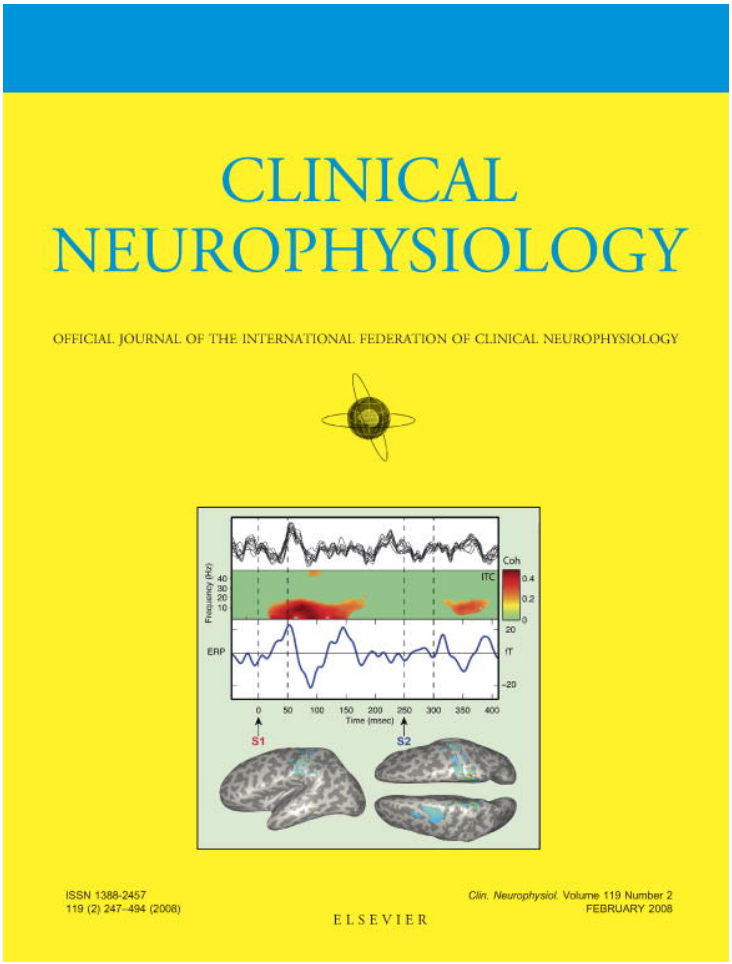

ELSEVIER

Clinical Neurophysiology 119 (2008) 409–417

www.elsevier.com/locate/clinph

Slow pattern-reversal stimulation facilitates the assessment of retinal function with multifocal recordings

Michael B. Hoffmann *, Johann-Joachim Flechner

Visual Processing Laboratory, Universitäts-Augenklinik, Leipziger Str. 44, 39120 Magdeburg, Germany

Accepted 6 October 2007

Abstract

Objective: The use of the multifocal pattern electroretinogram (mfPERG) for objective visual field testing is critically impaired by the small signal-to-noise ratios (SNRs) obtained. In order to explore ways to enhance mfPERG-SNRs and mfPERG-magnitude, the dependence of mfPERGs and multifocal visual evoked potentials (mfVEPs) on stimulation rate and stimulation mode is examined.
Methods: Using VERIS Science 5.1.10X (EDI, CA, USA) mfPERGs and mfVEPs were recorded simultaneously in two different experiments to stimulation at 52 locations comprising a visual field of 44° diameter. Firstly, in eight subjects the response magnitudes were compared for three pattern-reversal (PR) and two pattern-onset (PO) stimulus conditions, which differed in their maximal stimulation rate. Secondly, for equal recording durations the signal-to-noise-ratios (SNRs) of four PR stimuli with different stimulation rates were determined in eight subjects.
Results: Both mfPERG and mfVEP response magnitudes were substantially enhanced for the lower stimulation rates. The greatest effects were obtained for the mfPERG-N95 to pattern-reversal stimulation, which was by a factor of 5.2 ± 0.6 greater than that N95 for the standard condition ($p < 0.001$). mfPERGs for a comparatively low stimulation rate, i.e., reversing its contrast with a probability of 50% only every 53 ms, yielded the greatest SNRs (1.42-fold greater than for the standard condition; $p \leqslant 0.002$).
Conclusions: The enhancement of both mfPERG and mfVEP response magnitudes for slow stimulation suggests that retinal mechanisms contribute to this response enhancement and that slow pattern-reversal stimulation might facilitate simultaneous high-resolution mfPERG- and mfVEP-based visual field testing.
Significance: The study suggests that mfPERG-based assessment of retinal ganglion cell function can be improved with stimulation sequences that are 2–4 times slower than the standard multifocal stimulus.

Keywords: Human; Visual cortex; Retina; VEP; PERG; Multifocal; Onset; Reversal

1. Introduction

Electrophysiological recordings allow for an objective assessment of visual function in humans. Several methods are at hand to tap different stages of the visual pathway, the electroretinogram (ERG) targeting mainly the retinal photoreceptors and bipolar cells, the pattern-electroretinogram (PERG) targeting the retinal ganglion cells, and visual evoked potentials (VEPs) targeting the visual cortex (Bach and Kellner, 2000; Heckenlively and Arden, 2006). These methods can be combined with the multifocal technique, which enables one to record within a short time interval responses from a great number of distinct visual field locations (Sutter, 1985; Sutter, 1991; Sutter and Tran, 1992). Thus multifocal electrophysiological recordings open the possibility to obtain a detailed account of the visual field topography of visual function and dysfunction at various stages of the visual pathway. Accordingly, the multifocal electroretinogram (mfERG) contributes greatly to our understanding of retinal physiology and pathophysiology (Hood, 2000; Kretschmann et al., 2000; Seeliger et al., 2001). The use of multifocal visual evoked potentials

* Corresponding author. Tel.: +49 391 6713585; fax: +49 391 6713570.
E-mail address: michael.hoffmann@med.ovgu.de (M.B. Hoffmann).

