[Frontiers in Bioscience 14, 2730-2737, January 1, 2009]

Vision tests in the mouse: Functional phenotyping with electroretinography

Naoyuki Tanimoto[1], Regine L. Muehlfriedel[1], M. Dominik Fischer[1], Edda Fahl[1], Peter Humphries[2], Martin Biel[3], Mathias W. Seeliger[1]

[1]Ocular Neurodegeneration Research Group, Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tuebingen, Schleichstrasse 4/3, D-72076 Tuebingen, Germany, [2] Ocular Genetics Unit, Department of Genetics, Trinity College, Dublin 2, Ireland, [3] Center for Integrated Protein Science CIPS-M and Zentrum fuer Pharmaforschung -Department Pharmazie, Pharmakologie fuer Naturwissenschaften, Ludwig-Maximilians-Universitaet Muenchen, Butenandtstr. 5 -13, D-81377 Muenchen, Germany

TABLE OF CONTENTS

1. ABSTRACT

Electroretinography (ERG) is an established diagnostic technique in clinical ophthalmology and provides objective information about retinal function. This technique is also applied in basic research, where animal models of hereditary retinopathies have significantly contributed to our understanding of the composition of ERG responses in general and how retinal degenerative pathologies alter retinal function specifically. Indeed, electrophysiologic assessment of transgenic mice, which are genetically engineered to mimic human mutations that lead to retinal diseases, can be well compared with clinical data. Furthermore, limitations on examinations (e.g. length of measurement, range of light intensity) are much less of a concern when assessing mice compared to human patients. In order to measure and analyze retinal responses properly, several important aspects have to be considered. This paper focuses on these aspects, and shows exemplary ERG data which were obtained from normal wild-type mice and from transgenic mice with specific functional properties, namely *Rho*$^{-/-}$ (rod opsin knockout, cone function only), and *Cnga3*$^{-/-}$ (cone CNG channel deficient, rod function only) to illustrate rod and cone system contributions to ERG responses.

2. INTRODUCTION

Many patients with hereditary retinal disorders lose their visual function completely and early in life and there is currently no established therapeutic procedure. This is in part due to the fact that the pathophysiology of hereditary retinal diseases is still not fully understood. ERG is a valuable tool not only to diagnose, but to also to further investigate hereditary retinal disorders both in clinical ophthalmology and basic research. Because an in-depth analysis beyond the clinical examination can not be performed in patients, basic research often draws upon animal models such as monkeys, dogs, cats, rabbits, rats, mice, and zebrafish for detailed ERG analysis. To further our understanding of hereditary retinal disorders and underlying mechanisms, animals should be selected depending on the specific aim of the experiment. On one hand, retinae of larger animals have more similarities with human retina regarding its anatomy and function; on the other hand, animal care is cheaper and reproduction is faster in small animals. Thus, since effects of gene manipulation in small animals can be examined within a relatively short time period, animals such as rats, mice, and zebrafish are commonly used. In our laboratory, we frequently analyze mouse models with ERG because 1) the

Figure 1. Ganzfeld ERG recording setup. The ERG equipment consists of a Ganzfeld bowl, a direct current amplifier, and a PC-based control and recording unit.

configurations of ERG responses in mice are comparable to those in humans, 2) experimental procedures and animal care are relatively simple, and 3) they can be easily crossbred with other mouse models for further investigation (1, 2). In this paper, essential aspects for recording mouse ERG as well as the systematic interpretation of ERG responses are introduced.

3. PREPARATION OF MICE

Since many parameters including performance of investigator and environmental conditions such as humidity and noise level are different day by day, proper control animals should be included in every set of measurements. One should bear in mind that ERG parameters change through of life so that one should only compare mice of approx. the same age. To accurately and reproducibly detect even small alterations of retinal function, it is not sufficient to use random mice from the same strain (e.g. C57Bl/6, 129/Sv, etc.) as controls. Rather, wild-type and mutant littermates from heterozygote breeding pairs should be examined so that the genetic background is the same except for the gene of interest. This also allows to examine all mice with the same date of birth in one day. Usually, we measure 4~5 mutants and 4~5 littermate controls in the first experiment. Experiments should not be conducted in a blinded fashion, as it is more important that control and mutant mice are analyzed alternately to avoid bias due to changes in experimental conditions even within one set of measurements. Before each ERG experiment, full dark adaptation of the animals is required in order to examine the maximal performance of the rod system. For this purpose, overnight dark adaptation (longer than 12 hours) is done. This length of dark adaptation is usually sufficient even for mouse lines, which show delayed dark adaptation (3, 4).

