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Signal Pathways in the Electroretinogram

Jan Kremers
University of Erlangen-Nürnberg
Germany

1. Introduction

The electroretinogram (ERG) is an electrical potential of retinal origin elicited by a visual stimulus. The basis of the ERG is the same photochemical process that leads to the neural response of the retina and the visual system[1]: photon absorptions in the photoreceptoral opsin pigments[2]. In addition, it is a purely retinal response that reflects the retinal physiology. It is not surprising that the ERG received substantial attention because it is a potentially useful way to study the function of the retina and its disease-related changes. Indeed, it still is a very important tool in the clinic. In addition, there was hope that the ERG might be related to the visual responses of retinal neurones whose signals are transmitted to the brain for visual perception and motor responses. However, even though the ERG is known for nearly 150 years now, the similarities with visually relevant neuronal signals and with visual perception were disappointingly small. In the 1970s, Armington (1974) wrote in his book "The Electroretinogram" when discussing the link between ERGs and psychophysics (chapter 7): "The electroretinogram is unique because its components allow the experimenter to follow several separate retinal activities, while recording is performed with a minimum of discomfort to the subject. Furthermore, the subject may make verbal reports or judgements regarding the same stimulus, which was used to elicit the electroretinogram. It is thus possible to relate the visual appearance of a stimulus to the underlying physiological processes. The full potential for doing this in a sophisticated manner, however, has not been yet realized." As a result, ERGs were mainly used in studies that had a clinical interest. It was treated as an epiphenomenon of the responses of the visual pathways without showing a direct relationship with them. In this chapter, I will argue that under well chosen circumstances, which can be created owing to modern stimulus and recording techniques, a correlation with the physiological properties of the major pathways projecting to the brain and the related visual performances can be obtained.

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[1] in this chapter I will mainly confine myself to the situation in mammals and more specifically primates, but the basic mechanism is probably similar in all vertebrates; in invertebrates the differences are possibly larger.

[2] I neglect for the time being that light absorption in the melanopsin containing retinal ganglion cells may also elicit a detectable ERG signal see: FUKUDA, Y., TSUJIMURA, S., HIGUCHI, S., YASUKOUCHI, A. & MORITA, T. (2010): The ERG responses to light stimuli of melanopsin-expressing retinal ganglion cells that are independent of rods and cones. *Neurosci Lett* 479**,** 282-286..

2. Historical overview of the ERG and stimulus types

The electroretinogram was discovered twice independently by Holmgren and by Dewar and McKendrick around 1870 (for a historical overview see de Rouck (2006)). The stimuli that initially were most often used, were high intensity flashes, probably because they were relatively easy to deliver with the early techniques and a single stimulus elicited a response that was large enough to be measured. Improvements in the electrodes and amplifiers and the advent of averaging techniques enabled recording of smaller response components. At a later stage, other stimulus techniques (such as trains of flashes to elicit a flicker ERG or long flash ERGs, pattern ERGs etc) became available so that other features of the ERG could be studied. This chapter is not meant to give a complete overview of all ERG types and their properties. Instead, a short description of the flash and the conventional flicker ERGs is given, because it provides a context for comparison the results of the work performed in my lab.

The responses elicited by flashes have a complex waveform with different components. The responses to a flash stimulus contain a-, b- and c-waves that each have different cellular origins (Frishman, 2006). With flashes that last long enough, the responses to stimulus onset and offset can be separated and compared and it was shown that the two responses are quite different. Whereas the response to stimulus onset is similar in appearance (but certainly not identical) to the response to the short flash the response to stimulus offset displays a cornea positive peak called the d-wave. Systems that are linear or that contain simple contrast dependent nonlinearities have mirror imaged responses to stimulus on- and offset. Obviously, this is not the case in the flash ERG indicating that its signal pathway contains substantial nonlinearities, which makes interpretation of the signals difficult.

As an alternative to the flash ERG, the response to a repetition of pulses (called the flicker ERG) can be measured. The response waveform with pulse trains at about 30 Hz is much simpler than the flash ERG and therefore might also be easier to interpret. The flicker ERG can have certain properties that are comparable to perceptual properties. Already in the 70s of the 20th century, it was found that the spectral sensitivities of the flicker ERGs at relatively high temporal frequencies were similar to the spectral luminosity function (Padmos & van Norren). After refining this method, the correlation was so strong that inter-species and inter-individual differences in the spectral luminosity function could be retraced in the flicker ERG. This technique has been employed extremely successfully by Gerald Jacobs and his colleagues (Neitz & Jacobs, 1984; Jacobs *et al.*, 1987; Neitz *et al.*, 1991; Jacobs & Deegan Ii, 1993b, a; Jacobs *et al.*, 1993a; Jacobs & Neitz, 1993; Jacobs *et al.*, 1996a; Jacobs *et al.*, 1996b; Jacobs *et al.*, 1996c; Jacobs & Deegan Ii, 1997; Jacobs, 1998; Jacobs & Deegan Ii, 1999; Jacobs *et al.*, 2004).

Through the development of light emitting diodes (LEDs) and their use in creating stimuli, recordings and interpretation of the flicker ERG could be further improved. First, the stimulus waveform can be chosen because the LEDs can be controlled at a high temporal resolution. For instance, pure sine-wave modulation can be generated at all relevant temporal frequencies. A sine-wave contains only one frequency component whereas a pulse train has considerable higher harmonics. This can make the interpretation of the ERG responses elicited by sine-waves easier. A second possibility that can be achieved with LEDs is that sine-waves and other periodic waveforms can be modulated around a mean luminance. Stimulus strength can be varied by changing the modulation depth without changing the mean luminance and chromaticity of the stimulus and therefore without changing the state of adaptation. The stimulus strength can be quantified by Michelson contrast C :

$$C = \frac{(L_{max} - L_{min})}{(L_{max} + L_{min})} \qquad (1)$$

L_{max} is the maximal and L_{min} is the minimal intensity of the output. In contrast, flash trains are mostly delivered upon a steady background. Changing the stimulus strength is achieved by changing the pulse intensity and therefore is confounded with changes in the state of adaptation. In that case the stimulus strength is quantified by Weber fraction:

$$F = \frac{\Delta L}{L_{back}} \qquad (2)$$

ΔL is the flash intensity and L_{back} is the intensity of the background. A third advantage of the use of LEDs is that many differently coloured diodes are available, with which a multidimensional stimulus space can be created with according flexibility enabling, for instance, the stimulation of single photoreceptor types. Thus, the retinal signal flow originating in the different photoreceptor types can be studied. To be able to do this optimally, at least four differently coloured light sources are necessary to study the human retina. In the next section, a description is given of how photoreceptor isolating stimuli can be made and how this method can be extended to conditions in which the stimulus strength in each photoreceptor type can be quantified and how more than one photoreceptor types can be stimulated simultaneously.

3. Cone isolating stimuli

In the early days, isolation of photoreceptor responses was achieved by using a selective adaptation paradigm. A background light was chosen that adapted the photoreceptor systems that were not of interest and a flashed stimulus was delivered having a wavelength at which the photoreceptor of interest was sensitive. This method was used before by Stiles to describe psychophysically the photoreceptor spectral sensitivities of π mechanisms (Stiles, 1939, 1953, 1959, 1978). However, although the response will be strongly dominated by one photoreceptor type, isolation is never complete. In addition, this method has the disadvantage that strongly varying adapting fields have to be used to isolate the responses of different photoreceptor types. As a result, the state of adaptation is also variable. The state of adaptation can have a strong influence upon the cone driven signals (see below), indicating that measurements that are performed at different states of adaptation cannot be compared quantitatively.

A new stimulation method that does not have the above-described disadvantages is the silent substitution method. After more data became available on photopigments and photoreceptor physiology, Donner and Rushton (1959) described a new method of isolating single photoreceptor mechanisms which they called the 'spectral compensation' method. This method is based upon the principle of univariance (Naka & Rushton, 1966), stating that each photoisomerization leads to an identical response of the photoreceptor independent of the wavelength of the photon that was absorbed by the photopigment. As a result, the replacement of one stimulus by another will not lead to a change in photoreceptor excitation if the number of isomerizations is not altered (for a review see Kremers, 2003). This method was later renamed into 'silent substitution' and has been developed further by Estévez and Spekreijse (Estévez & Spekreijse, 1974, 1982) and by Smith and Pokorny and colleagues (e.g.

