# Temporal Properties of Flicker ERGs in Rabbit Model of Retinitis Pigmentosa

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Submitted: June 1, 2017
Accepted: September 28, 2017

Citation: Okado S, Ueno S, Kominami T, et al. Temporal properties of flicker ERGs in rabbit model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2017;58:5518–5525. DOI: 10.1167/iovs.17-22332

**Purpose.** We determined the effects of a remodeled inner retina on the flicker electroretinograms (ERGs) in a rabbit eye at an advanced stage of inherited retinal degeneration.

**Methods.** Six wild-type (WT) and four rhodopsin P347L transgenic (Tg) rabbits were studied at 18 months of age. Flicker ERGs were elicited by sinusoidal stimuli at frequencies of 3.906 to 50.781 Hz. To block the ON and OFF retinal pathways, 2-amino-4-phosphonobutyric acid (APB), and 6-cyano-7-nitroquinoxaline-2, 3(1H, 4H)-dione (CNQX), respectively, were injected intravitreally. The amplitudes and phases of the fundamental components of the pre- and postdrug ERGs were analyzed. The postsynaptic APB (ON-) and CNQX (OFF-) sensitive components were determined by examining the phases and amplitude vectors.

**Results.** The temporal properties of the Tg rabbits were different from those of the WT rabbits and had unique features; at 3.906 Hz, the amplitude was depressed but it increased by more than 3.5-fold at 15.625 Hz. The reduction of the amplitude at 3.906 Hz in Tg rabbits was caused by a cancelation of the ON and OFF components by a phase difference of 180°. On the other hand, an increase in the amplitude at 15.625 Hz in Tg rabbits was caused by the summation of the ON and OFF components, which had an approximate 120° phase difference.

**Conclusions.** The temporal properties of the flicker ERGs of Tg rabbits were affected markedly by the remodeling of the retinal neurons. Evaluations of the flicker ERGs in RP eyes must be done with careful considerations of the current findings.

Keywords: flicker ERG, remodeling, retinal ON bipolar cell, retinitis pigmentosa, retinal OFF bipolar cell

Retinitis pigmentosa (RP) is a hereditary retinal disease that causes severe visual impairments. The genetic mutations in patients with RP cause degeneration of the rod photoreceptors followed by degeneration of the cones. The death of the photoreceptors leads to a gradual deconstruction of the morphology of the retina and functional reprogramming of the middle and inner retina, which have been reported as a remodeling of the retina in humans[1,2] and animal models of RP.[3–6]

Flicker electroretinograms (ERGs) have been used to evaluate the residual cone function in patients with RP, and the results showed a reduction in the amplitudes and a prolongation of the intrinsic times.[7–13] Flicker ERGs have been reported to have larger contributions from the postreceptoral components, viz., the depolarizing ON bipolar cell pathway (ON pathway) and the hyperpolarizing OFF bipolar cell pathway (OFF pathway), with a small contribution from the photoreceptors in primates.[14,15]

Thus, the amplitudes and implicit times of the flicker ERGs in RP patients are believed to be due not only to the degeneration of the photoreceptors but also to signals from the inner retina which has been remodeled in eyes with RP.[12,16]

Several studies have used harmonic analyses of the flicker ERGs to determine the temporal properties of the ERGs.[17–20] In addition, a "vector analyses" method was developed by Kondo and Sieving, which modified the harmonic analyses method.[15,21] In the vector analyses, flicker ERGs elicited before and after pharmacologic blockades of the ON and OFF pathways of the retina were analyzed by Fourier transformation. The obtained fundamental components of the ERGs are shown in the phase and amplitude vector. These analyses revealed the effects of a subtraction and summation of the ON and OFF pathways, and how they contribute to the amplitudes of the flicker ERGs.

We determined how the remodeled inner retina in transgenic (Tg) rabbit eyes altered the contribution of the photopic ON and OFF pathways to the flicker ERGs. To accomplish this, we used rhodopsin Pro347Leu Tg rabbits, which were generated by BAC transgenesis, as a model of RP in humans.[22] This animal model was shown to have a progressive degeneration of the rods followed by that of the cones.[6,22] Full-field ERGs showed that the rod components of the ERGs were reduced to only 5% by 48 weeks, while the cone components remained at 35% of the wild-type (WT) at the same age. At 18 months of age, the Tg rabbits had very few nuclei in the outer nuclear layer and there is a remodeling of the inner retina accompanied by an augmented of the OFF component and abnormal responses of the third-order neurons.[23] We showed

that the pattern of summation and subtraction of the ON and OFF pathways of the flicker ERGs in Tg rabbits was different from that in WT rabbits.

## Materials and Methods

### Animals

All experimental procedures adhered to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research and the Guidelines for the Use of Animals of the Nagoya University Graduate School of Medicine. The Nagoya University Animal Experiment Committee approved this project (Approval Number, 28405).

Six WT (WT1-6) and four TG (Tg1-4) rabbits whose background was the New Zealand White (NZW) strain were studied at 18 months of age. The generation of this Tg rabbit was described in detail.[22] Before beginning the experiments, we confirmed that scotopic ERGs were not elicited by 0.01 log cd-s/$m^2$ from the Tg rabbits, which indicated that the rod function essentially was absent.

### ERG Recordings

The procedures of recording ERGs have been reported in detail.[23] Briefly, the rabbits were anesthetized with an intramuscular injection of ketamine and xylazine. ERGs were recorded with a bipolar contact lens electrode (Gold Lens; Doran Instruments, Littleton, MA, USA) after the pupils were fully dilated. The signals were amplified and band pass-filtered between 0.3 and 1000 Hz and digitized at 2000 Hz. A total of 20 to 30 ERGs were averaged with a computer-assisted signal averaging system (Power Lab; AD Instruments, Castle Hill, Australia).