4. RECORDING EQUIPMENT AND SETTINGS

Normally, ERG responses in mice are recorded by evenly stimulating the whole retina, which is called Ganzfeld ERG. To generate an evenly spread stimulus, light is directed into a Ganzfeld bowl, whose inner wall is highly reflective. As mentioned above, this method was first established for use in clinical ophthalmology (5). The equipment produced for clinical application can also be used for ERG measurements in mice (Figure 1). The ERG equipment which we use consists of a Ganzfeld bowl, a direct current amplifier, and a PC-based control and recording unit (Multiliner Vision; VIASYS Healthcare GmbH, Hoechberg, Germany). In addition to this equipment, we use a small box, on which the anesthetized mouse lies. The body temperature has to be stabilized with a heating pad, because retinal functions are very sensitive to this variable (6). This heating pad is placed only under the mouse body, whereas the mouse head should be placed on a transparent plate in order to allow equal amounts of light stimuli to reach the eyes from all directions. Disposable stainless needle electrodes are applied subcutaneously at the forehead region and the back near the tail as a reference and a ground electrode, respectively. On the small box, two arms are attached, where each arm features a gold ring electrode at the free end. Position of the electrodes can be adjusted in all three dimensions of space through the joints of each arm in order to make contact with the corneae. The mouse should be placed well into the center of the Ganzfeld bowl, since mouse eyes are anatomically oriented to the sides and not to the front as in primates. It is preferable that ERG responses are obtained from both eyes simultaneously, because one can verify proper position of active electrodes by comparing ERG responses between right and left eyes. Also, this internal control helps to detect artifacts within a series of measurements. Two things have to be checked if ERG responses are divergent between both eyes. The first one is the position of electrodes. Spatial relationship between electrode and cornea can change during measurements due to heavy breathing. Secondly, changes in eyes which are not related to the inherent phenotype of examined animals but occurred spontaneously and hence mostly unilaterally. Changes in the anterior segment, such as corneal lesions, pupil abnormality, and cataract, can be checked with the naked eye or using a convex lens. However, changes in vitreous and retina can be examined only with special imaging techniques (7). In our laboratory, eyes are examined morphologically by scanning-laser ophthalmoscopy immediately after ERG measurements. For comprehensive phenotyping, functional data must always be compared with morphological data just as in clinical diagnostics in ophthalmology. ERG recording from both eyes is also valuable in case of experiments with therapeutic aims, where the untreated eye can be used as internal control (8, 9). In addition to Ganzfeld ERG, multifocal ERG can be also performed in mice (10).

In our laboratory, mice are anesthetized with subcutaneous injection of a mixture of ketamine, xylazine, and physiological saline. Ketamin and xylazine are given at 66.7 mg/kg body weight and 11.7 mg/kg body weight, respectively. The pupils are dilated with tropicamide eye drops (Mydriaticum Stulln, Pharma Stulln, Stulln, Germany). Additional usage of phenylephrine and/or atropine eye drops may help to dilate pupils. Impedance for all four electrical circuits (electrodes) mentioned above have to be checked during the positioning of electrodes and

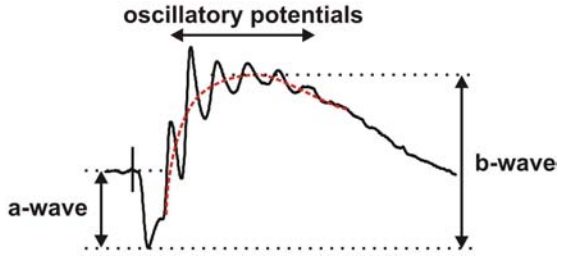

Figure 2. Diagram of a scotopic flash ERG response. Like in human ERG responses, the positive deflection (b-wave) immediately follows the negative one (a-wave). In mice, the track of the b-wave is not seen clearly because of the large oscillatory potentials (OPs). Therefore, the b-wave has to be pictured as the red dashed curve which approximately runs through the midpoints of all OPs. Midpoints are defined as mean value between adjacent minimum and maximum of oscillations.

shortly before measurements. Impedance of active electrode is dependent mainly on the contact between electrode and cornea and on the size of the mouse. Large impedance (i.e. >10 megohms) means that the contact is insufficient and electrodes should be repositioned. When the impedance is too small (i.e. <6 megohms), the electrode might press too strongly on the corneal surface causing insufficient ocular circulation and/or corneal abrasions. The contact between electrode and cornea also influences fluctuation of the baseline ERG signal. Therefore, optimized impedance and stable baseline are required for reliable ERG response acquisition.

ERG signals recorded through active electrodes are amplified. This amplification determines the shape of responses which are displayed on the monitor and available for further analysis. Generally, the first negative deflection after light flash is called a-wave, and the following positive deflection is called b-wave, which features characteristic oscillatory potentials (OPs) superimposed onto the signal (Figure 2). To gain such a signal, the bandpass filter of the amplifier should be adjusted to selectively amplify signals within the range of 0.3 and 300 Hz just as it is recommended for recordings of human ERG (5). By changing this filter setting, one can extract specific components of the ERG signal, such as the OPs (amplification between 75 and 300 Hz).

