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Interpretation of the mouse electroretinogram

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Abstract

The mouse electroretinogram (ERG) consists of a complex set of signals or “waves” generated by multiple types of retinal cell. The origins of these waves are reviewed briefly for the C57BL/6J mouse. The differences in the properties of these waves are described for 34 strains of mice and 11 F1 hybrid mice, as is the way that inter-strain genetic polymorphisms can be exploited in order to help pin-point the genes responsible for ERG differences. There are certain technical difficulties, some subtle, that can arise in recording the ERG and these are classified and illustrated in order to facilitate their diagnosis. Forward genetic screens are described, along with abnormal mice that have been generated in a large screen. Several means are suggested for determining if a mouse having an abnormal ERG is a mutant.

Keywords

Mouse; Inheritance; Retina; Genotype; Strain variation; Albino; Degeneration; C57BL/6J;
Forward genetics; Genetic transmission

Introduction

Although long recognized as the experimental mammalian genetic model system of choice, it is only recently that the mouse has been exploited for the study of the visual system. A plethora of functional and behavioral studies in the last five years has finally put to rest the incorrect notion that the mouse is not a ‘visual animal’. This article is intended for those who wish to make use of the genetic variation available among the inbred strains and mutants of the mouse to study the visual system. The electroretinogram (ERG) is a noninvasive way to evaluate the function of specific layers or neurons of the retina that also permits the animal to breed after being tested. The origin of the ERG is complex and has been reviewed extensively [1–3] but for convenience will be summarized here.

The electroretinogram results from the currents that flow within the eye as a result of the light-induced activity of neuronal, glial and retinal pigment epithelial cells and can be analyzed into component ‘waves’ that result from specific cells or sets of cells. The identity of the component waves and the means by which they are measured are explained in Fig. 1A. Photoreceptors are the source of the negative-going a-wave [4–8]. The polarity of this

wave can be explained in part by the fact that it results from the cessation of a standing photocurrent that flows constantly in the dark. Rod bipolar cells are the source of the b-wave in the dark-adapted retina [9–12]. The b-wave can be detected with less luminous stimuli than the a-wave in part because bipolar cell signals represent an amplification of the rod signals and result from a convergence of rods onto bipolar cells [10]. However, the b-wave is not the most sensitive to dim stimuli. Rather, the scotopic threshold response (STR) is the most sensitive [13]. The STR is the result of inner retinal activity, but its precise cellular origin has not been determined. The STR in C57BL/6J mice is biphasic [13, 2]. The c-wave is a slow, usually positive-going signal, that originates from two opposing sources [14–16]: the retinal pigment epithelium and retinal glial cells. The retinal pigment epithelial cells respond to reduced [K⁺] in the interphotoreceptor space during illumination and tend to produce a positive potential while the retinal glial cells produce the negative slow PIII component that opposes the potential resulting from the retinal pigment epithelium. The c-wave results from the sum of these two processes and can therefore have either polarity. However, in C57BL/6J mice the polarity is usually positive. Oscillatory potentials (OP) originate in the inner retina [17] and are quite variable in the mouse. The OP responses are shown in Fig. 1 but will not be considered further.

Materials and methods

Mice were obtained from the Jackson Laboratory or were bred at Northwestern University. All experiments were performed in accordance with the ARVO guidelines for the use of animals in research. During anesthesia body temperature was maintained between 36 and 37 °C.

Recordings were made using a “Frishman-Robson” device [13] with DTL fibers. It is key that all preparation was done with infra-red illumination and image converters. A minimum of 2 h prior dark adaptation was used [18]. Alternatively, the mouse was dark-adapted after electrode placement.

