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ORIGINAL RESEARCH ARTICLE

An extended 15 Hz ERG protocol (2): data of normal subjects and patients with achromatopsia, CSNB1, and CSNB2

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Abstract The amplitude versus flash strength curve of 15 Hz electroretinograms (ERGs) shows two minima. The minima are caused by interactions between the primary and the secondary rod pathways (first minimum), and the secondary rod pathway and the cone-driven pathway (second minimum). Furthermore, cone pathway contributions cause higher-order harmonics to occur in the responses. We measured 15 Hz ERGs in 20 healthy subjects to determine normal ranges and in patients to verify our hypotheses on the contributions of the different pathways and to investigate the clinical application. We analyzed the amplitudes and phases of the 15, 30, and 45 Hz components in the ERGs. The overall shape of the 15 Hz amplitude curves was similar in all normal subjects and showed two minima. The 30 and 45 Hz amplitude curves increased for stimuli of high flash strengths indicating cone pathway contributions. The 15 Hz amplitude curve of the responses of an achromat was similar to that of the normal subjects for low flash strengths and showed a minimum, indicating normal primary and secondary rod pathway function. There was no second minimum, and there were no higher-order harmonics, consistent with absent cone pathway function. The 15 Hz ERGs in CSNB1 and CSNB2 patients were similar and of low amplitude for flash strengths just above where the first minimum normally occurs. We could determine that in the CSNB1 patients, the responses originate from the cone pathway, while in the CSNB2 patients, the responses originate from the secondary rod pathway.

Keywords ERG · 15 Hz flicker · Rods · Cones · Primary rod pathway · Secondary rod pathway · Cone pathway · Congenital stationary night blindness · Achromatopsia

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Introduction

Several studies have provided evidence that primary and secondary rod pathways [1–3] can be distinguished by 15 Hz flicker electroretinograms (ERGs) [4–7]. In the primary and most sensitive rod pathway, rod signals travel via rod ON bipolar cells and all amacrine cells to either cone ON bipolar cells and ON ganglion cells or to cone OFF bipolar cells and OFF ganglion cells. In the secondary rod pathway, rod signals travel via gap junctions between rod and cone

pedicles and are then further transported to cone ON or OFF bipolar cells and ON or OFF ganglion cells. The amplitude versus flash strength curve of 15 Hz ERGs shows a minimum. At the same flash strength, the phase versus flash strength curve shows a change of roughly 180° (henceforth called amplitude and phase curves). The current model is that the minimum is caused by the interaction of the two rod pathways. This phenomenon can potentially be used in the clinic to examine the two rod pathways in patients. In a previous study [8], we investigated cone pathway contributions (cone signals that travel via ON or OFF cone bipolar cells to ON or OFF ganglion cells, respectively) to 15 Hz ERGs to get more insight into the possible origin of the 15 Hz responses. In the present study, we will further investigate the clinical possibilities.

Evidence that the minimum in the 15 Hz ERG amplitude curve is caused by the interaction of rod pathways only was provided in a study by Rüther et al. [9] who recorded responses of an achromat. The 15 Hz responses in the achromat showed a minimum, comparable to that of a normal subject, albeit at 0.4 log units higher flash strength. However, the significance of this shift could not be verified due to the lack of information about the normal range. Scholl et al. [10] recorded 15 Hz ERGs in 22 healthy subjects. The flash strength at the minimum as measured in the normal subject by Rüther et al. (−0.73 log scot td·s) fell outside the normal range determined by Scholl et al. (mean: −1.64 log scot td·s, standard deviation: 0.17 log scot td·s). There is no explanation for this discrepancy; the two studies used the same equipment. Also in other studies [11–13], the flash strengths at which the minima occurred in healthy individuals did not correspond to the normal range determined by Scholl et al.

In the clinic, 15 Hz ERGs have been used to examine the primary and secondary rod pathways in several retinal disorders [11–15]. These studies showed that 15 Hz ERGs may provide an insight into the dysfunction of signal transmission in retinal disorders. However, the studies differ in their interpretation of 15 Hz responses in patients with congenital stationary night blindness (CSNB) type 1. Genetic studies have revealed that CSNB1 is caused by a defect in ON bipolar cells [16–19]. Because of the defect in the rod ON bipolar cells, the primary rod pathway does not function in CSNB1 patients. However, in the secondary rod pathway, rod signals can also travel via cone OFF bipolar cells. The literature provides only inconclusive data about whether the secondary rod pathway is functionally reduced in CSNB1 patients or completely inactive. Scholl et al. [14] examined 11 CSNB1 patients and compared their responses to their normal range. Responses were found for flash strengths above that of the minimum: they were reduced in amplitude, but still significantly above noise, and advanced in phase. The suggestion was that these responses originate from the secondary rod pathway using the cone OFF bipolar cells. Van Genderen et al. [13] recorded similar 15 Hz responses in an autosomal recessive (ar-) CSNB1 patient with a TRPM1 mutation and concluded likewise. In contrast, an earlier study by Rüther et al. [9] used the 15 Hz ERGs of a CSNB1 patient to determine the cone threshold. As the CSNB1 patient did not exhibit rod responses in the standard ERG, they assumed that both rod pathways were dysfunctional and that the responses could thus only originate from the cones.

In the accompanying study [8], we investigated cone pathway contributions to dark-adapted 15 Hz ERGs in five healthy subjects. We extended the 15 Hz protocol to higher flash strengths and analyzed the 15, 30, and 45 Hz components present in the ERGs. We found that when we used short flash flicker stimuli, we could recognize cone pathway contributions by the appearance of higher harmonics (30 and 45 Hz components) in the ERGs. We also found that for flash strengths where both the secondary rod pathway and the cone pathway are active, a second minimum appeared in the 15 Hz amplitude curve that was accompanied by a small shift in phase. We concluded that for flash strengths below that of the second minimum, the secondary rod pathway dominates, while at higher flash strengths, the cone pathway dominates.