5. RECORDING PROTOCOLS AND INTERPRETATIONS

When studying animal models of hereditary retinal diseases, one of the first and important questions that usually has to be answered is whether the rod or the cone system (or both) are affected. Therefore, protocols which are used routinely must give an answer to this question. However, even in normal healthy eyes, the contributions of rod and cone systems to the ERG are not yet fully understood. In this chapter, our routine protocols are introduced with actual ERG responses obtained not only from a wild-type mouse but also from a *Rho*$^{-/-}$ mouse (rod opsin knockout, cone function only (11)) and a *Cnga3*$^{-/-}$ mouse (cone CNG channel deficient, rod function only (12)). All mice were measured at postnatal week (PW) 4. This time point is particularly important when using the *Rho*$^{-/-}$ mouse line as an all-cone model, since no viable rod signal can be detected in *Rho*$^{-/-}$ mice at this age, whereas cones are still functionally intact. Consequently, the *Cnga3*$^{-/-}$ mouse line is currently a unique model which shows pure rod system responses regardless of light intensity. Use of these transgenic animals for ERG recordings allows dissecting the contribution of rod and cone system to the ERG signal and sets an ideal background to investigate consequences of rod and cone function loss in the interpretation of ERG data.

ERG recordings are divided into two groups according to the frequency of stimuli, namely single flash and flicker ERG. Even in "single" flash ERG, a series of light flashes is used to calculate an average response in order to minimize fluctuations of the baseline and increase the signal to noise ratio. In single flash ERG, inter stimulus intervals (ISI) must be long enough for photopigments to fully regenerate before iterative stimuli. With increasing stimulus intensity, this regeneration process takes longer; hence, the ISI must be adjusted accordingly. However, it is also important that a series of ERG measurements from one mouse is performed in a timely fashion to minimize time-dependent variables (e.g. level of anesthesia). In our laboratory, we normally measure one mouse within one hour from the start of anesthesia, which allows us to examine 8~10 mice (4~5 mutants and 4~5 controls) in one day. Mice of the two groups should be measured alternately to avoid any bias associated with the time of day.

ERG measurements are also divided into dark-adapted (scotopic) and light-adapted (photopic) measurements, the latter being performed in the presence of a static background light. Exposed to static background light of 30 cd/m^2, rods are usually saturated and cannot react to light stimuli. However, there are exceptions. If rods are desensitized, the static background light becomes relatively dark for rods and is no longer "rod-saturating". In this case, rods can also react to light flashes in spite of the presence of background light. A typical photopic session begins after a 10 minute exposure of the retinae to background light in order to reach a stable level of the photopic responses (13).

5.1. Single flash intensity series

In the scotopic single flash intensity series, we use white-Xenon flash stimulation ranges from -4 to 1.5 log cd*s/m^2. It is divided into ten steps of 0.5 and 1 log cd*s/m^2. Ten responses are averaged with an ISI of either 5 seconds (for -4, -3, -2, -1.5, -1, and -0.5 log cd*s/m^2) or 17 seconds (for 0, 0.5, 1, and 1.5 log cd*s/m^2). In the photopic single flash intensity series, the protocol starts at the intensity of -2 log cd*s/m^2, but steps of light intensity and ISI are exactly the same as those in the scotopic single flash intensity series.

Typical ERG responses obtained from a wild-type mouse in the scotopic single flash intensity series are shown in Figure 3 (Figure 3A, left). The responses up to

