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TECHNICAL NOTE

Absence of ocular interaction in flicker ERG responses reflecting cone opponent and luminance signals

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Abstract The aim of the study was to investigate whether there is an ocular interaction in the flicker ERG responses reflecting luminance and cone opponency in normal human subjects. Flicker ERGs were recorded from one dilated eye of 10 healthy volunteers. Each subject was tested twice: once with and once without occluding the opposite eye. Red and green LEDs were modulated in counterphase in a Ganzfeld stimulator. ERG responses were recorded for different ratios of the modulation in the red and green LEDs and at 12 and 36 Hz. The amplitudes and phases of the fundamental components were compared between the conditions with and without occlusion. The 12-Hz flicker ERGs reflected activity of the cone opponent channel, whereas the 36-Hz data reflected luminance activity. There were no significant differences between the conditions with and without occluding the opposite eye for any of the stimulus protocols. Ocular interaction is absent in flicker ERGs reflecting cone opponent and luminance activity.

Keywords Ocular interaction · Electroretinogram · Luminance · Cone opponency

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Introduction

For decades, the electroretinogram (ERG) has been experimentally used to obtain a better understanding of the visual receptors and their associated neural structures [1]. The ERG is also an important non-invasive clinical tool used to investigate the normal function and the dysfunction of the human retina [2]. The International Society for Clinical Electrophysiology of Vision (ISCEV) has standardized ERG protocols in order to provide comparable results among different clinical and research centers. The standard protocols specify electrode placement, stimulus, and background luminance, signal amplification, data analysis, among other aspects [3, 4].

The human ERG is recorded at the cornea using surface electrodes, and the individual response from the eye can be measured when the other eye is also stimulated (binocular stimulation) or when the other eye is occluded (monocular stimulation). It has

always been implicitly assumed that binocular and monocular measurements yield identical results and there is no ocular interaction on the level of the electroretinogram. Ocular interactions might be caused by indirect stimulation of the contralateral eye or by neuronal feedback. To our knowledge, this issue, however, has not been subject of direct investigation.

To study whether ocular interactions are present, we chose to use the flicker ERG since its responses originate to a large extent in post-receptoral neurons [5–7]. Recent studies in our laboratory showed that heterochromatic stimuli in which red and green LEDs are modulated in counterphase can reflect activity in the magnocellular luminance channel at high temporal frequencies (>24 Hz) and in the parvocellular L-M cone opponent channel at around 12 Hz [8]. Although flicker ERGs probably originate in bipolar cells [5], we now have the possibility to study responses in pathways that remain separate at least up to the visual cortex using the ERG. A neuronal ocular interaction that is different in distinct retino-geniculate pathways might possibly be revealed in the heterochromatic flicker ERG.

It is the purpose of the present study to examine whether ocular interactions influence flicker ERG measurements by comparing ERG responses obtained in monocular and binocular conditions.

Methods

The study was performed in accordance with the tenets of the declaration of Helsinki. Participants were ten healthy volunteers (8 men and 2 women) aged from 29 to 56(mean = 43 ± 11). One eye was dilated with a drop of mydriaticum (0.5% tropicamide), and corneal ERG responses were measured with a DTL fiber electrode (UniMed Electrode Supplies) attached at the outer to the inner canthus of the eye. The reference and ground skin electrodes were attached to the ipsilateral temple and the forehead, respectively. Under certain circumstances involving pattern ERG, contralateral reference electrode positioning and the use of non-corneal active electrodes ERG-signals from the contralateral eyes can be recorded [10–13]. We, however, regard this type of cross-talk as an artifact rather than an interaction with a physiological basis.

The tested eye was randomly chosen. Each subject was tested twice: once without occluding the opposite eye (binocular stimulation; called the "no occlusion" condition) and once with occlusion of the opposite eye (monocular stimulation; the "occlusion" condition). One subject was tested eight times at the same session (four times without occlusion and four times with occlusion). The tests were performed in random order (six subjects were first tested without occlusion and four were first tested with occlusion).