In the present study, we have applied the extended 15 Hz protocol to 20 healthy subjects to (1) investigate whether previous results [8] can be reproduced in a larger group of subjects, (2) determine the range of flash strengths where the minima and maxima appear, and (3) determine the 95% range in amplitude and phase per flash strength. Furthermore, we measured 15 Hz ERGs in an achromat, four CSNB1 patients, and three CSNB2 patients and compared the results with those of the normal subjects.

Methods

Subjects

This study included 20 healthy volunteers: 11 women, 9 men; 8 were emmetropic, 11 were myopic (less than five diopters), and one woman was highly myopic (D 11). Her responses were similar to the responses of the other subjects. The age of the 20 subjects ranged from 22 to 58 years, the median was 35 years. All volunteers gave their informed consent.

An achromatopsia patient (patient 1, aged 40 years) participated in this study. Diagnosis was based on ERG measurements according to ISCEV standards [20] and ophthalmological examination. The ERGs showed no cone activity. The patient was completely color blind and suffered from photophobia, but her visual field and funduscopy were normal. Her refractive error was S +3.75, C −3.00 on both eyes, and her visual acuity was 0.1. The ERGs of four patients with CSNB1 (patients 2–5, aged 16, 7, 6, and 9 years, respectively) and three with CSNB2 (patients 6–8, aged 42, 11, and 6 years, respectively) were also included in this study. Diagnoses were based on clinical examinations, standard ERG measurements [20], and dark adaptation curves (Goldmann-Weekers dark adaptometer), which corresponded with the distinct features of CSNB1 and CSNB2 as described by Miyake et al. [21]. Their visual fields and funduscopy were normal. Two of the CSNB1 patients (patients 2 and 3) had genetic mutations in the TRPM1 gene; their 15 Hz ERG responses had been presented previously [13]. Patient 6 had a mutation in CACNA1F. The other patients were not genetically tested. All patients gave their informed consent.

Apparatus and stimuli

Stimuli were produced by a Ganzfeld stimulator (Standalone Colordome system, Diagnosys LLC, Impington, Cambridge, UK). The bowl was homogeneously illuminated by 4 ms white flashes at a frequency of 15 Hz. Flashes were produced by LEDS. We recorded ERG responses to a series of flash strengths of −1.90 to 2.10 log scot td·s (assuming a pupil diameter of 8 mm) in 20 logarithmic steps (approximately 0.2 log scot td·s). We used the same protocol for the patients. However, for some of the younger ones (patient 3, 5, 7, and 8), we reduced the protocol by increasing the flash strength steps to 0.4 log scot td·s.

Procedure

Subjects' pupils were dilated with 0.5% tropicamide. We used DTL fiber electrodes [22] on both eyes. The ends of the fiber were attached to the skin, as close as possible to the left and right corners of the eye, so that the fiber itself floated on the cornea. A reference electrode was placed on the forehead just above the nose, and a ground electrode was positioned on the left temple. At the start of the experiment, subjects dark-adapted for 20 min.

Data analysis

The ERG responses were recorded at a sample frequency of 1,024 Hz for approximately 25 s and stored using the computer program Eemagine EEG (Advanced Neuro Technology BV, Enschede, The Netherlands). We averaged approximately 250 ERG responses over 0.267 s time intervals (four periods). The program automatically rejected responses if the amplitude exceeded ±250 μV. We applied Fourier transformation on the responses and retrieved the amplitude and the phase of the 15, 30, and 45 Hz components (i.e., the first, second, and third harmonics). We investigated the correlation between the responses of the left and the right eye in the normal subjects. Because the two eyes do not provide independent data we used only right eye responses in further analyses.

We determined the 95% ranges of the amplitude data and phase data of each component per flash strength. As the amplitude data were not normally distributed, we used a gamma distribution [23]. The phase data were rejected where the signal-to-noise ratio was below 4.55 (1% significance level). For the signal-to-noise ratio, we divided the amplitude of the 15, 30, or 45 Hz components by the average amplitude of the neighboring frequencies (11 and 19 Hz, 26 and 34 Hz, 41 and 49 Hz, respectively) [24]. The remaining phase data were analyzed by means of circular statistics [25, 26]. Using circular statistics, we also tested whether or not the phase data were uniformly distributed between 0° and 360° (the Rayleigh test). The absolute phase was determined by minimizing the difference with the average phase for the surrounding

flash strengths. We also determined the 95% ranges of flash strengths where the minima and maxima occurred. To determine the flash strength of the minima and maxima in each 15 Hz amplitude curve, the curves were first smoothed by a triangle window of three points to improve the signal-to-noise ratio. Interindividual differences were further investigated by correlation analysis.

The phases and amplitudes of the 15, 30, and 45 Hz components in the responses from both eyes of the patients were compared with the normal ranges. The absolute phase was determined by minimizing the difference with the phase found in the responses to surrounding flash strengths. The phase data are not plotted for flash strengths where we were not able to determine a normal range. Some of the responses of patients 4 and 5 were strongly affected by eye blinks. These responses were removed from the data set.

Results

We determined the correlation between the 15 Hz amplitude data of the left and right eye responses for each subject. We found a strong correlation coefficient that was 0.98 ($P < 0.05$) on average. The ratio between the amplitude data of the left and right eyes ranged from 0.67 to 1.34.

Normal data

The 15 Hz amplitude and phase curves of each subject's right eye responses are plotted in Fig. 1. Each amplitude curve showed two minima and one maximum. The first 15 Hz minimum was always accompanied by a strong steepening in phase decrease of roughly 180° (referred to as a phase shift). The second 15 Hz minimum was also always accompanied by a phase shift, but this shift was much smaller. Apart from these similar trends, the curves showed several interindividual differences. The 15 Hz amplitudes to stimuli of equal flash strength differed greatly between subjects. The trend of the phase shifts, smooth or more abrupt, also differed between subjects, see Fig. 1.