### Visual Stimulation

Rabbits were placed in a Ganzfeld bowl (Model 2503SH; LKC Technologies, Gaithersburg, MD, USA) and stimulated with light-emitting diodes (LEDs) providing white stimuli (modified version of LS200; Mayo Corporation, Aichi, Japan). The luminance of the LED light was measured with an integrating radiometer (40X-Spotmeter; United Detector Technology, Hawthorne, CA, USA). The LEDs were controlled by a digital function generator (WF1945, NF Corporation, Tokyo, Japan) which controlled the intensity and frequency of the stimulating light.

Flicker ERGs were elicited by sinusoidally modulated stimuli whose maximal intensity was 2.40 log cd/$m^2$ and minimal intensity was 0.00 cd/$m^2$. The stimuli were presented on a constant white background of 40 cd/$m^2$. We chose these stimulus intensities from the results of earlier studies.[15] The ERGs were recorded before and 60 to 90 minutes after the drug injections[5,23] because the drugs have been reported to affect the retina within 30 minutes.[24] We confirmed that the drugs had affected the retina by the waveform of the ON-OFF ERGs at the beginning of the experiments.

### Drug Injections

The drugs that were injected intravitreally were 2-amino-4-phosphonobutyric acid (APB; Sigma-Aldrich Japan, Tokyo, Japan) and 6-cyano-7-nitroquinoxaline-2, 3 (1H, 4H)-dione (CNQX; Sigma-Aldrich Japan). APB was injected into the vitreous cavity of the eye followed by an injection of CNQX. The intravitreal concentrations were 4 mM for APB and 0.4 mM for CNQX assuming that the vitreous volume of the NZW rabbit is 1.5 mL. The drugs were dissolved in PBS, and 0.05 mL was injected into the mid-vitreous. Six eyes of six WT and four eyes of four Tg rabbits were analyzed after injecting APB following a CNQX injection. To evaluate the effect of intravitreal injections of the drugs on the flicker ERGs, we injected PBS into the eyes of 2 WT rabbits. The ERGs appeared not to be affected by the PBS injection. Representative ERGs recorded before and after the sham injections are shown in Supplementary Figure S1.

### Waveform Analyses

The fundamental components of the pre- and post-drug flicker ERGs elicited by each frequency of stimulation were assessed by harmonic analysis as described in detail.[15,25] Because there are 2048 Fast Fourier Transform (FFT) points, the FFT analyses could be performed using Excel (Excel 2013; Microsoft, Inc., Redmond, WA, USA). The sampling frequency (2000 Hz) was divided by the number of FFT points (2048) to produce a factor of approximately 0.977. Flicker ERGs were recorded for 13 frequencies ranging from 3.906 ($4 \times 0.977$) to 50.781 ($52 \times 0.977$) Hz in steps of 3.906 Hz ($4 \times 0.977$). Because of equipment limitations, we were not able to determine the stimulus frequencies beyond the third decimal place, and the frequencies were rounded off accordingly. We did not analyze the higher harmonics. The phase lag of the fundamental component relative to the stimulus was determined and drawn on polar plots clockwise from the positive *x*-axis. The phase lag relative to the stimuli sine wave was presented on polar plots with negative values shown clockwise from the 0° polar axis. Because FFT analysis yields phases between −360° and 360°, we extrapolated the absolute response phases beyond this limit by comparisons of the phases of adjacent temporal stimulus frequencies as in the studies of Kominami et al.[25]

### Statistical Analyses

Two-factor factorial ANOVA followed by the Tukey-Kramer test was used to determine the significance of the differences in the amplitudes and phases of the ERGs components between WT and Tg rabbits. $P < 0.05$ was considered statistically significant.

### Retinal Histology

Two WT and two Tg rabbits were euthanized after the ERG recordings. Eyes were enucleated and fixed in Davidson's fixative for 6 hours and then transferred to 10% neutral buffered formalin. The tissues were trimmed and embedded in paraffin, sectioned vertically through the optic nerve, and stained with hematoxylin and eosin.

## Results

### Representative ERGs

Representative ERGs of the WT and Tg rabbits before and after APB and APB+ CNQX at four frequencies ranging from 3.906 to 46.875 Hz are shown in Figure 1. The predrug ERGs were composed of several components with different shapes at the lower stimulus intensities, but became simpler and more sinusoidal at 46.875 Hz. The amplitudes of the ERGs of the Tg rabbit were smaller than that of the WT rabbit at 3.906 Hz; however, it became comparable to that of the WT rabbit at other frequencies (Supplementary Fig. S2A).

After the APB injection, the ERGs were composed of the signals of the photoreceptor potentials and the OFF pathway. The amplitudes of the post-APB ERGs of the Tg rabbit at 3.906 and 15.625 Hz were larger than that of the WT, and the