1388-2457/$32.00
doi:10.1016/j.clinph.2007.10.005

(mfVEPs) was initially hampered by small and variable signals. These problems were overcome with refined analysis strategies and by recording from multiple electrodes. As a result, a technique for an objective visual field assessment based on cortical signals emerged (Baseler et al., 1994; Hoffmann, 2007; Hood and Greenstein, 2003; Hood and Zhang, 2000; Klistorner et al., 1998). In contrast, the multifocal pattern-electroretinogram (mfPERG) has up to date received only little attention as the practicability of this approach is greatly reduced by the small signal-to-noise-ratios (SNRs) obtained. Some studies assessed retinal ganglion cell function with mfPERGs. In these studies signals from great expanses of the retina had to be pooled to obtain sizable signals (Harrison et al., 2006; Klistorner et al., 2000; Stiefelmeyer et al., 2004). Thus the signal-to-noise-ratio of the recordings was increased, but at the expense of the advantage of the multifocal approach, namely the high spatial resolution at which the visual field is sampled. Clearly, before mfPERGs can contribute substantially to the field of non-invasive electrophysiology in humans, it is necessary to explore ways to enhance the SNRs of the individual mfPERGs. This might be achieved by increasing the magnitude of the mfPERG-signals.

Which strategies are at hand to increase the signals obtained with the multifocal technique? Previously this issue was addressed in mfVEP studies, namely by slowing down the stimulation sequence or by changing the stimulation mode. The standard stimulus to record mfVEPs to pattern stimulation is a pattern-reversal stimulus which reverses its contrast with a probability of 50% every 13 ms (Hoffmann, 2007; Hood and Greenstein, 2003). Fortune et al. reported that reducing the stimulation rate, e.g., to a 50% probability of a contrast reversal within 104 or 208 ms, leads to increased mfVEP-amplitudes (Fortune and Hood, 2003). This effect was sizable in some subjects, a quantitative account on the effect size, however, was not provided in the study. The influence of the stimulation mode on mfVEP size has received more attention. Several studies showed that pattern-onset-offset stimulation leads to increased mfVEPs particularly in the central visual field (Hoffmann and Seufert, 2005; Hoffmann et al., 2003; Klistorner and Graham, 2005). Combined with reduced stimulation rates the increase can be substantial (James, 2003; James et al., 2005; Maddess et al., 2005), James reported mfVEPs to slow pattern-onset-offset to be 15-fold greater than those to the standard pattern-reversal stimulus (James, 2003). As a potential mechanism for this increase in response amplitudes, contrast-adaptation and contrast-gain control mechanisms were suggested. It is not clear whether these mechanisms would reside in the visual cortex or at its input stages, namely the retina or LGN.

The aim of the present study was twofold: (1) The assessment of the dependence of mfPERGs on stimulation rate and stimulation mode. Can mfPERG-amplitudes and mfPERG-SNRs be enhanced by deviating from the standard multifocal stimulation parameters? (2) Comparison of the dependence of mfPERGs and mfVEPs on stimulation rate and mode. Similar dependences would indicate that the mechanisms enhancing mfVEPs might be of retinal origin. To address these questions simultaneous mfPERGs and mfVEPs were recorded.

2. Methods

2.1. Subjects

Subjects aged 20–39 with normal vision (visual acuity $\geqslant 1.0$), if necessary with refractive correction, gave their written consent to participate in the study. Eight subjects participated in Experiments 1 and 2. For each Experiment half of the subjects were male. The procedures followed the tenets of the Declaration of Helsinki (World Medical Association, 2000) and the protocol was approved by the Ethical Committee of the University of Magdeburg, Germany. Subjects were instructed to view a central fixation mark, a cross of 2° diameter, during the experiments. To reduce contamination of the recordings with blink-artifacts stimulation segments were kept short (14 s, see below) and subjects were instructed to blink between two such segments.

2.2. Stimuli

VERIS Science 5.1.10X (EDI: Electro-Diagnostic Imaging, Redwood City, CA, USA) was used for stimulus delivery and electrophysiological recordings. Stimuli were presented at a frame rate of 75 Hz. The stimulus display, a circular dartboard-checkerboard pattern, was viewed from a distance of 36 cm and covered 44° of visual angle. The stimulus consisted of 52 elements. The elements were arranged in five rings spanning following eccentricity ranges: 0.0–3.5°, 3.5–8.0°, 8.0–12.5°, 12.5–17.5°, and 17.5–22.0°. Each ring comprised 12 elements, apart from the central ring, which comprised four elements. An element consisted of a 4×4 checkerboard. The 52 fields of this display were stimulated independently according to an m-sequence. M-sequences consist of a pseudo-random succession of 0 and 1 states. For pattern-reversal stimulation these two states were represented by two contrast inverted checkerboard fields. For pattern-onset-offset stimulation state 0 was represented by a succession of grey fields, while state 1 was represented by a succession of a single checkerboard pattern-frame and as many grey frames as required for the given stimulation rate.

Two experiments with simultaneous mfPERG and mfVEP recordings were conducted: Experiment 1 – comparison of pattern-reversal and pattern-onset-offset responses at different stimulation rates. Experiment 2 – determination of the most effective pattern-reversal mfPERG stimulus by comparing pattern-reversal responses to different stimulation rates for the same recording duration. The specific stimulation parameters for the two experiments are given in Table 1. Pattern-onset-offset stimulation can be affected by luminance intrusions, while pattern-reversal stimulation with symmetrical patterns is robust to

Table 1
Overview of the stimulus conditions used in the three experiments of the study

	Duration of one state	Elements in m-sequence	Recording duration	No. of recording	Pattern contrast (%)	Mean luminance (cd/m^2)	Stimulated eye
Experiment 1							
PR-1f	1 frame	2^{13}-1	1′19″	1	87	47	bin
PR-2f	2 frames	2^{13}-1	3′38″	1	87	47	bin
PR-8f	8 frames	2^{13}-1	14′34″	1	87	47	bin
PO-2f	2 frames	2^{13}-1	3′38″	1	87	47	bin
PO-8f	8 frames	2^{13}-1	14′34″	1	87	47	bin
Experiment 2							
PR-1f	1 frame	2^{16}-1	14′34″	1	96	47	bin
PR-2f	2 frames	2^{15}-1	14′34″	1	96	47	bin
PR-4f	4 frames	2^{14}-1	14′34″	1	96	47	bin
PR-8f	8 frames	2^{13}-1	14′34″	1	96	47	bin

