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Multifocal Oscillatory Potentials in the 'Two Global Flash' mfERG in High and Normal Tension Primary Open-Angle Glaucoma

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Abstract

Purpose: In a previous study using the 2 flash mfERG, 90% of normal tension glaucoma (NTG) patients and 85% of high tension primary open angle (POAG) patients could be correctly classified as abnormal while 80% of the control subjects were correctly classified as normal. The purpose of this study was to analyse whether glaucomatous changes contribute to alterations of multifocal oscillatory potentials.

Methods: MfERGs were recorded from 20 NTG and 20 POAG patients and compared to those of 20 controls. The mfERG array consisted of 103 hexagons. Each m-sequence step started with a focal flash that could be either dark or light (m-sequence: 2^13, Lmax: 200cd/m^2, Lmin: 1cd/m^2), followed by two global flashes (Lmax: 200cd/m^2) at an interval of ~26ms. Signals were recorded with a bandpass filter at 10-300Hz. Oscillatory potentials were obtained through offline bandpass filtering at 100-300 Hz (VERIS 5.1). Focal scalar products (SP) were calculated for the response to the focal flash, the direct component at 10-40 ms (DC) and the following two components induced by the effects of the preceding focal flash on the response to the global flashes at 40-70ms (IC-1) and at 70-100 ms (IC-2). For each epoch, eight small group averages were analyzed.

Results: Overall, OPs had a larger SP in control subjects than in glaucoma subjects. In both, the response to the direct component, DC, and in the second induced response, IC2, OPs differed significantly between the control group and the glaucoma patients (repeat measure ANOVA).

Conclusion: Small areas of impaired mfOPs can be detected in both NTG and POAG in the 2 global flash multifocal OP.

Keywords: mfERG; mfOP; Glaucoma; Global flash paradigm

Introduction

Open angle glaucoma, affects at least 1.7% of the population over 40 years of age in industrial countries [1]. In this disease an increasing loss of ganglion cell fibers results in a progressive optic atrophy with an increased cup/disc ratio and an irreversible visual field loss [2]. In an attempt to detect early glaucomatous dysfunction, the mfERG has been studied as a possible diagnostic tool for the past decade. In experimental glaucoma, nerve fiber cell damage induced in the primate results in a marked reduction of amplitude in the mfERG [3-5].

In humans, initial studies that describe changes in the mfERG secondary to glaucoma show only a small reduction in amplitude and an increase in latencies [6-9] in POAG patients when compared to a control group. However, changes in stimulation parameters have lead to an increased sensitivity of the mfERG to detect glaucomatous dysfunction, mainly through enhancing adaptive components in the mfERG, which are generally attributed to the inner retina.

For example, with an increase in the stimulus base interval, a small induced response component resulting from the response to the following stimulus in the m-sequence cycle becomes apparent. At a stimulus base interval of ~54 ms there is no overlap between this induced component and the m sequence response [10]. Under these conditions, oscillatory potentials become apparent in the induced component [11] and the sensitivity to detect NTG increases to about 85% [12]. In the monkey, OPs elicited by such a stimulus are increasingly affected with glaucomatous damage [13].

Adaptive mechanisms, can also be enhanced by interposing bright global flashes into the stimulation sequence, as suggested by Sutter et al. [14]. When global flashes are introduced into the stimulus sequence, the mfERG sensitivity to detect retinal dysfunction in glaucoma increases to 50% with the use of 3 global flashes [15] and to about 75% with a specificity of 83% with the use of a single global flash [16].

The multifocal oscillatory potentials (mfOPs) may also be affected in glaucoma when a global flash stimulus is used. Following a laser induced focal ganglion cell fiber layer defect in the primate, a one global flash mfERG showed fewer and smaller high frequency oscillations, especially in the response to the global flash but also to the focal flash [16]. Recently we obtained similar results using a mfERG stimulus with two global flashes instead of one. With this stimulus, the response to the first global flash was abnormal in 90% of the NTG patients and 85% of the POAG patients while 80% of the control subjects were correctly classified as normal [17]. The purpose of the present paper is to analyse whether multifocal oscillatory potentials contribute to the glaucomatous changes observed in the 2 global flash mfERG.