Results

Variation of the ERG among inbred strains of the mouse

In this section the absence of the ERG that occurs in strains that possess the *rd* mutation will not be considered (see Discussion). Although the C57BL/6J strain is generally used for physiological, genetic and behavioral studies of vision in the mouse, other strains are used for certain purposes. For example, homologous recombination, which is essential for substituting a mutant allele of a gene for the wild-type allele, can be done best using embryonic stem cells derived from the 129S1/SvImJ strain. In order to map a mutation genetically it is necessary to perform crosses with a ‘counterstrain’ that has DNA polymorphisms but still has a relatively normal electroretinogram. The 129S1/SvImJ strain and the DBA strain are both useful for this purpose. Since albinism affects vision by increasing retinal illumination through light reflected from the back of the eye, it is also important to keep in mind that albinism affects the ERG, and a number of inbred strains are albino. Not surprisingly, the ERG varies among inbred strains [19]. This can be seen in Fig. 2 in which the scotopic ERG is shown for three of 34 inbred strains that were studied. A number of differences are evident. First, the c-wave is more pronounced and tends to be more positive for C57BL/6J than for either 129S1/SvImJ or A/J. Second, the amplitude of the a-wave, relative to that of the b-wave, is greater for C57BL/6J than for the other strains. Third, the time course of the b-wave in response to bright stimuli is much more prolonged for A/J. We have quantified the values of the various waves of the ERG for 34 strains and 11 F1 hybrids, and the results are contained in Supplemental Tables I and II. The naturally-occurring variation in the ERG among inbred strains requires that interpretation be done

with this variation in mind. For example, in the ERG of test cross mice produced for genetic mapping is likely to be influenced by variation in the background genetic composition of the test cross mice, particularly in the F2 generation.

“Abnormal” ERGs that are commonly encountered

The normal ERG of the C57BL/6J strain will be used as a basis for comparison in this section.

Responses with a transient appearance—Albino strains of mice and C57BL/6J mice that have been light-adapted both display ERG waves that appear more transient than those of dark-adapted C57BL/6J mice. This is shown in the first row of Fig. 3a. The light-adapted ERG, however, has a smaller amplitude than the ERG of most albino strains.

Responses with inverted appearance—There can be several reasons for the appearance of such responses, including known pathological conditions [23], but in our experience only one type of inverted appearance is heritable. In the absence of the b-wave, the a-wave dominates in the early phase of the ERG and creates an initial negative-going ERG. This occurs for mutant mice in which the pathway to the rod bipolar cell is attenuated, which occurs in the “nob” series of mutations that affect either nyctalopin, the rod photoreceptor synaptic Ca channel, or the bipolar cell mGluR6 receptor [24–27]. Comparison of the “no b-wave” and “inverted responses” records shows that in the latter the peak of the b-wave appears inverted but that an a-wave occurs with normal polarity early in the response. We have never found inverted responses of this type to be heritable (see below) and have also found that such responses often, but not always, become normal upon retesting. Inverted c-wave responses often, but not always, have normal positive polarity in C57BL/6J mice when retested.

Distorted ERG waves—We have found two principal sources for artifactual distortion of the ERG waves. The first occurs when excess saline is applied to the cornea and the excess somehow finds its way to the contact between the DTL fiber and the metal wire with which it makes contact. The ensuing liquid junction potential is large and unstable and causes the baseline to shift, often imparting large displacements to the waves. The second source of distortion is improper placement or poor electrical contact with the cornea. Both of these sources of distortion can be usually be remedied by drying the cornea, rewetting the DTL fiber, and replacing the contact lens; this procedure is best learned using visible light but can be done with practice using infrared light and image converters.

Identifying a genuinely unresponsive mouse—The ERG recording will appear to have a ‘flat line’ appearance under three conditions: when the connections to the amplifier are not made, when the mouse does not respond, and when the stimulus has been inadvertently omitted. The first condition will result in a recording that has noise generated by the amplifier and will have a peak-to-peak amplitude (in the bandwidth of 0.1–1 kHz) of only a few microvolts. However, if either the connection is made properly and the mouse is unresponsive or the stimulus is inadvertently not applied, the recordings will not be as quiet as when the connections are not made at all. The appearance of these two cases is shown in Fig. 3b.

