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The 2-global flash mfERG in glaucoma: attempting to increase sensitivity by reducing the focal flash luminance and changing filter settings

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Abstract

Purpose   To test a new 2-flash multifocal electroretinogram (mfERG) paradigm in glaucoma using a reduced light intensity of the m-frame flash as opposed to the global flash, as it has been suggested that this may increase the responses induced by the global flash, which has been the part of the mfERG response where most changes have been noted in glaucoma.

Methods   A mfERG was recorded from one eye of 22 primary open angle glaucoma (POAG) patients [16 normal tension glaucoma (NTG), 6 high tension glaucoma (HTG)] and 20 control subjects. A binary m-sequence ($2^{13}-1$, L_{max} 100 cd/m^2, L_{min} <1 cd/m^2), followed by two global flashes (L_{max} 200 cd/m^2) at an interval of 26 ms (VERIS 6.0^{TM}, FMSIII), was used. The stimulus array consisted of 103 hexagons. Retinal signals were amplified (gain = 50 K) and bandpass filtered at 1–300 Hz. For each focal response, the root mean square was calculated. We analyzed 5 larger response averages (central 15° and 4 adjoining quadrants) as well as 8 smaller response averages (central 10° and 7 surrounding response averages of approximately 7° radius each). Three epochs were analyzed: the direct component at 15–45 ms (DC) and the following two components induced by the effects of the preceding focal flash on the response to the global flashes at 45–75 ms (IC-1) and at 75–105 ms (IC-2). Statistical analysis was performed using linear mixed effects models adjusted for age.

Results   Responses differed significantly between POAG patients and controls in all central response averages. This difference was larger for the central 10° than for the response average of the central 15°. While these observations held true for all response epochs analyzed, the DC differed least and the IC-1 most when POAG was compared to control. For POAG, the most sensitive differential measure was IC-1 of the central 10° with an area under the ROC curve of 0.78. With a cutoff value of 12.52 nV/deg^2, 80 % of the POAG patients (100 % HTG, 69 % NTG) were correctly classified as abnormal, while 77 % of the control subjects were correctly classified as normal. When the results of the mfERG were compared to the visual fields, there was a tendency for the mfERG to decrease as the mean defect increased. However, this correlation was only significant in the superior nasal quadrant when the IC-1 of the mfERG was compared to the corresponding area of the visual field.

Conclusion   When compared to findings from previous studies, reducing the luminance of the m-frame flash in the 2-global flash paradigm did not increase the sensitivity and specificity of the mfERG to detect glaucoma further.

Keywords   MfERG · Glaucoma · POAG · Global flash

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Introduction

Glaucoma is one of the most frequent causes of visual impairment and blindness worldwide. In the population over 40 years old, 1 out of 40 people has glaucoma. This means that 60 million people worldwide are affected, and among these, 8.4 millions are bilaterally blind [1]. The population is getting older, so in the future the occurrence of problems caused by glaucoma will increase.

The Early Manifest Glaucoma Trial (EMGT) has suggested that a decrease of the IOP delays visual impairment and therefore the course of disease [2]. Thus, irreversible blindness could be avoided by early diagnosis and treatment. Analysis of different medical databases shows that glaucoma screening of groups at risk may even be cost-effective [3].

Glaucoma can be asymptomatic for a long period of time. Early recognition of glaucoma therefore relies on examinations. In the past, it was assumed that structural loss precedes functional damage. Quigley et al. postulated that an increased cup-to-disc ratio precedes a detectable field loss. He demonstrated that the disc glia are less susceptible to damage than axons, which means that an early cup enlargement must then represent nerve fiber loss [4]. It was thought that at least 25–35 % nerve fiber loss is needed before an abnormality in the visual field can be detected [5]. A recent study by Hood et al. compared structural and functional measures of glaucomatous damage by comparing a functional test [standard automated perimetry (SAP)] with a structural test [optical coherence tomography (OCT)]. The relationship between these two tests was described by a simple linear model. The model predicted that both the SAP sensitivity and the OCT thickness of the retinal nerve fiber layer (RNFL) decrease linearly with retinal ganglion cell loss. It predicted that the structural test (OCT) shows a statistical significance in detecting glaucomatous damage earlier than the functional test. However, if the functional test reaches statistical significance first, this patient was born with a thicker RNFL. Therefore, he had a greater reserve of nerve fibers before the RNFL dropped below the 5th percentile of normal due to glaucomatous damage [6].

In an attempt to increase early detection of glaucoma, where ganglion cell damage occurs, electroretinographical methods have been applied. Nearly 20 years ago, Sutter and Tran introduced the multifocal electroretinogram (mfERG). This method allows simultaneous but independent stimulation of multiple retinal areas. Through cross-correlation, individual electroretinographic responses are then calculated from the overall response recorded, for each stimulated retinal area. This allows an objective topographic examination of retinal function with a high resolution.