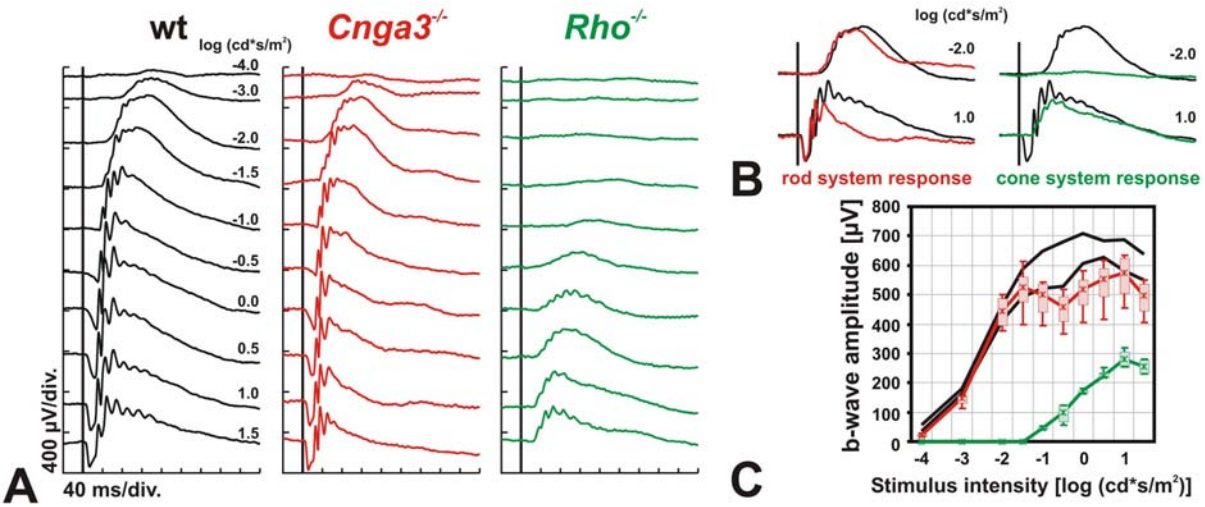

Figure 3. Electroretinographic data of dark-adapted (scotopic) single-flash ERG intensity series. (A) Representative ERG responses obtained from a wild-type, a *Cnga3*$^{-/-}$, and a *Rho*$^{-/-}$ mouse. Stimulus intensities are indicated in the panel. Vertical line crossing each trace shows the timing of the light flash. (B) Overlay of selected waveforms illustrates contributions of the rod and cone systems to the responses. (C) Scotopic b-wave amplitude vs. stimulus intensity (log) function. Data are given as box-and-whisker plots showing 5 and 95% quantiles (whiskers), 25 and 75% quartiles (box), and the median (marked by a cross). The black lines delimit the range given by the 5 and 95% quantile of wild-type eyes. ERG responses at -2.0 log cd*s/m^2 and below are generated exclusively by the rod system.

approximately -2 log cd*s/m^2 intensity are composed only of a positive component called b-wave. An earlier negative component, the a-wave, is detected only in the higher intensity range above -2 log cd*s/m^2. The slope of the negative component becomes steeper with increasing flash intensity. OPs, a number of higher-frequent oscillations on top of the b-wave, change in size as well as in number with increasing stimulus intensities, and at high intensities of 1.0 and 1.5 log cd*s/m^2, several OPs exist on the b-wave. The ERG responses from a *Cnga3*$^{-/-}$ (Figure 3A, middle) and a *Rho*$^{-/-}$ (Figure 3A, right) mouse elucidate the rod and cone system contributions under the given conditions. In the low intensity range up to -2.0 log cd*s/m^2, *Rho*$^{-/-}$ mice show no ERG response, while there is no remarkable difference between wild-type and *Cnga3*$^{-/-}$ mice (Figures 3B and 3C), illustrating that ERG responses up to this stimulus intensity are evoked exclusively by the rod system. The response at -2.0 log cd*s/m^2 is comparable to the "rod ERG" of the human ERG standard (5). From -1.5 log cd*s/m^2, the b-wave amplitude in *Cnga3*$^{-/-}$ mice does not increase as in wild-type mice with increasing stimulus intensities (Figure 3C). The overlay of the waveforms from a wild-type and a *Cnga3*$^{-/-}$ mouse at high stimulus intensity indicates that the b-wave in *Cnga3*$^{-/-}$ is reduced not generally but locally at the trailing edge of the b-wave. The initial part of the b-wave as well as the a-wave do not change remarkably, meaning that they are generated almost exclusively from the rod system in mice (12, 14). In *Rho*$^{-/-}$ mice, the scotopic flash ERG responses show the cone system activity in the dark adapted state, which is usually masked by the large rod system response (Figure 3A, right). Single flash responses under photopic conditions in a wild-type mouse have different configurations from those under scotopic conditions (Figure 4A, left), i.e. there is no substantial a-wave under photopic conditions. The photopic flash responses in *Rho*-/- mice are comparable to wild-type (Figures 4A right, 4B, and 4C), and *Cnga3*-/- mice show no response under given conditions, indicating that these responses are usually entirely cone-driven. In general, the duration of cone responses in mice is longer than in e.g. humans, leading to a lower flicker fusion frequency (see 5.2.).

Sometimes it is necessary to choose light stimuli outside of the standard stimulus range to elicit small residual retinal responses, which are not clearly seen in the standard examination protocol (2). For such additional photopic bright flash experiments, we use a Mecablitz 60CT4 flash gun (Metz, Germany) added to the Ganzfeld bowl, which increases the available intensity range at the high end from 2.0 to 4.5 log cd*s/m2. Mice do not encounter light stimuli in this range in their natural habitat, but additional protocols tailored to answer a specific question, such as whether a certain condition allows for a small but remaining retinal electrical activity or not, might lead to additional clues regarding retinal pathophysiology.