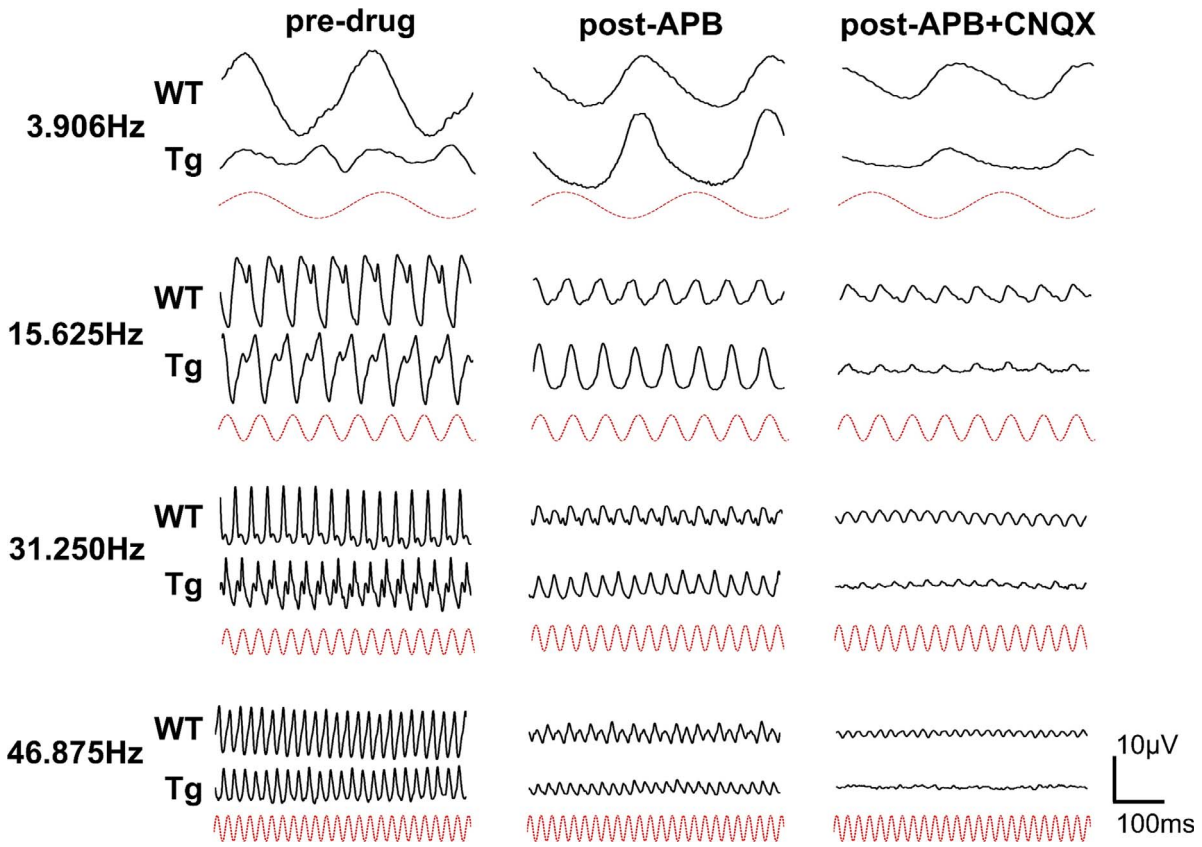


FIGURE 1. Representative flicker ERGs elicited by sine wave stimuli from WT and Tg rabbits before and after intravitreal injections of APB and APB+CNQX. The stimulus frequencies ranged from 3.906 to 46.875 Hz. The stimulus traces are shown in *red* beneath the ERGs.

amplitude of the ERGs at 31.250 Hz was comparable to that of the WT rabbits (Supplementary Fig. S2B).

After the APB+CNQX injection to isolate the photoreceptor component, the amplitude of the photoreceptor component was smaller in the Tg than in the WT rabbits at all frequencies (Supplementary Fig. S2C).

These results indicated that the temporal properties of Tg and WT rabbits were different before and after injection of the drugs. The vectors were analyzed to determine the mechanisms for these differences.

## Harmonic Analyses

The representative waveforms of the fundamental components after FFT filtering are shown in Supplementary Figure S3. The amplitudes (Figs. 2A, 2C, 2E) and phases (Figs. 2B, 2D, 2F) of the fundamental components of the flicker ERGs elicited by 13 frequencies from WT and Tg rabbits after Fourier transform are shown in Figure 2. In the WT rabbit, the predrug amplitude of the fundamental components was largest at 3.906 Hz and then decreased gradually with increasing stimulus frequencies to reach a plateau at 19.531 Hz. The amplitude then increased at 46.875 Hz. However, the predrug frequency-amplitude profile of Tg rabbits was different from that of WT rabbits. The amplitude of the fundamental components of Tg rabbits was smallest at 3.906 Hz and increased to the largest amplitude at 15.625 Hz, then gradually decreased.

The phases of the fundamental components of the Tg and WT rabbits at 3.906 Hz differed by nearly 150°, and the phase of the fundamental component of the Tg rabbits was delayed at all frequencies (Fig. 2B).

After the APB injection, the amplitudes of the flicker ERGs decreased monotonically with increasing stimulus frequencies for both types of rabbits (Fig. 2C). One interesting finding was that the post-APB amplitudes of the fundamental components were larger in the Tg than in the WT rabbits at the lower frequencies, although the difference was not significantly different. The phase of the fundamental components in the Tg rabbits was delayed at frequencies higher than 15.625 Hz (Fig. 2D).

After the APB+CNQX injection (photoreceptor component), the amplitude of the photoreceptor component was smaller in the Tg than in the WT rabbits at all frequencies. This indicated that the photoreceptor potentials of WT rabbits were much larger than those of Tg rabbits; the cone photoreceptors were significantly impaired during the course of retinal degeneration in the Tg rabbits. The photoreceptor component was largest at the lowest frequencies for both types of rabbits and gradually decreased with increasing stimulus frequencies. The phase of the fundamental components in the Tg rabbits was delayed at frequencies higher than 11.719 Hz.