When the mfERG was applied in glaucoma, conventional stimulation was not sensitive enough to reliably detect early retinal dysfunction in glaucoma [7–9]. Previous findings showed that interposing bright global flashes into the stimulation sequence increased the inner retinal contributions to the mfERG and therefore its sensitivity in glaucoma. With the use of 3 global flashes, the sensitivity to detect glaucomatous dysfunction was 50 % [10]. One single global flash increased the sensitivity to 75 % and the specificity to 83 %. The most sensitive parameter was the IC-1, the response induced by the effect of the preceding bright and dark elements in the m-frame on the response to the first global flash. Here, an oscillation of the induced components (ICs) of the temporal retina could be observed, which resulted in a small nasal-temporal response asymmetry. This asymmetry was significantly reduced in patients with glaucoma due to a selective loss of this oscillatory component in the temporal retina [11].

In another study using a 2-global flash mfERG, 90 % of the normal tension glaucoma (NTG) patients, 85 % of the high tension glaucoma (HTG) patients, and 80 % of the control group were correctly classified. In that study, the binary bright and dark elements in the m-frame (L_{max} 200 cd/m² and L_{min} <1 cd/m²) were followed by two global flashes with a brightness of 200 cd/m². Again, there was a significant difference in the IC-1. Neither the response to the bright and dark elements in the m-frame, the direct component (DC), nor the response induced by the effect of the preceding bright and dark elements in the m-frame on the response to the second global flash (IC-2) differed significantly between the groups examined [12].

Chu et al. modified a one-global flash paradigm to examine the adaptive function of the retina: Localized luminance differences between bright and dark elements of the m-frame were set at stimulus contrasts of 96, 65, 49, and 29 %. The peripheral IC showed a linear dependence on the luminance difference, while the peripheral DC was saturated at higher luminance

differences. In glaucoma, amplitudes of the peripheral DC were reduced at mid-luminance difference levels and therefore did not reach saturation level as soon. An adaptive index of the DC showed a good differentiation between healthy subjects and glaucoma patients with an area under the curve (AUC) of 0.986 (sensitivity: 93 %, specificity: 95 %) [13]. The total recording time needed to obtain this data was 6×8 min which does not seem feasible in the clinic. Chu et al. also showed that the IC-1 in glaucoma differs most from control at high luminance differences, whereas the DC differs more at medium luminance differences.

In the past, mfERGs have been obtained with a conventional stimulus but at low contrast (7, 9). Here, however, sensitivity of the response to the m-sequence frame, the DC, was not sensitive enough to reliably recognize early glaucomatous dysfunction.

A recent study by Shimada et al. [14] has confirmed the importance of different luminance and contrast behaviors on the DC and on the IC. With a focal flash intensity of 100 cd/m^2 and a global flash of 200 cd/m^2, the induced component is enhanced most, while the DC is still discernible with a reasonable signal-to-noise ratio. Inter-subject variability was largest in the absence of a global flash. In the global flash paradigm, inter-subject variability could be reduced with a lower focal flash intensity. Thus, this study suggests that in a global flash paradigm, reducing the light intensity of the m-frame flash as opposed to the global flash may increase the IC. In the present study, we therefore applied these parameters in an attempt to increase this inner retinal contribution and thus the sensitivity of the mfERG to detect glaucomatous damage.

For the standard multifocal ERG, ISCEV recommends a high pass cutoff between 3 and 10 Hz and low pass cutoff between 100 and 300 Hz [15]. Previous studies found significant differences between glaucoma and control when the bandpass filter was set at 10–300 Hz [7]. Applying the same filter setting, the sensitivity of the mfERG to detect glaucomatous retinal dysfunction was increased when global flash paradigms were introduced [10, 11].

In human glaucoma patients, a focal photopic negative response ERG (PhNR ERG) recorded with a low-frequency cutoff of 5 Hz showed significant reduction of amplitude associated with a local decrease in retinal sensitivity in POAG [16]. In experimental glaucoma in macaques, a glaucoma-sensitive low-frequency component (LFC) was identified under 25 Hz. Lou et al. [17] reported that the low-frequency band can provide information on retinal dysfunction in glaucoma in monkeys. This suggests that in POAG, a filter setting of 1–300 Hz might be more sensitive than a filter setting of 10–300 Hz.