Failure of light-adaptation to occur while measuring the cone ERG—To measure the cone ERG a steady adapting light is applied while a flashing stimulus is presented. The adapting light is often generated by an LED, and it is usually presumed that LEDs have very long lifetimes. We have witnessed the partial failure of LEDs at least five times in 4 years of screening. The failure was subtle: flashing stimuli were normal but a steadily applied

voltage did not produce a steady light from the LED. This gave the false impression that the cone ERG was much larger than it would have been had it been recorded properly. Problems of this type can be minimized by monthly calibration of the apparatus and re-calibration of the apparatus every time an unexplained abnormality appears in the electroretinogram.

Mice with reproducible ERG abnormalities that are not transmitted genetically

—It has not been generally appreciated that the mouse is useful to apply the ‘forward genetic’ approach in which genes that are important for vision are discovered. This approach starts with random or spontaneous mutagenesis of a gene the identity of which is not known, proceeds with the discovery of visually affected mutants by screening for mice with abnormal vision, and continues with the identification of the mutated gene and the study of the mechanism by which the mutated gene results in abnormal vision. This approach offers several advantages. (1) It requires no prior knowledge of the mechanism, components or genes involved. (2) A number of mutant alleles can often be isolated that alter gene function in a number of ways. (3) This approach usually identifies point mutations, which in some instances can be more informative than targeted null mutations because gain-of-function and dominant negative mutations can be isolated. (4) Finding a single essential gene opens the door to finding other genes in the affected pathway. (5) This approach parallels most closely natural mutagenesis.

Forward genetics has helped to identify proteins involved in mammalian vision. Over 80 genes that, when mutated, result in human retinal degeneration have been identified [28]. The following examples show the wide variety of essential retinal genes that have been identified using forward genetics in mammals. The retinal degeneration (*rd*) mutation in mouse occurs in a gene for the phototransduction cascade (*Pde6 rd1*) [29], the *rdy* mutation in rats disrupts the receptor tyrosine kinase *Mertk* and impairs phagocytosis of shed rod outer segments by the retinal pigment epithelium (RPE), resulting in degeneration of the retina [30]. The protein nyctalopin, essential for bipolar cell function, was identified by cloning the *nob* gene [31]. Mutation of nyctalopin eliminates the b-wave of the ERG [25] and results in congenital stationary night blindness (CSNB) in humans [32]. Genes have been identified that modify the effects of deleterious mutations. The tubby (*tub*) gene in the mouse, named for its effect on body weight, also results in retinal degeneration. However, when mice of the C57BL/6J strain bearing this mutation are intercrossed to mice of the AKR strain, some of the resulting homozygous mutant mice are spared. Those mice that are spared have inherited the AKR allele of a defined region of chromosome 2 [33], suggesting the presence of a modifying allele on chromosome 2. A catalog of visual mutants that has been produced by forward genetics can be found at the web site www.Neuromice.org.

While conducting a forward genetic screen for mice with an abnormal ERG we measured the ERG of over 20,000 third generation offspring (G3) of mutagenized mice and found over 70 that had an abnormal ERG that remained abnormal upon retesting. In order to determine if these mice possessed a mutation we bred each one with a wild-type C57BL/6J mouse to obtain G4F1 mice, and then bred the G4F1 mice with one another in order to obtain G5F2 mice. One-fourth of these mice would be expected to be homozygous for the mutation, so in order to have over 80% certainty of identifying one such homozygote we tested 20 G5F2 mice [34]. In most cases no G5F2 mice were identified that had the same abnormal ERG phenotype seen in the founder affected G3 mouse. There are several explanations for this observation. First, it is possible that in some cases more than one gene was mutated in the founder G3 and thus fewer than one-fourth of the G5F2 mice would be expected to be affected. Second, it is possible that the retina of the founder G3 mouse was afflicted with an injury or illness that was not detected by funduscopy that was performed for each G3 mouse [18].

We have classified the ERGs of the G3 founders that were not transmitted to G5F2 mice (“non-transmitted phenotypes”) into nine categories (Fig. 4). With the exception of the ‘large a-wave’ phenotype several examples of each non-transmitted phenotype were encountered. In order to be sure that technical problems were not responsible for the abnormal ERG that was measured, each of the G3 founder mice were re-tested. The results of the original test, repeat test, and G5F2 mouse ERG are shown for each of these nine categories in Supplemental Figs. 1A and 1B.

