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Slow-stimulated multifocal ERG in high- and normal-tension glaucoma

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Abstract

Purpose: To study the ability and sensitivity of the slow stimulation multifocal ERG (mfERG) to detect glaucomatous damage. *Methods:* Right eyes of 20 patients with normal-tension glaucoma (NTG), 15 patients with high-tension glaucoma (HTG) and 15 healthy volunteers underwent testing with the mfERG (VERIS 4.1^{TM}). The central 50 degrees of the retina were stimulated by 103 hexagons (m-sequence: 2^{13}-1, Lmax: 100 cd/m^2, Lmin: 1 cd/m^2, background: 50 cd/m^2). Each m-sequence step was followed by 3 black frames (Lmax: < 1 cd/m^2). Five response averages of the first order response component (KI) were analyzed: the central 7.5 degrees and the 4 adjoining quadrants. The amplitudes from the first minimum, N1, to the first maximum, P1, and from P1 to the second minimum, N2, were analyzed as well as the latencies of N1, P1, N2 and the latencies of 3 multifocal oscillatory potentials (mfOPs) with their maxima at about 73, 80 and 85 ms. *Results:* For each parameter the percentage of deviation from the mean of the control group was calculated. These values were then added for each individual to form a deviation index (DI). Seventeen patients (85.0%) with NTG and 3 patients (20.0%) with HTG showed a DI outside the normal range. The major changes were observed in the mfOPs of the NTG patients. MfOPs were then selectively filtered at 100–300 Hz and their scalar product was analyzed over an epoch of 68–105 ms. This confirmed that mfOPs differed significantly from the control in the central 7.5° and, for NTG, in the nasal field. With a logistic regression analysis the mfOPs had a sensitivity to differentiate 85% of the NTG patients and 73% of the HTG patients from normal. *Conclusions:* Under these conditions, the slow-stimulated mfERG can detect glaucomatous dysfunction in NTG (85.0%). The differences observed between NTG and HTG are in support of a different underlying pathomechanism.

Introduction

Open angle glaucoma (OAG) is the second leading cause of vision loss worldwide [1]. As early therapeutic intervention may prevent progression and blindness, it is important to detect glaucoma at an early stage. Diagnosis is especially difficult in normal-tension glaucoma (NTG), where the intraocular pressure, which is one of the risk factors for OAG is less than 22 mmHg, that is in the normal range.

The multifocal ERG (mfERG), which permits a topographic display of retinal function, has shown promise in the investigation of OAG. It has been reported that the mfERG response contains a so called retinal component (RC) of presumed outer retinal origin and an inner retinal contribution such as the optic nerve head component (ONHC), which is attributed mainly to the ganglion cell layer [2]. The ONHC and the RC differ in their luminance- and contrast-sensitivity [3]. As the ONHC saturates at about 60% contrast, whereas the RC tends to increase linearly with increasing contrast, attempts have been made to increase the inner retinal contribution through decreasing the stimulus contrast. How-

ever, mfERGs at a low contrast (50%) were not sensitive enough to reliably detect retinal dysfunction in individual patients with OAG [4, 5].

Recently it has been found, that naso-temporal asymmetries in the oscillation rich contributions to a special slow mfERG stimulus sequence are caused by the changes in the relative alignment of the ONHC and the RC [6]. Therefore this stimulus holds promise in the investigation of glaucomatous functional damage. In this study we tested it's sensitivity in different forms of open angle glaucoma.

Methods

The subjects consisted of 20 patients with different stages of normal-tension glaucoma (NTG), 15 patients with high-tension glaucoma (HTG) and a regulated intraocular pressure, as well as 15 healthy volunteers. Informed consent was obtained from all subjects after explaining the procedure. The Declaration of Helsinki was followed.