Figure 2 shows the amplitude and phase data of the 20 normal subjects, the average response and the 95% normal ranges. On average, the first 15 Hz minimum occurred at −1.1 log scot td·s and was accompanied by a phase shift of approximately 137°. At higher flash strengths, the averaged 15 Hz amplitude curve showed a second minimum at 1.2 log scot td·s. This minimum was accompanied by a smaller change in phase of approximately 85°. The 95% ranges of the 15 Hz amplitude data increased with flash strength. Rayleigh tests [25, 26] showed that the 15 Hz phase data were not randomly distributed ($P < 0.01$) for all flash strengths.

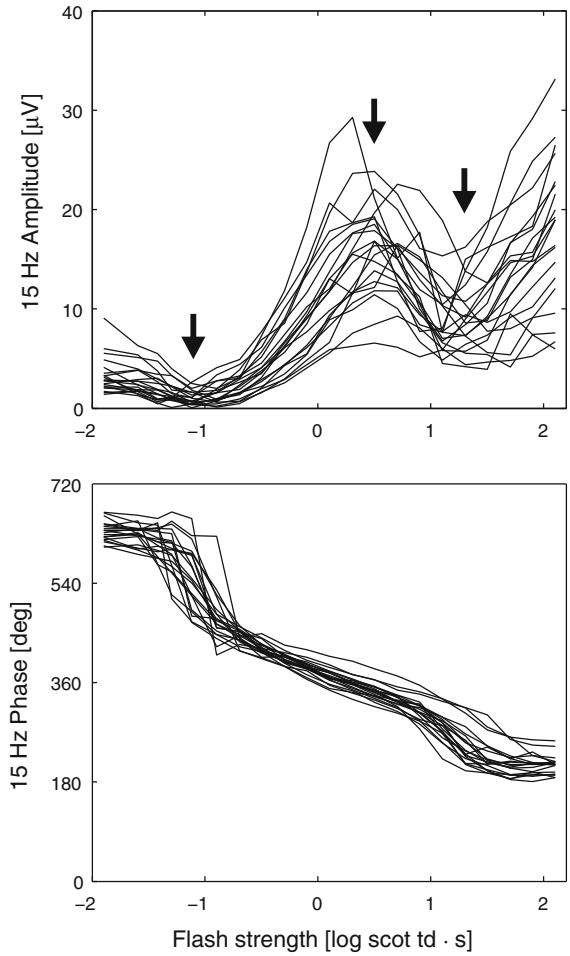

Fig. 1 The 15 Hz amplitude curves (*top*) and the 15 Hz phase curves (*bottom*) of the right eye responses of 20 normal subjects. All amplitude curves showed two minima and one maximum. All phase curves showed steepening in phase decrease at a flash strength of that of the minima. The *arrows* point at the average flash strength at which minima and maxima and phase changes occurred

The amplitudes of the 30 Hz components were close to zero for stimuli of low flash strengths. However, the averaged 30 Hz amplitude curve showed a first minimum at the same flash strength as the first 15 Hz minimum. This minimum was accompanied by a phase

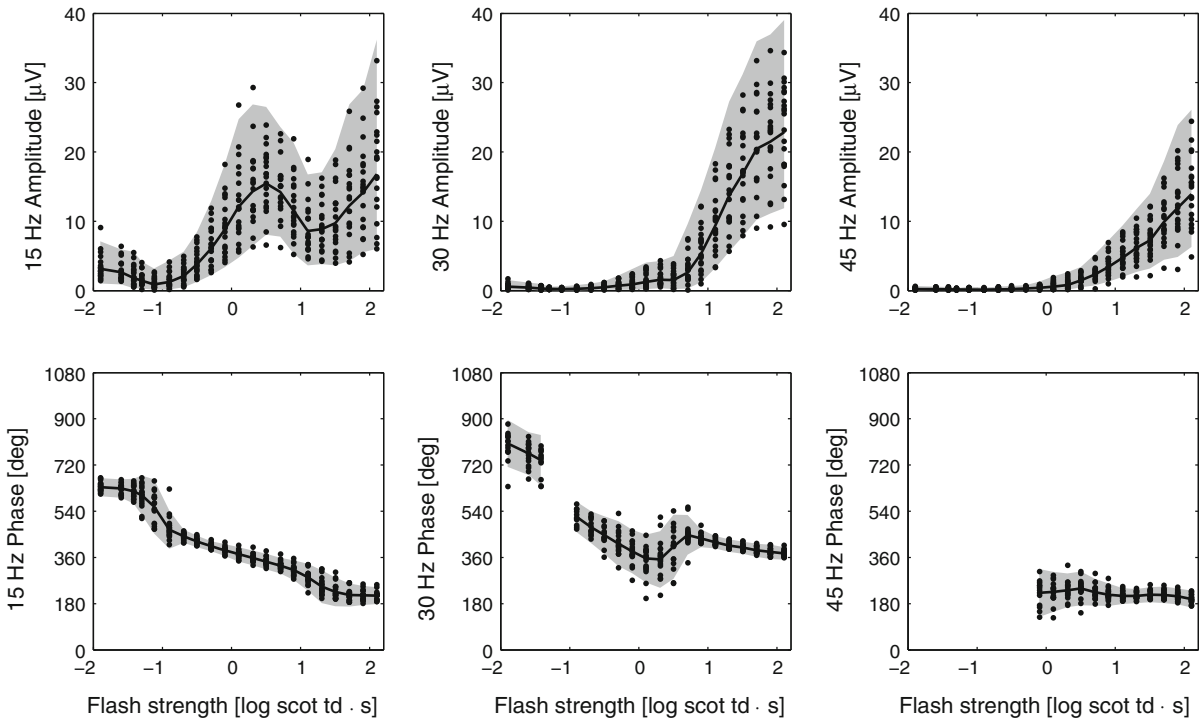

Fig. 2 The amplitude data (*top*) and the phase data (*bottom*) of the 15 Hz (*left*), the 30 Hz (*middle*) and the 45 Hz (*right*) components in the right eye responses of 20 subjects (*black dots*), the average response (*black line*) and the 95% ranges for normal subjects (*gray*)

shift of roughly 126°. Rayleigh tests showed that the 30 Hz phase data were not randomly distributed ($P < 0.01$) except for the data close to the first minimum (−1.3 and −1.1 log scot td·s). At a flash strength of 0.5 log scot td·s, a second minimum occurred that was accompanied by a phase increase of approximately 100°. This minimum was not clear in all subjects, but all 30 Hz phase curves showed a shift. After the second minimum, the 30 Hz amplitudes increased strongly.