Thus, compared to our previous "2-global flash" mfERG in glaucoma [12], the luminance of the focal m-frame flash was reduced from 200 to 100 cd/m^2. In addition, the mfERG responses analyzed in this study were recorded with a filter setting of 1–300 Hz in an attempt to include low-frequency components and thus increase the sensitivity of the mfERG to glaucoma.

Methods

The adapted 2-global flash mfERG was obtained in one eye of 22 patients (16 patients with NTG, 6 patients with HTG) and 20 control subjects. 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.

For glaucoma patients, the following inclusion criteria were applied:

1. A cup/disc ratio (CDR) of at least 0.5 with typical glaucomatous changes, such as a localized thinning of the neuro-retinal rim of the optic disc.
2. The presence of a glaucomatous visual field defect with a mean defect (MD) ≥ 2.2 dB or a loss variance (LV) ≥ 6 dB.
3. Before treatment with eye drops, a highest measured intraocular pressure (IOP) above 21 mmHg in HTG patients, and an intraocular pressure equal to or below 21 mmHg in NTG patients.

For both glaucoma and control subjects, the following exclusion criteria were applied:

The presence of systemic diseases such as hypertension, diabetes mellitus, Parkinson's disease or depression treated with medication; other ocular diseases such as refractive errors higher than 6 diopters of myopia or hyperopia, damage of the retina caused by an arterial or venous occlusion, previous eye surgery; eye diseases which decrease visual acuity,

such as cataract or corneal damage. If possible, the right eye was included, if it did not fulfill any exclusion criteria.

MfERG recording

Patients were adapted to ambient room light for 30 min before mfERG testing. The pupil of the tested eye was fully dilated (Tropicamide 0.5 %, Phenylephrin 1 %). After cleaning the skin with Everi (spes medica), a ground electrode was placed on the forehead, with the Ten 20[TM] conductive EEG-paste (Weaver and Company). To anesthetize the cornea, Proparacaine 0.5 % and Tetracaine 1 % eye drops were applied. Electrical responses were recorded monocularly with a bipolar Burian-Allen contact lens electrode (Hansen Ophthalmic Development Labs, Iowa City, IA), which was wetted with the hydroxy-propyl methylcellulose Methocel (OmniVision). The other eye was occluded during the recording.

The mfERGs were recorded with VERIS Science 6.1.2[TM] (Visual Evoked Response Imaging System, Electrodiagnostic Imaging, EDI). Refractive errors were corrected by refracting for best visual acuity, using the FMS III-fundus stimulator (EDI, San Mateo, CA). During the recording, the central 50° of the retina was stimulated with 103 hexagonal elements, which were scaled with eccentricity in order to take into account the retinal cone distribution. These hexagons flickered between black and white according to a binary m-sequence of $2^{13}-1$ (L_{max} 100 cd/m^2, L_{min} <1 cd/m^2), followed by two global flashes (200 cd/m^2) at an interval of 26 ms.

Retinal signals were amplified (gain = 50,000) and bandpass filtered at 1–300 Hz. The total recording time of 10 min and 55 s was divided into 16 segments. An infrared camera, included in the device, allowed monitoring of fixation during recording. Segments with poor signal were stopped and re-recorded. The artifact rejection technique, incorporated in the software, was applied twice. Spatial filtering was not used.

Visual fields were obtained with the program G2 of the Octopus perimeter (Octopus 101©, HAAG STREIT, Köniz, Switzerland). The G2 is a threshold static automated perimetry that includes 59 points within the center from 0° to 30°. The macula area is tested with a resolution of 2.8°. An additional 14 peripheral points in the 30°–60° area are screened as well. The stimulus size is equivalent to the Goldmann III/3e stimulus.

Each stimulus is presented for 100 ms. The background luminance is 4 apostilb (asb).

Response analysis

Figure 1 depicts one stimulus base interval: an m-frame (M), which can be light (100 cd/m^2) or dark (<1 cd/m^2) is followed by two global flashes (F, 200 cd/m^2) at an interval of 26 ms. For each focal response, the root mean square (RMS) was calculated for the following three epochs: the direct component at 15–45 ms (DC) and the following two response components, which are induced by the effects of the preceding focal flash on the response to the global flashes at 45–75 ms (IC-1) and at 75–105 ms (IC-2).