PR, pattern-reversal; PO, pattern-onset; hf, stimulated hemifield; rec, recordings; Stim eye, stimulated eye; Bin, binocular.

these intrusions (Bach and Hoffmann, 2006). Consequently, to avoid luminance artifacts the pattern contrast was set at 87% in the experiment in which pattern-onset-offset and pattern-reversal stimulation were used, i.e., Experiment 1. For Experiment 2, in which only pattern-reversal stimulation was used, a higher stimulus contrast (96%) could be used. For both experiments a mean luminance of 47 cd/m^2 was used, which is in accordance with the ISCEV-standards for PERG and VEP recordings requiring a minimum mean luminance of 40 cd/m^2 (Holder et al., 2007; Odom et al., 2004). For all conditions stimulation blocks were broken up into overlapping segments each lasting about 14 s. To avoid sequential effects, the order of the different conditions was counter-balanced between subjects.

2.3. Electrophysiological recordings

mfPERGs and mfVEPs were recorded simultaneously. mfPERGs were recorded separately for both eyes with a DTL-electrode (Dawson et al., 1979) referenced to the ipsilateral canthus. mfVEPs were recorded binocularly with gold cup recording-electrodes referenced to an electrode at the inion. The recording electrode was placed at Oz (American Encephalographic Society, 1994). The EEG was amplified by 100*k* with a physiological amplifier (Grass Model 12, Astro-Med, Inc., West Warwick, RI, USA), band-pass filtered (low and high frequency cut-offs: 3 and 100 Hz), and sampled at 1200 Hz.

2.4. Analysis

First order kernels and the 1st slice of the 2nd order kernels were extracted for pattern-onset-offset and pattern-reversal stimulation, respectively, using VERIS 5.1.10X. As the polarity of the 2nd order kernels is flipped by this software (Fortune and Hood, 2003; Sutter, 2001), the responses extracted with the 2nd order kernel are flipped back in relation to the software output for both the depiction of the traces and for the analysis. As recommended for mfERG analysis with VERIS 5, two iterations of the artifact removal available in VERIS were applied to the mfPERG-data obtained (settings for artifact removal–epoch: 0–600 ms to cover both epochs, that for the signal-magnitude-estimation and that for the noise-magnitude-estimation; included kernels: 1st order kernels and 1st slice of the 2nd order kernels for pattern-onset-offset and pattern-reversal, respectively). All subsequent analysis was performed with IGOR 5.01 Carbon (WaveMetrics Inc., Lake Oswego, OR, USA). Traces were digitally filtered (high pass cut-off: 3 Hz; low pass cut-off: 45 Hz).

For the quantification of the effect of the stimulation parameters on the multifocal potentials we optimized the signal-to-noise ratio by pooling signals from various visual field locations thus sacrificing the spatial resolution of the multifocal signals.

2.5. mfVEP Analysis

To assess the signal magnitude we determined the root-mean-square (RMS) of the signal in a signal time window (45–150 ms) and in a noise time window (425–530 ms). Subsequently, we evaluated the signal-to-noise ratio (SNR) using the mean noise-window SNR as described by Zhang et al. (Zhang et al., 2002). The SNR for each *i*th sector (of the $n = 52$ total sectors) of subject j was defined as

$$\mathrm{SNRij} = \mathrm{RMSij}(45\text{–}150\ \mathrm{ms})/[(\mathrm{iRMSij}(425\text{–}530\ \mathrm{ms})/n] - 1 \quad (1)$$

The denominator in (1) is the average of the individual RMS values in the noise time window for subject j. mfVEP-SNRs were determined for each visual field location and averaged subsequently as indicated for the specific analysis in Section 3.

2.6. mfPERG Analysis

In Experiments 1 and 2 mfPERGs were recorded from both eyes of the subjects. To avoid statistical problems with interocular correlations, the traces of both eyes of each subject were averaged and subsequently the number of

subjects, not the number of eyes, was used for all statistical tests. Prior to averaging, for one eye responses to the left and right hemifields were swapped to ensure that mfPERGs of the same hemi-retinae of the two eyes, i.e., the nasal and the temporal retinae, were averaged together. As the mfPERG traces obtained resemble the conventional PERG, peaks were determined for the mfPERG as suggested for transient PERGs by the ISCEV standard for PERG (Holder et al., 2007). Normally, the PERG waveform consists of a small initial negativity typically peaking at around 35 ms (N35), followed by a positivity typically peaking around 50 ms (P50), followed by a negativity typically peaking at around 95 ms (N95). As recommended by the ISCEV standard for PERG the amplitude measurements were made between peaks and troughs. Hence, the P50 amplitude was measured from the trough of N35 to the peak of P50, while the N95 amplitude was measured from the peak of P50 to the trough of N95. For mfPERGs the amplitude of the P50 and N95 peaks was determined from the average traces across the visual field locations as indicated for the specific analysis in Section 3. Additionally, RMS and SNR measures were taken in analogy to the mfVEP analysis (see above). For this purpose, the time windows were chosen for the signal epoch between 0 and 100 ms and for the noise epoch between 300 and 400 ms. SNR and RMS values were determined at each visual field location and averaged subsequently as indicated for the specific analysis in Section 3. For the assessment of the significance levels of the effects univariate ANOVAs were performed. Instead of conducting a repeated-measures-ANOVA we included 'subject' as a factor to account for inter-subject variability. Post hoc Fishers protected LSD test was used to determine the significance levels for the comparisons of the specific conditions.

