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High-frequency characteristics of L- and M-cone driven electroretinograms

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ABSTRACT

Electroretinograms (ERGs) elicited by high temporal frequency (26–95 Hz) L- and M-cone isolating sine-wave stimuli were investigated in human observers for full-field (FF) and different spatially restricted stimulus sizes (70°, 50°, 30°, and 10° diameter). Responses to L- and M-cone isolating FF stimuli were maximal around 48 Hz and decreased gradually with increasing temporal frequency up to 95 Hz. The response maximum was shifted to about 30–32 Hz for both L- and M-cone driven responses obtained with spatially restricted stimuli. The M-cone driven responses could only be measured up to 54 Hz with 70° stimuli. The response amplitudes for L- and M-cones and L-/M-cone amplitude ratios decreased with decreasing stimulus size. The ERG response phases to L- and M-cone isolating stimuli decreased with increasing temporal frequency and were about −160° apart for all stimulus sizes up to 34 Hz. Further increase in the temporal frequency displayed a positive correlation between stimulus size and L-M phase difference. The ERG data indicate that the responses evoked by high temporal frequency cone isolating stimuli reflect two mechanisms, one that is more centrally located and displays a maximum at about 30–32 Hz and a peripheral mechanism that is sensitive to higher temporal modulations. We propose that the peripheral mechanism (FF ERGs) reflects magnocellular activity, whereas the central mechanism (ERGs with spatially restricted stimuli) is based on a parvocellular activity up to about 30 Hz.

1. Introduction

Electroretinograms (ERGs) elicited by L- and M-cone isolating sine-wave stimuli generated using the silent substitution paradigm (Estevez & Spekreijse, 1974, 1982) provides an effective way to study the contribution of the L- and M-cone driven signals to the ERGs and their post-receptoral visual pathways (Kremers & Link, 2008; Kremers et al., 2000; Kremers, 2003). The silent substitution technique has an advantage over conventional chromatic adaptation techniques, where complete isolation of individual photoreceptor types cannot be achieved (Kremers, 2003; Stockman, MacLeod, & Johnson, 1993; Wald, 1964). Furthermore, adaptation changes the mode of operation of the retina prohibiting a direct comparison between the results obtained under different adaptation conditions.

Full-field (FF) ERGs elicited by cone isolating sine-wave stimuli at intermediate temporal frequencies (8–12 Hz) comprise characteristics which resemble those of post-receptoral cone opponent chromatic mechanism in healthy human observers (Barboni, Pangeni, Ventura, Horn, & Kremers, 2011; Kremers & Link, 2008; Kremers, Rodrigues, Silveira, & Filho, 2010; Parry et al., 2012). In this frequency range, L- and M-cone driven responses are nearly equal in magnitude resulting in L-/M-cone response amplitude ratios close to unity. With cone isolating high temporal frequency modulations (> 20 Hz), FF-ERGs show properties that reflect the characteristics of the post-receptoral luminance mechanism (Barboni et al., 2011; Kommanapalli, Murray, Kremers, Parry, & McKeefry, 2014; Kremers & Link, 2008; Kremers et al., 2010). At these frequencies, L-cone driven signals are consistently larger than M-cone driven signals resulting in L-/M-cone response amplitude ratios that can be as large as 10:1 in observers with normal L- and M-cone function (Kremers et al., 2000; Kremers, 2003). The L-/M-cone response amplitude ratios estimated using ERG data are similar to those obtained psychophysically (Kremers et al., 2000).

The primate visual system comprises two major psychophysical pathways for the detection of luminance and red-green chromatic modulations (Barboni et al., 2011; Kremers et al., 2010; Lee, Pokorny, Smith, Martin, & Valberg, 1990; Lee, Sun, & Zucchini, 2007). The physiologic basis of the psychophysical red-green chromatic pathway contains L-/M-cone-opponent retinal ganglion cells that transmit their signals to the parvocellular (PC) layers of the lateral geniculate nucleus (LGN) (Kremers & Link, 2008; Lee et al., 1990; Lee, 2011). The retinal pathway of the psychophysical luminance mechanism include non-opponent retinal ganglion cells which receive synergistic inputs from L-

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and M-cones and transmit their signals to the magnocellular (MC) layers of the LGN (Kremers & Link, 2008; Lee et al., 1990; Lee, 2011).

Recently, we found that the ERGs to L- and M-cone isolating sine-wave and sawteeth stimuli have distinct spatial properties when they reflect the activity of different post-receptoral mechanisms (Jacob et al., 2015; Tsai et al., 2016). Responses to high temporal frequencies (> 20 Hz; reflecting magnocellular activity), the ERG response amplitudes were positively correlated with stimulus size. At intermediate temporal frequencies (8–12 Hz; reflecting parvocellular activity), L- and M-cone driven ERG responses were relatively independent of the stimulus size (Jacob et al., 2015). The difference in spatial properties of the ERGs reflecting different post-receptoral mechanisms was confirmed using red-green heterochromatic stimuli that did not isolate one cone type (Martins et al., 2016).

ERGs elicited by L- and M-cone isolating incremental (ON) and decremental (OFF) sawteeth stimuli (Tsai et al., 2016) also showed cone opponency. The ERGs elicited by L-ON and M-OFF stimuli resembled each other. Similarly, the responses to L-OFF resembled those to M-ON stimuli. The opponent responses were particularly clear when spatially restricted stimuli were used.