Stimulation with red (638 nm, bandwidth at half height: 19 nm; CIE coordinates: x: 0.6957, y: 0.2966) and green (523 nm, bandwidth at half height 36 nm; CIE coordinates: x: 0.2016, y: 0.7371) LEDs was controlled by a RetiPort system (Roland Consult, Germany). The stimulator was a Ganzfeld (Q450 SC Roland-Consult, Germany) with six differently colored LED arrays; of which only the red and green LED arrays were activated. The mean luminance of each LED array was 100 cd/m^2, resulting in a retinal illuminance of about 10^4 td (with an approximately 8-mm-diameter pupil). The mean chromaticity was yellow with CIE coordinates: (0.5813, 0.4030).

Figure 1a shows the luminance outputs of red and green LEDs. They were sinusoidally modulated with three fractions of red modulation depth $R/(R + G)$: 0 (only the green LED is modulated with 100% contrast around the 100 cd/m^2 mean luminance; the red LED is constant at 100 cd/m^2); 0.5 (red and green LEDs modulated simultaneously in counterphase each with 50% contrast); and 1 (only the red LED modulated with 100% contrast; the green LED was constant at 100 cd/m^2). Figure 1b shows that the modulation of the output of the cone opponent system was the same in all three conditions. Furthermore, the phase of the cone opponent modulation was the same for all conditions (i.e. the stimulus was more reddish in the first half of a stimulus period and more greenish in the second half). Luminance modulation was large for the condition, in which $R/(R + G)$ equaled 0 and 1 and small for the condition $R/(R + G) = 0.5$. The luminance modulation followed the green LEDs at $R/(R + G) = 0$ and the red LEDs when $R/(R + G)$ equaled 1, so that they were 180° phase shifted relative to each other [8]. The sinusoidal cone opponent and luminance outputs had constant time-averaged values, indicating that the state of adaptation is the same for all stimulus conditions (assuming that the 12- and 36-Hz stimulus frequencies are too high for adaptation processes).