Shapiro *et al.*, 1996). It was used to create stimuli for psychophysical experiments (Zele *et al.*, 2006) and for single cell recordings (e.g. Yeh *et al.*, 1995). In the 1990s it was introduced in ERG measurements (Usui *et al.*, 1998a; Kremers *et al.*, 1999). Briefly, when using silent substitution, the number of photoisomerizations is in one or more photoreceptor types is not altered. Consider a monochromatic light of 535 nm that is replaced by another monochromatic light of 600 nm. When the two lights have equal energy than, according to the cone fundamentals (DeMarco *et al.*, 1992; Stockman *et al.*, 1993) the M-cones are about 3 times more sensitive to the 535 nm than to the 600 nm light. However, if the radiance of the 600 nm light is three times the radiance of the 535 nm light then the number of isomerizations in the M-cones is not changed by the replacement. Therefore this condition would be a silent substitution for the M-cones. The L-cones, however, are about as sensitive to the 535 nm as to the 600 nm light. Taking into account that the intensity of 600 nm is three times that of the 535 nm light, then the number of isomerizations increases by a factor of about three when the 600 nm light replaces the 535 nm light. If the radiances of the two lights are equal, then we would have a reversed situation with a silent substitution of L-cone and a threefold larger number of isomerizations in the M-cones when the 535 nm light replaces the 600 nm stimulus. Of course, the situation is normally more complicated because broadband sources are generally used instead of monochromatic lights. Nevertheless, the numbers of isomerizations can be calculated by multiplying the rod and cone fundamentals with the emission spectra of the light sources and integrating over wavelength. Furthermore, the human retina contains four receptor types and not just two. The latter problem can be solved when more light sources are used. Theoretically, four light sources are sufficient to be able to stimulate each photoreceptor type independently. For a more extensive explanation of the silent substitution technique I refer to Kremers (2003).

The silent substitution method can be more generalized because it is based upon the fact that the method actually allows to calculate the number of photoisomerizations in each photoreceptor type. The stimulus strength of photoreceptor can be calculated for each stimulus (and similar to the above-mentioned Michelson contrast expressed as cone contrast or rod contrast defined as:

$$PC = \frac{\left(PI_{max} - PI_{min}\right)}{\left(PI_{max} + PI_{min}\right)} \tag{3}$$

in which PC is photoreceptor contrast and PI are the numbers of photoisomerizations in the concerning photoreceptor. As mentioned above, the number of photoisomerizations is calculated by the integral of the multiplication of emission spectra of the stimuli and the absorption spectra of the photoreceptor types. With four differently coloured stimuli, this process of calculating stimulus strength in a particular photoreceptor type is a linear and one to one process. "Linear" means that if the stimulus contrasts are multiplied by a certain factor, the resulting photoreceptor contrasts are multiplied by the same factor. "One to one" means that each combination of stimulus contrasts gives a unique combination of photoreceptor contrasts. With a sufficient number independently coloured light stimuli it is also possible to calculate what stimulus condition has to be used to obtain a particular set photoreceptor contrasts. In the human retina, there are normally four photoreceptor types (one rod and three cone types). That means that theoretically four differently and independently coloured stimuli (independent means that the emission spectrum of one stimulus cannot be obtained by a combination of the other stimuli) are sufficient to obtain

every combination of photoreceptor stimulus strengths (in practise this dynamic range is limited because stimulus contrasts cannot be smaller than zero and larger than one). Silent substitution is a special case in which the contrast in one or more photoreceptor types is zero. Thus, using the silent substitution method it is possible to study the response pathway starting in one photoreceptor type. The method is, however, more powerful, because in principle every wanted combination can be obtained, so that the interactions between photoreceptor driven responses can be studied. I would like to stress once more that the different stimulus strengths are obtained by changing the stimulus contrasts. The mean outputs of the stimuli are not altered. In the next section, I will give an overview about the results of cone and rod driven ERG responses.

4. Cone and rod driven flicker-ERG responses

In collaboration with Tomoaki Usui I started to measure ERG response to L- and M-cone isolating stimuli (Usui *et al.*, 1998a, b; Kremers *et al.*, 1999). For a more complete review of the data with cone isolating stimuli I refer to Kremers (2003). We first explored the influence of stimulus strength of a 30 Hz modulation on the response. A response was defined as the fundamental component (the component at the stimulus frequency) out of the Fourier analysis on the recordings. At this temporal frequency, the complete response was mainly determined by the fundamental component (to which I also refer as the first harmonic component). We found that the response amplitude depended linearly on contrast (Usui *et al.*, 1998a). This was surprising because often ERG components in responses to flash stimuli have a more complex dependency on stimulus strength. In my opinion, this has two important consequences. First, it shows that the flicker ERG displays fundamental properties of the visual system without distortion by nonlinear processes. Second, the data imply that cone and rod contrast are an adequate measure to quantify stimulus strength. With a linear relationship between response amplitude and stimulus strength, the contrast gain (which is the increase in response amplitude per increase in cone contrast) is identical to the slope of the linear regression through the data. Furthermore, the results of the response properties at a pre-defined threshold do not depend on the threshold criterion.

We further found that the response phase increased (i.e. the time delay decreased) with increasing contrast. However, the phase relationship could be different when a retinal disorder is present (Usui *et al.*, 1998b). These data show that the response phases might be a sensitive indicator of retinal diseases. Indeed in subsequent experiments on e.g. patients with retinitis pigmentosa, Stargardt's disease, Morbus Best it turned out that the response phases were altered (Scholl & Kremers, 2000; Scholl *et al.*, 2000; Scholl *et al.*, 2001). Because the phases of L- and M-cone driven responses were often differently altered, this could lead to changed responses when the two were simultaneously stimulated.

When the L- and M-cones were simultaneously stimulated, the responses could be described by a vector addition of the L- and M-cone driven signals. As mentioned above, it is possible to construct such stimuli and the stimulus strength in each photoreceptor type can be theoretically chosen. In addition, all stimuli are presented at the same state of adaptation so that the results of the different measurements (L-cone isolation; M-cone isolation; simultaneous L- and M-cone modulation) can be directly compared. A vector addition means that the signals driven by the two cone types are linearly added at each time instant. Thus, any delay difference between the two cone driven signals is accounted for.

In conclusion, the flicker ERG, measured with high temporal frequency stimuli, display characteristics that can be related to L- and M-cone driven retinal pathways. However, the

question is, if these pathways only reflect photoreceptor properties or if post-receptoral pathways also play a role. For instance, the above-mentioned vector addition can be the result of an interaction of independent signals at the electrode. Alternatively, they may also reflect the properties of post-receptoral pathways. In the next section, I will argue that the flicker ERG does indeed reflect the properties of post-receptoral mechanisms. In addition, I will argue that these post-receptoral mechanisms are probably pathways of the retino-geniculate system that are important for different aspects of visual perception.

5. Post-receptoral responses in retino-geniculate pathways

The stimuli used for measuring ERG responses to isolated and combined photoreceptor stimulations were used before to describe the responses of cells belonging to different retino-geniculate pathways. Because the signals in the retino-geniculate pathways are transmitted to the visual cortex, where they are further processed for visual perception and motor reactions, a correlation of ERG signals with those of the retino-geniculate pathways would indicate that the ERGs can be discussed in a perceptual context.

In primates, three major retino-geniculate pathways are well described and the photoreceptor inputs to these post-receptoral mechanisms are known (Dacey & Lee, 1999; Silveira et al., 2005; Lee, 2011). In the retina, the magnocellular pathway contains diffuse bipolar cells and parasol ganglion cells that project to the magnocellular layers of the lateral geniculate nucleus (LGN). Physiologically, these cells are characterized by a high sensitivity to luminance stimuli, a high temporal resolution and large receptive fields. The magnocelular pathway receives additive input from the L- and M-cones. The L- and M-cone input weights are most probably determined by the numbers of L- and M-cones, which, in humans, varies between individuals. S-cone inputs are either absent or very small. At low illuminances, they receive rod input. The magnocellular pathway is most probably responsible for luminance vision, motion perception, vernier acuity and probably also other psychophyical tasks.

The parvocellular pathway also only receives input from L- and M-cones but not from S-cones. There are contradictory results concerning rod inputs. In contrast to the magnocellular pathway, the L- and M-cone interact subtractively (i.e. antagonistically) at low temporal frequencies. This is caused by the antagonistic input to receptive field centres and surrounds which receive differently weighted L- and M-cone inputs[3]. It is not clear whether the receptive field centres and surrounds are cone selective or receive a mixture of L- and M-cone input (Buzas et al., 2006; Jusuf et al., 2006). Another difference compared to the magnocellular pathway is that there is psychophysical evidence that L- and M-cones have about equal input independent of their densities in the retina (Krauskopf, 2000; Kremers et al., 2000) and state of adaptation (Kremers et al., 2003). Midget bipolar and midget ganglion cells are the anatomical retinal substrate of the parvocellular pathway. Physiologically, the retinal ganglion cells are characterized by cone opponency and thus by red-green colour sensitivity, small receptive fields and low temporal resolution. These cells are most probably involved in red-green colour vision, form perception and others.

The koniocellular pathway is a heterogeneous pathway containing cells with different physiological properties. One important sub-type are the blue-on cells. These cells receive

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[3] Owing to a latency difference between receptive field centres and surrounds there may be an additive interaction at high temporal frequencies.

strong excitatory S-cone input and inhibitory signals from L- and M-cones. Anatomically the blue cone bipolar cells are responsible for transmitting S-cone signals. Diffuse bipolar cells transmit the inhibitory L- and M-cone signals (Martin *et al.*, 1997). Retinal ganglion cells belonging to this pathway are anatomically described as small-field bistratified cells (Dacey & Lee, 1994). The blue-on pathway is responsible for blue-yellow colour vision. Probably there are many other cell types that receive S-cone input, although these cell types probably belong to minor pathways.