The following two group averages were calculated and compared between POAG and control subjects:

1. Five large response averages shown in Fig. 2 were analyzed. These include the central 15° and 4 adjoining quadrants. In glaucoma patients, these quadrant averages were also correlated with the corresponding visual field areas.
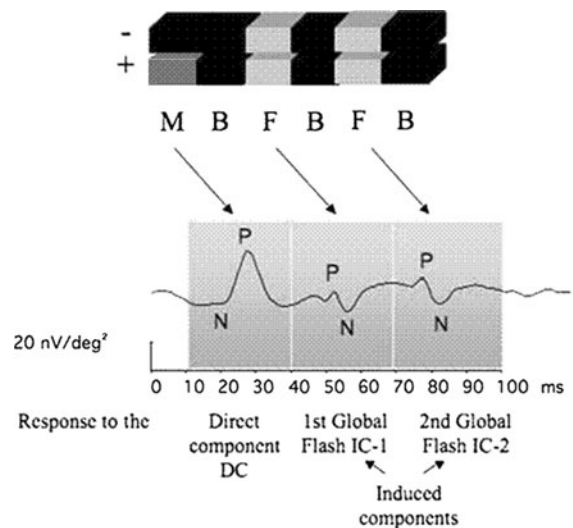

Fig. 1 The stimulus sequence (*top*) and its resulting overall response (*below*). An m-frame (*M*) which can be a light focal flash (100 cd/m^2) or a dark frame (<1 cd/m^2) is followed by two global flashes (*F*) (200 cd/m^2) at an interval of 26 ms. Thus, one stimulus base interval consisted of the following sequence: MBFBFB where *B* is a dark interposed frame (1 frame ≈ 13.33 ms). The resulting response is shown below. It consists of a response to the m-frame stimulus, the direct component (DC) and two following induced components, induced by the effects of the preceding focal flash on the response to the global flashes at 45–75 ms (IC-1) and at 75–105 ms (IC-2)
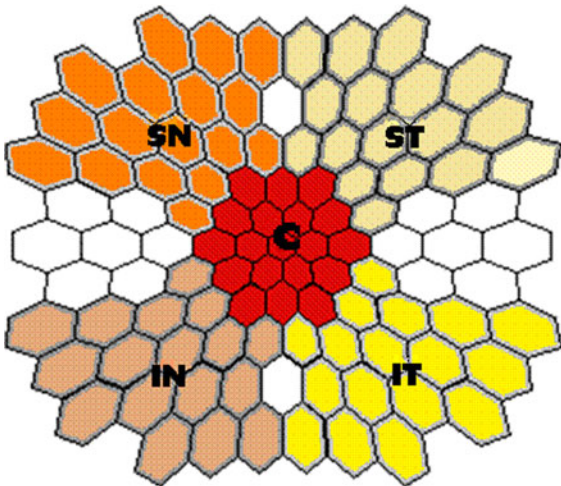
Fig. 2 The field view of the 5 large areas in which responses were averaged to generate the 5 large response averages analyzed. These include the central 15° (*C*) and the 4 adjoining quadrants: *ST* superior temporal quadrant, *SN* superior nasal quadrant, *IT* inferior temporal quadrant, *IN* inferior nasal quadrant
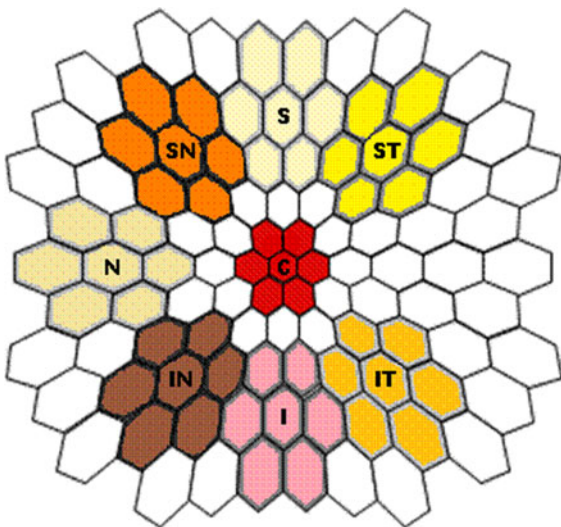
Fig. 3 The field view of the 8 smaller areas in which responses were averaged to form smaller response averages, that is the central 10° (*C*) and 7 surrounding response averages of approximately 14°: *ST* superior temporal area, *S* superior area, *SN* superior nasal area, *N* nasal area, *IT* inferior temporal area, *I* inferior area, *IN* inferior nasal area. These were also analyzed in order to not miss small areas of retinal dysfunction caused by glaucomatous damage. The temporal area in the field view, that is, the nasal part of the retinal region, was excluded as it contained the blind spot

2. Eight smaller response averages depicted in Fig. 3: the central 10° and 7 surrounding response averages of approximately 14° diameter each. These

were additionally analyzed in order to not miss small retinal dysfunctions caused by glaucomatous damage.

Statistical analysis

To predict the mfERG responses expressed as RMS values, a linear mixed effects model was performed. Fixed factors were disease status, location, epoch, and age; subject was a random factor. Results are expressed as differences of means with corresponding 95 % confidence intervals and *p* values.