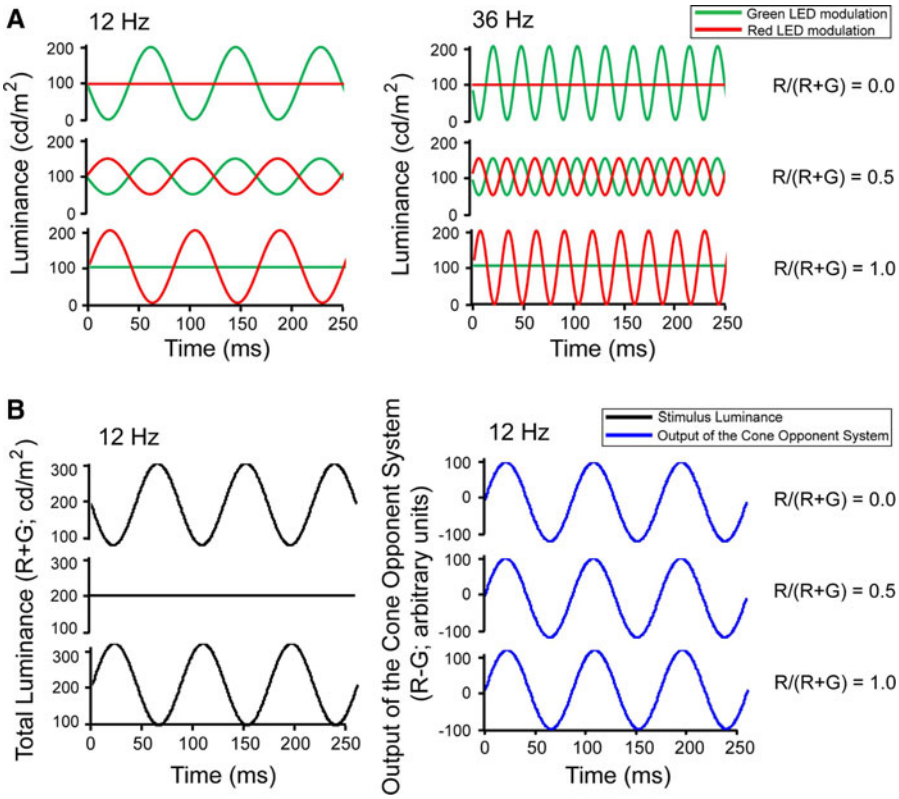

Fig. 1 Sketch of stimuli. **a** the luminance outputs of red and green LED arrays were sinusoidally modulated, and three fractions of red modulation depth $R/(R + G)$ were presented: 0 (only the green LED is modulated; the red LED is constant at 100 cd/m^2); 0.5 (red and green LEDs modulated simultaneously in counterphase); and 1 (only the red LED modulated; the green LED was constant at 100 cd/m^2). The modulation depth in the two LED arrays was around the mean luminance **b** *Left column*—12 Hz luminance output (*black line*) in stimulus coordinates is obtained by the addition of the red and green LED outputs. Luminance modulation has the same contrast at $R/(R + G)$ values of 0 and 1 but not when $R/(R + G) = 0.5$. The phase of the luminance output is 180° shifted between $R/(R + G)$ 0 and 1. The time-averaged luminance output is 200 cd/m^2 for all three stimulus conditions. *Right column*—the output of the cone opponent system of the 12-Hz stimulus (*blue line*) is expressed as the subtraction of the red and green LED outputs. This subtraction results in identical sinusoidal cone opponent modulations for all three conditions. Furthermore, cone opponent modulation has the same phase in all conditions

The signals were recorded with the RetiPort system. They were amplified 100,000×, filtered between 1 and 300 Hz, and sampled at 1024 Hz. The ERG responses were averages of 48 (12 Hz) and 24 sweeps (36 Hz), each lasting 1 s, resulting in 1-s recording episodes. The recordings were Fourier analyzed (using MATLAB, TheMathWorks, USA), and the ERG responses were defined as amplitudes and phases of the first (fundamental) harmonic component. An estimate of noise was obtained by averaging the amplitudes of the adjacent lower (i.e. 11 and 35 Hz, respectively) and upper (i.e. 13 Hz and 37 Hz, respectively) frequencies for all stimulus conditions. The phases were disregarded when the signal-to-noise ratios were less than 3. The comparisons between different test conditions were made using Wilcoxon matched pairs test, with a 95% confidence interval. Spearman correlation coefficients were also considered for the statistical analyses.

Results

Figure 2 shows individual recordings at 12 and 36 Hz. In agreement with previous data [8, 9], at 12 Hz, the responses display relatively constant amplitudes and phases for the different protocols, indicating that they reflect red–green opponent