Methods

Subjects and mfERG recording parameters and procedures were described previously [17].

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Briefly

Between April 2004 and April 2006 mfERG recordings were obtained from 20 patients with NTG, 20 with POAG and a control group of 20 normal subjects. The tenets of the Declaration of Helsinki were adhered to. The study was approved by the institutional review board of the University of Basel. Informed consent was obtained from patients and subjects after explanation of the nature and possible consequences of the study.

Inclusion criteria for glaucoma patients were a cup disc ratio of at least 0.5 as measured with the HRT (Heidelberg Retina Tomograph, Heidelberg Engineering, Heidelberg, Germany), localized thinning of the neuro-retinal rim of the optic disc, and the presence of a glaucomatous visual field defect. For POAG patients the highest IOP ever measured was > 21 mmHg, while for the NTG patients this had to be < 22 mmHg.

Exclusion criteria were the presence of other ocular or systemic diseases, such as diabetes mellitus or hypertension as well as refractive errors exceeding ±6 diopters. The right eye of each subject was included unless it did not fulfill the inclusion criteria or meet any of the exclusion criteria. In this case, the left eye was included, if it fulfilled the eligibility criteria.

For mfERG recording, patients were adapted to ambient room light for 30 minutes. Prior to recording, the pupil was maximally dilated (Tropicamide 0.5%, Phenylephrin 1%) and the cornea was anesthesized (Proxymetacain Hydrochlorid). Electrical responses were recorded monoculary via a bipolar Burian-Allen contact lens electrode (Hansen Ophthalmic Development Labs, Iowa City, IA), that was wetted with a drop of Thilo-Tears SE^{R}. The other eye was occluded during the recording. The ground electrode was placed on the forehead. Subjects were refracted for best visual acuity at 40 cm. The distance between the subject and the screen was adjusted to compensate for changes in stimulus size induced by the refractive lens.

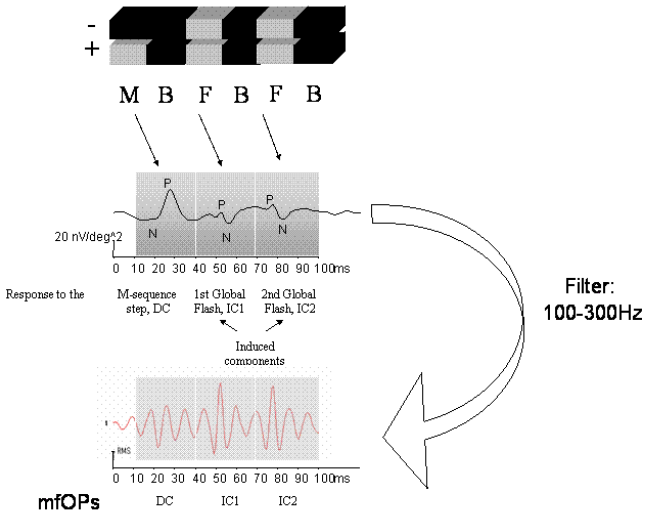

Figure 1: Figure 1 depicts the stimulus sequence of the mfERG (top), an example of the resulting retinal response elicited (middle) and the resulting mfOPs (bottom). **Top:** Each stimulus started with a focal flash that could be either light or dark (Lmax: 200cd/m^2, Lmin: 1cd/m^2), followed by two global flashes (F, Lmax: 200cd/m^2) at an interval of ~26ms. A dark frame (B, Lmax: ≤ 1cd/m^2) separated each step in the stimulus sequence. The mfERG first order response component is calculated by adding the focal mean response to a stimulus base interval starting with a light m-sequence stimulus and subtracting those starting with a dark m-sequence stimulus. **Middle:** The resulting mfERG response, filtered at 10-300 Hz, consists of three epochs: the response to the focal flash at 10-40 ms (direct component, DC) and the following two components induced by the global flashes at 40-70 ms (IC1) and at 70-100 ms (IC2). **Bottom:** Filtering the mfERG response at 100-300Hz results in mfOPs for each of these epochs: DC, IC1 and IC2.