The 45 Hz components were random for low flash strengths; Rayleigh tests showed that the phase data were not randomly distributed ($P < 0.01$) above −0.1 log scot td·s. When the flash strength increased, the 45 Hz amplitude curve increased gradually. Over the entire range of flash strengths, the 45 Hz phase curves did not show shifts.

The flash strength at which minima and maxima occurred differed between subjects, see Fig. 3. The differences between subjects could have been caused by variations in the strength of the flash at the retina, for instance, by differences in pupil diameter. We therefore determined correlation coefficients, between the flash strengths of the first minimum and the maximum (not significant), between the flash strengths of the maximum and the second minimum (0.61, $P < 0.01$), and between the flash strengths of the first and the second minima (not significant). An example of two 15 Hz amplitude curves that show differences in the positions of the minima and maxima is given in Fig. 4. In subject 1, the first minimum in the 15 Hz amplitude curve occurred at a higher flash strength while the second minimum occurred at a lower flash strength compared to these minima in subject 2. Also note that the first 15 Hz phase shift changed abruptly in subject 1 but smoothly in subject 2.

The amplitude of the responses also varied greatly between subjects, resulting in broad 95% ranges. These differences could have been caused by variations in the conductance of the DTL electrode and eye system. We therefore correlated the amplitude data for flash strengths as far as possible from the minima (0.6 log units). The data of two subjects could not be used in these analyses as their minima occurred too close to the end of the range of stimuli. We determined correlation coefficients between the amplitude of 15 Hz components at several flash strengths: at

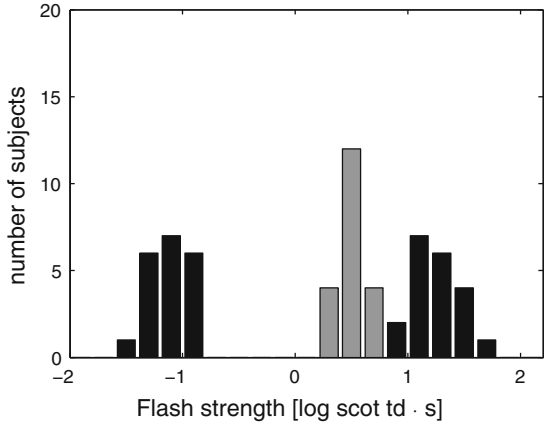

Fig. 3 The flash strengths at which the minima and maxima occurred in the right eye response of the 20 subjects. The first minima occurred between −1.4 and −0.9 log scot td·s, the median at −1.1 log scot td·s; the maxima occurred between 0.3 and 0.7 log scot td·s, the median at 0.5 log scot td·s; and the second minima occurred between 0.9 and 1.7 log scot td·s, the median at −1.3 log scot td·s

0.6 log units below that of the first minimum and at the maximum (0.53, $P < 0.05$), at the maximum and 0.6 log units above that of the second minimum (0.68, $P < 0.01$), and at 0.6 log units below that of the first minimum and above that of the second minimum (0.61, $P < 0.01$). An example of the responses of two subjects which differed in amplitude is given in Fig. 5. The 15 Hz amplitude curves of the responses in subjects 3 and 4 presented in this figure are comparable except between the two minima. Note that the phase curves were similar.

We also determined the correlation coefficients between the amplitudes of the 15, 30, and 45 Hz components in the responses to the highest flash strength (2.1 log scot td·s). The correlation between the 15 and 30 Hz components was 0.92 ($P < 0.01$), between the 30 and 45 Hz components 0.73 ($P < 0.01$), and between the 15 and 45 Hz components 0.74 ($P < 0.01$). Figure 6 shows the 15, 30, and 45 Hz amplitude curves of the responses in subjects 5 and 6. These amplitude curves were representative for all subjects and show the increase in the three components at high flash strengths.

Patient data

Figure 7 shows the amplitude and phase curves of the ERGs measured in both eyes of the achromat. The 15 Hz amplitude curves responses showed a first

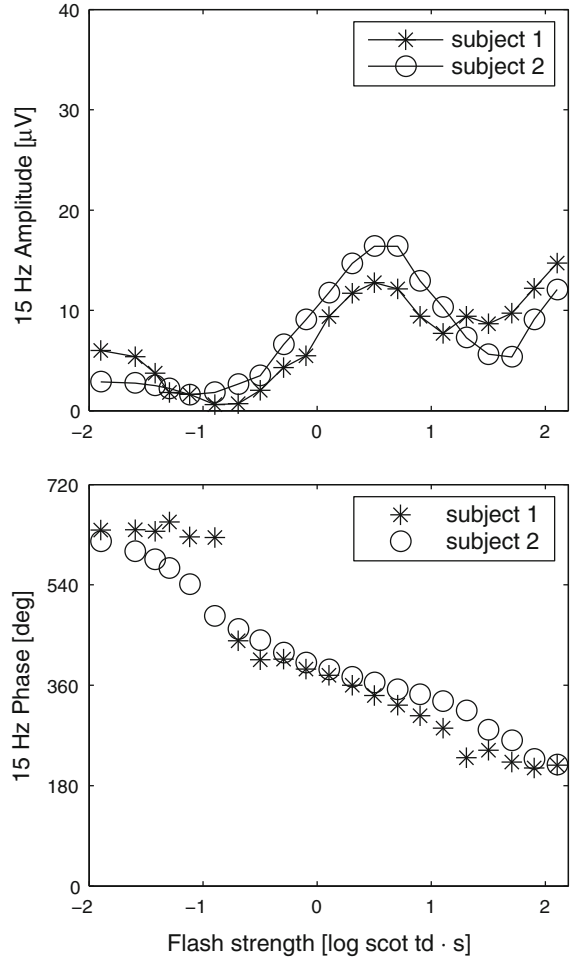

Fig. 4 The 15 Hz amplitude *curve* and the 15 Hz phase *curve* belonging to two normal subjects. In subject 1, the first minimum occurred at −0.7 log scot td·s and the second minimum at 1.1 log scot td·s. In subject 2, the first minimum occurred at −1.1 log scot td·s and the second minimum at 1.7 log scot td·s. The phase shift occurred abruptly in subject 1 but smoothly in subject 2. The flash strength at which these phase shifts occurred corresponded with the flash strength of the minima in amplitude