3. Results

3.1. Experiment 1 – Effect of stimulation mode and stimulation rate

In the first experiment the effect of stimulation mode and stimulation rate on mfPERGs and mfVEPs was assessed. For a qualitative assessment of the effects examples of the traces obtained for pattern-reversal and pattern-onset-offset stimulation at different rates are given for one subject in Fig. 1. Traces averaged across the entire visual field are shown for mfPERGs. Due to the inverted polarities of mfVEPs to upper compared to lower field stimulation, it is not applicable to average mfVEPs across the entire visual field. Therefore averages across the upper and lower visual hemifields are shown for mfVEPs and for better comparability also for mfPERGs. It is evident that pattern-reversal and pattern-onset-offset mfPERGs are greater for low than for high stimulation rates. Pattern-onset-offset stimulation elicits comparatively small PERGs, while the greatest mfPERGs are obtained for slow pattern-reversal stimulation (PR-8f stimulus). There is no strong dependence of pattern-reversal mfVEP-amplitudes on stim-

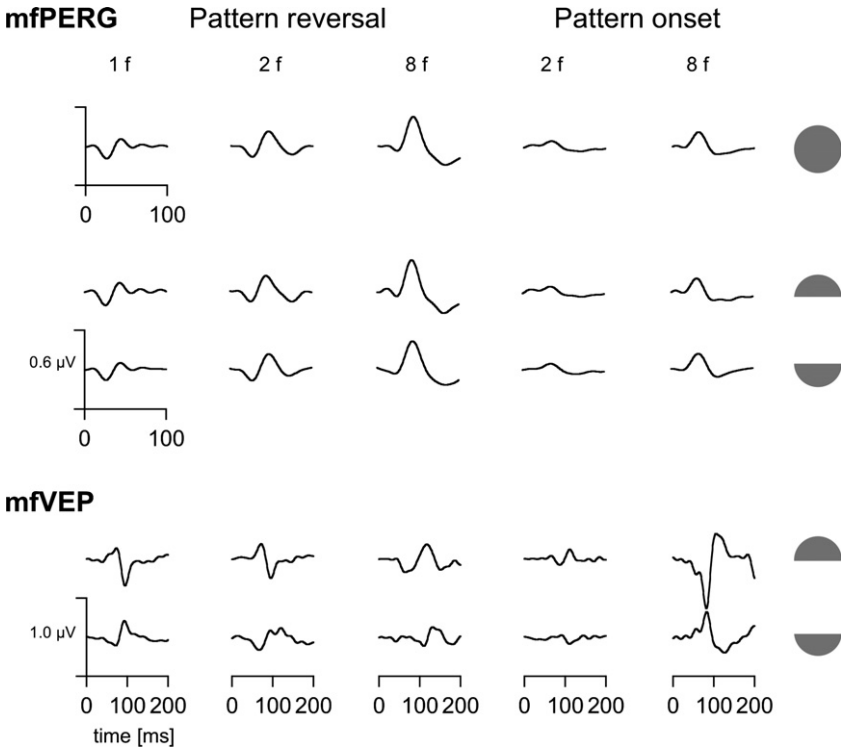

Fig. 1. Example of the mfPERG- and mfVEP-traces obtained in Experiment 1 for a single subject using pattern-reversal and pattern-onset stimulation at different stimulation rates as described in Table 1 (1f – fast; 8f – slow). Traces were averaged across the entire visual field and across the upper and lower visual hemifields as indicated by shading in the rightmost column.

ulation rate. In contrast mfVEPs to pattern-onset-offset are clearly increased for lower stimulation rate. Greatest mfVEPs are obtained for slow pattern-onset-offset stimulation (PO-8f stimulus).

In Fig. 2 the averaged data from the eight subjects are depicted for a quantitative assessment of the effects. P50, N95, and mfVEP-SNR depended on stimulus-condition (univariate ANOVA; $P < 0.0001$). Post hoc tests for P50 amplitudes and N95 amplitudes revealed consistently increased responses for low stimulation rates ($P \leqslant 0.027$). For pattern-reversal stimulation P50 is greater for the 8f- than for the 1f-condition by a factor of 1.80 ± 0.15 and N95 is greater by a factor of 5.21 ± 0.59 (mean $\pm$ SEM). For pattern-onset-offset stimulation P50 is greater for the 8f- than for the 2f-condition by a factor of 2.77 ± 0.33 and N95 is greater by a factor of 2.48 ± 0.17 (mean $\pm$ SEM). Similar dependences were evident for the mfPERG-SNRs.

Multifocal VEP-SNRs for pattern-reversal stimulation are greater for the 8f- and 2f- than for the 1f-condition by a factor of 1.57 ± 0.16 and 1.55 ± 0.10, respectively ($P = 0.048$ and $P = 0.040$, respectively). For pattern-onset-offset mfVEP-SNRs are by a factor of 1.92 ± 0.15 greater for the 8f- than for the 2f-condition ($P < 0.001$).

Finally pattern-reversal and pattern-onset responses were compared. mfPERG P50 and N95 were greater for pattern-reversal than for pattern-onset-offset stimulation ($P < 0.001$). Different results were obtained for mfVEPs. mfVEP-SNRs for pattern-reversal stimulation were smaller than those for slow pattern-onset-offset stimulation, i.e., the 8f-condition ($P < 0.001$), but did not differ significantly from the fast pattern-onset-offset stimulation, i.e., the 2f-condition.

3.2. Experiment 2 – Determination of the most effective pattern-reversal rate for mfPERGs

In Experiment 1, mfPERG-magnitudes were greatest for pattern-reversal stimulation with the lowest rate used, i.e., the 8f-condition. However, different recording durations were required to obtain responses for the same number of stimulus presentations for the various stimulation rates (see Table 1). Equal recording durations would result in a greater number of stimulus presentations for higher stimulation rates. This is of importance as the efficacy of a stimulus depends on the resulting signal-to-noise-ratio, which increases with an increase of the number of stimulus presentations. Therefore, Experiment 2 was designed to test, whether slow pattern-reversal is still the more effective stimulus than fast pattern-reversal, if both are recorded for the same duration. As pattern-onset-offset stimuli were not tested in this experiment an additional pattern-reversal condition could be included. Thus the dependence of mfPERGs on stimulation rate was determined for four stimulation rates, namely for the 1f-, 2f-, 4f- (not used in Experiment 1), and 8f-condition.