When correlating ERG signals with properties of post-receptoral retino-geniculate pathways it is important to keep the above mentioned properties in mind.

6. Flicker-ERG signals reflecting retino-geniculate pathways

As mentioned, many measurements showed that the responses of the flicker ERG at high temporal frequencies are correlated with activity of the luminance pathway. Both have identical spectral sensitivities and the same inter-individual variability can be found in the two. It is not clear if this correlation reflects a causal relationship between the two (Fig. 1, left). The alternative explanation could be that, if all L- and M-cones contribute to the flicker ERG and if the latencies between their signals are not too large, then their signals are summed just as in the luminance channel. The correlation between the ERG and the luminance pathway would then merely be the result of analogous cone signal processing without sharing the same signal pathways (Fig. 1, middle).

However, we found that cone signal weights both in the flicker ERG and in psychophysical luminance pathway strongly depend on the state of adaptation (Kremers *et al.*, 2003). In conclusion, adaptation has the same effect in both pathways. Since adaptation probably involves post-receptoral processing, it is also probable that the luminance pathway and the pathway leading to an ERG signal really share these post-receptoral mechanisms.

The question whether there is a causal relationship between the signal flow in the flicker ERG and in the retino-geniculate pathways is very important because, if the ERG indeed directly reflects the activity of the retino-geniculate pathways, it then can possibly be used for non-invasive studies of the electrophysiological properties of the retino-geniculate pathways in human subjects. This would increase the value of the flicker ERG tremendously beyond its pure clinical application.

If the flicker ERG can reflect activity of the magnocellular luminance pathway then possibly, under other stimulus conditions, it may also reflect activity of the parvocellular red-green chromatic pathway (Fig. 1 right). In 2006, my colleagues and I began to explore the possibility if the responses of the red-green chromatic (parvocellular) pathway can be detected in the ERG. In the next sections, I will describe the results of two experiments showing that indeed there are stimulus conditions at which the flicker ERG is directly related to activity in the red-green chromatic system.

7. Flicker ERGs and the red-green chromatic system

7.1 Methods

ERGs were measured in human observers using full field modulation at different temporal frequencies. For details in recording conditions, I refer to the original publications (Usui *et al.*, 1998a, b; Kremers *et al.*, 1999; Kremers *et al.*, 2003; Kremers & Link, 2008; Kremers *et al.*, 2010). Briefly, ERGs were recording using DTL electrodes with skin electrodes on the ipsilateral temple as reference and on the forehead as ground. The signals were amplified,

band-pass filtered (generally between 1 and 300 Hz) and digitized at a frequency of at least 1 KHz. Within the two described experiments, the mean luminance and chromaticity was constant in all stimulus conditions so that the retina was always in the same state of adaptation and the results could be compared with each other. The states of adaptation were differed between the experiments. In these experiments, luminance and chromatic modulations were varied in the different stimulus conditions.
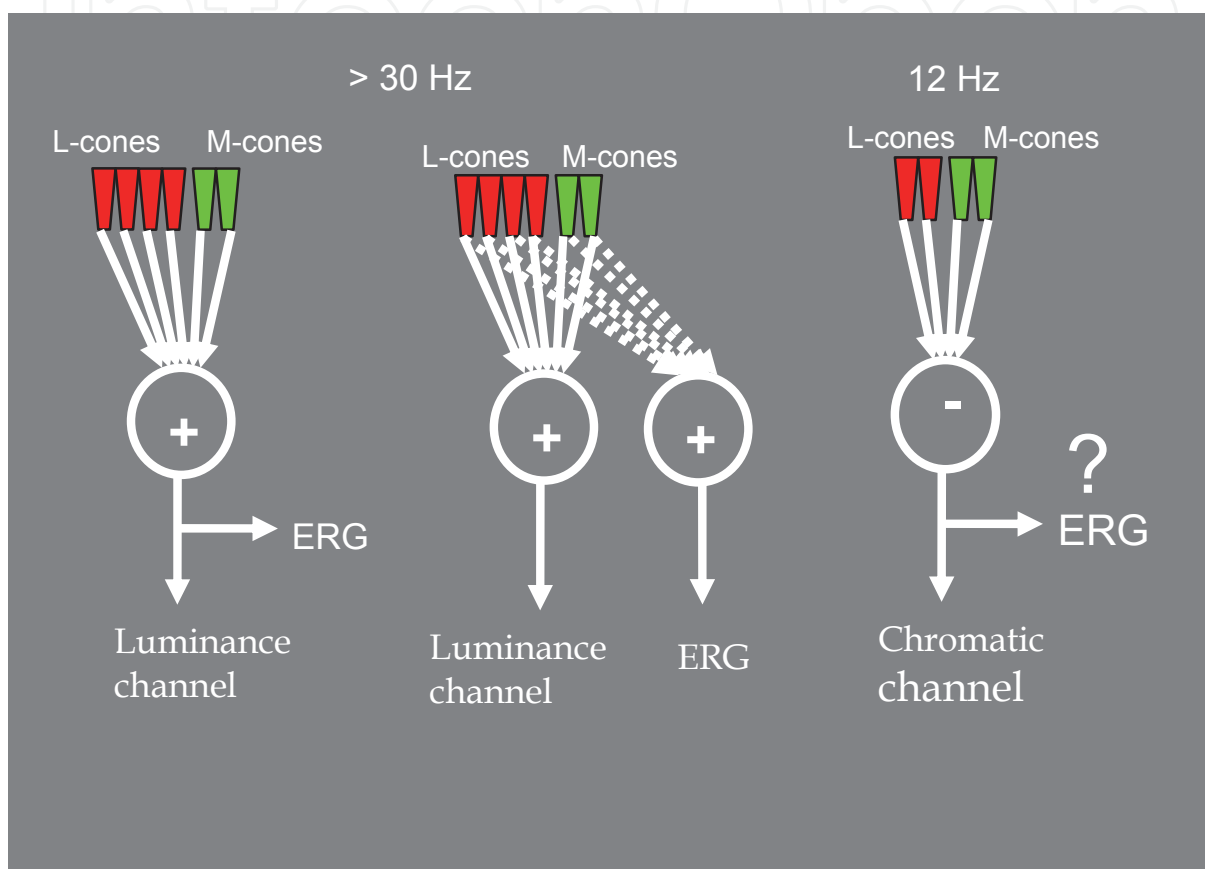

Fig. 1. Possible ideas concerning the relationship between the origins of the flicker ERG and the activity in major retino-geniculate pathways that are responsible for different aspects of visual perception. A correlation between the magnocellularly based luminance channel and the flicker ERGs using high temporal frequency stimuli is well established. The question is if this correlation is the result of the two pathways sharing common mechanisms (left graph) or of two separated pathways that more or less by accident process cone driven signals in an analogous manner (middle graph). If there is a direct causal relationship between the two, it could be expected that under certain stimulus conditions, the flicker ERG also reflects the activity of the parvocellular pathway, which is the physiological basis for red-green chromatic vision but, until recently, there was not much evidence for that proposal (right graph). Observe, that the L-/M-ration is about unity in the red-green chromatic channel whereas the L-/M- ratio is, on average, larger than one in the luminance channel.

Healthy subjects with normal colour vision participated in the experiments. In some experiments, as indicated, deuteranopic subjects and glaucoma patients participated. The glaucoma patients had no or only minor visual field defects and were basically diagnosed on the appearance of the optic nerve heads.

7.2 Experiment 1
7.2.1 Stimuli
On a CRT screen, L- and M-cone isolating stimuli were created. In addition, L- and M-cone excitations were modulated simultaneously at different relative strengths (expressed in cone contrast). In all conditions, the L- and the M-cones were modulated in counter-phase. The stimulus conditions are displayed in Fig 2 (Kremers & Link, 2008). An L-/M-cone modulation ratio of 1 indicates equal modulation strength for the L- and M-cones. A ratio of 2 indicates that the L-cones were modulated at twice the strength at which the M-cones were modulated; with a ratio of 0.5 the M-cone modulation strength was twice the L-cone modulation strength, etc. The rods were not modulated using the above-described silent substitution method. Because a CRT monitor contains three light sources (the red, green and blue phosphors), the excitation of only three photoreceptors can be controlled. Thus, S-cones were modulated at various contrasts at the different stimulus conditions. We repeated the measurements under conditions at which the S-cones were not modulated (S-cone silent substitution) and rods were modulated at various contrasts. The results of the S-cone silent measurements were basically similar and are not shown here. At each relative strength of L- and M-cone modulation, responses were measured at stimulus strengths for which

$$L_C^2 + M_C^2 = const \qquad (4)$$

in which Lc and Mc are the L- and M-cone contrasts. When depicted in a coordinate system with M-cone contrast on the abscissa and L-cone contrast on the ordinate (as in Fig. 2), these stimuli had equal distances to the origin. In pre-experiments it was found that for all conditions, the ERG amplitude depended approximately linearly on the stimulus strength. For additional information I refer to the original publication (Kremers & Link, 2008). The measurements were repeated at four different temporal frequencies (12, 18, 24 and 30 Hz).