To discriminate between POAG and control, receiver operating characteristics (ROC) curves with corresponding AUC (area under curve) values were estimated for selected locations and epochs.

A *p* value <0.05 is considered significant. All analyses were done using R version 2.12.0 [18].

Results

Table 1 shows the subjects' clinical characteristics. The control group included 7 men and 13 women, aged 27–73 years [mean 51.4 (SD 14.7) years]. The POAG group comprised 16 men and 6 women, aged 53–74 [mean 63.6 (SD 6.4) years]. Snellen visual acuity was ≥0.8 in all participants and did not differ significantly between the groups.

The mean IOP was slightly higher in the control group [14.8 mmHg (SD 2.7)] than in the glaucoma group [11.2 mmHg (SD 2.1)]. IOP did not differ significantly between the NTG and the HTG group. During the study, all participants had an IOP of ≤20 mmHg.

The mean CDR was 0.27 (SD 0.1) in the control group and 0.79 in the POAG group. Again, there was no significant difference between NTG and HTG group.

The mean absolute value of MD did not differ between the NTG [6.13 dB (SD 4.07, median 4.2)] and the HTG group [6.37 dB (SD 4.61, median 3.9)].

Table 2 depicts the difference of the means for the responses from the 5 larger and 8 smaller group averages (Figs. 2, 3) for control subjects and POAG patients. Table 2 also summarizes these findings for both HTG and NTG compared to control. As an example, Fig. 4 illustrates the response averages for glaucoma patients (right boxplots) compared to control (left boxplots) for the 5 larger response averages.

Table 1 Describes the subjects parameters

	Total	f	m
Control	20	13	7
POAG	22	6	16
NTG	16	6	10
HTG	6	0	6

	Control	POAG	NTG	HTG
VA (Snellen)				
Mean	1.02	0.96	0.97	0.93
SD	0.09	0.10	0.10	0.10
Median	1.00	1.00	1.00	1.00
p value	0.07			
logMAR				
Mean	−0.08	0.02	0.02	0.03
SD	0.25	0.05	0.04	0.05
median	0.00	0.00	0.00	0.00
p value	0.05			
IOP (mmHg)				
Mean	14.80	11.18	11.19	11.17
SD	2.28	2.48	2.10	3.54
Median	15.00	10.50	11.50	0.50
p value	<0.001			
CDR				
Mean	0.27	0.79	0.80	0.76
SD	0.10	0.11	0.12	0.11
Median		0.80	0.80	0.75
p value	<0.001			
Age (years)				
Mean	51.75	63.59	63.63	63.50
SD	14.77	6.35	5.8	8.12
Median	52.00	63.00	63.50	61.00
p value	0.02			
MD (dB)				
Mean		6.19	6.13	6.37
SD		4.11	4.07	4.61
Median		4.20	4.20	3.9

Within the POAG group, NTG did not differ significantly from HTG. IOP was slightly higher in the control group. As expected, the CDR was significantly smaller in the control group. There was also a significant difference in age between control and POAG subjects. This was taken into account during the statistical analysis

The top row presents IC-2, the middle row IC-1, and the bottom row DC. The columns illustrate the individual response averages.

Both control subjects and POAG had significantly larger central than peripheral responses. This held true for all response epochs analyzed. The biggest difference between patients and control subjects could be seen in the central 15° where POAG differed significantly from control in all epochs examined. The comparison of the peripheral large as well as the peripheral small response averages did not show a significant difference between patients and controls.

The difference between patients and control subjects was larger in the central 10° than in the central 15°. The IC-1 epoch differed most and the DC epoch least, when POAG was compared to control. Even though we only included 6 HTG patients, we tried to also assess whether our results would differ if HTG and NTG were looked at separately. HTG differed more from the control group than NTG. Again, IC-1 differed most. In the central 10°, all epochs examined differed significantly from the control group, while in the central 15° this only held true for the HTG group. Here, NTG only differed significantly in the IC-1.

Figure 5 shows the area under the ROC curve for the IC-1 epoch of the central 10°, when POAG is compared to the control group. An ROC provides the ability of a test to differentiate between two groups. The best sensitive discriminatory power for POAG could be shown in the IC-1 of the central 10° with an area under the ROC curve of 0.78. 80 % of the POAG (100 % HTG, 69 % NTG) were correctly classified as abnormal, while 77 % of the control subjects were correctly classified as normal when a cutoff value of 12.52 nV/deg^2 was used.