During recording, the central 50 degrees of the retina were stimulated with a Veris scientific 4.8 (Visual Evoked response Imaging System, VERIS EDI, San Mateo, California). The stimulus array consisted of 103 hexagons displayed on a monochrome monitor. The stimulus hexagons were scaled with eccentricity in order to take into account the retinal cone distribution and thus to achieve approximately equal focal response signals in the controls [18].

Figure 1 depicts the stimulus sequence used: Hexagons flickered between black and white according to an m-sequence of 2^13 (frame rate: 75 Hz). Each m-sequence step started with a focal flash that could be either light or dark (Lmax: 200cd/m^2, Lmin: ≤ 1cd/m^2), (M), followed by two global flashes (F, Lmax: 200cd/m^2). A dark frame (B, Lmax: ≤ 1cd/m^2) separated each flash in the sequence. Thus one stimulus base interval consisted of the following sequence: MBFBFB, with a stimulus base interval of ~80 ms and a contrast of 99%. The background was set at 50cd/m^2. Retinal signals were amplified (100 000) and bandpass filtered at 10-300Hz. The total recording time of 10 min 55 sec duration was divided into 32 segments. Segments with contaminated signals were discarded and re-recorded. The artifact rejection technique, incorporated in the software, was applied twice [18]. Spatial filtering was not used.

Figure 1, middle panel, also shows the resulting overall response average to this stimulus. It consists of the three epochs that were analyzed: the response to the focal stimulus, found at 10-40 ms (direct component, DC) and the following two components induced by the effects of the focal stimulus on the following global flashes at 40-70 ms (induced component 1, IC1) and at 70-100 ms (induced component 2, IC2).

The lower trace in Figure 1 shows the mfOPs that were then obtained by filtering the data at 100-300 Hz using VERIS. Again, the 3 different epochs can be distinguished: the mfOPs of the direct component, mfOPs-DC, and those of the IC1 and IC2. The resulting mfOPs are shown at the bottom of Figure 1.

Response analysis

For the mfOPs, focal scalar products (SP) were calculated for the response to the focal flash, that is, the direct component at 10-40 ms (DC) and the following two components induced by the effects of the preceding focal flash on the response to the global flashes at 40-70ms (IC-1) and at 70-100 ms (IC-2). The corresponding focal templates for the calculation of each focal SP were derived from the control subjects.

Figure 2 (top) shows a trace array of OPs obtained from a control. As the expression of OPs varies across the retina, small group averages containing only 8 focal responses were averaged together. Figure 2 (bottom) shows the areas over which responses were averaged to form eight response averages. For analysis of these eight group averages, a repeated measure ANOVA was performed in order to take into account the possible correlation of locations across subjects. To account for multiple testing the Tukey posthoc test was applied.

Results

As previously reported, neither age nor visual acuity differed between the three groups studied. Mean age was 53.9 (SD 13.1) years in the control group, 56.6 (SD 8.1) years in the NTG group and 61.0

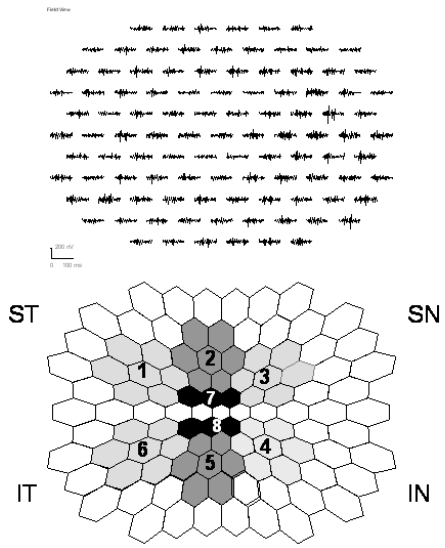

Figure 2: Figure 2 top depicts an example of the OPs (field view) obtained from the left eye of a control subject. The lower part of figure 2 shows the areas over which the focal SP values were averaged to form 8 response averages- in this example also for a left eye.