7.2.2 Results
For the different stimulus conditions (selective L- and M-cone stimulation and different counter-phase combinations of the two), the ERG amplitudes and phases (defined as the amplitudes and phases of the first harmonic components, which dominated most responses, indicating that the responses were mainly sinusoidal in shape) were determined. The mean amplitudes and phases for measurements performed at 12 and 30 Hz in one observer are displayed in Figure 3. It is obvious that the response data at the two temporal frequencies were quite different. At 30 Hz, the response amplitude to selective M-cone stimulation (L-fraction 0) was substantially smaller than the L-cone response (L-fraction 1). In addition, the phase changed relatively strongly as a function of L-fraction. At 12 Hz, the responses to L- and M-cone selective stimuli had similar amplitudes and phases. Because the stimuli were constructed to modulate L- and M-cones in counterphase, this means that the L- and M-cone driven ERG responses in fact differed by about 180 deg.
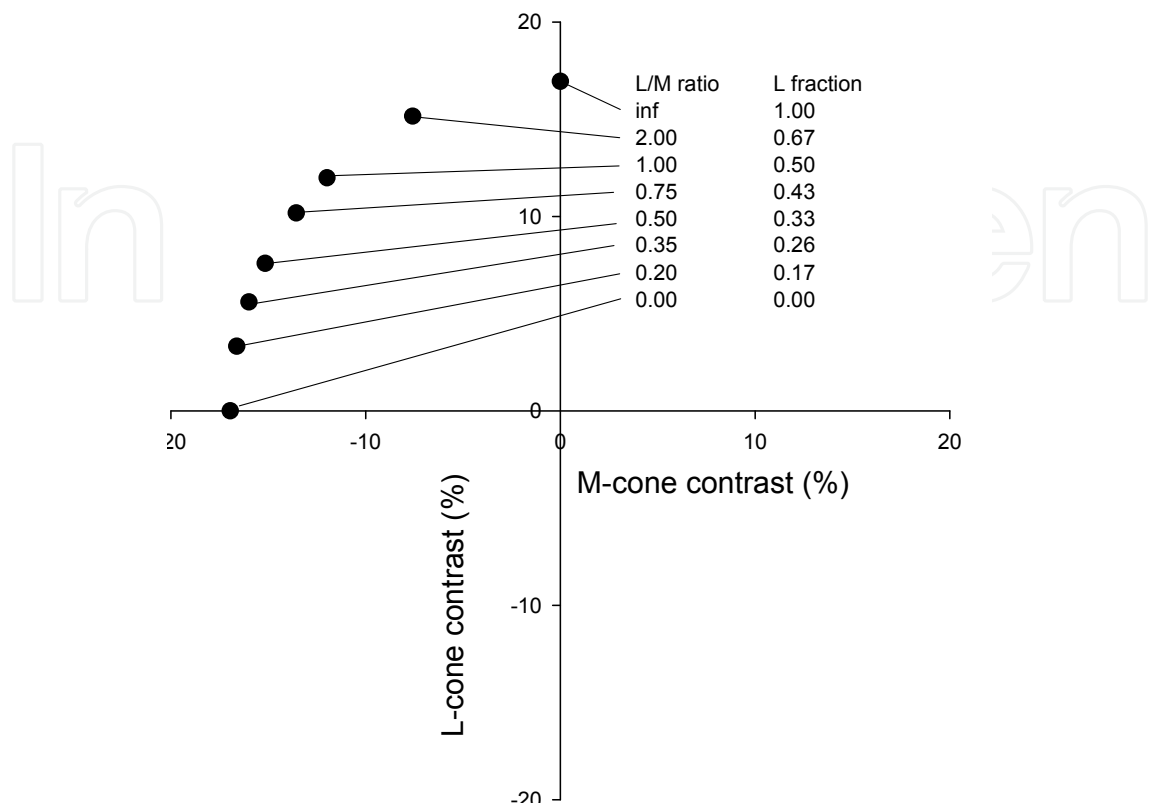
Stimuli for Experiment 1

Fig. 2. Schematic description of the stimuli used in experiment 1. A modulating stimulus is produced on a CRT screen. Eight stimuli were constructed. The rods were not stimulated by any of the stimuli. The stimulus strength for the M- and L-cones (in terms of cone contrast) are given by the X- and Y-values respectively. When the two were modulated simultaneously, they modulated in counter-phase.

The curves are fits of a linear model to the data. The model is identical to the vector summation model mentioned above and assumes that the L- and M-cone driven responses have different delays and weightings before they are added; this model assumes that L- and M-cone driven responses are added at each time. With sine wave responses, as is basically the case here, responses with an amplitude and a time delay, can be expressed by a vector, the length of which depicts the response amplitude. The phase of the response is reflected by the angle of the vector with the X-axis. In the vector addition model, the vector reflecting the response to a simultaneous L- and M-cone stimulation, is equal to the addition of the vectors reflecting the response to the selective L- and M-cone stimulation. For a more detailed description of a vector addition, see Kremers (2003). In the fits, amplitudes and phases were simultaneously considered. There were four free parameters: weights (amplitudes) and phases of the L- and M-cone driven ERG signals. Therefore, from the fits L-/M- amplitude ratios in the ERGs and their relative phases can be estimated. These were estimated for all subjects and all four temporal frequencies. Figure 4 shows the average L-/M-ratios and phase differences for six different observers measured at the different temporal frequencies. The phase difference increases with decreasing temporal frequency. At 12 Hz, the phase difference between the cone inputs is approximately 180° indicating

cone opponent inputs. Observe also that the inter-individual variability is substantially smaller at 12 Hz. The L-/M-ratio decreases with decreasing temporal frequency and is about unity at 12 Hz.
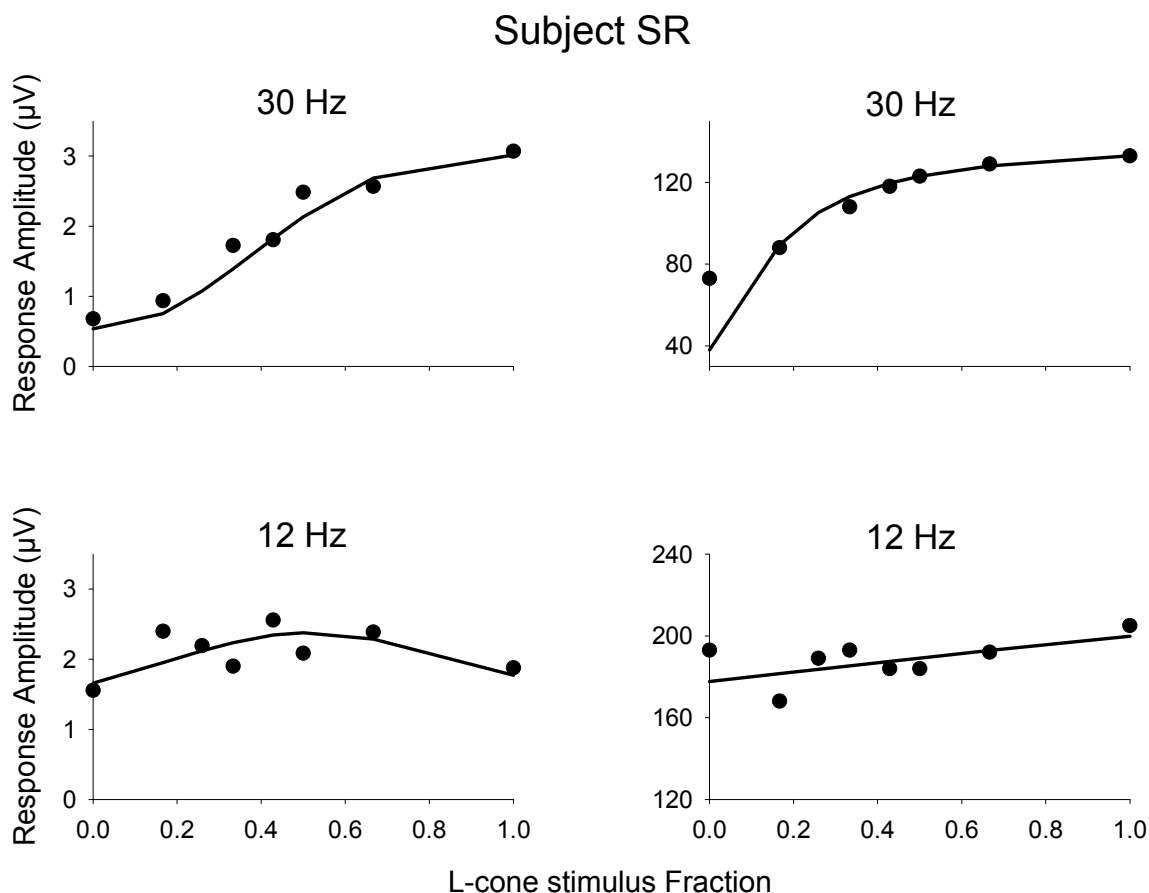

Fig. 3. Response amplitudes (left plots) and phases (right plots) displayed as a function of L-cone stimulation fraction (see Fig. 2 for explanation of stimulus conditions) in one subject measured at 30 Hz (upper plots) and 12 Hz (lower plots). The data are representative for the results obtained in all subjects with normal colour vision. At 30 Hz, the response amplitude to L-cone isolating stimuli (L-cone stimulus fraction equals 1) is larger than the response amplitude to M-cone isolating stimuli (L-cone stimulus fraction equals 0). At 12 Hz stimulation, the response amplitudes are all similar. In addition, the response phases at these two conditions are more similar at 12 Hz. Owing to the counter-phase stimulation of L- and M-cones this means that the L- and M-cone driven signals are about 180 degrees apart at 12 Hz.