The above-described data suggest that cone-opponent ERG signals are mainly elicited by the central retina, whereas luminance reflecting ERGs are larger in the peripheral retina.

At high temporal frequencies (30 Hz and higher), we previously found that the L/M ratios depend on temporal frequency and that there is an interaction with spatial stimulus configuration, indicating that at these temporal frequencies the post-receptoral mechanisms change or that they alter their properties. In the present study, we therefore investigated the post-receptoral processes that influence ERGs at frequencies of 26 Hz and higher to full-field and spatially restricted stimuli. A critical experiment to investigate this question may be to compare the responses to high frequency modulations using full-field stimuli and stimuli that only stimulate the central retina. ERGs evoked by L- and M-cone isolating high temporal frequency (between 26 and 95 Hz) stimuli were explored for 5 different stimulus configurations. The data show that the full-field ERGs reflect magnocellular activity for the entire frequency range employed in the present study, whereas the frequency range at which the ERGs reflect PC activity can be extended to about 30 Hz with the spatially restricted stimuli.

2. Methods

2.1. Subjects

In the present study, ERGs were recorded from seven healthy human observers aged between 29 and 58 years. Two experimental procedures were employed (see the section: 2.3 Visual Stimulation). Experiments 1 and 2 were conducted with four and three subjects respectively. Of the seven participants, one subject participated in both experiments. All subjects underwent a complete ophthalmological investigation to exclude the presence of any ocular abnormalities. All participants had normal color vision as established with the anomaloscope (Oculus Optikgerate GmbH, Wetzlar, Germany). A written consent was obtained from all participants prior to the experiments, which followed the tenets of the Declaration of Helsinki. The protocols were approved by the institutional ethical committee (Medical Faculty of the University of Erlangen-Nuremberg, Germany).

2.2. ERG recordings

Monocular ERGs were recorded from a randomly chosen eye of each subject using the RETIport system (Roland Consult, Germany). The pupil of the chosen eye was dilated with a drop of 0.5% tropicamide (Pharma Stulln GmbH, Stulln, Germany). A fibre electrode (Dawson, Trick, & Litzkow, 1979) placed over the lower conjunctiva and attached close to the inner and outer cantus, served as the active electrode. Two gold cup electrodes filled with an electrode paste (Ten20 conductive, D. O. Weaver & Co., Aurora, Colorado, USA) placed at the ipsilateral temple and on the forehead, after the skin was cleaned with Nuperp abrasive skin preparing gel (D. O. Weaver & Co.), served as reference and ground electrodes respectively. The impedance of the electrodes was maintained below 5 KΩ. Subjects were asked to fixate at the central red light emitting diode (LED) in the Ganzfeld stimulator (Q450SC, Roland Consult). The measured ERGs were amplified (10^6 times), bandpass filtered between 1 and 300 Hz, and sampled at 1024 Hz. For each stimulus condition, ERGs were averaged over 80–160 epochs each lasting one second. The signals to the initial two seconds of each trial were discarded to avoid onset artifacts.

2.3. Visual stimulation

The visual stimuli were generated using the RETIport system (Roland Consult, Germany) and presented using an integrating Ganzfeld bowl (Q450SC, Roland Consult). The Ganzfeld bowl was equipped with six different LED arrays (primaries) each with a different wavelength. The mean luminance, waveform, contrast, relative phase, and frequency of each LED array could be controlled independently with the RETIport software (Roland Consult, Germany). In the present study, four primaries (red, green, blue and amber) were used. The output of each LED array was sinusoidally modulated. The output peak wavelength ± half-bandwidth at half-height was 638 ± 9 nm for red, 523 ± 19 nm for green, 469 ± 11 nm for blue, and 594 ± 8 nm for amber respectively.

The isolation of responses of single photoreceptor types was achieved by using the triple silent substitution stimulation paradigm (Estevez & Spekreijse, 1974, 1982; Kremers, 2003). The silent substitution method allows controlling L-cone, M-cone, S-cone, and rod photoreceptor modulations independently. The individual photoreceptor types can be stimulated with known strength defined as the Michelson contrast (Donner & Rushton, 1959; Kremers, 2003; Shapiro, Pokorny, & Smith, 1996) around the mean excitation which results in the same mean luminance and chromaticity, and thus the identical states of adaptation for all stimulus conditions. For the current study, two sets of experiments were performed. In experiment 1, L- and M-cone modulation contrast was 10%. The mean chromaticity of the stimuli was white with CIE1931 coordinates: x = 0.3531, y = 0.3181. The individual luminances of the four primaries were 49 cd/m^2 for red, 67 cd/m^2 for amber, 124 cd/m^2 for green, and 44 cd/m^2 for blue. In order to achieve 10% M-cone modulation, it was required to allow −2% S-cone and 2% rod modulation contrast in the M-cone isolating stimuli (negative contrasts indicate a counterphase modulation). It has been shown in the previous study from our group that the ERG responses from S-cones and rods are significantly smaller than M-cone driven responses even if S-cone and rod modulation contrasts are higher than M-cone modulation contrast (Kremers & Pangeni, 2012). In experiment 2, the L- and M-cones modulation contrast was 18%. The individual luminances of the four primaries were 80, 160, 40, and 4 cd/m^2 for red, amber, green and blue respectively. Eccentricity dependent variations in preretinal filtering (e.g. owing to the macular pigment) and in photoreceptor absorption spectra may lead to residual modulation in the photoreceptor type that was meant to be silenced. We have previously calculated that the expected residual responses are probably small in comparison with those originating in the isolated cone type (Huchzermeyer & Kremers, 2017). The mean chromaticity of the stimuli was reddish with CIE1931 coordinates: x = 0.5951, y = 0.3857. The mean luminance in the two experiments was 284 cd/m^2 i.e. 14,000 photopic Td retinal illuminance, assuming an 8 mm pupil diameter.