Inclusion criteria for both groups of glaucoma were the presence of glaucomatous visual field defects (octopus d32). For patients with normal-tension glaucoma the highest intraocular pressure (IOP), measured by Goldman applanation tonometry, was less than 22 mmHg and the cup disk ratio (CDR) was 0.5 or higher. For patients with HTG the highest intraocular pressure (IOP) recorded on Goldman applanation tonometry, was over 22 mmHg. Other ocular diseases were excluded.

MfERGs were recorded of the right eyes using VERIS^{TM}. The mfERG signals were recorded monocularly with the help of a Burian-Allen bipolar contact lens electrode. The ground electrode was on the forehead. The pupils were dilated, the cornea was anesthetized. Refractive errors were corrected for best visual acuity at a viewing distance of 40 cm, the viewing distance was then adjusted to keep the image size constant [7].

During recording, the central 50 degrees of the retina were stimulated by 103 hexagons where each hexagon flickered according to a slow m-sequence stimulation. Figure 1 shows the stimulus sequence where each m-sequence step (M) with a luminance of either 100 or <1 cd/m^2 was followed by 3 black frames (B) with a luminance <1 cd/m^2. This four frame stimulus sequence

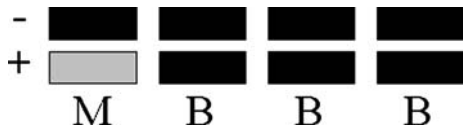

Figure 1. This figure depicts the stimulus sequence applied (MBBB-Sequence). The luminance of the m-sequence step (M) was either 100 or <1 cd/m^2, luminance of the interposed black frames (B) was <1 cd/m^2. The background was set at 50 cd/m^2. Each frame lasted 13.33 ms, resulting in a stimulus base interval of 53.3 ms.

(MBBB) re-occurred every 53.3 ms. The length of the m-sequence was 2^{13}-1. Total recording time was 7 min 17 s. To enhance the signal-quality each recording was split into 16 or 32 cycles of about 27.29 or 13.65 s. Contaminated segments were discarded and re-recorded. The raw signals were filtered (10–300 Hz) and amplified (gain = 100 000). 16 samples were obtained per display frame (sampling interval: 0.83 ms). An artifact elimination technique [8] was applied once. The first order response component (KI) was analyzed. For each location KI can be described as the difference between the mean local response to all the bright m-sequence stimuli and the mean focal response to the black m-sequence stimuli occurring in a stimulus cycle and taking into account the entire stimulus base interval (Figure 1).

Results

Table 1 summarizes the clinical information of the 20 NTG patients included in the study. Mean age was 50.8 years, mean Snellen visual acuity (VA) was 1.05. The mean CDR of the NTG patients was 0.73. Mean visual field parameters (Octopus d32) were as follows, mean sensitivity: 20.50 dB, mean defect: 6.98 dB and loss variance: 24.35 dB^2.

Table 2 depicts the clinical data of the 15 HTG patients included in the study. Here, mean age was 58.0 years, mean VA was 0.87. The mean CDR of 0.71 compared well to that of the NTG group. Mean visual field parameters (Octopus d32) of the HTG patients were as follows, mean sensitivity: 17.81 dB, mean defect: 8.64 dB and loss variance: 33.31 dB^2.

Figure 2 shows each subject's overall response average for the control group (left), the NTG group (middle) and the HTG group (right). The mean overall response, is shown at the bottom.