The stimuli used in these experiments are combinations of chromatic and luminance modulations. Previous psychophysical data suggest that flicker detection of these types of stimuli is mediated by the (parvocellularly based) red-green chromatic channel at low temporal frequencies and by the (magnocellularly based) luminance channel at high temporal frequencies (Kelly & van Norren, 1977; Kremers *et al.*, 1992). More importantly, the L-/M-sensitivity ratio for flicker detection is about unity (i.e. the sensitivity to L- and M-cone stimuli are equal) when the red-green chromatic channel mediates flicker detection. The L-/M-sensitivity ratio is on average larger than one (but with substantial inter-

individual variability) when the luminance channel mediates flicker detection (Miyahara *et al.*, 1998; Krauskopf, 2000; Kremers *et al.*, 2000; Kremers *et al.*, 2003).

The cone opponent input and the amplitude ratio of about one (with smaller inter-individual variability) in the 12 Hz flicker ERGs suggest that these responses reflect activity of the parvocellularly based red-green chromatic channel. At 30 Hz, the phases between L- and M-cone driven signals are smaller than 180° [see also Usui *et al.* (1998a) and Kremers *et al.*(1999)]. In addition, the individual L-/M-ratios can be correlated with the psychophysically determined L-/M-ratios for luminance mediated flicker detection (Jacobs & Neitz, 1993; Jacobs *et al.*, 1996c; Kremers *et al.*, 2000). Both probably find their origin in the ratio of L- to M-cone numbers in the retina (Brainard *et al.*, 2000; Kremers *et al.*, 2000). There is a large inter-individual variability in the L- to M-cone numbers but with a general bias towards L-/M-ratios larger than unity (Hofer *et al.*, 2005). This is reflected in the L-/M-ratio in the high temporal frequency ERG and in the psychophysical luminance channel. In conclusion, the 30 Hz flicker ERG seems to reflect magnocellularly based activity of the luminance pathway. In the described experiments rods were not stimulated indicating that rod intrusion cannot explain the results. To confirm this interpretation of the data, an additional experiment was conducted (experiment 2).

ERG
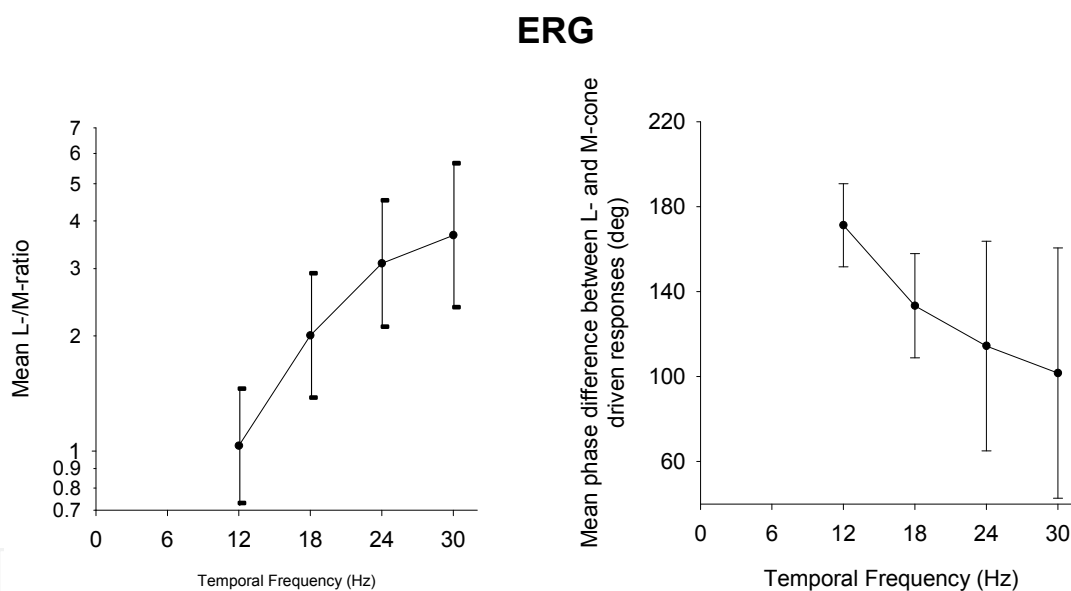
Fig. 4. L-/M-ratio (left) and L-M phase difference plotted versus temporal frequency. The L-M/-ratio decreases with decreasing temporal frequency and is about unity at 12 Hz. The L-M phase difference increases with decreasing temporal frequency and is about 180 degrees at 12 Hz.

7.3 Experiment 2
7.3.1 Stimuli

In a Ganzfeld bowl with differently coloured light emitting diodes (LEDs), the red and green LEDs were modulated in counter-phase at varying different ratios while leaving the overall modulation unchanged (Kremers *et al.*, 2010). The stimulus condition is expressed as the fraction of red LED (R) contrast of the total red and green modulation contrast (R+G). A red fraction of zero (R/(R+G)=0) means that only the green LEDs were modulated while the red LEDs were steady at its mean luminance. A red fraction of one (R/(R+G)=1) means that

only the red LEDs were modulated while the green LEDs were steady at its mean luminance. A red fraction of 0.5 (R/(R+G) = 0.5) indicates that the red and the green LEDs were modulated at equal contrast. The upper panel in Fig. 5 displays the calculated response amplitudes (defined as a contrast in excitation analogous to rod and cone contrasts as a definition for the responses in the rods and cones respectively) of the luminance and red-green chromatic systems as a function of R/(R+G). In addition, the response phases of the luminance and chromatic channels are displayed in the lower graph. The luminance modulation in the stimulus strongly depends on the stimulus condition whereas chromatic modulation is the same for all conditions. For the luminance system we assumed here a V_λ-like spectral sensitivity. As a result, the minimum is at an R/(R+G) of 0.5. Inter-individual variability in the spectral luminosity function (related to the above-mentioned individual differences of L-/M-ratios) results in a variability of this minimum. Dichromats are expected to have a spectral luminosity functions that are identical with the L- (deuteranopes) or M-cone (protanope) fundamentals. The minima of the luminance based responses therefore coincide with the silent substitution points of the L- and M-cone (i.e. those points where the L- and M-cone contrasts equal 0) in deuteranopes and protanopes respectively.
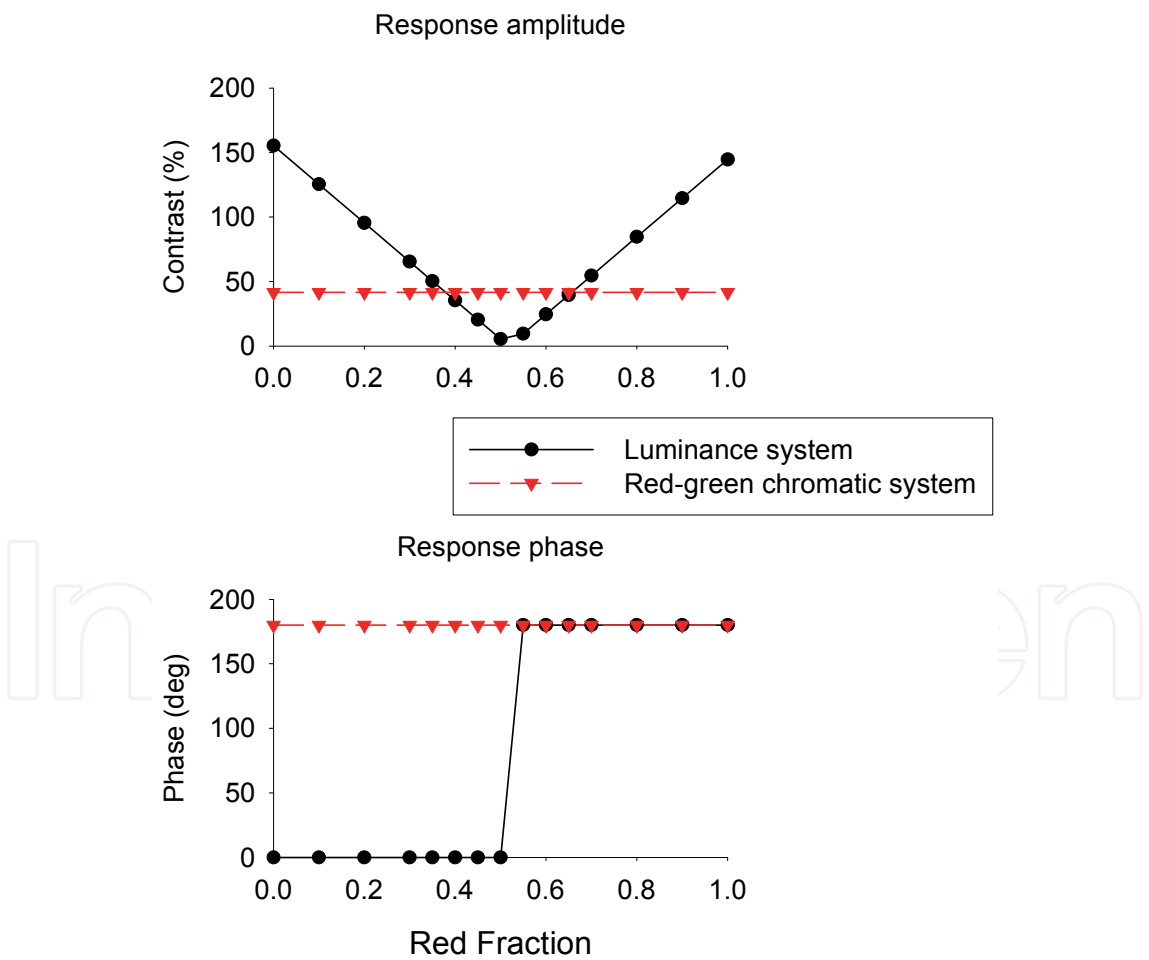
Fig. 5. Theoretical response amplitudes (upper plot) and phases (lower) of the luminance and red-green chromatic pathways given as a function of the stimulus conditions used in experiment 2.