5.2. Flicker frequency series

Dynamic properties of the ERG can be assessed by repetitive stimulation (flicker) of a certain frequency. In flicker frequency series, stimulus intensity remains the same, whereas stimulus frequency is increased stepwise. Under scotopic conditions, we use 0.5 log cd*s/m^2 (Figure 5) stimulus intensity, which is comparable to the combined-ERG in the standard human ERG (5). Steady-state responses within the time frame of 500 ms are averaged either 20 times (for 0.5, 1, 2, and 3 Hz) or 30 times (for 5 Hz and higher frequencies until 30 Hz). At the intensity of

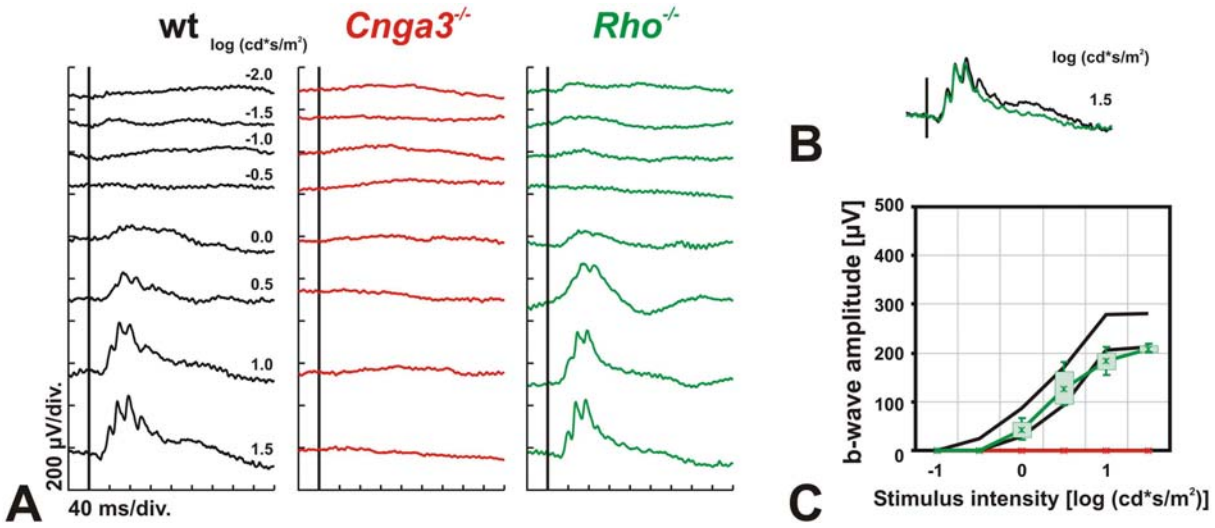

Figure 4. Electroretinographic data of light-adapted (photopic) single-flash ERG intensity series. (A) Representative ERG responses obtained from a wild-type, a *Cnga3*$^{-/-}$, and a *Rho*$^{-/-}$ mouse. Stimulus intensities are indicated in the panel. Vertical line crossing each trace shows the timing of the light flash. (B) Overlay of selected waveforms. (C) Photopic b-wave amplitude vs. stimulus intensity (log) function. Data are given as box-and-whisker plots showing 5 and 95% quantiles (whiskers), 25 and 75% quartiles (box), and the median (marked by a cross). The black lines delimit the range given by the 5 and 95% quantile of wild-type eyes. The photopic flash responses of *Rho*$^{-/-}$ mice are comparable to wild-type, indicating that these responses are usually entirely cone-driven.