In experiment 1, the ERG responses elicited by the L- and M-cone isolating sine-wave stimuli were recorded for five different spatial stimulus configurations (FF and 70°, 50°, 30°, and 10° diameter stimuli) each at 12 different temporal frequencies (26–48 Hz in the step of 2 Hz). The spatial stimulus configurations were created by using black cardboard field stops that were positioned at 3 cm distance from the subject's eye (Aher et al., 2018; Jacob et al., 2015). In experiment 2, the L-cone and M-cone driven ERG responses were measured only for the FF and the 70° stimulus size. The frequency range was extended: 30 different temporal frequencies between 26 Hz and 95 Hz were employed (from 26 Hz to 80 Hz in steps of 2 Hz, except 50 Hz, and from 80 Hz to 95 Hz in steps of 5 Hz). The experiments were performed in a darkroom. To avoid fatigue, the experiments were carried out in several recording sessions of maximally 1.5 h. Although the stimuli were no silent substitution conditions for melanopsin containing internally responsive retinal ganglion cells, their responses can most probably be neglected because of the high temporal frequencies of the stimuli.

2.4. Data analysis

The ERG responses were Fourier analyzed with a self-written MATLAB (Mathworks, Massachusetts, USA) program. The first harmonic (fundamental) components (f Hz) were defined as the responses and their amplitudes and phases were obtained. The noise level was estimated by averaging the response amplitudes at $f - 1$ Hz and $f + 1$ Hz (e.g. for 30 Hz, the average of response amplitudes at 29 Hz and 31 Hz were estimated as the noise) (Meigen & Bach, 1999). The response phases were discarded when the signal (Amp (f)) to noise ((Amp $(f - 1)$ + Amp $(f + 1)$)/2) ratio (SNRs) was below two. Furthermore, L-/M-cone driven response amplitude ratios were discarded if the SNR of one of the two was smaller than two. Meigen and Bach (1999) actually recommended a ratio of 2.82, but we noticed that response phases were generally in the expected range with SNRs above two, indicating that the ERG signals contained substantial responses when the SNRs were between 2 and 2.82.

The Fourier analysis returns the phases between −180° and 180°. The absolute phase can differ by multiples of 360°. To estimate correct phases of the responses at a particular temporal frequency, it was assumed that the phases obtained from each subject did not differ more than 180°. In addition, the phases were assumed to decrease with increasing temporal frequency of the stimulation.

3. Results

3.1. Experiment 1

In experiment 1, the ERG responses to temporal frequencies between 26 and 48 Hz were measured for different stimulus sizes. Fig. 1 displays the averaged L- and M-cone driven ERG response amplitudes (panels A and B respectively) and L-/M-cone response amplitude ratios (panel C) plotted as a function of temporal frequency. With FF stimuli, the L- and M-cone driven ERG response amplitudes increased for the whole frequency range employed in this experiment with consistently larger L-cone driven than M-cone driven responses and thus with L/M amplitude ratios that were consistently larger than unity. With smaller stimuli, the L- and M-cone driven ERG response amplitudes were maximal at about 32 Hz. The response amplitudes of L- and M-cone driven signals decreased when stimulus size decreased and the responses were not or barely measurable with 10° stimuli.

The L/M amplitude ratios obtained with the FF stimuli was around 3. Although L/M amplitude ratios were relatively variable, they clearly decreased as the stimulus size decreased. These findings are in agreement with previously published data from our group where L/M amplitude ratios at high temporal frequencies (> 30 Hz) were reported to be larger for FF stimuli than for spatially restricted stimuli (Jacob et al., 2015). A larger L/M ratio at FF was also found for some components in the responses to sawtooth stimuli (Tsai et al., 2016).

In Fig. 2, the L-cone (panel-A) and M-cone (panel-B) driven ERG response phases and the L-M phase difference (panel-C) are plotted as a function of temporal frequency for different stimulus sizes. The L- and M-cone driven ERG response phases were relatively advanced for the FF stimuli as compared to the spatially restricted stimuli. Between 34 and 38 Hz, the phase decrease was less strong than at other frequencies. The phase difference between L- and M-cone driven ERGs elicited by spatially restricted stimuli increased for frequencies above 34 Hz. The phase difference for FF stimuli was relatively constant. This is indicative for two response mechanisms may be involved: one that dominates below 34 Hz and in FF conditions; the other that can mainly be observed with spatially restricted stimuli and with temporal frequencies of 34 Hz and higher.