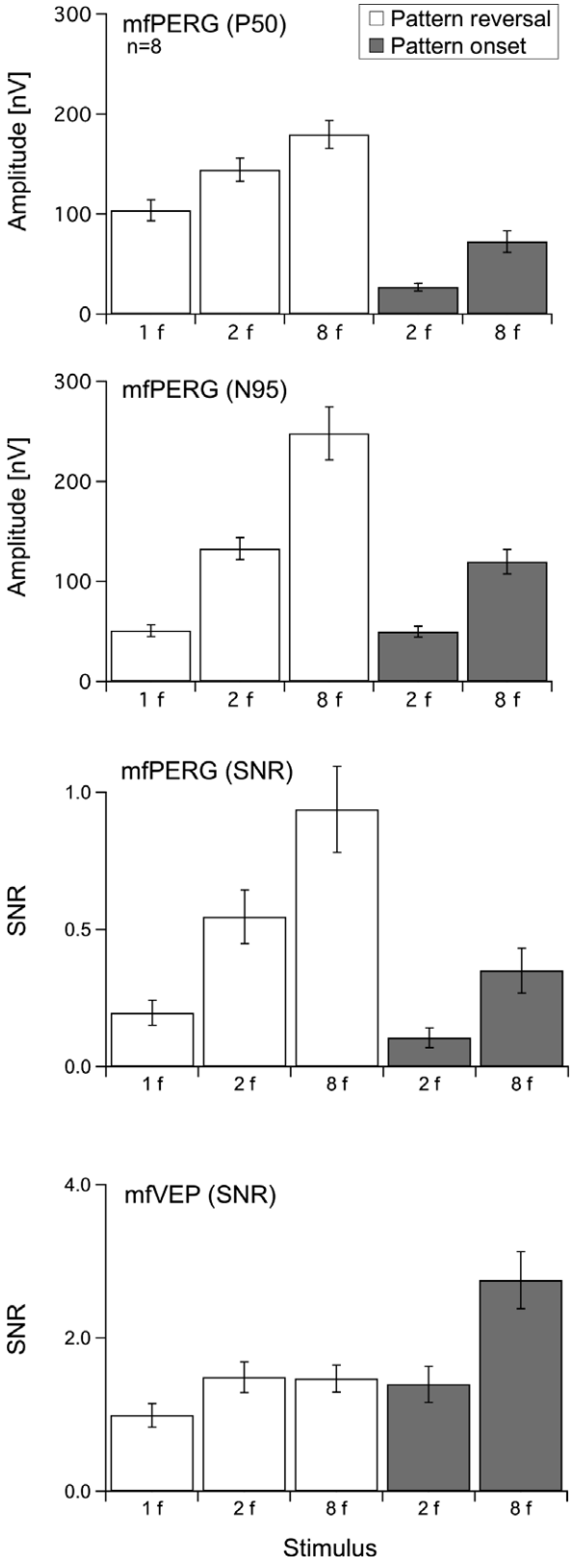

Fig. 2. Dependence of mfPERG-P50, -N95, and -SNR, and mfVEP-SNR on stimulation mode and rate (mean $\pm$ SEM; $n = 8$ subjects) for Experiment 1. mfPERG- and mfVEP-magnitudes were increased for low stimulation rates.

Due to the longer recording durations for most stimulus conditions in Experiment 2 and the resulting increase of signal-to-noise-ratios, responses can be grouped according to stimulation eccentricity for the analysis. Examples of the responses to pattern-reversal stimulation at different rates are given for one subject in Fig. 3 as an average across each eccentricity range for a qualitative assessment of the effects (black traces). Greatest mfPERGs are obtained for the 4f and the 8f pattern-reversal stimulus. This is also reflected by the analyses across all subjects, i.e., the RMS analysis illustrated in Fig. 4A and the single peak analysis illustrated in Fig. 5. A univariate ANOVA showed that RMS values depended on stimulation rate ($P < 0.001$). Post hoc tests revealed a significant increase of the mfPERG-RMS values with decreasing stimulation rate. Specifically, RMS values were significantly greater for the 4f- and 8f-conditions than for the 1f- and 2f-conditions, and for the 2f- greater than for the 1f-condition ($P < 0.001$). These findings are in accordance with Experiment 1. Finally, there was no significant difference of the mfPERG-RMS values for the 8f- and the 4f-condition (not used in Experiment 1). The single-peak analysis (see Fig. 5) indicates that these effects are evident particularly for the N95, while the P50-amplitude is greater for the 8f-, 4f-, and 2f-conditions only in comparison to the 1f-condition. The RMS-values obtained for the mfVEPs are illustrated in Fig. 4B. Only mfVEP-RMS values obtained for the 1f-condition differed significantly from the other conditions, indicating smaller RMS-values for the 1f-condition (univariate ANOVA: $P < 0.001$; post-hoc comparison: $P < 0.001$).

An evaluation of the SNR-values is essential to compare the efficacy of low and high stimulation rates. Thus it can be assessed, whether the effect of the amplitude enhancement for low stimulation rates is overridden by relatively reduced noise levels obtained for high stimulation rates as a consequence of the higher number of stimuli presented. This effect of the different noise levels is indicated in Fig. 3 by the grey traces, which are scaled relative to the respective noise estimates for the different stimulus conditions. For a quantitative account the mean SNR-values obtained for mfPERGs and mfVEPs are illustrated in Fig. 4C and D. This allows for a comparison of the efficacy of the different stimulation rates for mfPERG- and mfVEP-recordings. Greatest mfPERG-SNR-values are obtained for the 4f-condition (univariate ANOVA: $P < 0.001$; post hoc comparison: $P < 0.002$). On average mfPERG-SNR-values for the 4f-condition are by a factor of 1.42 ± 0.12 greater than for the 1f-condition. Accordingly, the higher number of stimulus presentations for high stimulation rates, e.g., in the 1f-condition, does not override the effect of amplitude increase evident for the lower stimulation rates used in the 2f- and 4f-conditions. For mfVEPs the smallest SNR-values are obtained for the 8f-condition (comparison of 8f vs 4f: $P < 0.001$) and the greatest for the 1f- and 2f-conditions (univariate ANOVA: $P < 0.001$; post-hoc comparisons: $P < 0.001$; mfVEP-SNR-values for the 1f- and 2f-conditions do not differ significantly from each other). Consequently, for mfVEPs the relative noise reduction in recordings with high stimulation rates, i.e., for the 1f-condition, does override the moderate amplitude increase observed for lower stimulation rates.