Table 1. Characteristics of the NTG patients examined

NTG ID	Gender	Age [years]	Visual acuity log MAR	CDR (cup disk ratio)	MS (mean sensitivity) [dB]	MD (mean defect) [dB]	LV (loss variance) [dB^2]
NTG 01	m	61	0.15	0.5	18.8	8.2	34.4
NTG 02	f	46	−0.1	0.9	19.8	7.5	19.5
NTG 03	f	40	0	0.8	23.0	4.5	7.3
NTG 04	f	62	−0.08	0.5	20.3	5.5	28.5
NTG 05	f	33	−0.1	0.8	26.1	2.0	6.5
NTG 06	f	52	−0.08	0.7	18.3	19.3	16.4
NTG 07	f	56	0.15	0.9	14.9	11.7	51.4
NTG 08	m	70	0	0.7	16.9	8.8	30.4
NTG 09	f	18	−0.1	0.8	26.0	2.9	11.0
NTG 10	f	29	−0.1	0.8	25.1	3.3	18.4
NTG 11	m	63	0	0.7	21.4	4.8	17.8
NTG 12	m	26	−0.08	0.5	23.2	5.4	10.4
NTG 13	f	65	0	0.75	11.9	14.1	80.8
NTG 14	m	63	0	0.5	16.8	9.3	57.3
NTG 15	f	65	0	0.8	22.0	3.9	21.1
NTG 16	f	35	0	0.75	23.0	5.0	17.5
NTG 17	f	53	0	0.7	24.9	1.8	6.6
NTG 18	m	50	0	0.65	24.1	2.9	9.1
NTG 19	f	64	0	0.9	18.9	7.2	22.0
NTG 20	f	65	0	0.9	14.6	11.4	20.6
Mean ± SD		50.80 ± 15.53	−0.02 ± 0.07	0.73 ± 0.14	20.50 ± 4.08	6.98 ± 4.49	24.35 ± 19.10

Table 2. Characteristics of the HTG patients examined

HTG ID	Gender	Age [years]	Visual acuity log MAR	CDR (cup disk ratio)	MS (mean sensitivity) [dB]	MD (mean defect) [dB]	LV (loss variance) [dB^2]
HTG 01	m	49	−0.1	0.8	20.0	7.1	28.6
HTG 02	m	36	0	0.8	17.9	10	53.2
HTG 03	f	56	0	0.9	15.9	10.7	85.7
HTG 04	f	71	0	0.9	10.8	14.7	66.1
HTG 05	m	64	0	0.8	24.8	1.3	12.3
HTG 06	f	65	0	0.5	23.3	2.7	24.6
HTG 07	m	53	0	0.6	24.3	2.5	20.7
HTG 08	f	71	0	0.9	16.2	9.4	47.2
HTG 09	f	59	0.15	1.0	0.9	25.5	16.4
HTG 10	f	64	0.05	0.9	14.9	11.2	48.2
HTG 11	f	39	−0.08	0.1	25.1	2.6	18.9
HTG 12	f	68	−0.10	0.4	24.0	1.7	8.5
HTG 13	m	56	1.0	1.0	2.3	24.3	31.2
HTG 14	m	58	0	0.7	23.9	2.6	23.0
HTG 15	f	61	0	0.4	22.9	3.3	15.1
Mean ± SD		58.0 ± 10.5	0.06 ± 0.27	0.71 ± 0.26	17.81 ± 7.89	8.64 ± 7.83	33.31 ± 22.17

The response to the MBBB stimulus consists of a first minimum, N1, followed by a maximum, P1, and then a second minimum, N2. Approximately one base interval later, the response average contains 3 multifocal oscillatory potentials (mfOPs) with their peaks at about 73, 80 and 85 ms. A marked difference can be observed between the mfOPs of the NTG-response average

