult mouse to the same stimulus flash presented at baseline and then as a function of time following the offset of the bright bleaching light.

Analysis

- 5 Measure a-wave amplitude as described under Basic Protocol 1.
- 6 Plot a-wave amplitude as a function of time following the offset of the bleaching light. In Figure 4B, this is plotted in μV units, but response amplitude can also be expressed relative to the baseline response, a convention which should decrease variability between animals. Recovery is measured as time required to reach the pre-bleach baseline.

Alternate Protocol 3

FLASH ERG-BASED ANALYSIS OF RPE FUNCTION—As noted in Basic Protocol 1, the strobe flash ERG can be used to monitor the function of the initial stages of the rod and cone visual pathways. With a minor modification of that protocol, it is also possible to measure the ERG c-wave. The c-wave reflects the sum of signals generated by non-neuronal cells. As reviewed in Steinberg et al. (1985), one is a positive polarity component generated by the apical membrane of the RPE in response to the decrease in subretinal $[\text{K}^+]$ induced by rod photoreceptor activity. The second is a negative polarity component generated by Müller glial cells (also referred to as ‘slow PIII’), that is now known to reflect in large part Kir4.1 channel activity (Kofuji et al. 2000; Wu et al., 2004a). These components combine to define the c-wave recorded at the corneal surface and the overall response is positive because the RPE component is larger than that generated by Müller glial cells (Wu et al., 2004a).

Materials / Initial Preparation

- 1 Overnight dark adaptation, pupil dilatation, and other aspects of animal preparation as described under Basic Protocol 1. Make two changes to the usual recording settings. First, extend the length of the acquisition sweep to

approximately 2–4 sec, as the c-wave is slower to appear than the a- or b-waves. Second, use the lowest possible setting for the high-pass filter (i.e., the setting nearest to 0 Hz).

Recording

- 2 Present a high luminance stimulus to the dark-adapted mouse.
- 3 Figure 5 presents a series of ERG obtained from a wild-type adult mouse to three flash stimulus levels (0.0, 0.6, 1.4 log cd s/m²). In each case, the responses obtained to a series of successive stimuli are superimposed. The main ERG components are indicated for the uppermost set of ERGs.

Analysis

- 4 Measure c-wave amplitude from the preceding trough to the c-wave peak (see arrows in Figure 5). Across the stimulus range used here, the c-wave grows in amplitude with increasing flash luminance, but peaks at a somewhat later time.

A c-wave protocol takes only a few minutes to complete, and can therefore be readily interleaved into the Basic Protocol described above. Where it is of interest to measure slow PIII separately, this component can be revealed genetically, by using one of the many ‘no b-wave’ genetic mouse models that lack the ERG b-wave (Pardue and Peachey, 2014; cf., Samuels et al., 2010).

Basic Protocol 2

DIRECT-CURRENT COUPLED-ELECTRORETINOGRAM (DC-ERG) ANALYSIS OF RPE FUNCTION

The RPE plays multiple critical roles in support of retinal function (Strauss, 2005). Consistent with this, multiple hereditary retinal disorders have been linked to mutations in genes expressed in the RPE (retnet.org). If a long duration stimulus is used, the mouse ERG contains components with time courses that are substantially slower than the b-wave or c-wave (Kikiwada, 1968). In order of appearance, these are the fast oscillation, the light peak and the off-response. Each is generated by the RPE, secondary to light-induced retinal activity (Steinberg et al., 1985). To better evaluate mouse models for genes expressed in the RPE, we adapted an *in vivo* direct current ERG (dc-ERG) procedure (Kikiwada, 1968) to monitor these responses in anesthetized mice (Wu et al., 2004b). The value of this analysis is supported by subsequent application, where mouse models have been identified in which dc-ERG abnormalities were documented in the face of normal ERG a- and b-waves (e.g., Edwards et al., 2010; Patil et al., 2014; Collin et al., 2015; Saksens et al., 2016). Basic Protocol 2 describes the approach used to record the mouse dc-ERG.

Materials—Mouse subject

Anesthetic

ketamine HCl (100 mg/ml)

xylazine (100 mg/ml)

0.9% saline

0.3 cc syringe

Eye drops

1% tropicamide

2.5% phenylephrine HCl

1% cyclopentolate HCl

1% proparacaine HCl

1% carboxymethylcellulose

Long-wavelength darkroom light

1 mm diameter glass capillary tube (BF100-50-10, Sutter Instruments, Novato CA).