minimum in both eyes at a flash strength well within the normal range. These minima were accompanied by a phase shift, although the absolute phases differed slightly from normal. The 15 Hz amplitude curves also showed a maximum occurring at a flash strength well within the normal range and accompanied by maxima in the 30 and 45 Hz amplitude curves. For higher flash strengths, the 15, 30, and 45 Hz amplitude curves decreased to noise. The 15 Hz phase curves did not show a second phase shift. The 30 and 45 Hz phase curves showed a strong linear decrease that deviated

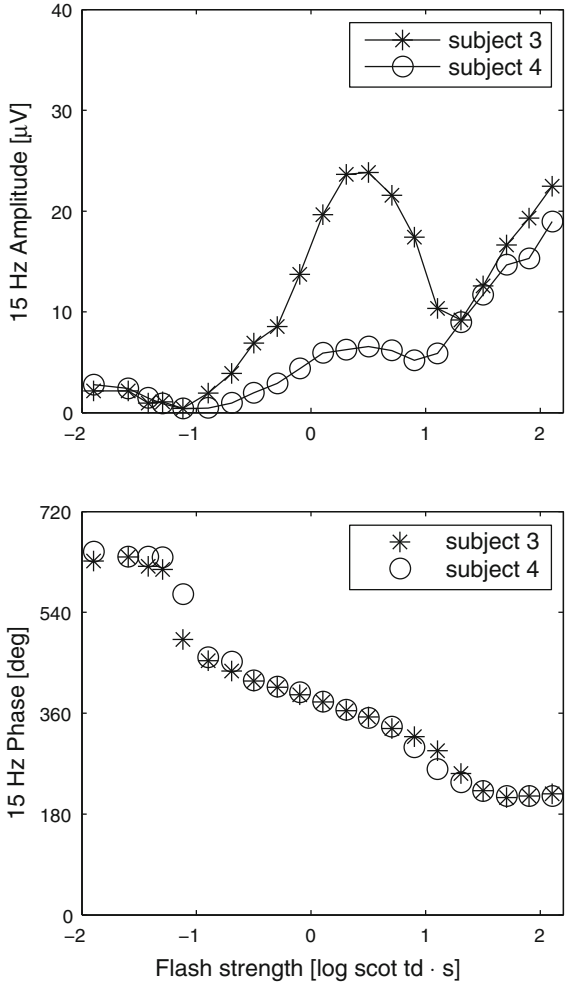

Fig. 5 The 15 Hz amplitude curves and the 15 Hz phase curves belonging to two normal subjects. The 15 Hz amplitude curves of these two subjects were comparable except at flash strengths between the two minima. The 15 Hz phase curves were very similar

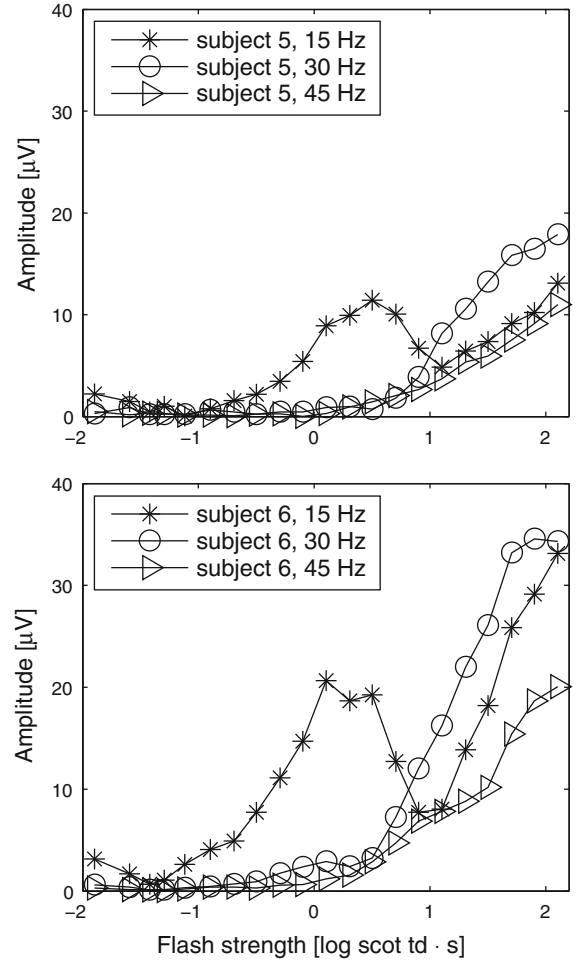

Fig. 6 The 15, 30, and 45 Hz amplitude curves belonging to two normal subjects. At high flash strengths, the 15, 30, and 45 Hz components increased uniformly within each subject

increasingly for higher flash strengths from those of the normal subjects.

Figure 8 shows the amplitude and phase curves of the ERGs measured in both eyes of the four CSNB1 patients. The 15 Hz amplitude curves did not show a first minimum or a phase change; the responses were of minimal amplitude for low flash strengths and the phase data varied greatly between subjects. For higher flash strengths, the 15 Hz amplitude curve increased monotonously and the data were within the normal range above flash strengths where normally the second minimum occurs. However, no minima or maxima could be distinguished. The phase decreased slightly but linearly in contrast to the trend seen in normal subjects. The absolute phases at the highest flash strength were advanced compared to normal subjects. The amplitude data and phase data of the 30 and 45 Hz components were approximately within the normal range at all flash strengths.