(SD 10.7) years in the POAG group (ANOVA p=0.126). Snellen visual acuity was ≥ 0.8 in all participants. At the time of the study, IOP was under 21 mmHG in all patients. Mean IOP was 11.7 (SD 2) mmHg in the control group, 13.5 (SD 1.8) mmHg in the NTG group and 14.4 (SD 3.5) mmHg in the POAG group. Mean cup-disc-ratio was 0.33 (SD 0.06) in the control group, 0.65 (SD 0.11) in the NTG group and 0.61 (SD 0.14) in the POAG group. Mean MD was 5.25 (SD 3.4) dB in the NTG group and 5.94 (SD 3.05) dB in the POAG group. MD also did not differ between quadrants [17]. The control group differed from the NTG and POAG groups in IOP and cup-disc-ratio, but the NTG and the POAG groups did not differ significantly in IOP, cup-disc-ratio or MD.

The overall response averages of the mfOPs were very small and showed a large variability. For the DC, mean SP values were: 0.9 nV/deg^2 (SD 1.4) for the control group, 0.2nV/deg^2 (SD 1.1) in NTG and 0.07nV/deg^2 (SD 1.2) in POAG. For IC1 these values were: 0.2nV/deg^2 (SD0.4) for the control group, 0.04nV/deg^2 (SD 0.3) in NTG and 0.25 nV/deg^2 (SD 1.2) in POAG. For IC2 mean SP values were: 0.5nV/deg^2 (SD 0.96) for the control group, 0.19 nV/deg^2 (SD 0.89) in NTG and 0.14nV/deg^2 (SD 0.91) in POAG.

Figure 3 shows the mean and ±2SE of the mean of the eight response averages analyzed. In the direct component, the control subjects differed significantly from NTG patients (p: 0.014, ANOVA, Tukey) and the POAG patients ((p: 0.00, ANOVA, Tukey). NTG patients and POAG patients did not differ significantly (p:0.28, ANOVA, Tukey). In the first induced component, SP appeared somewhat larger in the POAG patients. However, there was also a large variability in the responses of this patient population. Thus the statistical analysis did not show a significant difference between the 3 groups. In the second induced component, the control subjects again differed significantly from NTG patients (p: 0.023, ANOVA, Tukey) and the POAG patients ((p: 0.008, ANOVA, Tukey). NTG patients and POAG patients did not differ significantly (p:0.98, ANOVA, Tukey). Here, there was also a significant effect between locations (p:0.019, ANOVA, Wilks lambda).