7.3.2 Results

In Fig. 6, the 36 Hz and 12 Hz responses are displayed for three different trichromatic subjects. Obviously, the 36 Hz ERG responses closely correspond to the (magnocellularly based) activity of the luminance channel whereas the 12 Hz ERGs is more reminiscent of the response of the (parvocellularly based) red-green chromatic system (cf. Fig. 5). This experiment confirms the results of experiment 1 that the 36 Hz ERGs reflect magnocellular activity whereas the 12 Hz ERGs mainly reflect parvocellular activity. The results at intermediate temporal frequencies (data not shown) could be described as a linear vector addition of the magno- and parvocellular activity. The advantage of this experiment in comparison with experiment 1 was that larger stimulus contrasts and thus also larger ERG
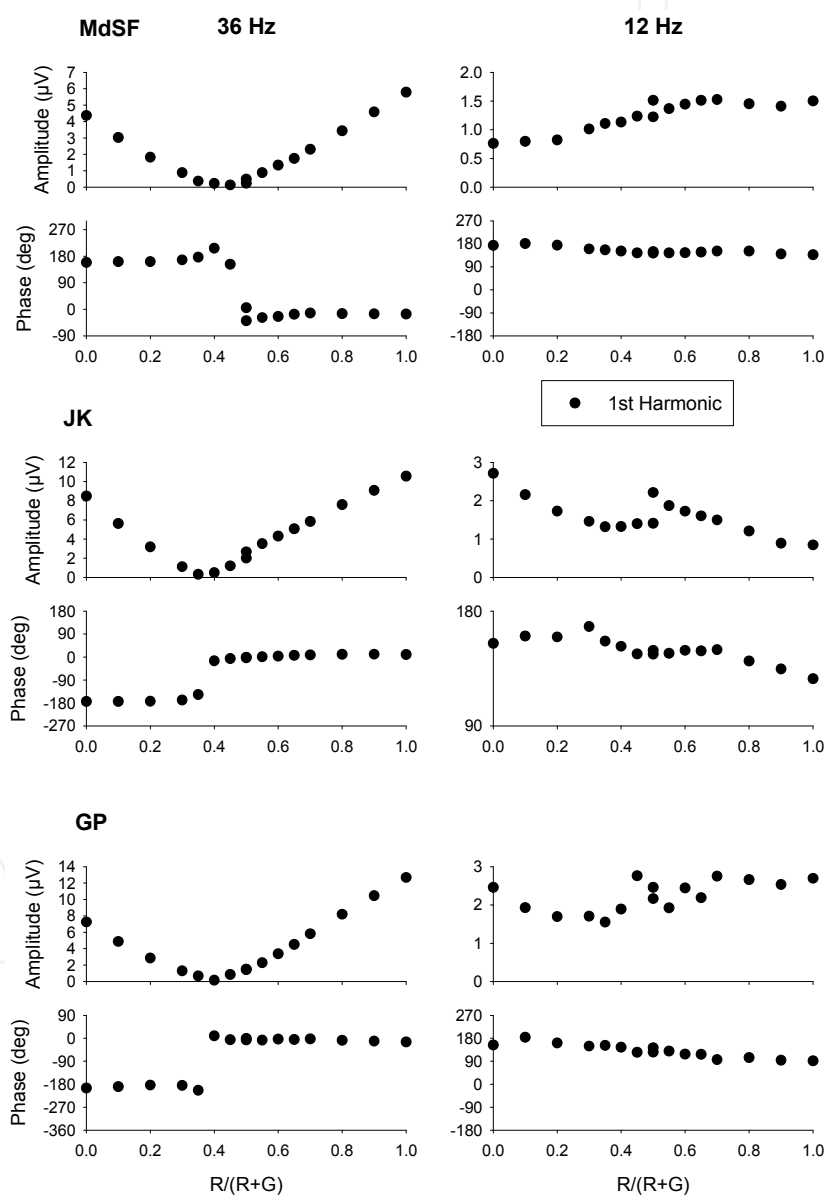
Fig. 6. Measured response amplitudes and phase in flicker ERGs at 36 Hz (left plots) and 12 Hz (right plots) for three different colour normal subjects obtained in experiment 2. Observe the resemblance of the response properties at 36 Hz with those of the luminance pathway and at 12 Hz with those of the red-green chromatic pathway (see fig. 5).

signals could be obtained. This was possible because the narrow band emission spectra of the LEDs allow larger contrasts than the phosphors of a CRT screen. However, in contrast to experiment 1 rods and S-cones responded to the stimuli as well. In principle, rod or S-cone intrusion could lead to similar results. To exclude this explanation of the data, the experiment was repeated in a deuteranope who has normal rods and S-cones but no L-cones and no functional parvocellularly based red-green colour system. If the above described effects were caused by rod and S-cone intrusion then the same results would be obtained in the measurements with the deuteranope. If the ERGs indeed reflected activity of the magno- and parvocellular pathways then it could be expected that the 12 Hz responses would depend on the different stimulus conditions in a similar manner as the 36 Hz responses. The later case was confirmed experimentally (see Figure 7). Therefore, we can conclude that the data in the trichromats were dominated by activity of the red-green chromatic pathway at 12 Hz. In addition, as expected (see above), the minima at 36 Hz and 12 Hz coincided with the silent substitution condition of the L-cones confirming that this subject had no functional M-cones.
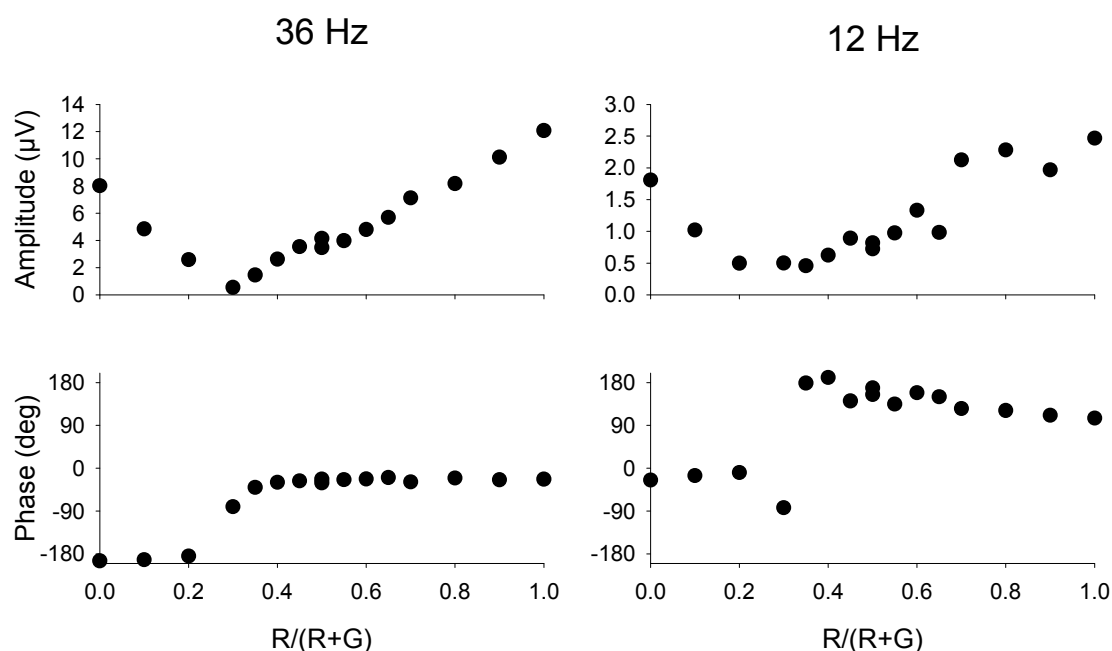
Fig. 7. The same responses as displayed in Fig. 6 for a deuteranopic subject. The responses at 36 Hz are similar to those of colour normal subjects although the stimulus condition for a minimal response is shifted towards lower values of R/(R+G) in comparison with the data of colour normals and coincides with the silent substitution condition for L-cones. At 12 Hz, the data are noisier but otherwise similar to those obtained at 36 Hz. The data show that the results obtained in the colour normals cannot be attributed to intrusion of rod and/or S-cone driven signals but to the presence of a red-green chromatic signal that is absent in the deuteranope.