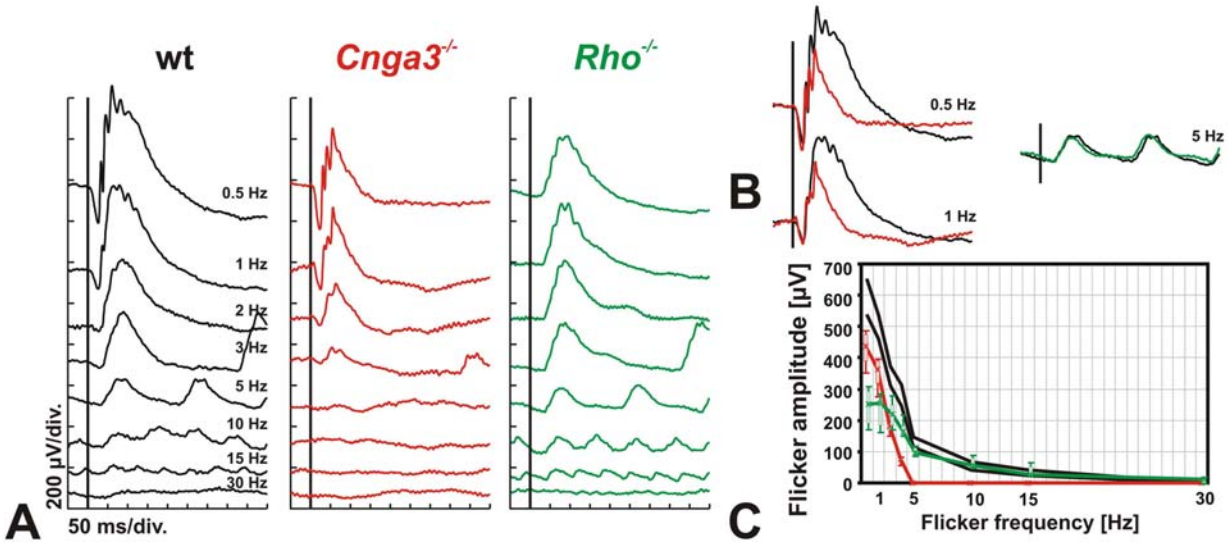

Figure 5. Electroretinographic data of dark-adapted (scotopic) flicker ERG frequency series at the intensity of 0.5 log cd*s/m^2. (A) Representative ERG responses obtained from a wild-type, a *Cnga3*$^{-/-}$, and a *Rho*$^{-/-}$ mouse. Stimulus frequencies are indicated in the panel. (B) Overlay of selected waveforms. (C) Flicker amplitude vs. frequency function. Data are given as box-and-whisker plots showing 5 and 95% quantiles (whiskers), 25 and 75% quartiles (box), and the median (marked by a cross). The black lines delimit the range given by the 5 and 95% quantile of wild-type eyes. Any response above ~3Hz is entirely cone-driven.

0.5 log cd*s/m^2, responses are generated both from the rod and cone systems if stimulus frequency is low. The rod-driven response becomes smaller with increasing stimulus frequencies because the signal has no time to return to baseline before the subsequent light stimulus, and flicker responses vanish at about 5 Hz (Figure 5A, middle). In addition, there is no remarkable difference between flicker responses in wild-type and *Rho*$^{-/-}$ mice at 5 Hz and above (Figures 5A, right and left, 5B, right, and 5C), proving that any response above ~3Hz is entirely cone-driven, if a standard flash (0.5 log cd*s/m^2) is used. In other words, cone system function can be partially assessed using standard scotopic flicker protocol before photopic sessions. The same series of flicker stimuli is also applied under

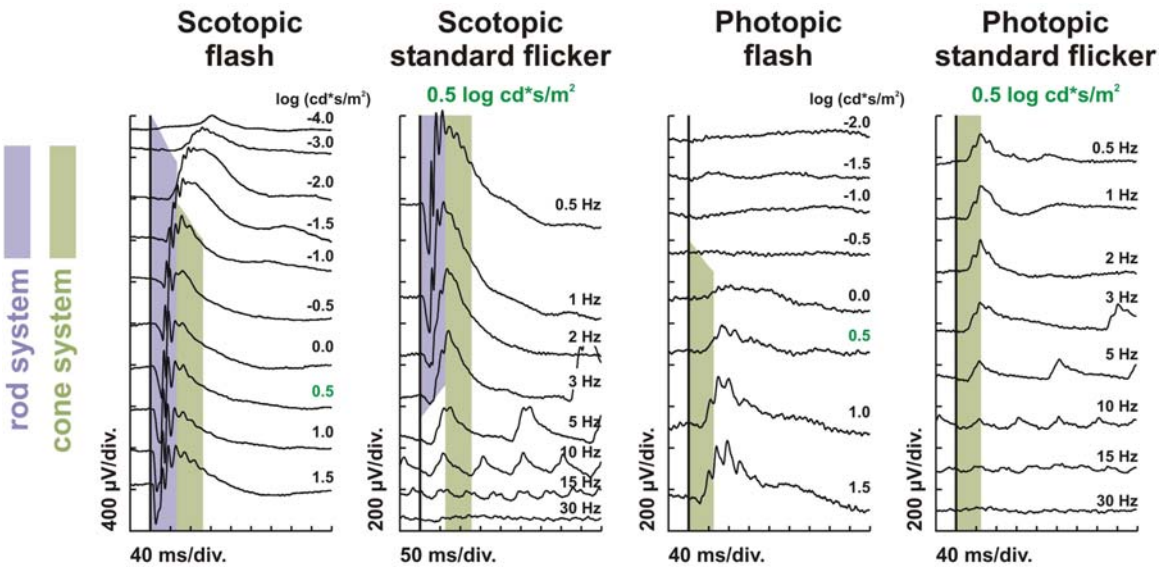

Figure 6. Summary of rod and cone system contributions to flash and flicker ERG responses. Rod and cone system contributions to the ERG responses are illustrated with blue and green bands, respectively. Note that this is the situation, where rods are not strongly desensitized.

photopic conditions. Due to the background light, *Cnga3*$^{-/-}$ mice show no response, and responses in wild-type and *Rho*$^{-/-}$ mice are comparable (data not shown).

5.3. Cone and rod system contributions

Most alterations of retinal function can be appropriately analyzed by applying the flash and flicker protocols described above. Therefore, we routinely use these protocols especially when we analyze mice with unknown retinal phenotype. After that, additional protocols tailored to answer a specific question might be applied. As written in the section 3, age and genetic background of mice have to be considered for ERG analysis.