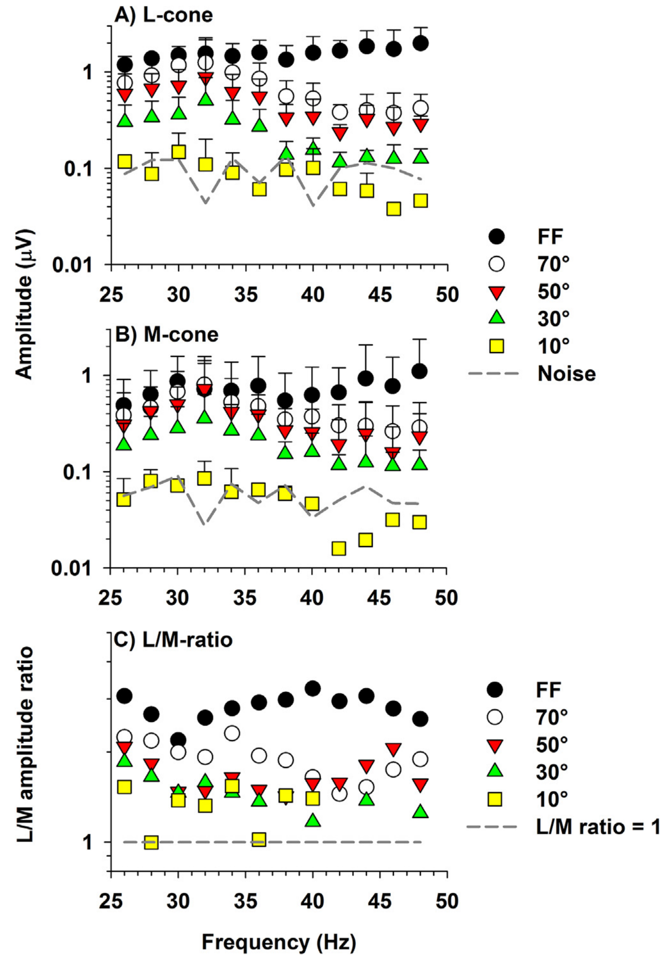

Fig. 1. Averaged response amplitudes (+1 s.d.) for L-cone (panel-A) and M-cone (panel-B) driven ERGs obtained in experiment 1 plotted as a function of temporal frequency for different stimulus sizes. The dashed dark-gray lines in the amplitude plots indicate mean noise. The L/M amplitude ratios are given in the panel-C. The dashed-dark-gray line indicates unity. The response amplitudes and L/M amplitude ratios are plotted in a logarithmic scale.

The data of experiment 1 indicate that FF and spatially restricted stimuli elicited ERGs with different properties. Responses to spatially restricted stimuli showed similar characteristics independently of stimulus size. To study the response characteristics for FF and spatially restricted stimuli in more detail, we performed a second experiment where only two stimulus sizes (FF and 70° diameter, at which the response amplitudes and thus the SNR was maximal) were used but in which the frequency range was increased to 26–95 Hz.

3.2. Experiment 2

In Fig. 3 (panels A and B), the L- and M-cone driven ERG response amplitudes obtained in experiment 2 with the FF and 70° diameter stimuli are plotted as a function of temporal frequency. In order to obtain the ERG responses at a wide range of frequencies, the cone

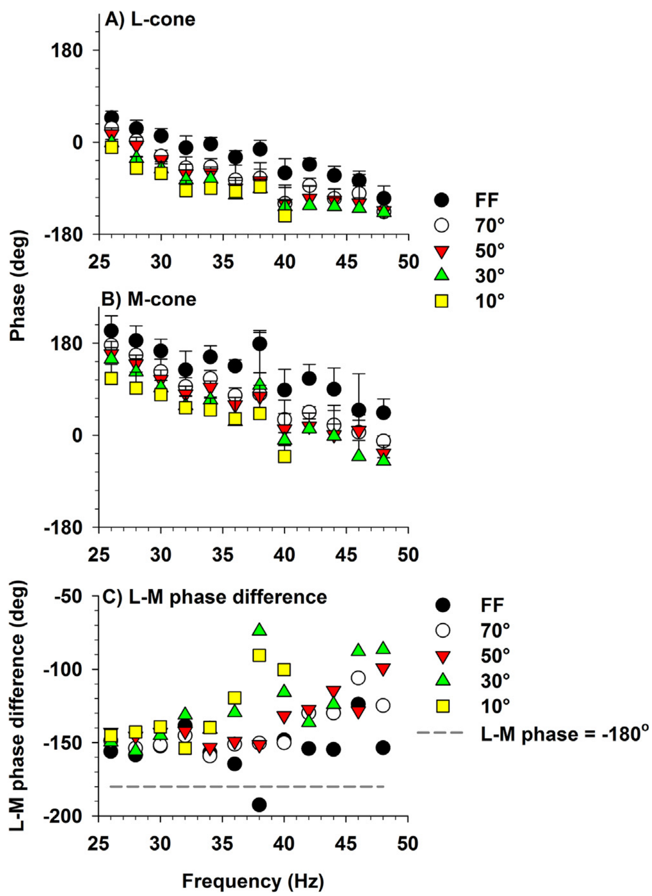

Fig. 2. Averaged response phases (+1 s.d.) for L-cone (panel-A) and M-cone (panel-B) driven ERG responses plotted as a function of temporal frequency for five different stimulus configurations. Panel-C shows the averaged L-M phase difference. The dashed dark-gray line in panel-C represents the L-M phase difference corresponding to −180°.