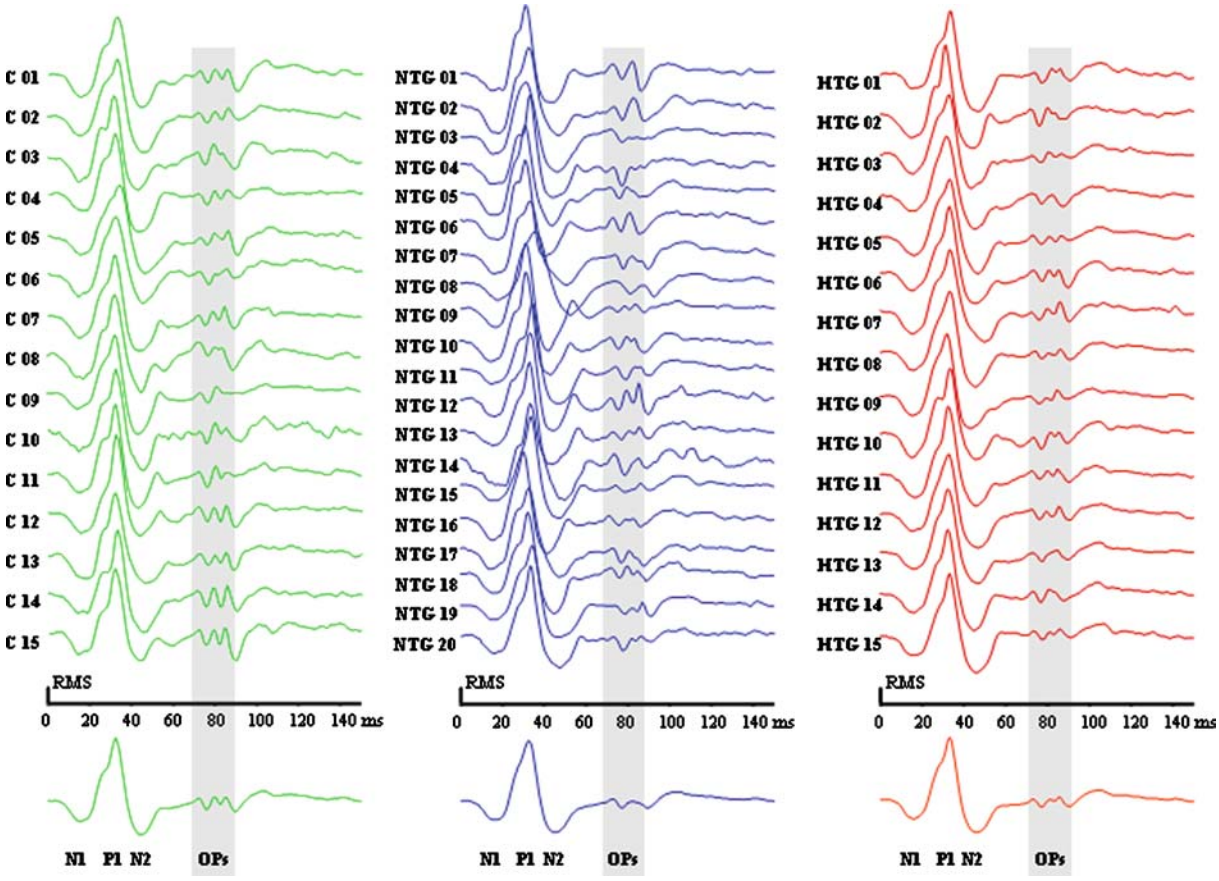

Figure 2. This figure shows each subject's overall response average for the control group (left), the NTG group (middle) and the HTG group (right). The mean overall response, is shown at the bottom. The response to the MBBB stimulus consists of a first minimum, N1, followed by a maximum, P1, and then a second minimum, N2. One base interval later, 3 multifocal oscillatory potentials (mfOP) can be observed. In order to allow a better comparison of the waveforms, responses were normalized to have an equal root mean square (RMS). There is a marked difference between the mfOPs of the NTG-response average and the mfOPs of the control- or HTG-response average.

and the mfOPs of the control- or HTG-response average.

In order to take into consideration the naso-temporal variation of the mfOPs [6], five response averages were formed. Figure 3 (top) depicts these 5 response averages that consisted of the central 7.5 degrees (center) and four adjoining quadrants A–D. Quadrant A constitutes the response average from the upper temporal field, quadrant B from the upper nasal field, quadrant C from the lower nasal field and quadrant D from the lower temporal field.

Figure 3 (bottom) shows the resulting traces of the 5 response averages analyzed. For each response average, the response of the control group is shown at the top, the middle trace represents the average of the NTG patients and the bottom trace the HTG patients. While the central response average shows mfOPs in NTG, HTG and in the control group, the mfOPs appear diminished in all field quadrant averages of the NTG-group.