Hanks' Balanced Salt Solution (1×)

Ag/AgCl wire (Catalog No. 64-1318, Warner Instruments, Hamden, CT)

Electrode Holders (Catalog No. 64-1035, Warner Instruments, Hamden, CT)

Pipette tips covered with black electrical tape

Bleach

2 X-Y-Z positioners

Heating pad

Optical system

Light source

Neutral density filters (and holder) to control stimulus luminance

Shutter and controller (Uniblitz, Rochester, NY)

Lenses to focus stimulus onto a light guide, the other end of which is positioned in front of the test eye

DC amplifier

Initial preparation

- 1 There are several steps to electrode preparation:
 - a. Mount two Ag/AgCl wires on two electrode holders and soak them in bleach overnight.

- b.** Thread each chlorided wire into a 1-mm-diam glass capillary tube that is then filled with Hanks' Balanced Salt Solution.
 - c.** Shield the two capillary tubes using pipettes covered in black electrical tape.
- 2** Dark-adapt mice. As noted in Basic Protocol 1, dark adaptation should be for at least 2 hours. Overnight is preferable. *Once dark adaptation has been initiated all animal procedures should be performed under dim long wavelength illumination.*
- 3** Anesthetize mouse, as described in Basic Protocol 1.
- 4** Dilate pupils and anesthetize corneal surface as described in Basic Protocol 1.
- 5** Place mouse on heating pad.
- 6** Place the two electrodes in contact with the test and reference eye.
- 7** Position the light guide in front of the test eye and shield the reference eye.

Recording

- 8** After initial preparation, record amplifier output to monitor the stability of the preparation.
- 9** After a stable level is achieved, present a stimulus flash. Figure 6 presents a recording made to a 7-minute duration stimulus. The major components of the dc-ERG are indicated. In practice, a single recording is made from a given mouse, which is then returned to its home cage for recovery.

Analysis

- 10** Figure 6 indicates the method for measuring the different components of the dc-ERG. Measure the c-wave from the baseline to the c-wave peak. Note that the c-wave recorded using this protocol is larger than that obtained to a strobe flash, reflecting the prolonged integration time of the c-wave generators (Witkovsky et al., 1975). The c-wave is followed by a negative polarity component termed the fast oscillation, which has slower kinetics. Measure the fast oscillation from the c-wave peak to the fast oscillation trough. The positive polarity light peak has still slower kinetics. Measure the light peak from the FO trough to the asymptote. The off-response is generated at flash offset. In the mouse, the off-response is of positive or negative polarity depending upon flash strength (Wu et al., 2004). Measure the off-response from the light peak asymptote to the peak or trough of this component. Note that this differs from the behavior seen in other species, where the off-response has a negative polarity across all flash levels (e.g., cats: Linsenmeier and Steinberg, 1982).

Commentary

Background Information

The mouse ERG protocols described above have been developed to allow a data set to be obtained from an individual mouse within the timeframe provided by the anesthesia protocol, and our approach is to test a series of mice using a standard protocol within a single recording session that lasts less than 40 min. Where possible we run experimental and control animals interleaved. Other laboratories use a more classical physiology approach wherein a smaller number of animals are tested in an extended experimental session involving prolonged anesthesia (e.g., Saszik et al., 2002). The isolated and perfused mouse retina provides a useful preparation for pharmacological studies (Vinberg et al., 2014).

The mouse ERG protocols described here are focused on assessment of function of the outer retina. Other protocols have been described that measure function of the inner retina and/or ganglion cells in the form of the scotopic threshold response (Saszik et al., 2002) or pattern ERG (Chou et al., 2014).

The ERG examples presented here were obtained from adult mice. But since the early work of Keeler and colleagues (1928), it is clear that a reproducible response can be obtained from mice near or even before eye opening (Bakall et al., 2003; Takada et al., 2004).

Critical Parameters & Troubleshooting

There are several parameters that require attention for a robust and reproducible ERG analysis. The mouse ERG is dependent upon body temperature (e.g., Mizota & Adachi-Usami, 2002; Kong & Gouras, 2003), so body temperature must be maintained to obtain stable recording conditions within and between animals. The corneal ERG is dependent upon electrode position (Cringle et al., 1986), so this requires careful and reproducible positioning of the electrodes. We check electrode position at the completion of a recording session to ensure that the correct position has been maintained. At the completion of the recording session, it is valuable to (a) apply methylcellulose gel to the corneal surface to prevent drying and (b) to maintain mice on a heating pad until they are fully recovered. If mice awake before the completion of the recording session, this indicates that the initial anesthetic has worn off. Supplemental anesthesia can be administered during light adaptation period in Basic Protocol 1.

Anticipated Results

Basic Protocol 1 will yield an overall measure of rod- and cone-mediated outer retinal function. In mouse models, several general types of changes can be seen (Peachey & Ball, 2003):

- i. a selective reduction of the dark-adapted ERG is indicative of decreased rod function;
- ii. a selective reduction of the light-adapted ERG is indicative of decreased cone function;
- iii. an overall decrease of all responses is indicative of photoreceptor degeneration;

- iv. a selective decrease of the b-wave, which relatively normal a-wave, is indicative of abnormal function at the photoreceptor-to-depolarizing bipolar cell function, or of a defect in depolarizing bipolar cell signal transduction, or abnormal perfusion of the outer plexiform layer (Pardue & Peachey, 2014).