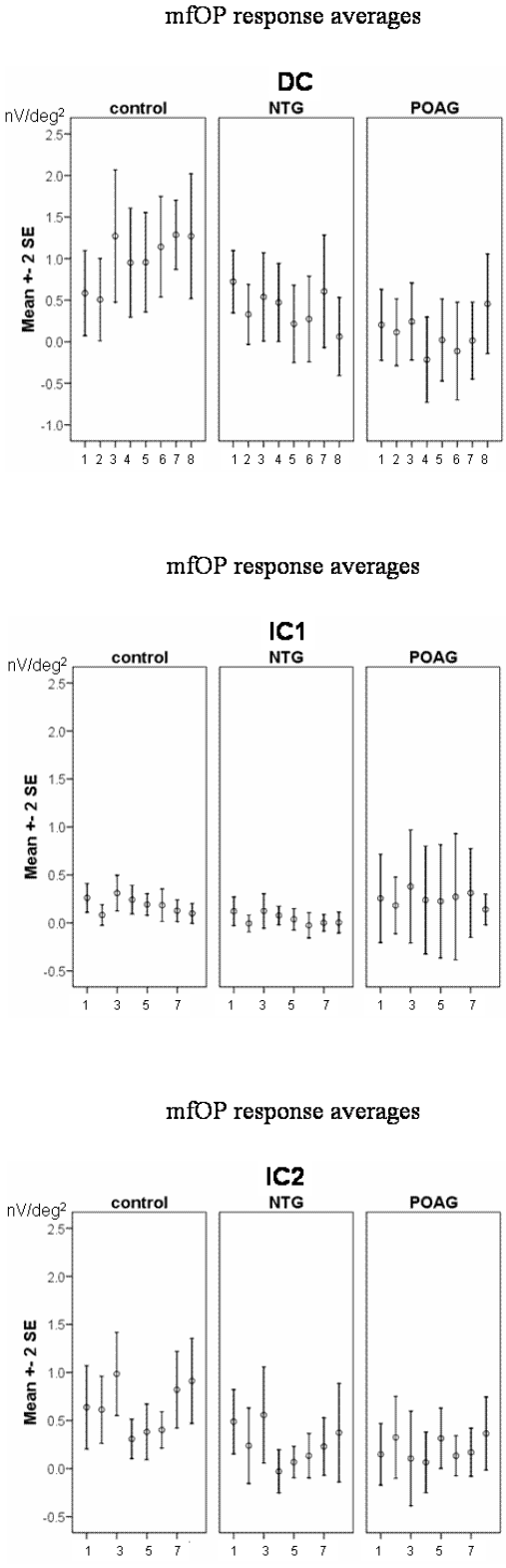

Figure 3: For each of the epochs examined (3a DC, 3b IC1, 3c IC2) figure 3 shows the mean and ± 2SE of the mean of the responses for the different groups (control, NTG and POAG) and for the 8 different response averages analyzed. In the DC and IC2 glaucoma patients differed significantly from the control. IC2 also demonstrated a significant effect of location.

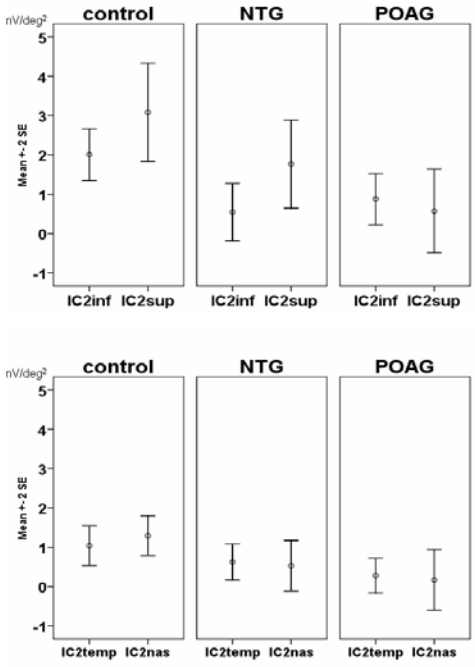

Figure 4: To assess the effect of location in IC2, figure 4a depicts the mean and ±2SEM for the superior (groups 1,2,3,7) and inferior (groups 4,5,6,8) response average while 4a shows the nasal (groups 3,4) versus the temporal (groups 1,6) response average.

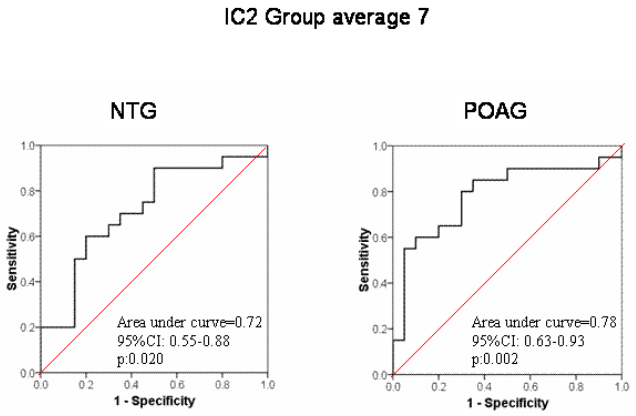

Figure 5: Figure 5 depicts the receiver operating characteristics (ROC) curves for the IC-2 of group average 7 for NTG patients (left) and POAG patients (right). If the values were aligned on the diagonal, this ability would be equal to chance. For a sensitivity and specificity of 100%, the ROC curve would follow the leftmost and the topmost margin of the graph. Thus, the area under the ROC curve is a measure of the ability of this parameter to differentiate between patients and controls. For NTG patients, the area under the ROC curve was 0.72 and for POAG it was 0.78, which is significantly better than chance (p≤0.02).