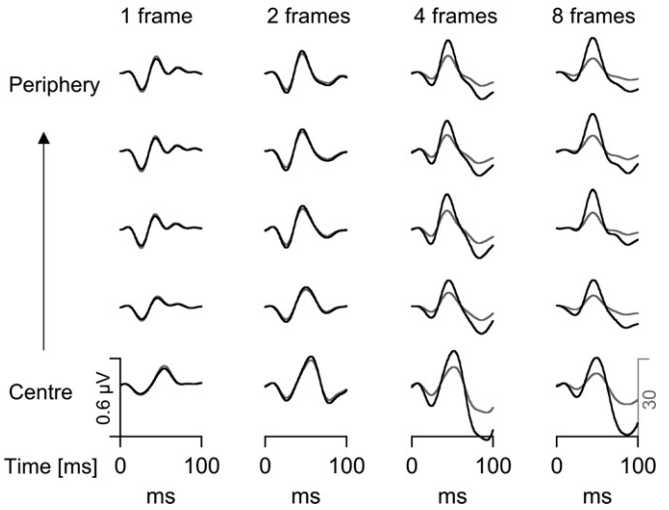

Fig. 3. Example of the mfPERG traces obtained in Experiment 2 for a single subject using pattern-reversal at different stimulation rates as described in Table 1. Traces obtained for the same visual field eccentricities were averaged. Stimulation rate decreases from the left to the right. Response magnitudes were increased for low stimulation rates (black traces). Grey traces are scaled relative to the noise estimate obtained for each stimulation condition (RMS in the noise time-window [nV] –1f: 17.5; 2f: 23.4; 4f: 38.9; 8f: 49.9).

4. Discussion

The main focus of the present study was to investigate how reducing the typical rate of multifocal pattern-reversal stimulation can enhance the efficacy of mfPERG-based visual field testing. In addition the dependences of mfPERGs and mfVEPs on stimulation rate and mode were compared.

4.1. Improving mfPERG-based objective visual field testing

The standard stimulus to record mfVEPs and mfPERGs to pattern stimulation is a pattern-reversal stimulus which reverses its contrast at a high rate, namely with a probability of 50% every 13 ms. In the present study, it was demonstrated that the mfPERG-magnitude is substantially enhanced, if lower stimulation rates are used. Greatest effects were obtained for the N95 amplitude, which was by a factor of 5.2 greater than that for the standard stimulus, SNRs for equal recording durations were by up to a factor of 1.4 greater. Consequently, it can be recommended for the recording of mfPERGs to use stimulation-rates that fall by a factor of 2 or 4 below the standard rate used. Slow pattern-reversal stimulation, particularly half as fast as the standard rate, still allows for an mfVEP-based visual field assessment. Thus not only the possibility of a mfPERG-based visual field test, but of a simultaneous mfPERG-

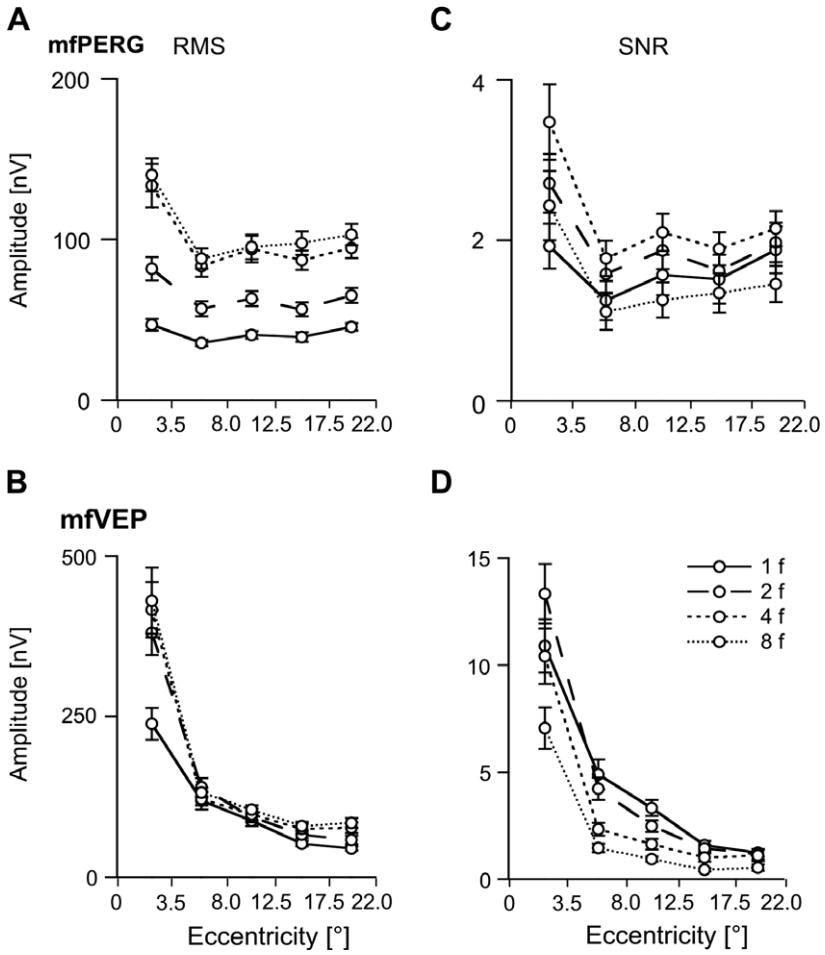

Fig. 4. Dependence of the RMS- and SNR-values of mfPERG and mfVEP (top and bottom rows, respectively) on stimulus eccentricity and stimulation rate as determined in Experiment 2 (mean ± SEM; $n = 8$). Largest mfPERG-SNR-values were obtained for 4f-stimulation, which results in intermediate mfVEP-SNR-values.

and mfVEPs-based visual field test might be opened by the use of slow stimulation sequences. This combined approach is expected to assist in the identification of the site of visual dysfunction in the visual pathway. For example, such an analysis might be relevant in glaucoma-patients. Here the visual field topography of visual field defects and of the reduction of the conventional PERG does not appear to be congruent (reviewed in Bach, 2001). As slowed mfPERGs facilitate an assessment of retinal ganglion cell function with a high spatial resolution, their combination with mfVEPs is expected to increase our understanding of the cause underlying such discrepancies.