Alternate Protocol 1 will yield an overall measure of the kinetics of rod photoreceptor deactivation. Abnormal deactivation will be observed as a decreased response to Flash 2 for ISIs for which control mice have equivalent responses to Flash 1 and Flash 2.

Alternative Protocol 2 will yield an overall measure of the time course of rod photoreceptor dark adaptation. Abnormal dark adaptation will be observed as a delayed time to achieve the baseline level (c.f., Saari et al., 2001).

Alternative Protocol 3 will yield a measure of RPE function, the c-wave. In interpreting changes in c-wave amplitude, bear in mind that the final waveform is defined by two opposing components: a positive wave generated by the apical membrane of the RPE and slow PIII, a negative wave generated by Muller glial cells. For example, a larger than normal c-wave can be observed in mice in which slow PIII is selectively reduced (Wu et al., 2004a).

Basic Protocol 2 will yield multiple measures of RPE function, namely the c-wave, fast oscillation, light peak and off-response. For Basic Protocol 2 and Alternative Protocol 3, it is important to bear in mind that these ERG components are generated secondary to rod photoreceptor activity. So, each component will be reduced in mice with decreased rod function. The clearest interpretation will be found in mice with normal a-wave amplitude, and decreased dc-ERG components (e.g., Collin et al., 2015; Saksens et al., 2016).

Time Considerations

The protocols described here were designed to be completed within 40 minutes, the time over which the anesthetic protocol described in Basic Protocol 1 will be effective.

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Figure 1. Dark-adapted ERGs and their measurement

(A) Series of dark-adapted ERGs obtained from a wild type (C57BL/6J) mouse to strobe flash stimuli that range from -3.6 to $2.1 \log \text{ cd s/m}^2$. The major components of the response are denoted (a-wave, b-wave). (B) Method for measurement of b-wave amplitude. For stimuli that do not evoke an initial negative component (e.g., $-2.4 \log \text{ cd s/m}^2$), b-wave is measured from the pre-stimulus baseline to the positive peak. For stimuli that evoke an initial negative component ((e.g., 0.6 or $1.4 \log \text{ cd s/m}^2$), the b-wave amplitude is measured from the negative trough. (C) Method for measurement of a-wave amplitude. To avoid contributions generated by the response of inner retinal neurons, and to avoid dependence

upon b-wave initiation, the a-wave is measured to a specific time point (vertical solid line). In each panel, the vertical dashed lines indicate time of flash presentation.

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Figure 2. Light-adapted ERGs and their measurement

(A) Series of light-adapted ERGs obtained from a wild type (C57BL/6J) mouse to strobe flash stimuli that range from -0.8 to 1.9 log cd s/m². The major component of the response is the b-wave. (B) Method for measurement of b-wave amplitude. The b-wave amplitude is measured from the negative trough to the positive peak.

Figure 3. Measurement of rod photoreceptor deactivation

(A) Series of trials in which two $1.4 \log \text{cd s/m}^2$ stimulus flashes were presented to the dark-adapted eye of a mouse. As noted on the left of each pair of waveforms, the interstimulus interval (ISI) varied from 64 s to 2 s across trials. Note that the amplitude of the response to the second flash declines with decreasing ISI, reflecting incomplete recovery of the rod photoresponse. (B) Ratio of the response to the second flash (R2) to that of the first flash (R1), plotted as a function of ISI.

Figure 4. Measurement of rod photoreceptor dark adaptation

(A) Responses obtained to 1.4 log cd s/m² stimulus flashes that were presented to the fully dark-adapted eye of a mouse (baseline, black trace), or at various times following the offset of a bleaching light (color traces). (B) Amplitude of the ERG a-wave obtained to 1.4 log cd s/m² stimulus flashes obtained at full dark adaptation (baseline) or at different time points during dark adaptation.

Figure 5. ERG c-waves evoked by strobe flash stimuli

Each trace indicates an ERG recording to a strobe flash stimulus (0.0, 0.6, or 1.4 log cd s/m²) presented to the dark-adapted eye. The recording epoch is extended to 2 s, allowing the slower c-wave to be seen after the initial a- and b-waves. For each stimulus condition, several recordings are superimposed. As indicated by the vertical arrows, the c-wave amplitude can be measured from the negative trough following the b-wave to the c-wave peak.

Figure 6. Representative dc-ERG obtained to a 7-minute duration 2.4 log cd/m² stimulus presented to the dark-adapted mouse eye. The major components of the dc-ERG and their measurement are indicated.