The rod and cone system contributions as demonstrated above are summarized and illustrated (Figure 6). Below a threshold of -2.0 log cd*s/m^2, pure rod system responses can be obtained in the scotopic flash intensity series. However, these contributions can change under some specific conditions, such as Rpe65 deficiency. Lack of this isomerase leads to Leber congenital amaurosis as it impairs the visual cycle at the level of retinal pigment epithelium mediated regeneration of the visual chromophore (15). As a result, the retina contains only trace amounts of vitamin A in form of 11-cis-retinal. In a RPE65 deficient mouse model (*Rpe65*$^{-/-}$ mice) there is no response up to -2.0 log cd*s/m^2, under scotopic conditions and responses appear only in the higher intensity range, a so-called “right shift” (15). Such findings in Rpe65-/- mice (with desensitized rods) seemed to be similar to those in Rho-/- mice in terms of sensitivity and response size. However, further investigations by crossbreeding with *Rho*$^{-/-}$ and *Cnga3*$^{-/-}$ mice revealed that they are produced not by the cone system but by the rod system (1). The hint was the comparison of response configurations (see Figure 2 of Ref. (1)). This illustrates the importance of precise analysis.

6. DATA ANALYSIS AND PRESENTATION

The ERG response is a sum of the electrical activities from both outer and inner retina (16). The negative a-wave represents both outer and inner retinal components, while the positive b-wave is shaped mainly by the bipolar cells of the inner retina. Since the neuronal activities of inner retina is dependent on photoreceptor function, b-wave analysis is generally a good starting point to check for overall retinal functionality. When determining the b-wave amplitude in mice, it has to be considered that mouse OPs are relatively large compared to human OPs, and the maximal positive excursion immediately following the a-wave may not resemble the peak of b-wave. In our laboratory, an imaginary curve (Figure 2, red dashed curve) is fitted to approximately run through the midpoints of all OPs (mean value between adjacent minimum and maximum of oscillations) to account for the contribution of OPs to the b-wave. We use this method for the b-wave analysis in both scotopic and photopic flash intensity series. But it is not applicable to the analysis of flicker ERG responses, because OPs and other response components merge. Thus, the size of flicker responses is measured from the minimum (trough) to the maximum (peak) of each response so that all responses of the frequency series are analyzed using the same definition.

Analyzed data (amplitude and latency) from all mice measured are graphed and compared among groups. Data should not be presented with mean and standard deviation, if it can not be assumed that they are not normally distributed. In our experience, the 5, 25, 50 (median), 75, and 95% quantiles provide a good overview of the signal distribution. To allow the assessment of the signal waveforms, one set of representative ERG responses from each group should also be shown (17-20). In addition, responses may be superposed so one can easily appreciate

changes in configuration (8, 12), such as the change in the trailing edge of b-wave in *Cnga3*$^{-/-}$ mice at high stimulus intensities (Figure 3B, other examples in Figures 4B and 5B).

7. SUMMARY AND PERSPECTIVE

In summary, a set of routine protocols for use with a Ganzfeld Xenon flash system in mice was presented. Mice with only rod function (*Cnga3*$^{-/-}$) and only cone function (*Rho*$^{-/-}$) were used to clarify the contributions of each system under various conditions. The combination of flash and flicker protocols provides further information for the interpretation of the functional status of the rod and cone systems in health and disease.

8. ACKNOWLEDGEMENTS

This work was supported by Deutsche Forschungsgemeinschaft (Se837/4-1 and 5-1) to MWS, and Tistou und Charlotte Kerstan Stiftung Vision 2000 to NT.

9. REFERENCES

1. Seeliger M. W., C. Grimm, F. Stahlberg, C. Friedburg, G. Jaissle, E. Zrenner, H. Guo, C. E. Reme, P. Humphries, F. Hofmann, M. Biel, R. N. Fariss, T. M. Redmond, A. Wenzel: New views on RPE65 deficiency: the rod system is the source of vision in a mouse model of Leber congenital amaurosis. *Nat Genet* 29, 70-74 (2001)

2. Wenzel A., J. von Lintig, V. Oberhauser, N. Tanimoto, C. Grimm, M. W. Seeliger: RPE65 is essential for the function of cone photoreceptors in NRL-deficient mice. *Invest Ophthalmol Vis Sci* 48, 534-542 (2007)

3. Wenzel A., V. Oberhauser, E. N. Pugh, Jr., T. D. Lamb, C. Grimm, M. Samardzija, E. Fahl, M. W. Seeliger, C. E. Reme, J. von Lintig: The retinal G protein-coupled receptor (RGR) enhances isomerohydrolase activity independent of light. *J Biol Chem* 280, 29874-29884 (2005)

4. Kim T. S., A. Maeda, T. Maeda, C. Heinlein, N. Kedishvili, K. Palczewski, P. S. Nelson: Delayed dark adaptation in 11-cis-retinol dehydrogenase-deficient mice: a role of RDH11 in visual processes in vivo. *J Biol Chem* 280, 8694-8704 (2005)

5. Marmor M. F., G. E. Holder, M. W. Seeliger, S. Yamamoto: Standard for clinical electroretinography (2004 update). *Doc Ophthalmol* 108, 107-114 (2004)

6. Kong J., P. Gouras: The effect of body temperature on the murine electroretinogram. *Doc Ophthalmol* 106, 239-242 (2003)