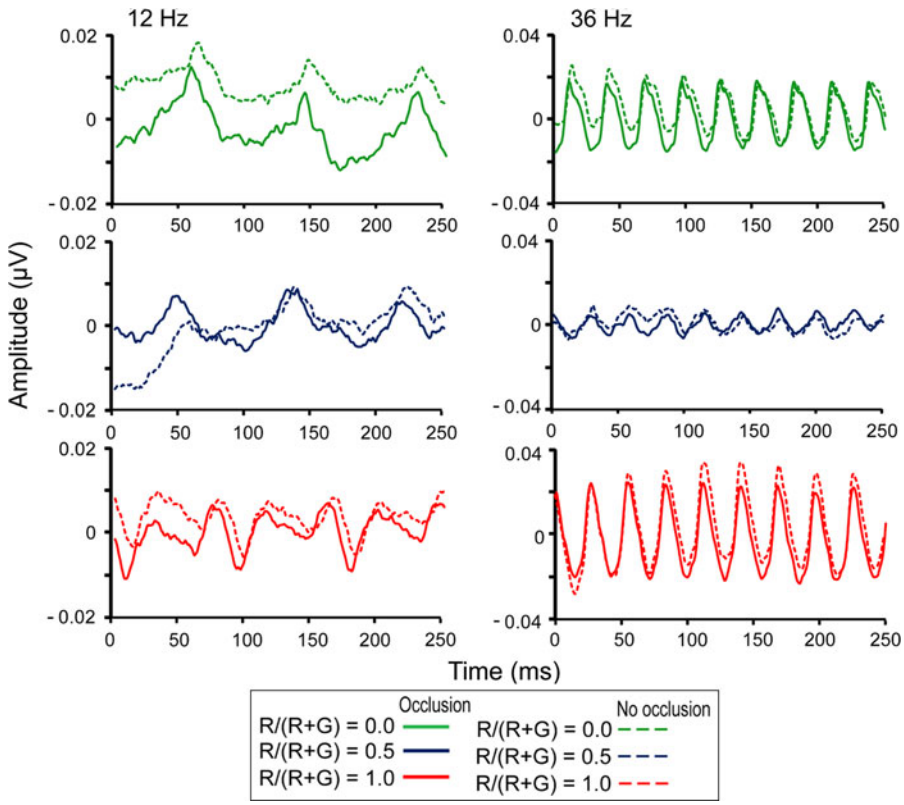

Fig. 2 Individual recordings for each protocol at 12 (*left*) and 36 Hz (*right*)

activity. The response amplitudes at 36 Hz changed strongly with the $R/(R + G)$ values. Moreover, the responses at $R/(R + G)$ 0 and 1 are in counterphase with each other at 36 Hz. In agreement with previous results [8, 9], this suggests that at this temporal frequency, the ERG reflects luminance activity.

Figure 3 shows the average (±one standard deviation) first harmonic response amplitudes and phases for the "no occlusion" (open circles) and "occlusion" (closed circles) conditions. As it was found previously [8], the first harmonic amplitudes and phases of responses at 12 Hz were relatively constant at the different stimulus conditions (Fig. 2 left). At 36 Hz (Fig. 2 right), there is a minimal response amplitude in condition $R/(R + G) = 0.5$, and the phases at conditions $R/(R + G) = 0$ and 1 were about 180° phase shifted. The averages and standard deviation were very similar in the "occlusion" and "no occlusion" conditions. There was no significant difference between the "no occlusion" and "occlusion" conditions at 12 Hz (amplitude, $P > 0.2$ and phase, $P > 0.4$) and also at 36 Hz (amplitude, $P > 0.6$ and phase, $P > 0.1$).

Figure 4 shows Bland–Altman plots in which the individual amplitude and phase differences measured in the "no occlusion" and "occlusion" conditions are plotted as a function of their averages. Negative differences indicate that the "no occlusion" amplitude is larger and the response phase is smaller. The dotted gray lines represent the linear regressions that are close to zero for both amplitudes (12 Hz, $R^2 = 0.002$ and 36 Hz, $R^2 = 0.077$) and phases (12 Hz, $R^2 = 0.003$ and 36 Hz, $R^2 = 0.086$).

Four "no occlusion" and four "occlusion" measurements were taken in one participant within the same test session. There was no significant first harmonic amplitude difference between "no occlusion" and "occlusion" (12 Hz, $P = 0.11$ and 36 Hz, $P = 0.37$), and the correlation between the conditions was significant (12 Hz, $P < 0.01$; 36 Hz, $P < 0.001$). Figure 5 shows these comparisons of the first harmonic amplitude response in each stimulus condition (upper: condition $R/(R + G) = 0.0$; middle: $R/(R + G) = 0.5$; lower: $R/(R + G) = 1.0$) at 12 Hz (left) and 36 Hz (right).