In every subject's mfERG, the amplitudes of N1P1 and P1N2 were analyzed as well as the corresponding latencies of N1, P1, N2 for each of the five response averages. In addition, the latencies of the 3 mfOPs with their maxima at about 73, 80 and 85 ms were measured. A reliable measurement of mfOP latencies was possible even in patients with NTG, as in an individual's group response averages the individual mfOP peaks were more clearly depicted than in

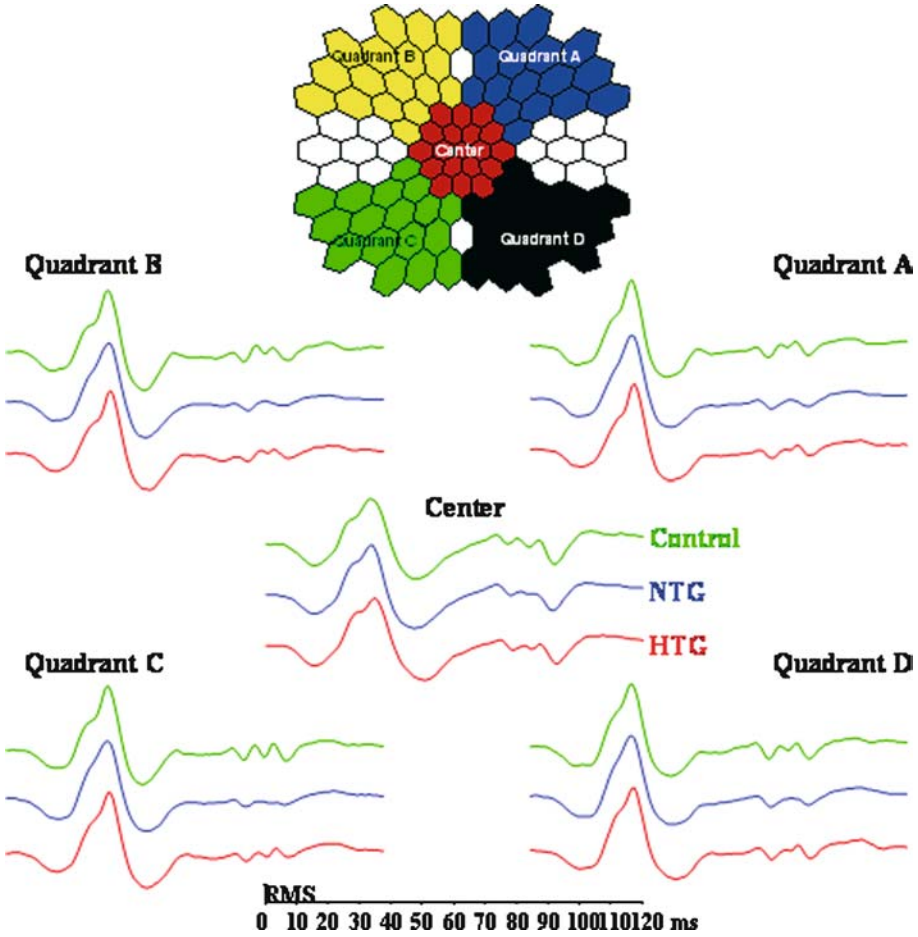

Figure 3. In the central 50 degrees responses of the central 7.5 degrees (Center) and the four adjoining quadrants (Quadrants A–D) were averaged as shown at the top. Quadrant A constitutes the upper temporal field, quadrant B the upper nasal field, quadrant C the lower nasal field and quadrant D the lower temporal field. Below the naso-temporal asymmetries of the respective mfERG responses averages are shown (center and the four quadrants). For each response average, the response of the control group is shown at the top, the middle trace represents the average of the NTG patients and the bottom response of the HTG patients. In order to allow a better comparison of the waveforms, responses were normalized to have an equal root mean square (RMS). In the quadrants the NTG-group again clearly differ in the range of the three mfOPs, that is between 70 and 90 ms.

the average of the 20 NTG patients shown in Figures 2 and 3. Table 3 shows the mean amplitudes N1P1 and P1N2 and the latencies of N1, P1, N2 as well as the 3 mfOPs for each response average. The standard deviation expresses the high inter-individual variability which results in an overlap between the groups that precludes the observation of a significant difference.