## Vector Model Analyses

From the data obtained after the harmonic analyses of predrug, post-APB, and post–APB+CNQX ERGs of each rabbit, we calculated the phase and amplitude vectors of the fundamental components at each frequency for each rabbit. The calculated vectors for both types of representative rabbits for temporal frequencies of 3.906, 15.625, and 31.250 Hz are shown in Figure 3. The vectors of the predrug ERGs are shown in black,

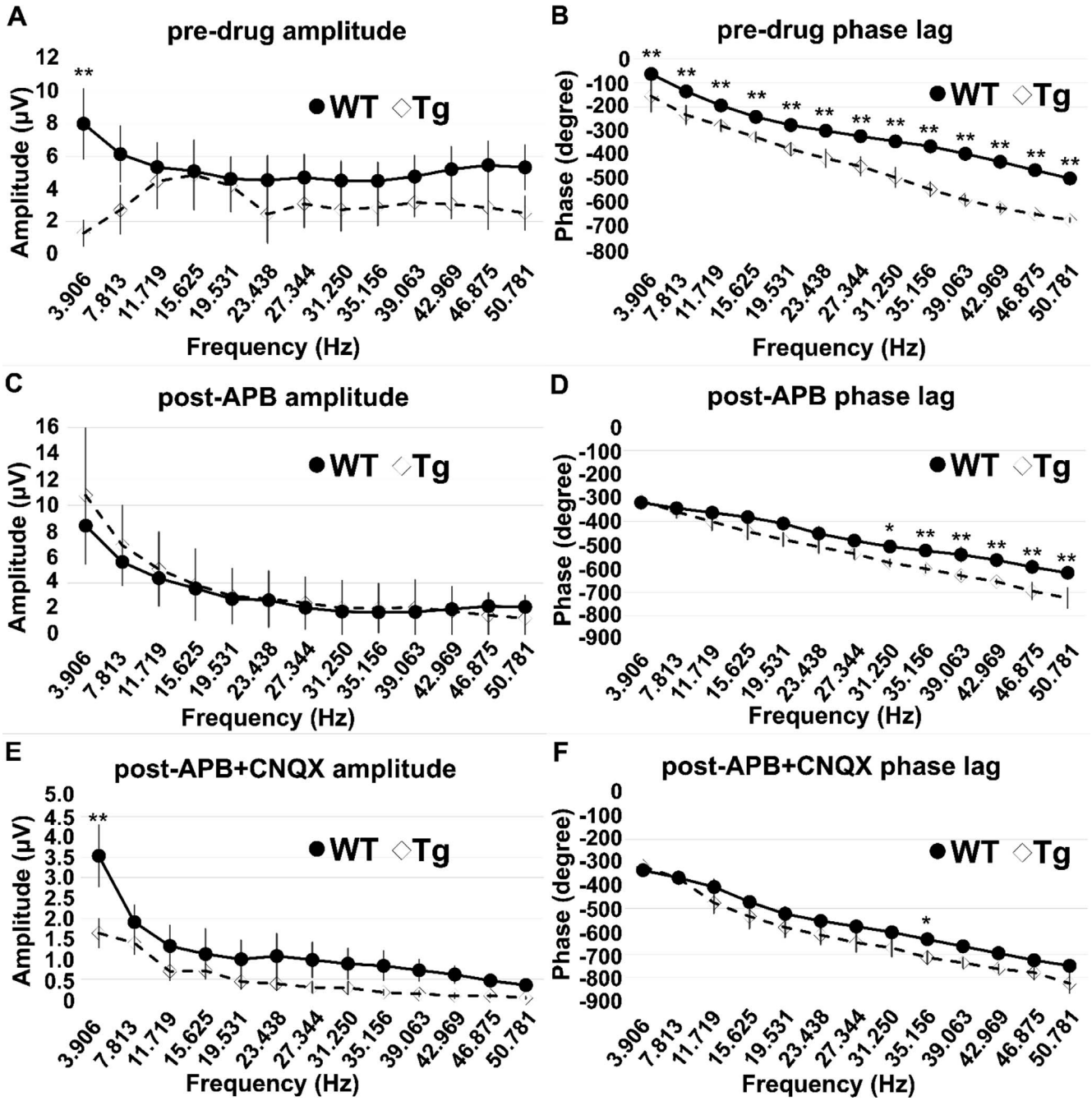


FIGURE 2. Plots of the amplitudes (**A**, **C**, **E**) and phases (**B**, **D**, **F**) of the fundamental components of the flicker ERGs before (**A**, **B**), after APB (**C**, **D**), and after APB+CNQX (**E**, **F**) from WT (•) and Tg (◇) rabbits. The amplitude profiles of the predrug ERGs of WT and Tg rabbit are different. For the Tg rabbit, the amplitude at 3.906 Hz was small, but it increased 3.5 times and reached maximum at 15.625 Hz (**A**). The means + SDs are shown. $^{*}P < 0.05$, $^{**}P < 0.01$. Two-factor factorial ANOVA.

those for the post-APB ERGs in green, and those of the post-APB+CNQX ERGs in yellow. To estimate the contribution of the ON pathway of the WT and Tg rabbits, we subtracted the vectors of the post-APB from the predrug ERGs (ON component). We also subtracted the vectors of the post-APB+CNQX (photoreceptor component) from those of post-APB ERGs to evaluate the OFF pathway (OFF component). The obtained vectors for the ON and OFF components are shown by red and blue arrows, respectively. The predrug fundamental components are composed of the summation of the vectors of the ON, OFF, and photoreceptor components.

## Vector Analysis at 3.906 Hz

The results of the phase and amplitude vector analyses of one representative WT rabbit and one Tg rabbit are shown in Figure 3A. For the WT rabbit, the length of the vector for the ON component was longer and approximately 200° out of phase from the OFF component. Consequently, the postsynaptic activity (ON+OFF component) was dominated by the ON component. The photoreceptor component (green arrow) was shifted approximately 90° from the ON component. Thus, the predrug component (black arrow) was slightly smaller than the ON component but larger than the photoreceptor component.