These experiments were also performed in patients with mild glaucoma. These patients either had no or mild visual field defects. In these experiments, only a subset of the above described stimuli was employed on a larger population of participants. The results of these measurements, shown in Fig. 8, with the healthy subjects were in agreement with the results of the more extended measurements, described above. The response amplitudes measured with the patients were very similar to those of the normal subjects. However, the response

phases differed significantly. Although the differences were not large, the small inter-individual variability in phase data made the phase parameter very useful for detecting differences between groups.
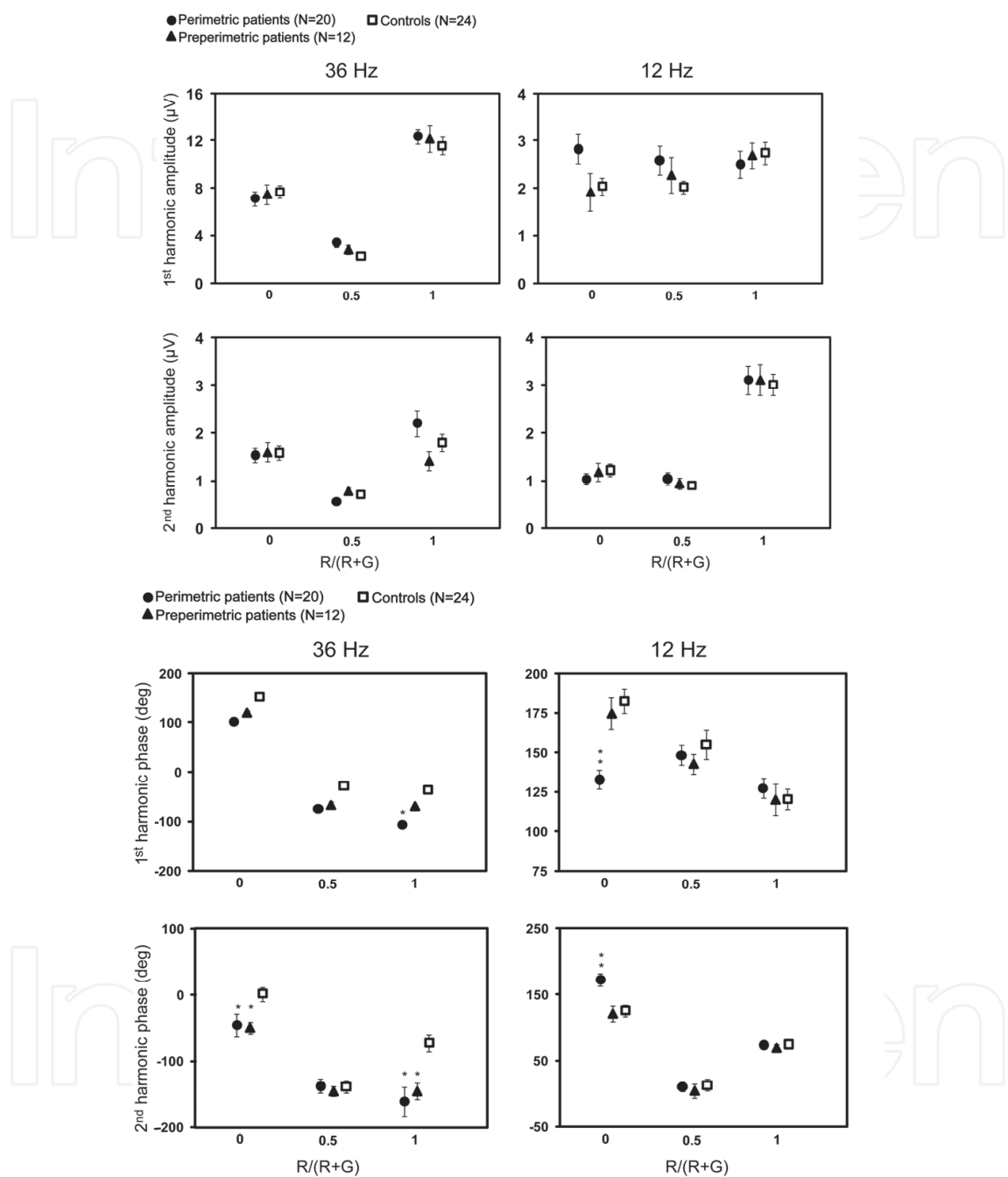

Fig. 8. Responses to a subset of stimuli displayed in Figs. 6 and 7 in larger group of normal subjects, preperimetric and perimetric glaucoma patients (i.e. glaucoma patients without and with visual field losses respectively). The largest differences between the perimetric patients and normals were found in the response phases rather than in the response amplitudes.

In conclusion, in two independent experiments we have shown that it is possible to record ERGs that reflect magnocellularly based luminance activity at high temporal frequency and

parvocellularly based red-green chromatic activity at 12 Hz. As I will discuss below, these data may have important implications for our understanding of the visual information processing in the retina. However, the ERG is an important clinical tool. The results with the glaucoma patients may be a starting point for studying the disease related functional changes in distinct retino-geniculate pathways.

8. Discussion

The individual L-/M-ratio in the ERGs with 30 Hz stimuli was measured in previous experiments by us and by others (Jacobs & Harwerth, 1989; Jacobs & Neitz, 1993; Jacobs *et al.*, 1996b; Jacobs *et al.*, 1996c; Brainard *et al.*, 1999; Kremers *et al.*, 1999; Brainard *et al.*, 2000; Kremers *et al.*, 2000; Kremers, 2003; Kremers *et al.*, 2003) and it was found that they correlated well with the individual L-/M-ratios in the psychophysical luminance channel. In addition, the spectral sensitivity of the high temporal frequency ERG corresponds well with the luminous efficiency function in different human individuals (Jacobs & Neitz, 1993; Kremers *et al.*, 2000) and in different primate species (Jacobs *et al.*, 1987; Jacobs & Harwerth, 1989; Jacobs, 1991; Jacobs & Deegan Ii, 1993b, a; Jacobs *et al.*, 1993a; Jacobs *et al.*, 1993b; Jacobs, 1996; Jacobs *et al.*, 1996a; Jacobs *et al.*, 1996b; Jacobs *et al.*, 1996d; Jacobs, 1997; Jacobs & Deegan Ii, 1997; Banin *et al.*, 1999; Jacobs *et al.*, 2002). It was further found that the L-/M-ratio was correlated with the ratio of L- to M-cone pigment content in the retina (Kremers *et al.*, 2000) and with the ratio of L- to M-cone numbers (Brainard *et al.*, 2000). Therefore, it seems that the high frequency ERG and the luminance channel share a similar type of post-receptoral processing by summing the information of all available L- and M-cones. However, does this mean that the two are intimately related or is the correlation between the two merely the result of an analogous processing in the two signal pathways without a closer relationship (see Fig. 1 for the alternative explanations)? This question was raised in part 6 of this chapter. The data of the two described experiments strongly suggest high temporal flicker ERG is indeed causally related to activity of the luminance channel. This is consistent with our previous finding that selective cone adaptation had similar effects on flicker detection thresholds mediated by the luminance channel and the high temporal frequency flicker ERG (Kremers *et al.*, 2003). The most parsimonious explanation for this observation is that the pathway leading to a high frequency flicker ERG and the magnocellular pathway share substantially parts of visual information processing mechanisms (Fig. 9 left graph). Furthermore, the flicker ERG, measured at a temporal frequency of about 12 Hz, is directly related to activity of the parvocellularly based red-green chromatic pathway (see Fig. 9 where the question mark in the right graph of fig. 1 has been replaced by an exclamation mark).

Based on these data, we conclude that the flicker ERG can reflect activity in the parvocellular and magnocellular retino-geniculate pathways and shares signal processing mechanisms with them. It has been previously proposed that flicker ERGs probably originate in bipolar cell activity (Bush & Sieving, 1996). Possibly, the flicker ERGs reflect activity of diffuse and midget bipolar cells rather than of retinal ganglion cells. That would implicate that the bipolar cells already have response properties that resemble those of the retinal ganglion cells. This is in agreement with the results of intracellular measurements from primate bipolar cells in which it was shown that the bipolar cells have centre-surround

structures (Dacey *et al.*, 2000). These findings may have important implications for basic and clinical science. It may now be possible to study some physiological properties of the two major retino-geniculate pathways objectively in human observers.