In order to reduce the inter-individual variability, the amplitudes of an individual's response averages were normalized to the amplitudes of this individual's overall response. For example, for each recording, the amplitude of N1P1 in quadrant A was divided by N1P1 of the overall response of the same recording. For each parameter (normalized amplitudes, latencies N1, P1, N2 and mfOP-latencies) the percentage of deviation from the mean of the control group was calculated. Adding these values resulted in a group deviation index for each of the five response averages. To obtain only one parameter that describes the mfERG response, an individual's 5 group deviation indices were added to form an overall deviation index.

Table 4 shows the resulting group deviation indices and the overall deviation index for the 20 NTG patients, while Table 5 depicts these values for the 15 HTG patients. The patients' data can

Table 3. This table shows the mean amplitudes N1P1 and P1N2 and the latencies of N1, P1, N2 as well as the 3 mfOPs. The standard deviation (SD) gives an indication of the inter-individual variability

		Center	A	B	C	D
N1P1 (nV/deg^2)	C	49.33 (SD 10.85)	28.43 (SD 7.75)	28.72 (SD 7.36)	29.84 (SD 8.03)	28.5 (SD 7.18)
	NTG	52.03 (SD 16.65)	34.35 (SD 18.04)	31.56 (SD 11.92)	33.48 (SD 11.66)	34.26 (SD 12.27)
	HTG	55.80 (SD 11.94)	36.03 (SD 9.36)	35.82 (SD 8.50)	35.28 (SD 7.29)	35.52 (SD 8.04)
P1N2 (nV/deg^2)	C	59.30 (SD 11.93)	33.13 (SD 9.46)	35.89 (SD 9.39)	35.65 (SD 9.57)	32.03 (SD 7.84)
	NTG	65.36 (SD 23.44)	39.95 (SD 18.04)	38.53 (SD 17.57)	39.43 (SD 16.27)	38.37 (SD 14.44)
	HTG	66.72 (SD 15.56)	42.41 (SD 11.02)	43.39 (SD 11.04)	42.07 (SD 9.20)	39.83 (SD 8.63)
N1 (ms)	C	15.78 (SD 1.07)	16.11 (SD 1.58)	15.99 (SD 1.47)	16.55 (SD 1.75)	16.71 (SD 2.40)
	NTG	16.21 (SD 1.98)	16.68 (SD 2.07)	17.00 (SD 1.98)	17.91 (SD 1.77)	18.38 (SD 1.95)
	HTG	15.50 (SD 0.88)	16.12 (SD 1.29)	16.27 (SD 1.26)	16.45 (SD 1.65)	17.99 (SD 1.40)
P1 (ms)	C	33.62 (SD 1.12)	32.29 (SD 0.91)	32.61 (SD 0.87)	32.45 (SD 0.72)	32.28 (SD 0.72)
	NTG	33.79 (SD 1.42)	32.91 (SD 1.73)	32.71 (SD 1.49)	32.30 (SD 1.79)	32.50 (SD 1.71)
	HTG	33.90 (SD 1.53)	32.87 (SD 0.82)	33.05 (SD 0.88)	32.59 (SD 0.88)	32.65 (SD 0.71)
N2 (ms)	C	48.01 (SD 1.77)	43.81 (SD 2.36)	44.03 (SD 1.60)	43.47 (SD 1.41)	44.26 (SD 2.12)
	NTG	47.35 (SD 3.60)	44.97 (SD 3.73)	45.59 (SD 3.39)	45.05 (SD 3.34)	44.92 (SD 3.57)
	HTG	50.25 (SD 1.77)	44.80 (SD 2.06)	45.46 (SD 1.70)	45.14 (SD 1.57)	46.52 (SD 1.75)
OP1 (ms)	C	73.79 (SD 0.74)	72.48 (SD 0.88)	72.87 (SD 1.07)	72.20 (SD 0.82)	72.48 (SD 0.77)
	NTG	74.93 (SD 2.14)	73.80 (SD 2.60)	74.35 (SD 2.30)	73.76 (SD 2.52)	73.21 (SD 2.65)
	HTG	75.08 (SD 0.63)	73.19 (SD 0.92)	73.89 (SD 0.88)	72.99 (SD 0.80)	72.92 (SD 0.61)
OP2 (ms)	C	81.97 (SD 3.25)	80.13 (SD 0.90)	80.21 (SD 0.72)	79.63 (SD 0.82)	80.25 (SD 0.98)
	NTG	82.36 (SD 1.49)	81.26 (SD 2.22)	81.93 (SD 2.83)	81.26 (SD 2.12)	81.98 (SD 2.43)
	HTG	82.13 (SD 0.99)	81.21 (SD 1.13)	81.24 (SD 0.93)	80.86 (SD 0.92)	81.08 (SD 0.92)
OP3 (ms)	C	87.59 (SD 1.12)	85.26 (SD 1.16)	85.42 (SD 0.98)	85.63 (SD 0.90)	85.52 (SD 0.91)
	NTG	87.64 (SD 2.04)	87.09 (SD 3.85)	87.38 (SD 4.28)	86.73 (SD 2.76)	87.67 (SD 3.78)
	HTG	87.75 (SD 1.2)	85.47 (SD 1.07)	86.63 (SD 1.80)	86.27 (SD 1.05)	86.85 (SD 2.94)