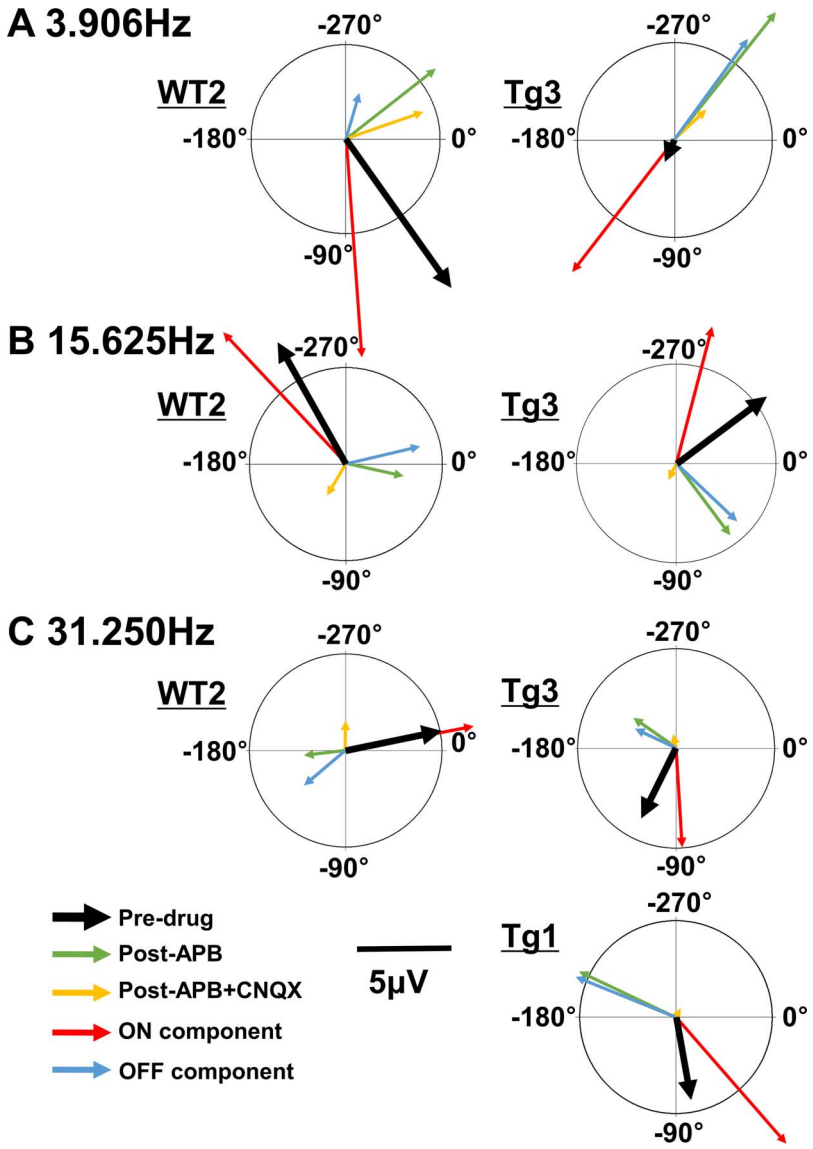


**Figure 3.** The vectors for the fundamental components of representative WT and Tg rabbits at 3.906 Hz (**A**), 15.625 Hz (**B**), and 31.250 Hz (**C**) sine wave flicker ERGs. *Black arrow*, predrug; *green arrow*, post-APB; and *yellow arrow*, post–APB+CNQX (photoreceptor component) ERGs. The ON component vectors (*red*) were calculated by subtracting the vector of the post-APB (*green*) from that of the predrug (*black*) vector. The OFF component vectors (*blue*) were determined by subtracting the vectors of the post–APB+CNQX (*yellow*) vectors from those of the post-APB (*green*) vectors. The predrug vectors (*black*) are composed of the summation of the vectors of the ON (*red*), the OFF (*blue*), and the photoreceptor components (*yellow*). Positive phase delays are plotted counterclockwise. Vector amplitudes are shown in absolute scale to the 5 μV calibration bar. At 3.906 Hz, the ON and OFF components of the Tg3 rabbits had similar lengths, and the phase of the ON component was approximately 80° out of phase with the OFF and photoreceptor components. This phase cancellation caused an almost complete elimination of amplitude of the predrug ERGs. At 31.250 Hz, the length of predrug vector was almost similar in the Tg1 and Tg3 rabbits; however, the contribution of ON and OFF components were different between the two rabbits.

For the Tg rabbit, the ON and OFF components had similar lengths, and the photoreceptor component was shorter than that of these components. The ON component was close to 180° out of phase from the OFF and photoreceptor components. This phase cancellation caused an almost complete elimination of the amplitude of the predrug response.

### Vector Analysis at 15.625 Hz

At 15.625 Hz, the ON and OFF components were nearly 120° out of phase in both WT and Tg rabbits. They added together to contribute to a large predrug vector. The contribution of photoreceptors was reduced especially for the Tg rabbit, but the lengths of the predrug components of both rabbits were similar. This indicated that the amplitudes of the flicker ERGs were more affected by the postreceptoral than the photoreceptor components.