Figure 9 shows the amplitude and phase curves of the 15 Hz ERGs measured in both eyes of the three CSNB2 patients. For the lowest flash strength, the responses were of minimal amplitude and the phase data varied greatly between subjects, so no first minimum could be distinguished. However, for flash strengths above that at which a first minimum normally occurs, the 15 Hz phase curves were within normal limits although the 15 Hz amplitude curves were still below the normal range. Minima could be

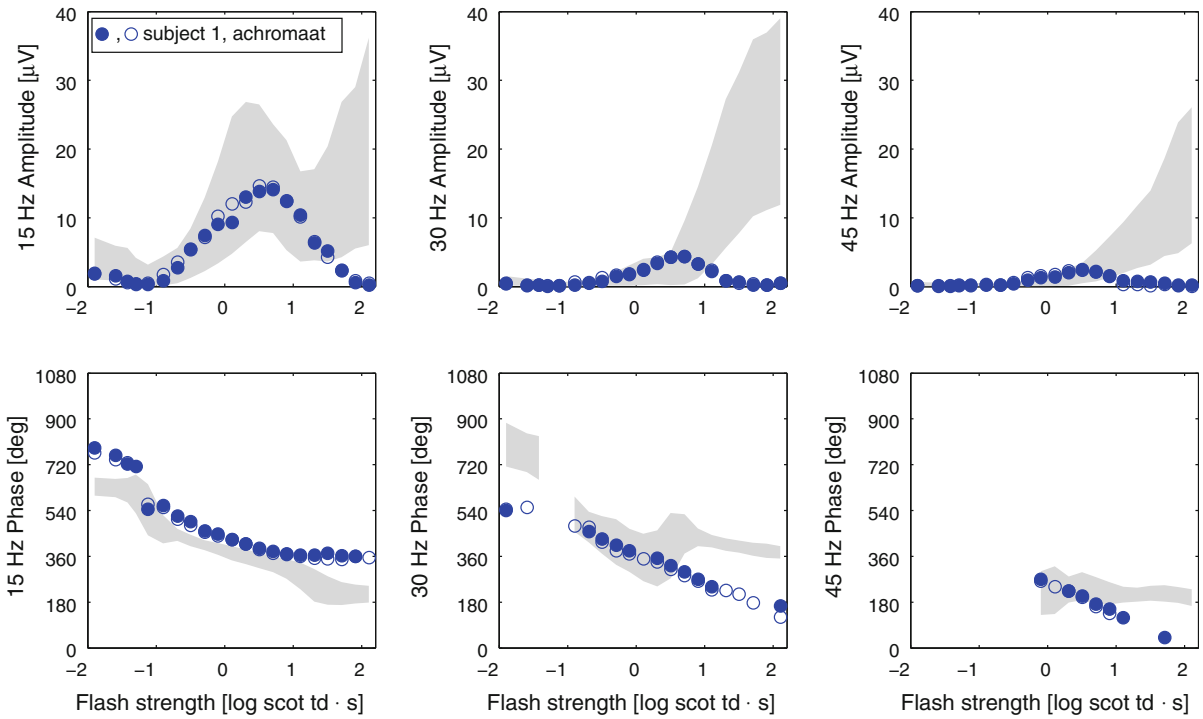

Fig. 7 The 15 Hz ERG responses of the right eye (*filled circles*) and left eye (*open circles*) of an achromat. The first minimum in the 15 Hz amplitude curve occurred at a flash strength of −1.1 log scot td·s in the right eye and −1.3 log scot td·s in the left eye, which were well within the normal range. These minima were accompanied by phase shifts. All amplitude curves showed a maximum but decreased to noise at higher flash strengths

distinguished, but not very clearly, in the 15 Hz amplitude curves at flash strengths where the second minima occurred in the normal subjects. These minima were accompanied by a phase shift corresponding to the phase data of the normal subjects. The 30 and 45 Hz amplitude curves were far below normal ranges and phase curves were abnormal.

Discussion

In our previous study [8], we argued that by means of an extended 15 Hz protocol, cone pathway contributions can be recognized by the occurrence of a second minimum in the 15 Hz amplitude curve and higher harmonics in the responses. In this study, we applied the extended 15 Hz protocol to 20 healthy subjects, in order to determine normal values. We found a high correlation in the amplitude of the 15 Hz components in the left and right eyes of each subject. However, we also found a fixed amplitude difference between the left and right eye responses; the ratio's varied from 0.67 to 1.34. We suggest that these differences are caused by differences in conductance of the DTL electrode and the eye system [27].

The 15 Hz amplitude curves belonging to the normal subjects all had the same overall shape and showed two minima and one maximum. However, there were interindividual differences: the flash strength at which the minima and maxima occurred, varied over a range of flash strengths of almost one log unit, and the amplitude data per flash strength showed broad 95% ranges. These differences could be caused by variations in retinal luminance (pupil size) or conductance (due to the media of the eye or the position of the DTL electrodes). However, correlation analysis showed that the interindividual differences could not be explained solely by overall shifts in flash strength or amplitude between subjects. We therefore suggest that interindividual variability in the density of rods, cones, (rod) bipolar cells, and gap junctions between rods and cones [28–30] also contribute to these differences.