be compared to the range of normal which is shown in the lower two rows. In Tables 4 and 5 deviation indices outside the normal range are highlighted in black. An overall deviation index outside the range of the control group could be observed in 17 NTG patients but only in three HTG patients. This corresponds to a sensitivity of 85% for NTG and only 20% for HTG. Figure 4 (left) shows a boxplot of the overall deviation index which graphically highlights these results.

Table 6 shows how often the individual parameters were outside the normal range for each of the 5 response averages analyzed. Thus, these tables demonstrate which of the analyzed parameters (amplitudes, latencies or mfOP latencies) are most effected in glaucoma. Overall, the mfOP latencies differed most between NTG patients and the control group. However, these differences were only seen in the peripheral response averages, while in the central 7.5 degrees no glaucoma patient showed mfOP latencies outside the normal range. The second column from the right in Table 6 summarizes the number of patients that showed a group deviation index outside the range of norm for each group response average. Here the two upper quadrants (quadrants A and B) differed most. These changes did not correlate with the changes observed in the visual fields (Tables 6, rightmost column).

In order to appraise our results in a less examiner dependent manner, we selectively filtered the data at 100–300 Hz in order to isolate the mfOPs from the underlying response components. Over an epoch of 68–105 ms we formed the scalar product (SP), using the waveform of the respective group average as a template [8]. The average scalar product was calculated for each of the 5 groups. In addition to including information on latency, the scalar product also includes information on changes in amplitude. In contrast to absolute measurements of amplitude and latency, the SP measurement is less susceptible to the influence of noise. In order to ensure a normalized distribution, the log of the SP values was formed and an analysis of variance

Table 4. Group and overall deviation indices are shown for the 20 NTG patients (NTG 01 to NTG 20). Values outside the range of normal, which is shown in the two lower rows, are highlighted. The indices describe the deviation from the mean of the control group for the parameter analyzed (normalized amplitudes, latencies and mfOPs). Individual deviation indices were then added for each response average to form a group deviation index. In order to obtain a single measure that describes the mfERG, the group deviation indices were added to obtain an overall deviation index for each subject