### Vector Analysis at 31.250 Hz

The results of the vector analyses of one representative WT rabbit and two Tg rabbits are shown in Figure 3C. The amplitude of the predrug vector (black vector) of WT was similar to those of Tg rabbits but the difference of the phase between the two types of rabbits was approximately 270°. The contributions of photoreceptors were small for both types of rabbits, similar to that at 15.625 Hz. The length of the ON component (red arrow) was relatively longer than that of the

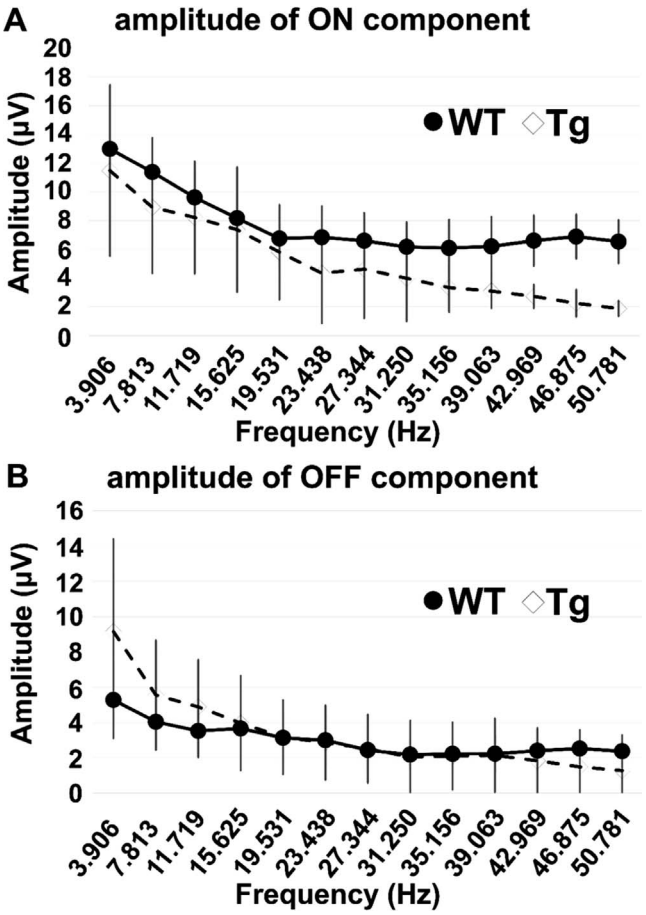


FIGURE 4. Mean amplitudes of the ON component (**A**) and OFF component (**B**) of WT (•) and Tg (◇) rabbits. The amplitudes of OFF component (**A**) were augmented in the lower frequencies (3.906–11.719 Hz). *Error bars*: standard deviation. $^{*}P < 0.05$, $^{**}P < 0.01$ (two-factor factorial ANOVA).

OFF components in both types of rabbits. Thus, the predrug wave tended to be affected by the ON components.

The length of the predrug vector was almost similar in the Tg1 and Tg3 rabbits; however, the amplitude of the ON and OFF components was much larger in Tg1 rabbits. These findings indicated that it is difficult to predict the contribution of the ON and OFF components from the predrug ERGs. The differences in the results of the vector analysis between two Tg rabbits were probably due to differences in the degrees of retinal degeneration and retinal remodeling. These findings indicated that the amplitude of the flicker ERGs in degenerated retinas is affected by the remodeled inner retina and does not always indicate the status of the photoreceptor degeneration.

### Amplitudes of ON and OFF Components as Function of Temporal Frequency

We calculated the amplitudes of the ON and OFF components of the WT and Tg rabbits obtained by the vector analysis (Fig. 4). The amplitude of the ON pathway was largest at 13.0 μV in WT and 11.5 μV in Tg rabbits at 3.906 Hz, and it decreased as the stimulus frequency increased. The amplitude of the ON component in the Tg rabbits was smaller than that in the WT rabbit at all frequencies although the differences were not significant at many frequencies. On the other hand, the amplitude of the OFF component in both types of rabbits was largest (5.3 and 9.2 μV) at 3.906 Hz, and the amplitudes of the Tg rabbits were larger than those of the WT rabbits at 3.906, 7.813, and 11.719 Hz, although the differences were not significant. The amplitude of the OFF components in the WT rabbits was smaller than those of ON components at all frequencies in the WT rabbits, but the amplitude of these two components were comparable at most of the frequencies in the Tg rabbits. These findings indicated that there was an increase of the OFF components in the flicker ERGs of Tg rabbits.

To estimate the effect of remodeling of the ON and OFF bipolar cells on flicker ERGs, we analyzed the ratio of the ON bipolar cell/photoreceptor amplitudes and the ratio of the OFF bipolar cell/photoreceptor amplitudes (Supplementary Fig. S4). The results showed the contributions of ON and OFF biplolar cells to the flicker ERGs in Tg rabbits were higher than those of WT rabbits at all frequencies, and the ratio became higher with increasing frequencies.

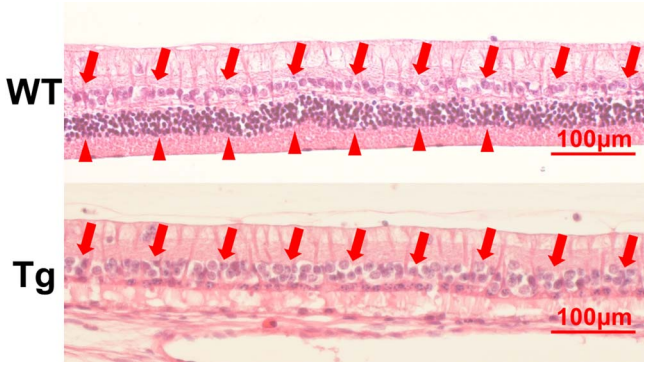


FIGURE 5. Retinal histology of WT and Tg rabbits at 18 months of age. Vertical retinal sections approximately 3 mm inferior to the optic nerve head. *Arrows* indicate the inner nuclear layer and *arrowheads* indicate the outer nuclear layer. *Scale bars*: 100 μm.

### Retinal Histology

The retinal histology in the area of the visual streak of WT and Tg rabbits at 18 months of age is shown in Figure 5. In WT rabbits, there were two to three rows of nuclei in the inner nuclear layer and five to seven rows of nuclei in the outer nuclear layer. On the other hand, there were very few scattered nuclei in the outer nuclear layer in Tg rabbits, and the nerve fiber layer to inner nuclear layer was relatively well preserved in Tg rabbits.