Discussion

There are three principal reasons why the ERG recorded from a mouse might differ from what the experimenter expects from a ‘normal’ mouse. (1) The strain background of the mouse might differ from that of the ‘normal’ mouse that the experimenter has in mind. Inter-strain differences in the ERG (Fig. 2, supplemental Fig. 1 supplemental Tables I and II) are a possible cause that needs to be considered. (2) Technical difficulties associated with inadvertent light-adaptation, electrode placement and stimulus and adapting lights are a second possibility, but careful examination of the waveform of the dark-adapted ERG in these instances (Fig. 3) might be helpful in arriving at a diagnosis. (3) It is possible that a mouse with an abnormal ERG, confirmed by retesting to eliminate technical difficulties as the culprit, has a mutation that is responsible for the phenotype. However, it is also possible that the mouse has either a multi-gene defect or an illness or injury that is not detectable by the investigator. Only by breeding for two generations and testing 20 or more second generation progeny can the latter possibility be examined further.

The mouse has not, until recently, been considered to be a “visual animal”, and this incorrect notion needs to be addressed. One reason that this belief was held is that the retinal degeneration (*rd*, now *Pde6b rd1*) mutation of the phosphodiesterase 6b enzyme occurs in many common laboratory strains and renders all of the mice in these strains incapable of normal responses to light [35]. Keeler, found the retinas of some mice to be deficient in photoreceptors [36]. This mutation was named rodless (*r*) and the mutant mice were distributed to many laboratories. A similar phenotype was found by Brückner in 1951 among wild mice from the Basel and Zurich areas that were probably interbred with some laboratory strains [37]; this mutation was named retinal degeneration (*rd*). The similarity between the phenotypes of the *r/r* and *rd/rd* mutants led to speculation that they might be the same mutation. This question was resolved in Baehr’s laboratory by using PCR to amplify DNA from archival microscope slides containing the *r/r* mutant retinas [38]. The result was that both *r/r* and *rd/rd* retinas, the latter from many strains, contain not only the same missense mutation but also the same polymorphisms (differences in one or more nucleotide that usually do not result in deleterious effects but can be used to ‘fingerprint’ the DNA to determine its origin). This led to the conclusion that the mutations are genetically identical and support the interpretation that the retinal degeneration mutation present in many laboratory strains has its origin in Keeler’s rodless mutation. Since so many strains are affected with the same blinding mutation, it is understandable that researchers would have dismissed the mouse as a model for visual studies beyond studies of degeneration.

In addition to the strains that carry the *rd* mutation, some strains of mice bear mutations in genes other than *Pde6b* that affect vision, strengthening the impression that the mouse is not a visual animal. For example, many laboratory strains are albino (*Tyr c*) or hypopigmented, so that under the bright illumination of a research laboratory, they may not be able to see properly. The fact that a number of common laboratory strains have genetic alterations impairing their vision does not mean, of course, that the majority of strains of mice without these mutations are also blind. However, it has undoubtedly contributed to the perception that all mice are blind or have poor vision.

Mice, of course, are not blind. C57BL/6J mice perform well in a number of behavioral tasks [39, 40] and their ERG is very similar to that of other mammals that are generally considered to be “visual animals”. It is hoped that the results presented in this paper will allow researchers to exploit the genetic variation of the mouse to better understand the visual system, using the ERG as a tool for studying the early steps of vision that occur in the retina.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ERG	Electroretinogram
OP	Oscillatory potential
RPE	Retinal pigment epithelium
STR	Scotopic threshold response

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Fig. 1.