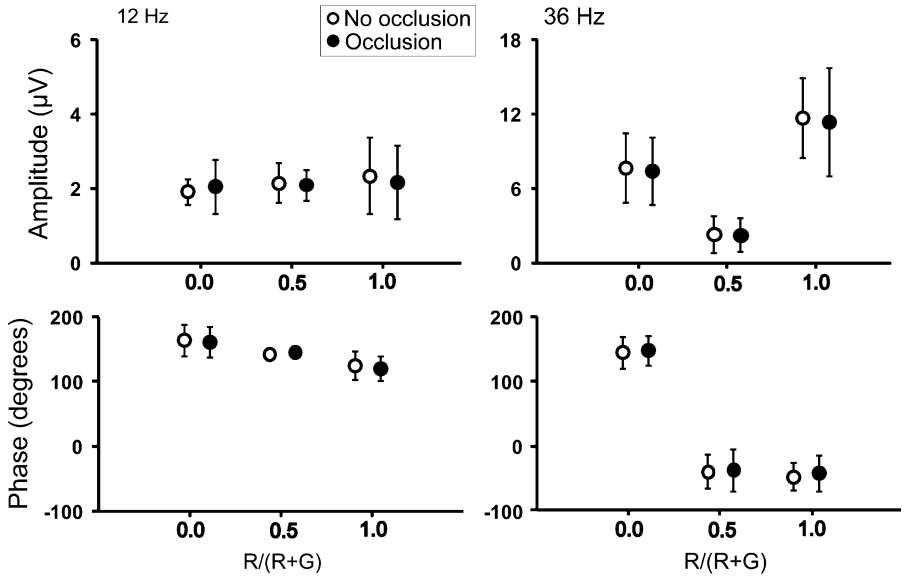

Fig. 3 Average (±SD) amplitudes (*upper plots*) and phases (*lower plots*) of the first harmonic responses in the "no occlusion" (*open circles*) and "occlusion" (*closed circles*) conditions measured at 12 Hz (*left*) and 36 Hz (*right*)

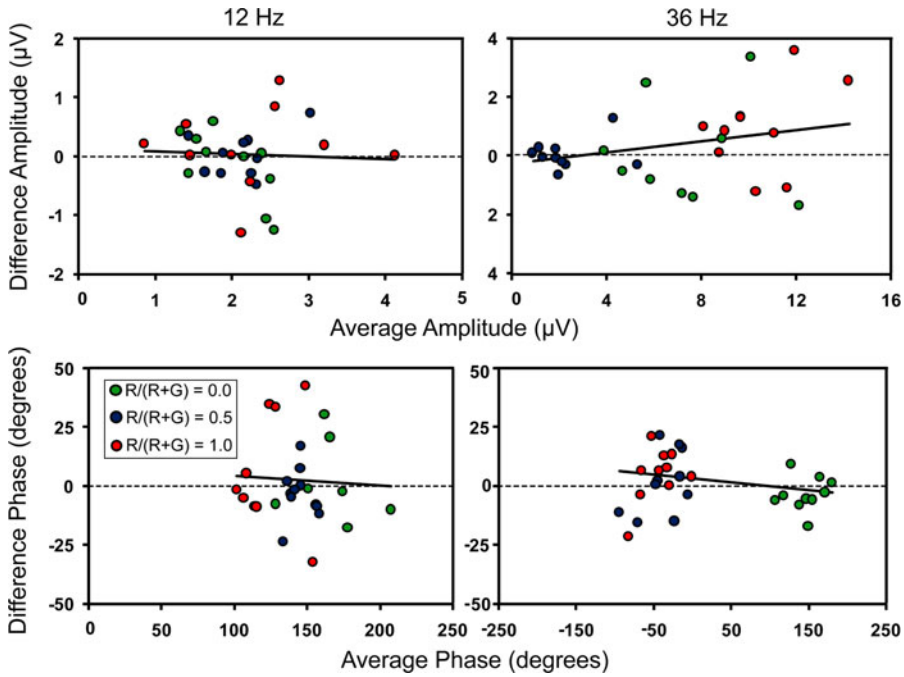

Fig. 4 Bland–Altman plot: individual amplitude and phase differences measured in the "no occlusion" and "occlusion" conditions as a function of their averages. *Negative differences* indicate that the "no occlusion" amplitude is larger and the response phase is smaller. *Black lines* represent the linear regressions that are close to zero for both amplitudes (12 Hz, $R^2 = 0.002$ and 36 Hz, $R^2 = 0.077$) and phases (12 Hz, $R^2 = 0.003$ and 36 Hz, $R^2 = 0.086$)

Discussion

To our knowledge, this is the first report in which ocular interactions in the flicker ERG responses are directly studied. Our data strongly suggest the absence of physiological ocular interactions in this recording condition: the responses measured in one eye are not influenced by equal and simultaneous stimulation of the other eye. As a result, ERGs obtained with monocular and binocular stimulation can be compared with each other.