4.2. Mechanisms influencing mfPERG- and mfVEP-magnitude

Stimulation rate has a profound influence on multifocal electrophysiological responses to pattern stimulation. Particularly for pattern-onset-offset mfVEPs an increase of amplitudes for lower stimulation rates was demonstrated in previous studies (James et al., 2005; Maddess et al., 2005). This effect might be associated with contrast-adaptation mechanisms. For low pattern-onset-offset rates a pattern that can be adapted to is less frequently present than for high rates. For high contrast patterns, contrast adaptation would, as a consequence, lead to reduced responses for the higher stimulation rates, while this effect should be smaller for low pattern contrasts. Indeed, Maddess et al. demonstrated that the dependence of pattern-onset-offset mfVEPs on stimulation rate is greatest for high stimulus contrasts (Maddess et al., 2005). Accordingly, they suggest that contrast-adaptation mechanisms might be of relevance for the dependence of pattern-onset-offset mfVEPs on stimulation rate. The fact that larger pattern-reversal responses are obtained for lower stimulation rates might be associated with a different mechanism, possibly with temporal low-pass response characteristics of the system.

As a consequence from the above, contrast adaptation and low-pass characteristics are potential mechanisms to explain increased responses for low stimulation rates. Simultaneous mfPERG and mfVEP recordings might help us to determine, whether such mechanisms take action at the retinal or at the post-retinal level. In the present study,

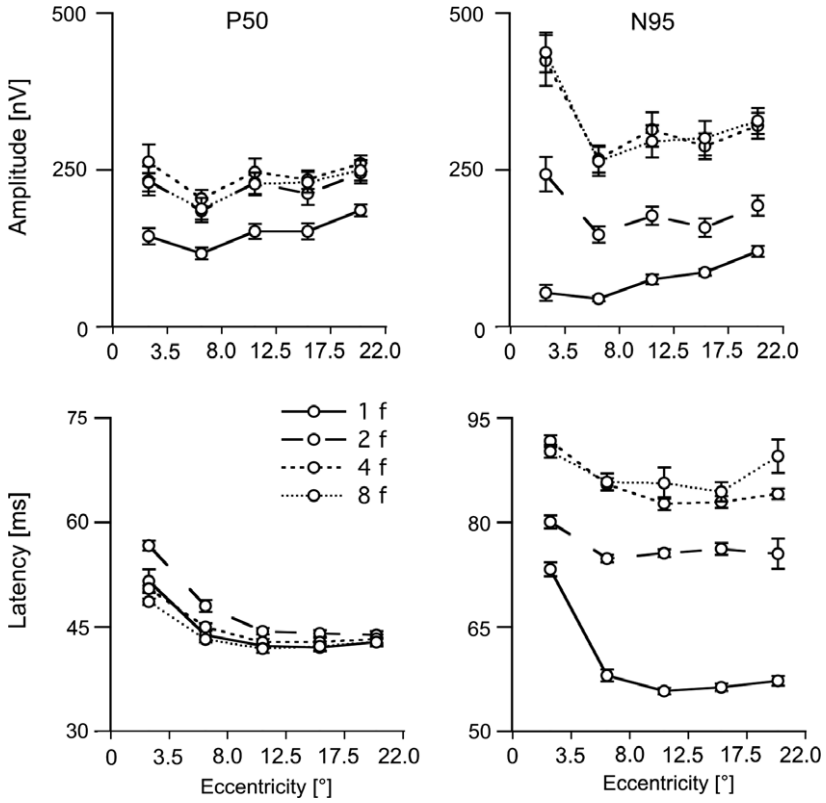

Fig. 5. Dependence of the pattern-reversal mfPERG P50- and N95-amplitude and latency (top and bottom row, respectively) on stimulus eccentricity and stimulation rate as determined in Experiment 2 (mean ± SEM; $n = 8$). Largest responses were obtained for the lowest stimulation rates (4f- and 8f-condition).

mfPERGs and mfVEPs were recorded simultaneously for different stimulation rates and two main findings for the settings in experiment 1 are: (a) an enhancement of both mfPERGs and mfVEPs to pattern-onset-offset for the lower compared to the higher stimulation rate; (b) a differential effect of pattern-reversal rate on mfPERGs and mfVEPs: For mfPERGs, there was a monotonic increase of pattern-reversal responses for decreasing stimulation rates. For mfVEPs, a saturating dependence was observed, i.e., increased responses were evident only relative to the responses obtained with the highest stimulation rate. Strikingly, these findings show that mfPERG response magnitudes both to pattern-reversal and to pattern-onset-offset stimulation were higher for lower stimulation rates. This is in accordance with the hypothesis that the mechanisms, that are associated with higher responses for lower stimulation rates, reside in the retina. But finding (b) also suggests that additional post-retinal mechanisms are at work, which have an additional impact on mfVEP magnitude.

The presence of additional post-retinal mechanisms acting on the magnitude of the cortical responses is also underlined by studies on conventional PERG and VEP. They demonstrated a linear or accelerating dependence of PERG amplitudes on stimulus contrast (Hess and Baker, 1984; Thompson and Drasdo, 1989; Zapf and Bach, 1999), while the dependence of pattern-VEPs on stimulus contrast tends to be saturating (Bach and Ullrich, 1997; Heinrich and Bach, 2002). This might also explain another feature observed in the multifocal responses of the present study, namely that pattern-onset mfPERGs are smaller than the corresponding pattern-reversal mfPERGs, while this was not observed for mfVEPs. A pattern of 100% contrast will induce a 100% contrast step for pattern-reversal stimulation (white and black checks become black and white, respectively). It will, however, only produce a 50% contrast step for pattern-onset (grey becomes black or white). This difference of pattern-onset-offset and pattern-reversal stimulation will be of relevance only for responses that depend strongly on contrast, namely the PERG (Zapf and Bach, 1999).

The presented data show increased mfPERG-SNRs for slowed stimulation. Thus the possibility of simultaneous mfPERG- and mfVEPs-based objective visual field testing might be opened. It is expected that such a combined approach helps to understand how subjective visual field defects are caused by the interplay of retinal ganglion cell dysfunction and reduced cortical responses.