The responses of all subjects to stimuli of high flash strengths contained strong higher-order harmonics as

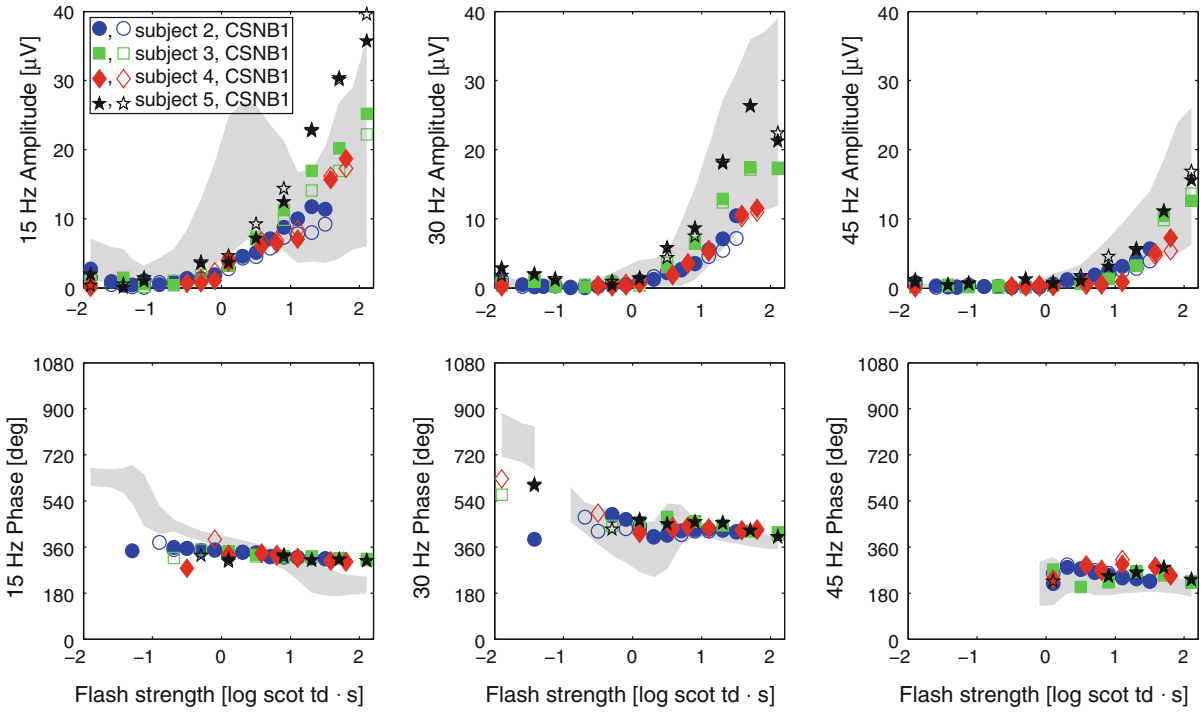

Fig. 8 The 15 Hz ERG responses of the right eye (*filled markers*) and left eye (*open markers*) of four CSNB1 patients. Only for flash strengths above approximately 0.1 log scot td·s could substantial responses be recorded. The amplitude curves showed no minima or maxima and the phase curves decreased monotonously

shown by the fast increase in the 30 and 45 Hz amplitude curves. In the previous study [8], we argued that these higher-order harmonics must originate from the cone pathway since cones are sensitive to high flicker frequencies while rods are not [31]. In this study, we found a high correlation between the amplitudes of the 15, 30, and 45 Hz components of the responses to stimuli of high flash strengths. This confirms that these higher harmonics are generated by one system only, i.e., the cone pathway.

In our data set, the first minimum occurred at flash strengths between −1.4 and −0.9 log scot td·s (the median at −1.1 log scot td·s). The normal study of Scholl et al. [10] (22 healthy subjects) found a minimum at a mean flash strength of −1.64 log scot td·s with a standard deviation of 0.17 log scot td·s. Thus, they also found a broad normal range for the position of the first minimum, but their mean value does not correspond to ours. Also in other studies, the minimum in 15 Hz responses of healthy subjects occurred at variable flash strengths [9, 11–13]. These differences may be caused by variations in calibration.

In this study, we recorded 15 Hz responses in an achromat. The ERG responses showed completely normal primary and secondary rod pathway function: at low flash strengths, the 15 Hz components showed a minimum in amplitude and a phase shift of approximately 170°. In contrast, the lack of increase in the 15, 30, and 45 Hz components in the responses higher flash strengths and the uniform phases showed that cone pathway contributions were absent. Interestingly, the data clearly showed a decrease in the responses of the secondary rod pathway at high flash strengths, indicating saturation. In our previous study [8], we discussed the existence of minima in the 30 and 45 Hz amplitude curves. We hypothesized that these minima were caused by interactions between nonlinear rod responses and linear cone responses. The data of the achromat provided evidence for this hypothesis by showing that 30 and 45 Hz components can originate from rods. The data also showed that a decrease in amplitude can be caused by saturation and does not need to involve interaction with another pathway.

In the achromat, the first 15 Hz minimum occurred at normal flash strengths. This is in contrast to the