## DISCUSSION

The contributions of the ON and OFF pathways to the temporal responses have been analyzed in mice,[19,25] rats,[26] rabbits,[27] and monkeys.[15] However, a search of PubMed showed that no studies have investigated the temporal properties and contributions of the ON and OFF pathways of degenerating retinas.

We analyzed the flicker ERGs elicited by a wide range of frequencies at an advanced stage of degeneration in Tg rabbits. We found a consistent delay of the phases of the fundamental component at all frequencies in the predrug Tg rabbits, and also unique temporal properties of the predrug amplitudes of the fundamental component in Tg rabbits. The amplitude was reduced at 3.906 Hz but it increased more than 3.5 times at 15.625 Hz. To determine the mechanism for this unexpected feature, we analyzed the responses by vector analysis. Using this rabbit model has an advantage in that it was easier to evaluate the OFF-bipolar component in the rabbits than in

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rodents that have a weak OFF-bipolar cell contribution to the cone ERGs.[25,28]

An estimation of the degree of residual cone function in Tg rabbits was determined by comparing the ERGs of Tg rabbits to those of WT rabbits after an injection of APB+CNQX in WT rabbits.[14] The amplitudes of the flicker ERGs in the Tg rabbits were approximately 40% to 60% of those in the WT rabbits at all frequencies tested. These results indicated that the cone function was disrupted consistently in Tg rabbits.

On the other hand, the amplitudes of the OFF component of the Tg rabbits appeared to be increased as has been reported in a porcine model of RP[3] and our Tg rabbit model.[23,29] The ratio of the amplitudes of the ON and OFF component/photoreceptors are shown in Supplementary Figure S3. In the Tg rabbits, the amplitudes of the ON and OFF components, and those of the photoreceptors were larger than those of the WT rabbits at all frequencies. These results agreed with those of previous reports.

An interesting finding determined by the vector model analysis was that the increase in amplitude of the OFF pathway canceled the amplitude of the ON pathway due to a phase difference of 180° at low frequencies. This led to a large reduction in the amplitude of the ERGs at 3.906 Hz in the Tg rabbits.

In addition, the amplitudes of the flicker ERGs of the Tg1, Tg3, and WT2 rabbits were not different at 32.50 Hz but the compositions of the ON, OFF, and photoreceptor components were completely different. Thus, the analyses of the amplitudes of the flicker ERGs do not simply reflect the cone photoreceptor function, but they were unexpectedly affected by the summation and subtraction of the signals of the ON and OFF pathways in the remodeled inner retina.

An earlier study compared the temporal properties of RP patients to those of normal subjects.[16] In normal subjects, the fundamental component had two main peaks at 3.7 and 41 Hz, while only four of 12 RP patients had a peak at 3.7 Hz. These results are in agreement with our results, and might indicate an unexpected subtraction of the ON and OFF pathways due to a remodeling of the retinal neurons in the inner retina of RP patients.

We evaluated the retinal histology of WT and Tg rabbits at 18 months of age. We found that the WT rabbits had five to seven rows of nuclei in the outer nuclear layer of the retina, but the Tg rabbits had only scattered nuclei in the outer nuclear layer.[23] It is assumed that similar histologic changes occur in the retina of RP patients, and these lead to the functional remodeling of the retina.

Flicker ERGs elicited by 30 Hz stimuli are part of the standard ERGs recommended to be recorded by the International Society for Clinical Electrophysiology of Vision (ISCEV).[30] The flicker ERGs are used to quantify the functional status of the retinal cone system in patients with retinal diseases.[9,31–33] Our results indicated that recording the flicker ERGs elicited by only one frequency of 30 Hz in RP patients may limit the collection of important information of the physiology of the retina. Thus, analyses of the ERGs for a wide range of frequencies might be more informative about the temporal properties of the cone system. In addition, the implicit times or phases of the major harmonics would be more suitable than the amplitude of fundamental components for evaluating the cone function.

There is a limitation in our study design. Our study was based on the data from four Tg animals because of the shortage of older Tg rabbits, which weakens the conclusions that can be made.

In conclusion, our results indicated that the temporal properties of 18-month-old Tg rabbits are significantly different from those of WT rabbits. Vector model analysis revealed an increase in the amplitude of the OFF component canceled by the ON pathway at low frequencies. Our data indicated that the interpretation of flicker ERGs in eyes with RP must be done cautiously, especially in the evaluation of the amplitudes, because the flicker ERGs are affected by unexpected summation and cancelation of the secondary changes of retinal neurons.

## Acknowledgments

Supported by Scientific Research C (16K11320 SU) from Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Disclosure: **S. Okado**, None; **S. Ueno**, None; **T. Kominami**, None; **A. Nakanishi**, None; **D. Inooka**, None; **A. Sayo**, None; **M. Kondo**, None; **H. Terasaki**, None