In agreement with previous data [8, 9], it has been confirmed here that the first harmonic 12-Hz flicker ERG component reflects cone opponent activity. The 36-Hz first harmonic component reflects luminance activity since the amplitudes and phases resemble those of the luminance channel. The absence of an ocular interaction at both temporal frequencies

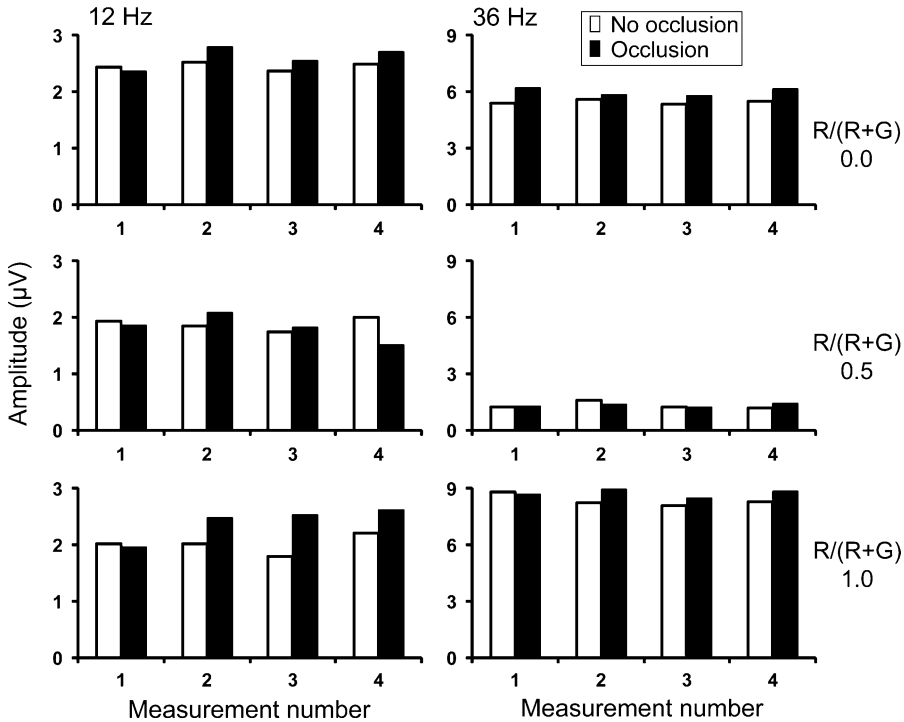

Fig. 5 Comparison of the first harmonic amplitude response in each stimulus condition (upper: condition $R/(R + G) = 0.0$; middle: $R/(R + G) = 0.5$; lower: $R/(R + G) = 1.0$) at 12 Hz (*left*) and 36 Hz (*right*). There was no significant first harmonic amplitude difference between the "no occlusion" and "occlusion" conditions (12 Hz, $P = 0.11$ and 36 Hz, $P = 0.37$); the correlation between the conditions was significant (12 Hz, $P < 0.01$; 36 Hz, $P > 0.001$)

suggests that neither in the parvocellular nor in the magnocellular pathways, a substantial neuronal feedback from post-retinal structures is present.

The ERG responses from healthy subjects recorded in the present study showed reliable signal-to-noise ratio in almost all measurements. Only two subjects displayed a signal-to-noise ratio smaller than 3 at 12 Hz, and in both cases the phases were disregarded from the data analysis. These low signal-to-noise ratios were found in the "no occlusion" condition. This is possibly due to the larger amount of light reaching the subject in the binocular stimulation compared to the monocular stimulation, which may increase the subjects' blinking reactions during the recordings, especially at lower temporal frequencies. Occluding the opposite eye (monocular stimulation) when measuring flicker ERG may be considered useful because it is more convenient for the subject.

The human ERG is a non-invasive technique that permits to study the physiology of the retina in vivo. Flicker ERG is recommended by the ISCEV standardization committee as one of the basic full field ERG protocols [4]. It can also give excellent estimates of the spectral sensitivity of cones in humans [14], and it now is possible to perform non-invasive measurements of basic electrophysiological properties of the luminance and cone opponent pathways on a retinal level in humans [8, 15]. Concerning the importance of human flicker ERG either for basic investigation on the retina or for clinical assessment of retinal disturbances, the present study investigated whether one eye can influence the responses of the opposite eye if both eyes are stimulated at the same time (binocular stimulation).

The present report shows that there is no significant difference between the flicker ERG responses in conditions with and without occlusion the opposite eye. In conclusion, ocular interaction is absent in flicker ERGs.

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