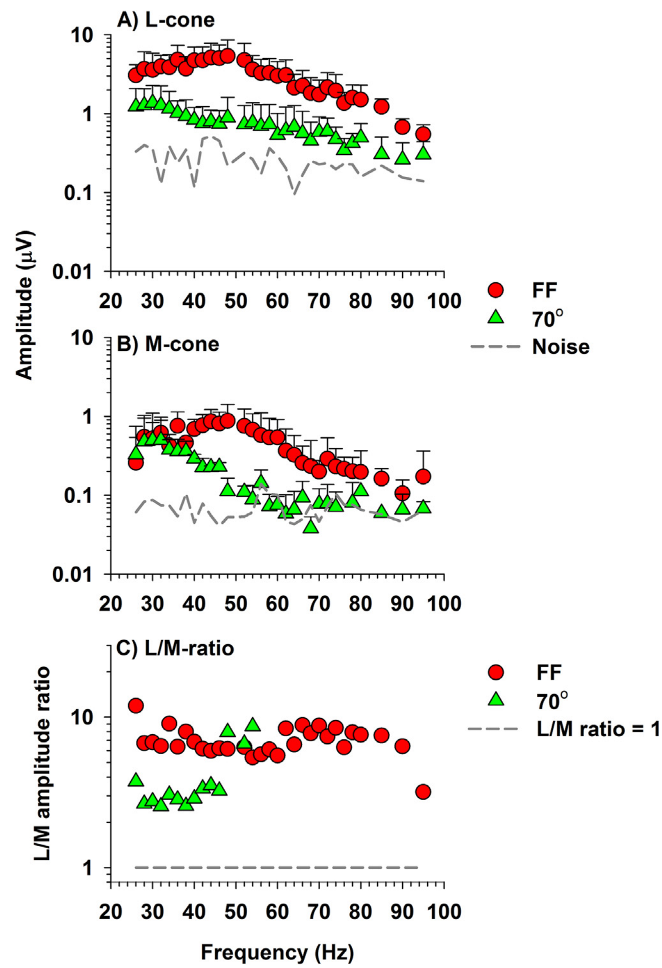

Fig. 3. Averaged response amplitudes (+1 s.d.) for L-cone (panel-A) and M-cone (panel-B) driven ERGs recorded in experiment 2 as a function of temporal frequency for FF (red-circles) and 70° (green-triangles) stimuli. The dashed dark gray lines in the panels A and B indicate mean noise. Panel-C displays the L/M amplitude ratios plotted as a function of temporal frequency. The dashed dark gray line indicates unity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contrast was increased to 18%. To be able to obtain this contrast, the mean chromaticity of the stimuli was changed as described in the Methods section. The L-cone driven ERG responses obtained in experiment 2 with FF stimuli were 2–3 times larger than those obtained in experiment 1 (cf. Figs. 1A and 3A). The FF M-cone driven responses were only slightly larger (cf. Figs. 1B and 3B) resulting in larger L/M amplitude ratios (cf. Figs. 1C and 3C). This may be caused by the different mean chromaticity used in experiment 2. With the FF stimuli, the L- and M-cone driven ERG responses were measurable up to 95 Hz with SNRs of about 4.9 and 2.4 respectively. In agreement with the results obtained in experiment 1, the L- and M-cone driven ERG response amplitudes were maximal at a higher frequency for FF compared to 70° stimuli. The FF responses reached a maximum at 48 Hz and further decreased monotonically up to 95 Hz. The M-cone driven response amplitudes were above the noise level up to 95 Hz.

The L- and M-cone driven response amplitudes with 70° diameter stimuli were maximal at about 30 Hz. The L- and M-cone driven responses were also up to a factor of 10 smaller than with FF stimuli and the M-cone driven ERGs were measurable only up to 54 Hz. Fig. 3 (panel-C) displays the L/M amplitude ratios for the FF and 70° diameter stimuli as a function of temporal frequency. For the FF stimuli, the ratios were between 3 and 10 and are in good agreement with ratios reported previously (Kommanapalli et al., 2014). Above 85 Hz, the L/M amplitude ratio seemingly decreased from about 9 to approximately 3 at 95 Hz. This may indicate that at these frequencies noise contributed substantially to the responses (i.e. the SNRs were considerably lower than at the other frequencies). Again in agreement with the results of experiment 1 the L/M ratios were smaller for the 70° stimuli.

In Fig. 4, the averaged L- (panel-A) and M-cone (panel-B) driven ERG response phases for the FF and the 70° diameter stimuli are plotted as a function of temporal frequency. The L- and M-cone driven response phase vs. frequency profiles for the FF stimuli were similar as reported previously (Aher et al., 2018; Kremers & Pangeni, 2012). The response phases for the L- and M-cone driven ERGs decreased monotonously with increasing temporal frequency for both FF and 70° diameter stimuli. Up to 48 Hz and in agreement with the results of experiment 1, the L- and M-cone response phases to FF stimuli were relatively phase advanced as compared to those obtained with the 70° stimuli. Interestingly, above 48 Hz, the L-cone driven response phases with the 70° diameter stimuli were phase advanced as compared to those with the FF stimuli. The L-M phase differences (Fig. 4; panel-C) for the FF and 70° diameter stimuli were close to −160° around 30 Hz and monotonously increased with an increasing temporal frequency. The increase was stronger for responses to 70° diameter stimuli. Above 36 Hz, the L-M phase difference with the 70° diameter stimuli was larger than those obtained with FF stimuli.