## References

1. Milam AH, Li ZY, Cideciyan AV, Jacobson SG. Clinicopathologic effects of the Q64ter rhodopsin mutation in retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 1996;37:753–765.
2. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res*. 1998;17:175–205.
3. Banin E, Cideciyan AV, Aleman TS, et al. Retinal rod photoreceptor-specific gene mutation perturbs cone pathway development. *Neuron*. 1999;23:549–557.
4. Jones BW, Watt CB, Frederick JM, et al. Retinal remodeling triggered by photoreceptor degenerations. *J Comp Neurol*. 2003;464:1–16.
5. Sakai T, Kondo M, Ueno S, Koyasu T, Komeima K, Terasaki H. Supernormal ERG oscillatory potentials in transgenic rabbit with rhodopsin P347L mutation and retinal degeneration. *Invest Ophthalmol Vis Sci*. 2009;50:4402–4409.
6. Ueno S, Koyasu T, Kominami T, et al. Focal cone ERGs of rhodopsin Pro347Leu transgenic rabbits. *Vision Res*. 2013;91:118–123.
7. Sandberg MA, Effron MH, Berson EL. Focal cone electroretinograms in dominant retinitis pigmentosa with reduced penetrance. *Invest Ophthalmol Vis Sci*. 1978;17:1096–1101.
8. Sandberg MA, Jacobson SG, Berson EL. Foveal cone electroretinograms in retinitis pigmentosa and juvenile macular degeneration. *Am J Ophthalmol*. 1979;88:702–707.
9. Berson EL, Sandberg MA, Rosner B, Birch DG, Hanson AH. Natural course of retinitis pigmentosa over a 3-year interval. *Am J Ophthalmol*. 1985;99:240–251.
10. Seiple WH, Siegel IM, Carr RE, Mayron C. Evaluating macular function using the focal ERG. *Invest Ophthalmol Vis Sci*. 1986;27:1123–1130.
11. Holopigian K, Greenstein V, Seiple W, Carr RE. Rates of change differ among measures of visual function in patients with retinitis pigmentosa. *Ophthalmology*. 1996;103:398–405.
12. Hood DC, Birch DG. Abnormalities of the retinal cone system in retinitis pigmentosa. *Vision Res*. 1996;36:1699–1709.
13. Zein WM, Falsini B, Tsilou ET, et al. Cone responses in Usher syndrome types 1 and 2 by microvolt electroretinography. *Invest Ophthalmol Vis Sci*. 2014;56:107–114.
14. Bush RA, Sieving PA. Inner retinal contributions to the primate photopic fast flicker electroretinogram. *J Opt Soc Am A Opt Image Sci Vis*. 1996;13:557–565.
15. Kondo M, Sieving PA. Primate photopic sine-wave flicker ERG: Vector modeling analysis of component origins using glutamate analogs. *Invest Ophthalmol Vis Sci*. 2001;42:305–312.
16. Falsini B, Iarossi G, Fadda A, et al. The fundamental and second harmonic of the photopic flicker electroretinogram: temporal frequency-dependent abnormalities in retinitis pigmentosa. *Clin Neurophysiol*. 1999;110:1554–1562.

17. Baker CL, Hess RF. Linear and nonlinear components of human electroretinogram. *J Neurophysiol*. 1984;51:952–967.
18. Odom JV, Reits D, Burgers N, Riemslag FCC. Flicker electroretinograms - a systems analytic approach. *Optom Vis Sci*. 1992;69:106–116.
19. Krishna VR, Alexander KR, Peachey NS. Temporal properties of the mouse cone electroretinogram. *J Neurophysiol*. 2002; 87:42–48.
20. Khan NW, Kondo M, Hiriyanna KT, Jamison JA, Bush RA, Sieving PA. Primate retinal signaling pathways: suppressing ON-pathway activity in monkey with glutamate analogues mimics human CSNB1-NYX genetic night blindness. *J Neurophysiol*. 2005;93:481–492.
21. Kondo M, Sieving PA. Post-photoreceptoral activity dominates primate photopic 32-Hz ERG for sine-, square-, and pulsed stimuli. *Invest Ophthalmol Vis Sci*. 2002;43:2500–2507.
22. Kondo M, Sakai T, Komeima K, et al. Generation of a transgenic rabbit model of retinal degeneration. *Invest Ophthalmol Vis Sci*. 2009;50:1371–1377.
23. Kominami T, Ueno S, Okado S, Nakanishi A, Kondo M, Terasaki H. Contributions of second- and third-order retinal neurons to cone electroretinograms after loss of rod function in rhodopsin P347L transgenic rabbits. *Invest Ophthalmol Vis Sci*. 2017;58:1417–1424.
24. Sieving PA, Murayama K, Naarendorp F. Push-pull model of the primate photopic electroretinogram: a role for hyperpolarizing neurons in shaping the b-wave. *Vis Neurosci*. 1994; 11:519–532.
25. Kominami T, Ueno S, Nakanishi A, et al. Temporal properties of cone ERGs of pikachurin null mutant Mouse. *Invest Ophthalmol Vis Sci*. 2016;57:1264–1269.
26. Qian H, Shah MR, Alexander KR, Ripps H. Two distinct processes are evident in rat cone flicker ERG responses at low and high temporal frequencies. *Exp Eye Res*. 2008;87:71–75.
27. Qian H, Alexander KR, Ripps H. Harmonic analysis of the cone flicker ERG of rabbit. *Exp Eye Res*. 2010;91:811–817.
28. Koyasu T, Kondo M, Miyata K, et al. Photopic electroretinograms of mGluR6-deficient mice. *Curr Eye Res*. 2008;33:91–99.
29. Jones BW, Kondo M, Terasaki H, et al. Retinal remodeling in the Tg P347L rabbit, a large-eye model of retinal degeneration. *J Comp Neurol*. 2011;519:2713–2733.
30. McCulloch DL, Marmor MF, Brigell MG, et al. ISCEV Standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol*. 2015;130:1–12.
31. Berson EL. Retinitis-pigmentosa - the Friedenwald lecture. *Invest Ophthalmol Vis Sci*. 1993;34:1659–1676.
32. Fujinami K, Lois N, Davidson AE, et al. A longitudinal study of Stargardt disease: clinical and electrophysiologic assessment, progression, and genotype correlations. *Am J Ophthalmol*. 2013;155:1075–1088.e13.
33. Yasuda S, Kachi S, Ueno S, Piao CH, Terasaki H. Flicker electroretinograms before and after intravitreal ranibizumab injection in eyes with central retinal vein occlusion. *Acta Ophthalmol*. 2015;93:e465–e468.

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