A clinical application was introduced above with glaucoma patients. I want to give two examples of basic vision science issues to which the ERG data may contribute. As mentioned above, the 12 Hz ERG data are consistent with psychophysical data showing that the L-/M-ratio in the red-green chromatic channel is about unity despite the generally larger L-/M-ratios and the large inter-individual differences in the luminance channel and in the cone numbers. Moreover, the L-/M-ratio remains at unity in different adaptation conditions although the same adaptation conditions have a large influence upon the responses in the luminance channel (Kremers *et al.*, 2003). This strongly suggests the presence of a sophisticated compensatory mechanism in the retina. I propose that this compensatory mechanism needs to develop in early lifetime of an individual through experience-based weighting and recalibrating of the cone input to the red-green chromatic system. The consequence of this viewpoint is that the cone signal inputs are transformed continuously. This proposal of a dynamic system is in contrast with ideas of a static and hard random wiring in the parvocellular pathway which suggests that the presence of two photoreceptor types with distinct absorption spectra, that are both connected to the midget bipolar cells, is sufficient for the existence of red-green colour vision. In a recent experiment it was found that dichromatic monkeys transfected with an extra opsin gene, express this gene and that the extra photopigment is used behaviourally (Mancuso *et al.*, 2009). It has been suggested that this behaviour is caused by colour vision. This would not be in line with my suggestion that colour vision is experience based. However, an alternative explanation of the monkey data is that the extra pigment has introduced retinal areas that are intrinsically dichromatic (so do not have extra colour vision) but have different spectral sensitivities. This proposal could also explain the behavioural data.

What would the functional and evolutionary advantage of such compensatory mechanism be? This is highly speculative but the answer may be found if we consider the fundamental difference between luminance and chromatic perception. Luminance perception is relative: we are able to see differences in luminances, meaning that we are able to recognize whether one structure is more luminant or brighter than the other, but it is not possible to state what the absolute luminance or brightness is. In contrast, colour perception can be given in absolute terms: we can recognize the colour of a structure directly without a comparison with another structure. We are able to see and identify whether a flower is red or some other colour. It is not necessary to say whether it is more reddish or more greenish than another structure. In addition, we are able to communicate this colour to another person without confusion (provided the two persons have normal colour vision). If somebody asks colour normal persons to pick the red flower in bouquet with a blue, a green, a yellow and an orange, all persons will pick the same flower without any dissent and they will pick correctly the requested flower. Thus, colour vision is absolute and very similar in different individuals despite the large variability in L- and M-cone numbers in their retinae and despite the variability in lighting conditions. That means that the colour system continuously is recalibrated. The proposed compensatory mechanism could be the basis for this. The ERG data may now contribute to these contemplations on basic questions.
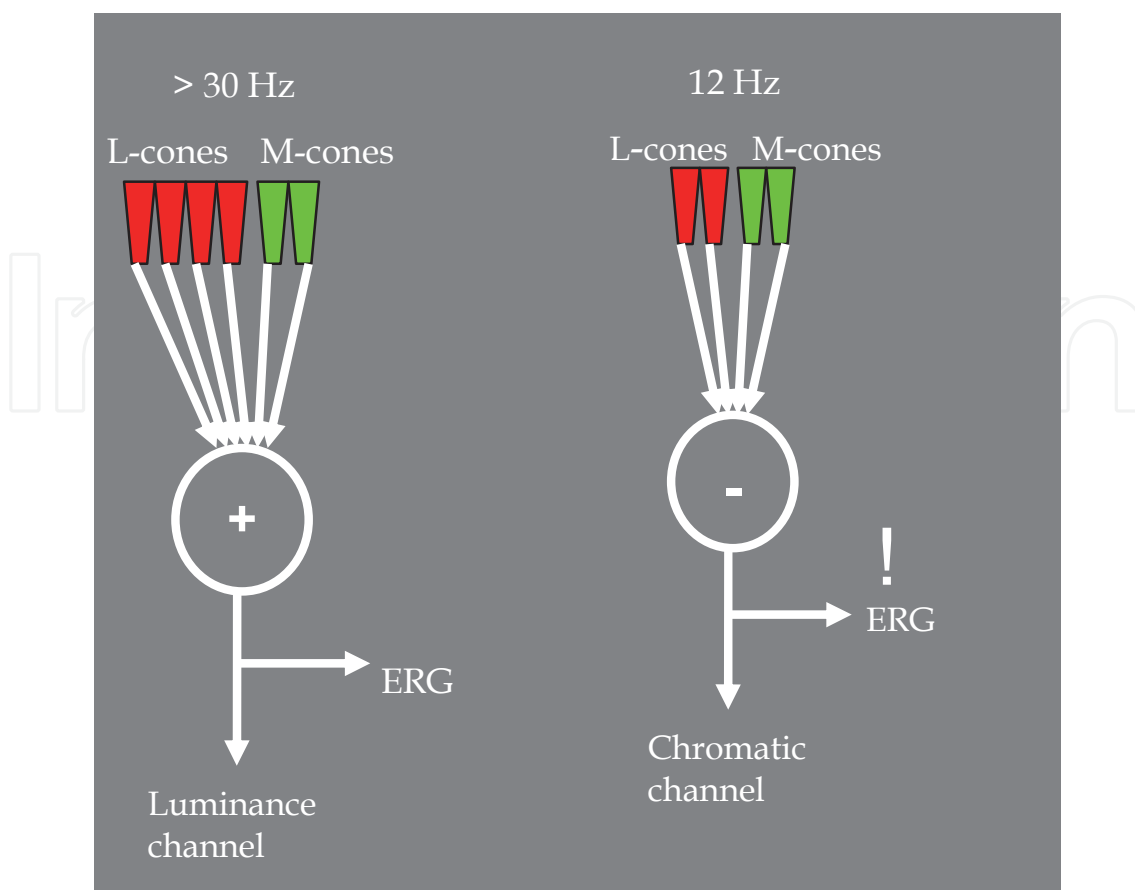
Fig. 9. The proposal based on the results of experiments 1 – 3. The activity of the flicker ERG directly reflects the activity of the luminance channel when high temporal frequency stimuli are used. It reflects the activity of the red-green chromatic pathway when stimulated at 12 Hz.

A second basic issue is that the ERG data can also be used to obtain information about dynamics of photoreceptor driven signals in the human retina. The ERG data can be compared with psychophysical data, but they provide additional information about response delays which can not easily be obtained from psychophysical experiments. The ERG data can be used to explain interesting observations. For instance, it was found that the phase differences between L- and M-cone driven ERG responses at high temporal frequency stimuli (and therefore reflecting luminance activity) were particularly large (1) in subjects with high L-/M-ratios (Kremers *et al.*, 2011), (2) in the periphery of the retina (Challa *et al.*, 2010) and (3) in retinitis pigmentosa patients (Scholl & Kremers, 2000). In all of these cases, the large phase differences were accompanied by a change in M-cone driven phases. The phases of the L-cone driven responses were much more stable and there was less inter-individual variability. Do the phase changes in the M-cone driven ERGs have the same causes in these three cases? If so, what could this cause be? The factor that these cases possibly have in common is a low number of M-cones. Normal subjects with high L-/M-ratios have lower numbers of M-cones. The number of cones decreases with increasing retinal eccentricity. Finally, the numbers of cones also decrease in RP patients. Possibly, if the M-cone numbers fall below a threshold the response phases change. This proposition implies that a reasonable number of cones of one type should be present so that their responses can be synchronized amongst each other and with the other cone types. Although

this idea is quite speculative, it provides a testable working hypothesis for future experiments. In addition, it provides a common solution for the three puzzling results given above. Finally, it may explain why L- and M-cone driven signals have different properties even though the L- and M-cones, and the postreceptoral pathways connected to them, are biochemically and structurally nearly identical.

9. Acknowledgment

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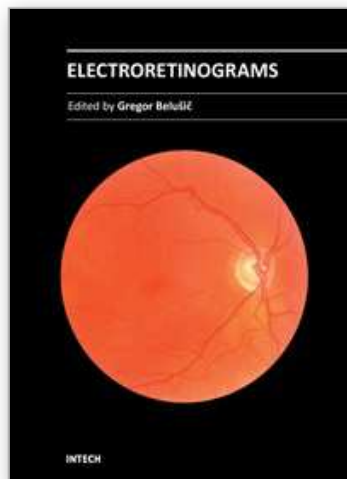
Electroretinograms

Edited by Dr. Gregor Belusic

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Electroretinography (ERG) is a non-invasive electrophysiological method which provides objective information about the function of the retina. Advanced ERG allows to assay the different types of retinal receptors and neurons in human and animal models. This book presents contributions on the recent state of the ERG. The book is divided into three parts. The first, methodological part, reviews standard methods and normatives of human ERG, reports about the advanced spatial, temporal and spectral methods of stimulation in human ERG, and deals with the analysis of the multifocal ERG signal. The second part deals with the ERG in different diseases of the human visual system and in diabetes. The third part presents the ERG in the standard animal models of human retinal disease: mouse, rat, macaque and fruitfly.

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