We also performed a control experiment with an annular stimulus in which the central 70° was blocked by black cardboard (data not shown). The ERG response amplitudes and phases were similar to those obtained with FF stimuli. This indicates that the responses to FF stimuli

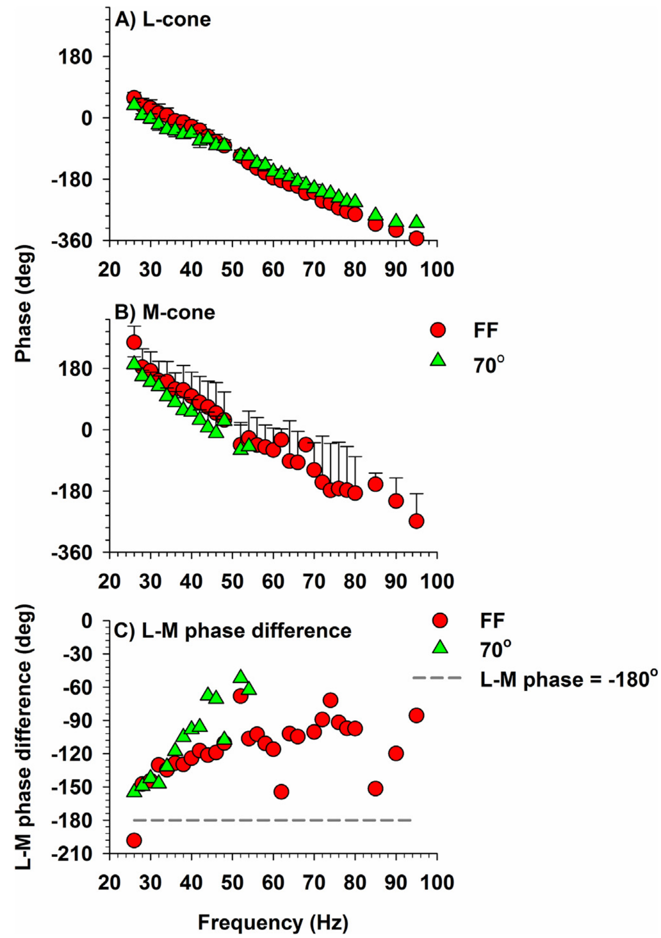

Fig. 4. Averaged response phases (+1 s.d.) for L-cone (panel-A; s.d.'s were of the size of the symbols) and M-cone (panel-B) driven ERGs recorded in experiment 2 as a function of temporal frequency for FF (red-circles) and 70° (green-triangles) stimuli. Panel-C shows the L-M phase difference. The dashed dark-gray line indicates the L-M phase difference of −180°. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are dominated by responses of the peripheral retina.

As stated earlier (Kommanapalli et al., 2014), the phase characteristics can be a sensitive indicator of changes in mechanisms underlying the ERG responses. We, therefore, analyzed the slopes of the phase vs. frequency plots by performing a regression analysis on a set of the five adjacent data points. The choice of including five data points in the local regression was a compromise between smoothing the slope data (for which large local window sizes are necessary) and the requirements of being able to capture physiologically determined slope changes and the inclusion of as many slope data as possible (for which small window sizes are optimal). The slope of the linear regression approximated the slope of the phase vs. frequency function at the median of the five frequencies. The linear regressions were performed for median frequencies between 30 and 76 Hz. Only regressions were included when the SNRs at all frequencies were larger than 2. Fig. 5 shows the slopes for the L- (left-panels) and M-cone (right-panels) driven response phases recorded in experiment 1 (top panels) and experiment 2 (bottom panels). The slope analysis was performed for all stimulus sizes used in experiment 1, but for the sake of clarity, only the FF and 70° stimulus size data are shown in Fig. 5 (top-panel). The slopes estimated for the recordings with the 50°, 30° and 10° stimulus sizes in experiment 1 exhibited a similar trend as those obtained with the 70° stimulus size. The data are clearest for L-cone driven response probably because of the larger responses and because slopes were available for a larger range of frequencies. The data indicate the presence of two transitions in underlying mechanisms: one transition occurs between 30 and 40 Hz and can mainly be discerned with smaller stimuli. The second transition occurs between 55 and 65 Hz and with FF stimuli. Interestingly, between 40 and 60 Hz (Fig. 5b), the slopes for L- and M-cone driven responses were significantly smaller for the 70° diameter stimuli than for the FF stimuli (paired *t-test*, $p < 0.001$). Above 60 Hz, the slopes for the L-cone driven response phases obtained with the FF and 70° diameter stimuli were similar and did not vary strongly with temporal frequency.

4. Discussion

In this study, we have examined the spatial properties of ERGs elicited by high temporal frequency L- and M-cone isolating sine wave stimuli in healthy human observers. The data indicate that the responses elicited by FF stimuli are fundamentally different from those that stimulate the central retina up to an eccentricity of at least 35° (i.e. elicited by stimuli with 70° diameter and smaller) because distinct dependencies of L- and M-cone driven ERG responses as a function of temporal frequencies were observed. They were maximal at around 48 Hz with FF and about 32 Hz with smaller stimuli. The observed maximum with FF stimuli is in good agreement with ERG data reported previously (Kommanapalli et al., 2014). We, therefore, propose that ERGs driven by L- and M-cone isolating high temporal frequency stimuli with different stimulus sizes have two different response mechanisms. One which is more centrally located on the retina and exhibits response maximum at 32 Hz and another one which is located at retinal periphery which is more sensitive to high temporal frequency stimulations.