To assess the association between the MD of the visual field and the mfERG, a linear regression was performed. The responses of the mfERG measures were converted to logarithmic scale. These values were then compared to the corresponding quadrants of the visual field. Results were calculated as regression slopes with corresponding 95 % confidence intervals and p values. There was a tendency for the mfERG to decrease as MD increases. However, this was only statistically significant in the superior nasal quadrant when the IC-1 of the mfERG was compared to the corresponding area of the visual field. Results are not shown.

When compared to the results of a previous 2-global flash paradigm [12], our results did not indicate a higher sensitivity to detect glaucomatous damage. As this might be due to patients being affected differently by their glaucoma, we compared the visual field

Table 2 The responses from the 5 larger and 8 smaller group averages (Figs. 2, 3) for control subjects and POAG patients also separately for HTG and NTG compared to control

Epoch	Comparison of the central area	Group averages	Difference of means ± 2 SEM	*p* value
DC	Glaucoma versus control	5 larger group averages	−1.48 ± 1.16	0.01
IC-1			−2.50 ± 1.16	<0.001
IC-2			−1.89 ± 1.16	<0.001
DC		8 smaller group averages	−2.46 ± 1.44	<0.001
IC-1			−4.77 ± 1.44	<0.001
IC-2			−3.77 ± 1.44	<0.001
DC	HTG versus control	5 larger group averages	−2.27 ± 1.62	0.01
	NTG versus control		−1.18 ± 1.22	*0.05*
IC-1	HTG versus control		−4.47 ± 1.62	<0.001
	NTG versus control		−1.75 ± 1.22	<0.001
IC-2	HTG versus control		−4.08 ± 1.62	<0.001
	NTG versus control		−1.06 ± 1.22	*0.08*
DC	HTG versus control	8 smaller group averages	−2.49 ± 2.06	0.02
	NTG versus control		−2.45 ± 1.52	<0.001
IC-1	HTG versus control		−6.46 ± 2.06	<0.001
	NTG versus control		−4.13 ± 1.52	<0.001
IC-2	HTG versus control		−6.87 ± 2.06	<0.001
	NTG versus control		−2.60 ± 1.52	<0.001

Averages are given in nV/deg^2. Both control subjects and POAG had significantly larger central than peripheral responses. The difference between PAOG and control was larger in the central 10° than in the central 15°. This held true for all response epochs analyzed. HTG differed more from the control group than NTG when they were looked at separately

The italicized numbers in the row of the *p* value highlight the comparisons which do not differ significantly

defects of the patients of the previous study of Palmowski-Wolfe et al. [12] to those of the patients in the present study. The overall MD of the present study: median 4.2 (5–95 % CI 4.37–8.01) did not differ from the MD of the previous study [12]: median 4.5, (5–95 % CI 4.48–6.49). The MD shows a bigger spread in the present study. We also compared the MD of the corresponding quadrants and did not find a difference between the studies ($p = 0.84$) (Table 3).

Discussion

Previous studies have shown that interposing bright global flashes into the stimulation sequence increases the inner retinal contributions to the mfERG and therefore its sensitivity in glaucoma detection [10–12]. Typically, changes are seen in the response to the global flashes.

Figure 1 shows that the mfERG response is derived by adding the responses which follow a bright m-frame flash and by subtracting those following a

dark m-frame. Therefore, a response to global flashes (full-screen flashes) will only be visible in the derived response if it is influenced differently by the response to the preceding focal flash. This is the only stimulus frame that differs in the individual stimulus base intervals. Thus, the presence of a response to a global flash—that is, an induced response component—demonstrates the presence of retinal adaptation which is presumed to be of inner retinal origin [12, 19].

In the present 2-global flash mfERG, the most prominent difference was seen in IC-1 where POAG differed significantly from control in the central 15° and even more in the central 10°. This held true for both NTG and HTG patients.