7. Seeliger M. W., S. C. Beck, N. Pereyra-Munoz, S. Dangel, J. Y. Tsai, U. F. Luhmann, S. A. van de Pavert, J. Wijnholds, M. Samardzija, A. Wenzel, E. Zrenner, K. Narfstrom, E. Fahl, N. Tanimoto, N. Acar, F. Tonagel: In vivo confocal imaging of the retina in animal models using scanning laser ophthalmoscopy. *Vision Res* 45, 3512-3519 (2005)

8. Min S. H., L. L. Molday, M. W. Seeliger, A. Dinculescu, A. M. Timmers, A. Janssen, F. Tonagel, N. Tanimoto, B. H. Weber, R. S. Molday, W. W. Hauswirth: Prolonged recovery of retinal structure/function after gene therapy in an Rs1h-deficient mouse model of x-linked juvenile retinoschisis. *Mol Ther* 12, 644-651 (2005)

9. Janssen A., S. H. Min, L. L. Molday, N. Tanimoto, M. W. Seeliger, W. W. Hauswirth, R. S. Molday, B. H. Weber. Effect of late-stage therapy on disease progression in AAV-mediated rescue of photoreceptor cells in the retinoschisin-deficient mouse. *Mol Ther* 16, 1010-1017 (2008)

10. Seeliger M. W., B. H. Weber, D. Besch, E. Zrenner, H. Schrewe, H. Mayser: mf ERG waveform characteristics in the RS1h mouse model featuring a 'negative' ERG. *Doc Ophthalmol* 107, 37-44 (2003)

11. Jaissle G. B., C. A. May, J. Reinhard, K. Kohler, S. Fauser, E. Lutjen-Drecoll, E. Zrenner, M. W. Seeliger: Evaluation of the rhodopsin knockout mouse as a model of pure cone function. *Invest Ophthalmol Vis Sci* 42, 506-513 (2001)

12. Biel M., M. Seeliger, A. Pfeifer, K. Kohler, A. Gerstner, A. Ludwig, G. Jaissle, S. Fauser, E. Zrenner, F. Hofmann: Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. *Proc Natl Acad Sci USA* 96, 7553-7557 (1999)

13. Peachey N. S., Y. Goto, M. R. al-Ubaidi, M. I. Naash: Properties of the mouse cone-mediated electroretinogram during light adaptation. *Neurosci Lett* 162, 9 –11 (1993)

14. Lyubarsky A. L., E. N. Pugh Jr.: Recovery phase of the murine rod photoresponse reconstructed from electroretinographic recordings. *J Neurosci* 16, 563-571 (1996)

15. Redmond T. M., S. Yu, E. Lee, D. Bok, D. Hamasaki, N. Chen, P. Goletz, J. X. Ma, R. K. Crouch, K. Pfeifer: Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet* 20, 344-351 (1998)

16. Granit R.: The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. *J Physiol* 77, 207-239 (1933)

17. Chen D., R. Opavsky, M. Pacal, N. Tanimoto, P. Wenzel, M. W. Seeliger, G. Leone, R. Bremner: Rb-mediated neuronal differentiation through cell-cycle-independent regulation of E2f3a. *PLoS Biol* 5, e179 (2007)

18. Schild A., S. Isenmann, N. Tanimoto, F. Tonagel, M. W. Seeliger, L. M. Ittner, A. Kretz, E. Ogris, J. Gotz: Impaired development of the Harderian gland in mutant protein phosphatase 2A transgenic mice. *Mech Dev* 123, 362-371 (2006)

19. Aartsen W. M., A. Kantardzhieva, J. Klooster, A. G. van Rossum, S. A. van de Pavert, I. Versteeg, B. N. Cardozo, F. Tonagel, S. C. Beck, N. Tanimoto, M. W. Seeliger, J. Wijnholds: Mpp4 recruits Psd95 and Veli3 towards the photoreceptor synapse. *Hum Mol Genet* 15, 1291-1302 (2006)

20. Samardzija M., J. von Lintig, N. Tanimoto, V. Oberhauser, M. Thiersch, C. E. Reme, M. Seeliger, C. Grimm, A. Wenzel: R91W mutation in Rpe65 leads to milder early-onset retinal dystrophy due to the generation of low levels of 11-cis-retinal. *Hum Mol Genet* 17, 281-292 (2008)

Abbreviations: CNG: cyclic nucleotide-gated (channel); ERG: electroretinogram; ISI: inter stimulus intervals; OPs: oscillatory potentials

Key Words: Animal model, Cone, Electroretinogram, Flash, Flicker, Rod, Review

Send correspondence to: Naoyuki Tanimoto, Ocular Neurodegeneration Research Group, Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tuebingen, Schleichstrasse 4/3, D-72076 Tuebingen, Germany, Tel: 49-7071-298-7778, Fax: 49-7071-29-4503, E-mail: naoyuki.tanimoto@med.uni-tuebingen.de

http://www.bioscience.org/current/vol14.htm