4.1. FF mechanism

L-cone driven responses were measurable up to 95 Hz with good signal to noise ratio with FF stimuli. This finding is in agreement with previously reported data (Kommanapalli et al., 2014). Furthermore, in agreement with previous data (Aher et al., 2018; Jacob et al., 2015) the FF L- and M-cone driven ERG response amplitudes were positively correlated with the stimulus size for the frequency range used in the present study. The FF responses were maximal at about 48 Hz. Furthermore, the L/M ratios were larger for FF stimuli (between 3 and 10) than for smaller stimuli, indicating that the retinal periphery is strongly L-cone dominated. The phase difference between L- and M-cone driven ERG responses to FF stimuli were about −160° at about 30 Hz and increased gradually with increasing temporal frequency. Moreover, the slopes of the phase plots changed between 55 and 65 Hz indicating a change in the mechanism underlying the responses.

We propose that L- and M-cone driven ERGs to FF stimuli at the employed high frequencies reflect the activity of magnocellular (MC) pathway. It has been frequently demonstrated that the L- and M-cone driven ERG responses to high temporal modulations (> 25 Hz) contribute to the post-receptoral luminance sensitive MC pathway (Challa et al., 2010; Kommanapalli et al., 2014; Kremers & Link, 2008). The L/M ERG amplitude ratios that are consistently larger than unity strengthened this notion because human observers display similar L/M ratios in psychophysical luminance sensitivity measurements (Kremers et al., 2000). Neurons of the MC pathway are thought to mediate psychophysical luminance detection (Kremers et al., 2000; Lee et al., 1990). The L/M ERG amplitude ratios obtained psychophysically for the luminance pathway correlate well with the L/M amplitude ratios reported in the present study with FF stimuli (Kremers et al., 2000; Kremers, 2003).

MC Ganglion cells respond maximally at around 50 Hz to luminance modulation (Lee et al., 1990), similar to those obtained with the FF ERG response maxima found in the present study. However, Solomon, Martin, White, Ruttiger, and Lee (2002) demonstrated that PC ganglion cells show a maximum at similar luminance frequencies. Therefore, our

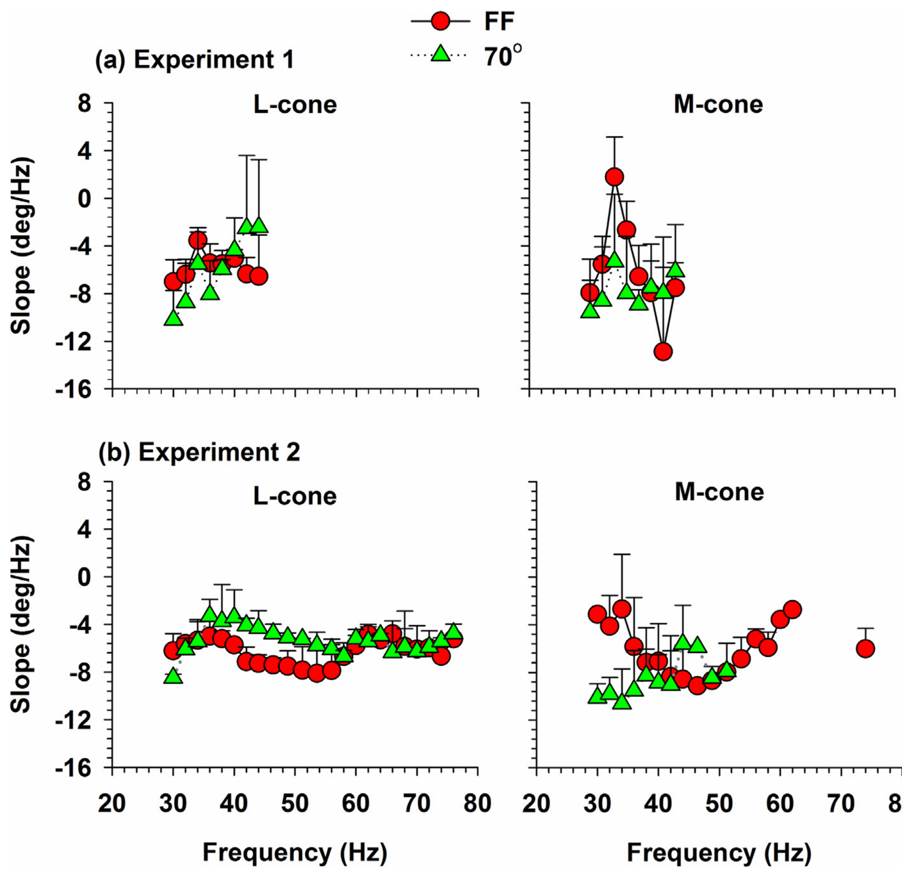

Fig. 5. Average slopes (+1 s.d.) of L-cone (left panels) and M-cone (right panels) driven ERG response phases recorded in experiment 1 (top row) and experiment 2 (bottom row) as a function of temporal frequency. Each data point on the graph represents the local slope of a linear regression on five data points.

hypothesis that high frequency L- and M-cone driven ERGs with the FF stimuli reflect the activity of the MC pathway is mainly based on the large L/M amplitude ratios obtained.