The components of the C57BL/6J mouse electroretinogram (ERG) and how they are measured. **(A)** Upper traces, the dark-adapted ERG evoked by stimuli of medium to high luminance. Middle trace, the a-wave, b-wave and c-wave are labeled for the response to a high luminance stimulus, showing how the amplitude of each wave was measured for this study. Lower drawing, sketches of the approximate time-courses of the a-, b- and c-waves in the middle trace, were they able to be recorded in isolation. **(B)** The dark-adapted ERG evoked by two very dim stimuli (0.000176 & 0.000281 cd s/m²). The biphasic scotopic threshold response (STR) is evoked in this strain and its amplitude is measured as shown. **(C)** The light-adapted ERG. A steady adapting light sufficient to saturate the rod pathway (0.5 cd/m²) was presented while a flashing stimulus was applied. The luminance of the flashing stimulus was 0.2 cd s/m². This same stimulus evoked a larger response with longer latency in the dark-adapted retina (see **A**). **(D)** Oscillatory potentials (OP) contribute to the mouse ERG and have been digitally filtered from all responses in this paper except for that shown here. Filtering these potentials makes it possible to focus attention on the other waves of the ERG. A response is shown with and without OP and below the OP time course is shown in the absence of the rest of the ERG.

Fig. 2.

Examples of inter-strain variation in the time course of the ERG. Note that the c-wave is more prominent for the C57BL/6J strain than for either 129S1/SvImJ or A/J. The A/J strain is also albino, and consistent with this some stimuli evoke more transient responses in A/J mice than in pigmented strains. Inter-strain variability is quantified in Supplemental Tables I and II. It should be pointed out that variation in the ERG or any other property of vision among strains can be used to help pin-point the genes that are responsible for the variation. The presently-used inbred laboratory strains used today derive from very few wild-caught mice (and in a few cases, wild mice) and thus their genomes are mosaics of the chromosomes of these founder mice in which the haplotype blocks are very small (<1 Mb) [20, 21]. The genetic diversity of the founder mice allows the DNA derived from them to be identified by characterizing their polymorphisms. The polymorphisms between many standard laboratory strains have been characterized [22] at over 150,000 locations in the genome. These polymorphisms have been used to identify known genes that affect vision. What is needed on the part of the investigator is to measure the phenotype in each of many strains and then apply these data to a mapping program designed for this purpose. The program establishes associations between the DNA segments of each strain (from the small number of founder mice) and the phenotype. A program for this purpose is available on the web (http://snpster.gnf.org/cgi-bin/snpster_ext.cgi). Thus, naturally-occurring variation among strains of mice provides a resource for vision researchers that can be exploited readily at the present time.

Fig. 3.

Technical difficulties that are often encountered when recording the ERG. (A) Top row. Transient responses are often associated with albino animals and light-adapted retinas, but the peak response amplitude from light-adapted retinas in normally sighted mice is less than in albino mice. Bottom Row. Inverted responses can either result from genuine genetic defects such as the mutations in nyctalopin or mGluR6 (lower left). However inverted b- and c-wave responses can also occur for reasons related to the condition of the mouse, a situation that can often be clarified by retesting the mouse. (B) Top row. Excess saline in contact with metal electrodes can cause large, unstable liquid junction potentials and poor electrical contact can cause distortion and instability of recording. Middle row. A mouse with advanced retinal degeneration will have no response whatsoever (middle left, *Noerg-1*, note higher amplification of trace) but the baseline of the recording will not be as quiet as when the amplifier is not connected to the mouse. A normal mouse that is not presented with a stimulus (middle right) will produce a recording with a similar baseline. Lower row. The cone or light-adapted ERG is recorded in the presence of a steady adapting light, in this case from an LED. However, some LEDs lose their ability to produce a steady light after some use and in this instance the light adaptation will be incomplete, resulting in a larger than normal cone ERG

Fig. 4.

Responses of G3 founders in a forward genetic screen that had reproducibly abnormal ERG responses. None of at least 20 G5F2 offspring of each of these mice were affected. It is possible that these mice either had a disease or injury that was not revealed by fundoscopy or that the abnormal ERG resulted from mutations in more than one gene. The classifications that are used in this figure are arbitrary and are included only to call attention to a distinguishing feature of the abnormal ERG. The mouse with the large a-wave (lower left) is one of two G3 siblings whose a-wave amplitude (see supplementary Tables I and II) was more than 2 SD greater than the mean. Neither sibling's phenotype was observed in the G4F2 mice. Each of these abnormal phenotypes was recorded a second time (see Supplementary Fig. 1)