In order to visually assess the effect of location, Figure 4a depicts the mean and ±2SEM for the superior (groups 1,2,3,7) and inferior (groups 4,5,6,8) response averages while 4a shows the nasal (groups 3,4) versus the temporal (groups 1,6) response average. From this it appears that a superior- inferior asymmetry seen in the control subjects and also to some extent in NTG gets smaller and reverses in POAG. Statistical analysis shows this to differ significantly between the control subjects and POAG (p= 0.008, ANOVA, Tukey) but not between the other groups (control versus NTG: p= 0.053, NTG versus POAG: p= 0.74).

The difference in the naso-temporal field responses seems much smaller, but again appears slightly different in the control subjects where nasal responses are on average larger (1.3 nV/deg2) than the temporal field response (1.0 nV/deg2) and in POAG where temporal responses are on average larger (0.28 nV/deg2) than the nasal field response (0.16 nV/deg2) (p= 0.03, ANOVA, Tukey). There was no significant difference between the control versus NTG group: p= 0.23 or NTG versus POAG: p= 0.6.

When looking at the mfOPs, the most sensitive parameter seemed to be the IC2 response of group average 7. Figure 5 shows the receiver operating curve for this parameter. For NTG the area under the curve was 0.72 and for POAG it was 0.78.

Discussion

In this study, significant changes were observed in the mfOPs of the direct component and the second induced component in NTG and POAG patients. The most sensitive parameter when looking at the mfOPs was the IC2 response of group average 7 where for NTG patients, the area under the ROC curve was 0.72 and for POAG it was 0.78, which is significantly better than chance (p≤0.02).

Interestingly, the response filtered at 10-300Hz had previously shown glaucomatous dysfunction in IC1 [17]. In contrast, there was no statistically significant group difference observed in the mfOPs of IC1. However, POAG mfOPs of IC1 appeared to have a somewhat larger SP albeit with a very large variability.

It is interesting to note that in the one global flash mfERG, not only do adaptive effects of the focal m-sequence stimulus influence the IC-response, but the global flashes also influence the response to the focal flash [19]. This also holds true for stimuli with 3 global flashes, where the response to the focal flash is greatly altered [15]. These nonlinear contributions, have been reported to be much larger in the IC than in the DC.

Of the nonlinear contributions to the mfERG, the optic nerve head component (ONHC) which has been attributed to the nerve fiber layer [20-23] is reflected in a large naso-temporal asymmetry of the mfERG response that may be diminished in glaucoma [22,24]. Indeed, the IC has been shown to contain a large naso-temporal asymmetry, while this is only slightly present in the focal flash response [15,16,19]. These adaptive mechanisms are generally attributed to the inner retina [15,16], another reason to apply a global flash paradigm that elicits induced components to the diagnosis of glaucoma.

In the present study, the mfERG analysis of the mfOPs, demonstrated a significant effect of location in IC2. However, this does not appear primarily due to changes in a naso-temporal asymmetry. The mfOPs appear to rather show a more pronounced superior-inferior difference in the SP analyzed which seems to be affected in glaucoma (Figure 3b). Visual field testing revealed no significant differences in the distribution of the mean defect of the different quadrants of the visual fields in either NTG or POAG patients. This is not a contradiction, as previous studies did not find mfERG changes co-localized to visual field defects, either for the no global flash mfERG [17,25]or for the pattern mfERG [26].

In conclusion, our results support previous findings, that interposing bright global flashes into the stimulation sequence, increases the sensitivity of the mfERG to detect retinal dysfunction in glaucoma [15,16,27]. While the second induced component of a

3 global flash mfERG had a 50% sensitivity to detect glaucomatous damage [15], this was increased to 75% with use of a single global flash mfERG [16]. The 2 global flash stimulus has been shown to have a comparable ability to correctly classify 90% of the NTG patients and 85% of the POAG patients as abnormal and 80% of the control subjects as normal [15].

A new finding of this study is that focal impairment of mfOPs can be detected in both NTG and POAG patients. Changes in the mfOPs contribute to the glaucomatous retinal dysfunction in the 2 flash mfERG. However, in this stimulus stetting, mfOPs alone do not reach the sensitivity level previously reported.

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