In glaucoma, the ganglion cells are primarily affected. Glovinsky et al. studied the pattern of foveal ganglion cell loss in glaucoma and observed that larger ganglion cells show a selective loss in experimental glaucoma in monkeys. These authors concluded that testing the function of large foveal ganglion cells would increase detection of early glaucoma damage. The physiological variability of cell density is larger in
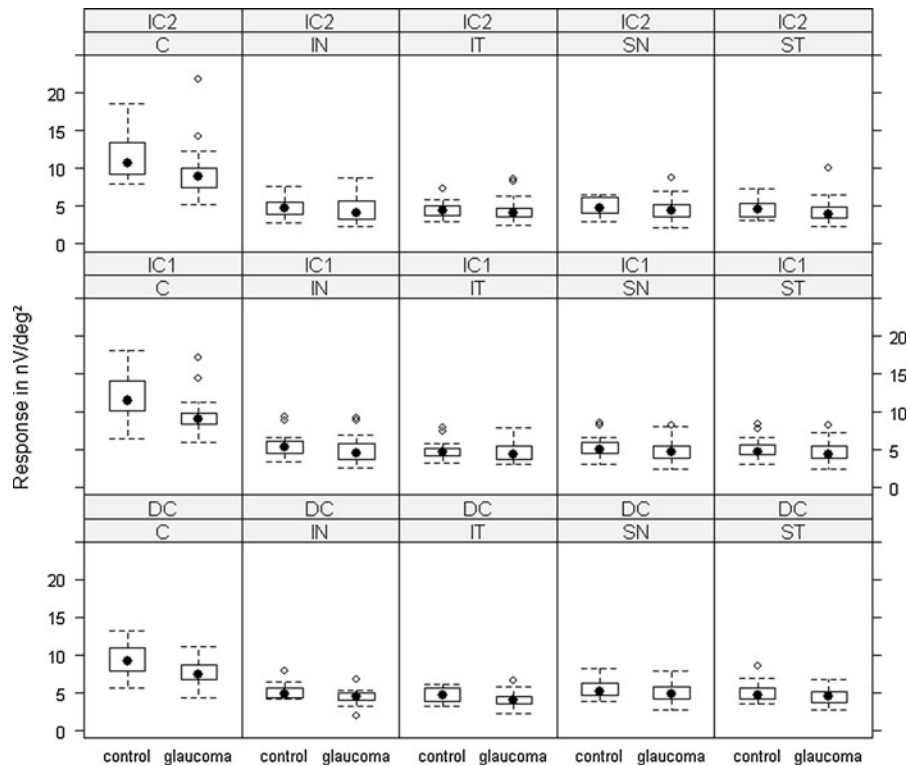
Fig. 4 Glaucoma patients (*right box plot*) are compared to the control group (*left box plot*) for the 5 larger response averages. Each boxplot shows the following: first quartile (*lower end of box*), median (*point in box*) and third quartile (*upper end of box*). The whiskers represent the lowest data point (*lower whisker*) still within 1.5 interquartile range (IQR) of the lower quartile and the highest data point (*upper whisker*) still within 1.5 IQR of the upper quartile. Outliers are shown as *open rectangles*. The *top row* presents IC-2, *the middle row* IC-1 and the *bottom row* DC. The columns illustrate the individual response averages: *C* central 15°, *IN* inferior nasal area, *IT* inferior temporal area, *SN* superior nasal area, and *ST* superior temporal area. POAG differed significantly from control in the central 15° in all epochs examined

the peripheral areas of the retina than in the central ones. So tests for detecting glaucomatous damage may be more precise if they involve the responses of the ganglion cells of the central area which thus becomes the area of maximum interest [20]. Interestingly, in our study, the smaller central response averages appeared to differ more between POAG and control than the larger central response averages. This may reflect a more focal glaucomatous damage in the patients that may be lost if larger areas are averaged together.

Our finding of a significant central loss in the RMS amplitudes of the DC, IC-1, and IC-2 of the 2-global flash mfERG is in agreement with previous reports of macular involvement in glaucoma [20–22]. It is also consistent with previous OCT findings of macular involvement in glaucoma, where a qualitative reduction in the thickness of the RGC layer by computer-aided manual segmentation procedure corresponds to local losses in visual field sensitivity [23].

When the results of the mfERG were compared to the visual fields, we saw a tendency for the mfERG to decrease as MD increased. However, this was only significant in the superior nasal field (quadrant) when the IC-1 of the mfERG was compared to the corresponding area of the visual field. The significance in the other areas may be impaired, as there is a wide range of mfERG responses in the lower range of MD, which is the range in which patients are expected to have a more localized field defect.

In the POAG group, HTG patients differed more from the control group than NTG patients. However, both groups did not differ in MD ($p = 0.87$), cup/disc ratio, or age. However, this needs to be viewed with caution, as only 6 HTG patients were included compared to 16 NTG patients.

For POAG, the best sensitive discriminatory power could be shown in the IC-1 of the central 10° with an area under the ROC curve of 0.78. With a cutoff value