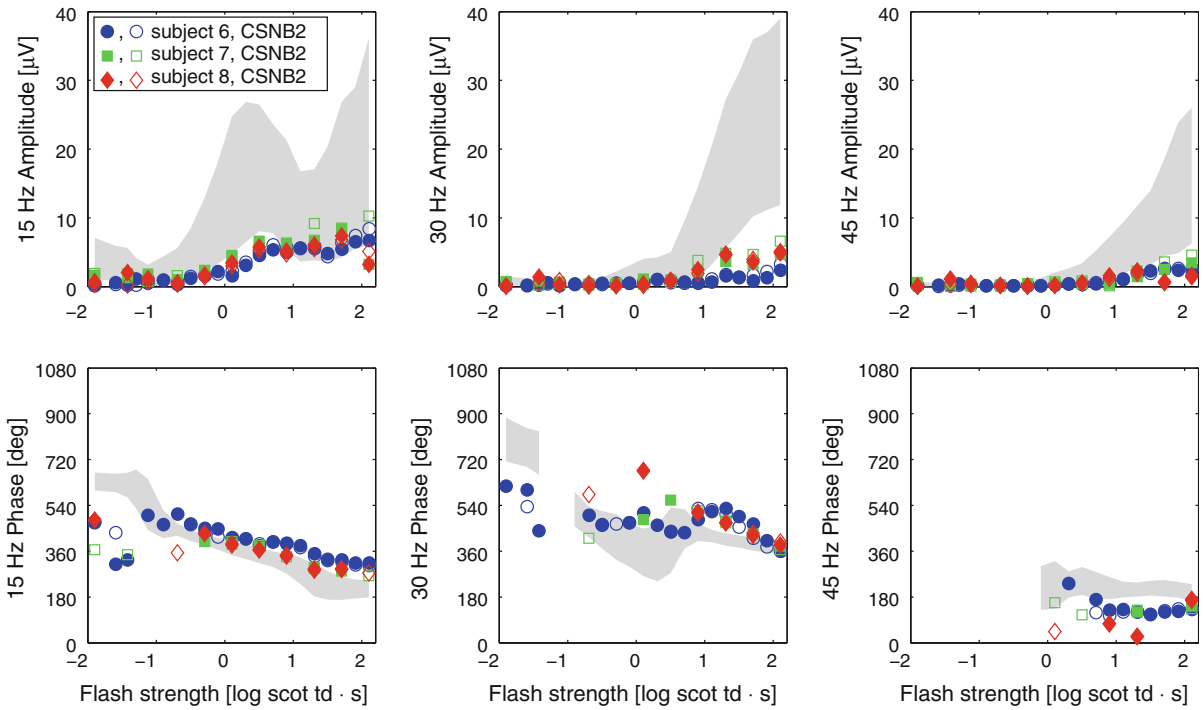

Fig. 9 The 15 Hz ERG responses of the right eye (*filled markers*) and left eye (*open markers*) of three CSNB2 patients. Only for flash strengths above approximately −0.9 log scot td·s could substantial responses be recorded. At higher flash strengths, the 15 Hz amplitude curves showed a maximum and a minimum, but not very clearly. However, the minimum was accompanied by a phase shift

conclusion drawn by Rüther et al. [9]. They found that the minimum in the achromat occurred at a flash strength of 0.4 log units above that of a normal subject. However, considering that normal ranges cover one log unit of flash strengths (found in this study and by Scholl et al. [10]), this difference may not be significant. Therefore, we argue that there is no difference between the 15 Hz ERGs of an achromat and normal subjects for low flash strengths and that the primary and secondary rod pathways function normally. In the literature, the functioning of the rods in achromatic patients is generally described as normal [32–36], although recently, abnormalities in rod driven ERGs have also been reported [37, 38]. Therefore, it would be interesting to measure 15 Hz ERGs in more patients with achromatopsia.

We also measured 15 Hz responses in four CSNB1 patients. From genetic studies, it is known that the gene defects in CSNB1 lead to absent ON bipolar function [14, 16–19]. Thus, the 15 Hz responses can originate from either the secondary rod pathway using cone OFF bipolar cells or the cone pathway using cone OFF bipolar cells. Previous studies [13, 14] suggested that the 15 Hz flicker responses to flash strengths just above that of the first minimum may originate from the secondary rod pathway. In our study, the amplitude curves showed no minima and the phase curves showed no shifts. This indicates that there was no transition from the secondary rod pathway to the cone pathway. Furthermore, the 15, 30, and 45 Hz amplitude curves increased linearly, which indicates that these responses originate from the cone pathway only. Nevertheless, responses could be recorded for flash strengths just above that of the minimum. This may indicate that, in the absence of rod pathway activity, cones respond to stimuli of lower flash strengths compared to normal subjects. In the literature, it has been described that rods can suppress cone flicker sensitivity [39–42]. The 15 Hz phase of the responses to stimuli of higher flash strengths differed from those of normal subjects. The phases may deviate because the cone signals can only travel via cone OFF bipolar cells.

In our previous study [8], we investigated the origin of 15 Hz responses. To explain our data, we developed a model that described the contribution of the different

pathways. The amplitude curves of rod pathway responses were described by Gaussian functions and the cone pathway responses by an exponential curve (for the range of flash strengths used in this study). The phase curves of the responses of all pathways were described to decrease linearly with the flash strength. The vector sum of the different pathway responses was effective in describing the data of the normal subjects. The data of the achromat supported our assumption on the contribution of the rod pathways. Similarly, the data of the CSNB1 patients provided evidence for our description of the cone pathway contributions.

The data of the CSNB2 patients did not differ greatly from the data of the CSNB1 patients. As in the CSNB1 patients, no 15 Hz responses could be distinguished for flash strengths below that of the first minimum. However, at higher flash strengths, amplitudes were just within the 95% range and phases were normal. Furthermore, the 15 Hz components showed a second minimum in the amplitude curve and a shift in the 15 Hz phase curve indicating a transition from the secondary rod pathway to the cone pathway. In contrast to the data of the CSNB1 patients, the 30 and 45 Hz components increased only slightly in amplitude at high flash strengths. This indicates that in CSNB2 patients, the secondary rod pathway and the cone pathway are both present but functionally reduced. This may also be true for the primary rod pathway. However, because of the very low amplitudes of the primary rod pathway responses, reduced functioning may result in responses that are indistinguishable from noise. Genetic studies have shown that the gene defects in CSNB2 lead to the malfunctioning of calcium channels in the photoreceptor synaptic terminal of both rods and cones [19, 43] which may explain the reduced functioning of all pathways.

In conclusion, we used a 15 Hz ERG protocol in this study to determine the functioning of the primary and secondary rod pathways in patients with retinal disease. Compared to earlier studies, we extended the protocol with stimuli of higher flash strengths. We analyzed not only the 15 Hz components in the responses, but also the 30 and 45 Hz components. We showed that by this method, secondary rod pathway responses can be distinguished from those of the cone pathway. This contributes greatly to the clinical application of the 15 Hz protocol. The findings made in the case of an achromat confirmed our suggestions concerning cone pathway contributions. The value of extending the protocol was shown by the findings made in the CSNB1 and CSNB2 patients. We recorded responses of low amplitude in all patients for flash strengths above where normally the first minimum occurs. From the analyses of the higher-order components, we concluded that, in the CSNB1 patients, the responses originate from the cone pathway, while in the CSNB2 patients, similar responses to the same stimuli originate from the secondary rod pathway.

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Conflict of interest None.

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