4.2. Central mechanism

The response amplitudes to spatially restricted stimuli were maximal at about 32 Hz (Figs. 1 and 3) and the L/M ERG amplitude ratios were smaller than for FF stimuli (except at frequencies above 40 Hz; Figs. 1C and 3C). These data indicate that the responses with spatially restricted stimuli are mediated by a different mechanism. However, there are several indications of an additional transition in the mechanism underlying the small field responses at frequencies between 30 and 40 Hz. These indications include a strong change in the phase slopes (Fig. 5B), a strong increase in the L-M response phase difference (Fig. 4C) and larger L/M ratios at frequencies above 40 Hz (resembling those obtained with FF stimuli; Fig. 3C). We propose a transition from PC-reflecting responses (L/M ratios close to unity; L-M phase differences close to 180° indicating cone opponency) at frequencies below 30 Hz to MC-reflecting ERGs (L/M ratios substantially larger than unity and resembling those of FF recordings; L-M phase differences that indicate considerably weaker cone opponency) at frequencies above 40 Hz.

We previously have found evidence that L- and M-cone driven ERGs to frequencies between 8 and 12 Hz reflect properties of the L/M-cone opponent PC pathway (Jacob et al., 2015; Kremers & Link, 2008; Kremers et al., 2000; Parry et al., 2012). These responses are also relatively strong with spatially restricted stimuli and display L/M amplitude ratios that are close to unity (Jacob et al., 2015). The present data indicate that the frequency range at which the ERGs reflect PC activity can be extended to about 30 Hz with spatially restricted stimuli. In previous experiments (Jacob et al., 2015; Martins et al., 2016) we found that PC-reflecting ERG amplitudes do not change with stimulus size down to 10° in diameter, whereas MC-reflecting ERGs decrease in amplitude with a decrease in stimulus size. This indicates that PC-reflecting ERGs are more centrally located in comparison with MC-reflecting ERGs. This might explain the extended range of PC-activity found in the present study.

We like to emphasize that the results of the present study on themselves only present weak support for our idea of ERGs that reflect activity of different post-receptoral retinal pathways and only make sense when regarded in the light of previous data. However, alternative explanations of the data should be considered. One alternative explanation for the optimal frequency with FF and central stimulus is that central and peripheral ganglion cells belonging to the same retinal pathway have different optimal frequencies (Solomon et al., 2002). Another possibility is that peripheral cone photoreceptors may respond to higher temporal frequencies than central cones (Tyler, 1985). These mechanisms may be a basis for the differences in optimal temporal frequency but they neither explain the changes in L/M ratios nor the phase shifts in the ERGs without additional assumptions.

The ERG responses to temporally modulating stimuli mainly arise in activity of the bipolar cells (Bush & Sieving, 1996). The proposal of MC- and PC-cell reflecting ERG responses implies that midget and diffuse bipolar cells have similar physiological properties as the ganglion cells belonging to the respective post-receptoral pathways. There is limited evidence for this from direct measurements. Bipolar cells have receptive fields with antagonistic (center-surround) inputs (Dacey et al., 2000). Furthermore, the centers of foveal midget bipolar cells receive input from a single cone (Dacey & Packer, 2003; Martin, 1998). The appearance of cone opponency at low temporal frequencies is thought to be a direct consequence of this (Wool et al., 2018) independent of whether the receptive field surrounds receive mixed cone input or have some degree of cone selectivity. The present data indicate that the latency difference between centers and surrounds, leading to the loss of opponency at higher temporal frequencies in retinal ganglion cells (Smith, Lee, Pokorny, Martin, & Valberg, 1992), is also present in midget bipolar cells. Our data also imply that midget and diffuse bipolar cells have similar temporal characteristics as the ganglion cells to which they are connected. Direct evidence for this is still lacking.

A further question is how ERG responses may be measured considering that they originate in the activity of On- and Off-center bipolar cells. The transmission from photoreceptors to depolarizing and hyperpolarizing bipolar cells involves different types of glutamate receptors and intracellular signal pathways (Peter, 2003). This may explain the presence of a luminance driven ERG signal (also in the conventional flash ERGs). For signals that are putatively derived from activity of midget bipolar cells an additional mechanism may be needed because in these cells there is an additional symmetry from depolarizing bipolar cells that receive L- and M-cone center input (and similarly for hyperpolarizing bipolar cells). A possible mechanism may be the different L- and M-cone packing densities. They may result in a similar density difference in the midget bipolar cells: a higher L-cone density may lead to a higher density of the midget bipolar cells with L-cone driven centers. This asymmetry may cause that the electrical combination of these cells can be registered at the ERG electrode. This assumption predicts that the cone opponent signal in the ERG is related to the L/M ratio. Future work will have to show if this prediction is true. We would like to emphasize that this is a possibly explanation for the presence (and magnitude)of a cone opponent ERG signal. The intrinsic L/M ratio and phase characteristics of this ERG signal would be similar to those of the midget bipolar cells.

5. Conclusion

The ERG responses elicited by cone isolating full-field high temporal frequency ($>$ 26 Hz) stimuli have fundamentally different characteristics than those originating in the central retina. Full-field ERGs probably reflect MC-activity. Spatially restricted stimuli elicit ERGs that reflect PC-activity up to about 30 Hz but are MC-pathway driven at frequencies 40 Hz and higher.

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Author contributions

Conceived and designed the experiments: AJA, JK. Performed the experiments: AJA, MMJ. Analyzed the data: AJA, JK. Wrote the paper: AJA, JK.

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