References

American Encephalographic Society. Guideline thirteen: guidelines for standard electrode position nomenclature. J Clin Neurophysiol 1994;11:111–13.

Bach M. Electrophysiological approaches for early detection of glaucoma. Eur J Ophthalmol 2001;11 Suppl 2:41–9.

Bach M, Hoffmann MB. Principles and practice of clinical electrophysiology of vision. In: Heckenlively JR, Arden GB, editors. The origin of the pattern electroretinogram. Cambridge London: MIT Press; 2006. p. 85–196.

Bach M, Kellner U. Elektrophysiologische diagnostik in der ophthalmologie. Ophthalmologe 2000;97:898–920.

Bach M, Ullrich D. Contrast dependency of motion-onset and pattern-reversal veps: interaction of stimulus type, recording site and response component. Vision Res 1997;37:1845–9.

Baseler HA, Sutter EE, Klein SA, Carney T. The topography of visual evoked response properties across the visual field. Electroenceph Clin Neurophysiol 1994;90:65–81.

Dawson WW, Trick GL, Litzkow CA. Improved electrode for electroretinography. Invest Ophthalmol Vis Sci 1979;18:988–91.

Fortune B, Hood DC. Conventional pattern-reversal veps are not equivalent to summed multifocal veps. Invest Ophthalmol Vis Sci 2003;44:1364–75.

Harrison WW, Viswanathan S, Malinovsky VE. Multifocal pattern electroretinogram: cellular origins and clinical implications. Optom Vis Sci 2006;83:473–85.

Heckenlively JR, Arden GB, editors. Principles and practice of clinical electrophysiology of vision. Cambridge London: MIT Press; 2006.

Heinrich TS, Bach M. Contrast adaptation in retinal and cortical evoked potentials: no adaptation to low spatial frequencies. Vis Neurosci 2002;19:645–50.

Hess RF, Baker CL. Human pattern-evoked electroretinogram. J Neurophysiol 1984;51:939–51.

Hoffmann MB, Seufert PS. Simulated nystagmus reduces pattern-reversal more strongly than pattern-onset multifocal visual evoked potentials. Clin Neurophysiol 2005;116:1723–32.

Hoffmann MB, Straube S, Bach B. Pattern-onset stimulation boosts central multifocal vep responses. J Vis 2003;3:432–9.

Hoffmann MB. Investigating visual function with multifocal visual evoked potentials. In: Lorenz B, Borruat F-X, editors. Essentials in neuro-ophthalmology (vol. 7/2). Berlin-Heidelberg-New York: Springer; 2007. p. 139–159.

Holder GE, Brigell MG, Hawlina M, Meigen T, Vaegan, Bach M. Iscev standard for clinical pattern electroretinography – 2007. Doc Ophthalmol 2007;114:111–16.

Hood DC. Assessing retinal function with the multifocal technique. Prog Retin Eye Res 2000;19:607–46.

Hood DC, Greenstein VC. Multifocal vep and ganglion cell damage: applications and limitations for the study of glaucoma. Prog Retin Eye Res 2003;22:201–51.

Hood DC, Zhang X. Multifocal erg and vep responses and visual fields: comparing disease-related changes. Doc Ophthalmol 2000;100:115–37.

James AC. The pattern-pulse multifocal visual evoked potential. Invest Ophthalmol Vis Sci 2003;44:879–90.

James AC, Ruseckaite R, Maddess T. Effect of temporal sparseness and dichoptic presentation on multifocal visual evoked potentials. Vis Neurosci 2005;22:45–54.

Klistorner AI, Graham SL. Effect of eccentricity on pattern-pulse multifocal vep. Doc Ophthalmol 2005;110:209–18.

Klistorner AI, Graham SL, Grigg JR, Billson FA. Multifocal topographic visual evoked potential: improving objective detection of local visual field defects. Invest Ophthalmol Vis Sci 1998;39:937–50.

Klistorner AI, Graham SL, Martins A. Multifocal pattern electroretinogram does not demonstrate localised field defects in glaucoma. Doc Ophthalmol 2000;100:155–65.

Kretschmann U, Bock M, Gockeln R, Zrenner E. Clinical applications of multifocal electroretinography. Doc Ophthalmol 2000;100:99–113.

Maddess T, James AC, Bowman EA. Contrast response of temporally sparse dichoptic multifocal visual evoked potentials. Vis Neurosci 2005;22:153–62.

Odom JV, Bach M, Barber C, Brigell M, Marmor MF, Tormene AP, et al. Visual evoked potentials standard (2004). Doc Ophthalmol 2004;108:115–23.

Seeliger MW, Jurklies B, Kellner U, Palmowski A, Bach M, Kretschmann U. [multifocal electroretinography (mferg)]. Ophthalmologe 2001;98:1112–27.

Stiefelmeyer S, Neubauer AS, Berninger T, Arden GB, Rudolph G. The multifocal pattern electroretinogram in glaucoma. Vision Res 2004;44:103–12.

Sutter EE. Multi-input ver und erg analysis for objective perimetry. In: Proceedings of the IEEE Inc. 7th ann. Conf., Eng Med Biol Soc, Chicago, 1985:414–19

Sutter EE. The fast m-transform: a fast computation of cross-correlations with binary m-sequences. SIAM J Comput 1991;20:686–94.

Sutter EE. Imaging visual function with the multifocal m-sequence technique. Vision Res 2001;41:1241–55.

Sutter EE, Tran D. The field topography of erg components in man – i. The photopic luminance response. Vision Res 1992;32:433–46.

Thompson D, Drasdo N. The effect of stimulus contrast on the latency and amplitude of the pattern electroretinogram. Vision Res 1989;29:309–13.

World Medical Association. Declaration of helsinki: ethical principles for medical research involving human subjects. JAMA 2000;284:3043–45.

Zapf HR, Bach M. The contrast characteristic of the pattern electroretinogram depends on temporal frequency. Graefes Arch Clin Exp Ophthalmol 1999;237:93–9.

Zhang X, Hood DC, Chen CS, Hong JE. A signal-to-noise analysis of multifocal vep responses: an objective definition for poor records. Doc Ophthalmol 2002;104:287–302.