ROC Curve
Epoche: IC1

AUC 78.2%
95%CI: 63.7-92.7%
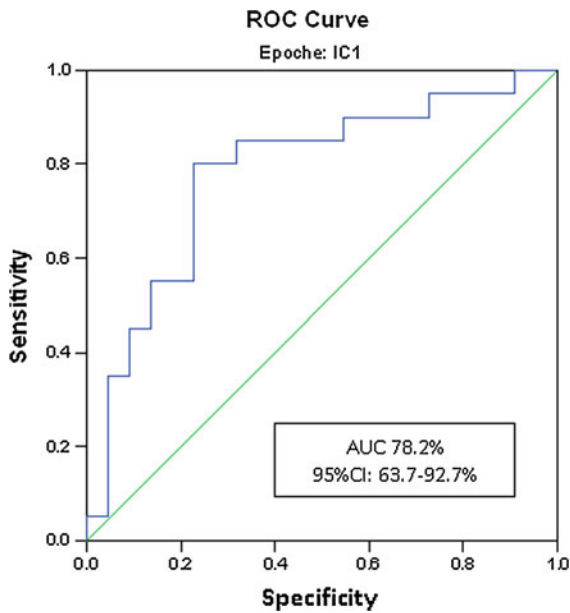
Fig. 5 The receiver operating characteristics (ROC) *curve* for the IC-1 epoch of the central 10°, when POAG was compared to the control group. An ROC gives information about sensitivity and specificity of a test and thus provides the ability of a test to differentiate between two groups. The best sensitive discriminatory power for POAG could be shown in the IC-1 of the central 10° with an area under the ROC curve of 0.78

of 12.52 nV/deg², 80 % of the POAG (100 % HTG, 69 % NTG) were correctly classified as abnormal, while 77 % of the control subjects were correctly classified as normal.

While previously POAG only differed from control in IC-1 [12], changing the luminance conditions of the 2-global flash paradigm revealed differences in all epochs: the DC, the IC-1, and the IC-2. However, when the IC-1, the most sensitive individual parameter, was analyzed in the central 10°, the current change in luminance conditions did not increase the sensitivity and specificity of the mfERG to detect glaucoma further.

This is not due to different stages of glaucoma, because when the visual field MD defects for all quadrants of both studies were compared, the POAG patients of both studies did not differ significantly ($p = 0.84$). However, these results may be influenced by differences in distribution of more localized field loss.

Two further differences exist between this study and the previous one [12] that might influence the results:

First, the differing stimulation characteristics of the FM-III stimulator and the CRT (cathode ray tube) monitor. The latter was used in our previous study and also in the study of Shimada et al. [14] which suggested the change in stimulus parameters. On the other hand, this is unlikely to be relevant, as the present study shows that when using an FMIII stimulator, glaucomatous dysfunction can be observed not only in the first induced component of the 2-global flash mfERG, but also in DC and IC-2. As described in the guidelines for clinical multifocal electroretinography of ISCEV, CRT and LCD (liquid crystal display) monitors differ from each other in the response time of displays [15]. The FMSIII system applied in our study uses a LCoS (liquid crystal on silicon) display which may be compared to an LCD stimulator. The amplitude and waveform of the mfERG can be affected by these different modes of stimulation. Kaltwasser et al. compared the suitability of a CRT and a LCD monitor in the mfERG. The pixel of a CRT monitor lights up with a very high intensity directly at the beginning of a frame. After 2–3 ms, luminance decays and remains dark until the next stimulus frame. In an LCD monitor, the liquid crystals need time to align themselves in a new orientation in the cell's electric fields. Therefore, in an LCD monitor, the pixel lights up slowly with a delay of 2–10 ms and luminance remains constant for the remaining frame length. At the end of the frame, light emission is slowly decreased to zero. The most

	MD ST	MD SN	MD IN	MD IT
Median				
Present study	4.95	6.50	2.45	2.75
Previous study	4.85	4.55	3.25	3.75
95 % CI				
Present study	4.25–8.26	5.27–10.70	3.27–9.14	2.14–6.41
Previous study	5.00–8.24	4.60–8.32	3.63–6.45	2.96–4.79
p value	0.76	0.90	0.46	0.51

Table 3 Comparing the MD (in dB) of the corresponding quadrants of the visual fields of the present to the previous study [12]

There is no significant difference between them ($p = 0.84$)

important observation in this study was an increase in N1 and P1 latencies while using the LCD monitor. Thus, a good mfERG response can be recorded with either stimulator, but because of the differences in the resulting responses, the reader needs to know which stimulation device was used [24]. As we do not compare mfERGs across studies, it is unlikely that the different stimulator used at present would greatly influence the difference in sensitivity to glaucoma observed.

Second, the different filter setting which was 1–300 Hz in the present study, but 10–300 Hz in the previous study [12]. A recent study has shown a glaucoma sensitive response in the lower frequencies of the 2-flash mfERG, the LFC [17]. Therefore, we changed the filter setting as described above.

In conclusion, reducing the luminance of the m-frame flash to 100 cd/m^2 in the 2-global flash paradigm and lowering the high pass filter to 1 Hz did not increase the sensitivity and specificity of the mfERG to detect glaucoma further, when compared to findings from previous studies.

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