ID	Group deviation indices – NTG					Overall deviation index
	Center	Quadrant A	Quadrant B	Quadrant C	Quadrant D	
NTG 01	**105**	**64**	41	**58**	**59**	**327**
NTG 02	50	**57**	**50**	29	41	**226**
NTG 03	45	32	36	**63**	53	**228**
NTG 04	49	**64**	**50**	34	47	**244**
NTG 05	**61**	34	**52**	**67**	**94**	**309**
NTG 06	27	**57**	41	48	**87**	**359**
NTG 07	31	**52**	**87**	**100**	**56**	**326**
NTG 08	**62**	**77**	**62**	**87**	**79**	**367**
NTG 09	46	**79**	25	43	35	**228**
NTG 10	24	27	22	14	38	124
NTG 11	50	**61**	38	46	38	**233**
NTG 12	33	27	37	21	41	158
NTG 13	53	23	21	23	36	156
NTG 14	**75**	**129**	**119**	**64**	45	**432**
NTG 15	49	36	39	37	29	**189**
NTG 16	49	47	30	42	31	**199**
NTG 17	52	35	**46**	32	48	**212**
NTG 18	25	**57**	**57**	43	31	**213**
NTG 19	52	**51**	40	26	26	**195**
NTG 20	44	**60**	**51**	**64**	**60**	**280**
Mean ± SD	49.0 ± 18.4	53.3 ± 24.3	47.2 ± 22.8	47.1 ± 22.5	48.7 ± 19.1	245.3 ± 76.0
Normal range						
Min	**14**	**8**	**8**	**12**	**16**	**83**
Max	**60**	**48**	**44**	**49**	**55**	**185**

(ANOVA) was performed. Age did not influence the results ($p = 0.95$). To adjust for multiple testing, the Tukey test was performed as a *post hoc* test.

Figure 5 depicts the boxplots of the scalar product for each response average showing a reduced SP in the mfOPs of glaucoma patients in all response averages. For patients with NTG, this reached a significance level in the nasal field (quadrant B, $p = 0.014$, and quadrant C, $p = 0.001$) as well as in the central response average ($p = 0.022$). HTG patients only differed significantly from the control group in the central response average ($p = 0.024$).

In order to test for sensitivity, we then performed a stepwise logistic regression using SPSS. For NTG patients, quadrants C and A contained the most relevant parameters, allowing 85% of NTG patients to be differentiated from normal (Table 7). For HTG patients, the central response average contained the most relevant parameters, allowing 73% of patients with HTG to be separated from normal (Table 8).

Discussion

A slow stimulation mfERG was applied in order to test it's ability and sensitivity to detect glaucomatous damage in NTG and HTG. When an 'overall deviation index' was calculated, glaucomatous retinal dysfunction in this MBBB stimulus derived mfERG, could be detected with a sensitivity of 85.0% in NTG but only 20% in HTG. Major changes were observed in an induced component, the three mfOPs, with an average latency of 73, 80 and 86 ms.

Table 5. Group and overall deviation indices of the 15 HTG patients (HTG 01 to HTG 15) are depicted in this table. As in Table 4, values outside the range of normal, which is shown in the two lower rows, are highlighted. The indices describe the deviation from the mean of the control group for the parameter analyzed (normalized amplitudes, latencies and mfOPs). Individual deviation indices were then added for each response average to form a group deviation index. In order to obtain a single measure that describes the mfERG, the group deviation indices were added to obtain an overall deviation index for each subject

ID	Group deviation indices – HTG					Overall deviation index
	Center	Quadrant A	Quadrant B	Quadrant C	Quadrant D	
HTG 01	22	20	**50**	21	40	153
HTG 02	28	34	13	25	29	128
HTG 03	45	18	11	25	**58**	156
HTG 04	54	21	30	46	38	**188**
HTG 05	24	29	41	45	28	166
HTG 06	48	22	38	22	46	175
HTG 07	28	47	27	44	36	182
HTG 08	43	28	25	36	27	159
HTG 09	**63**	46	41	24	39	**213**
HTG 10	19	32	28	42	26	146
HTG 11	22	27	**56**	41	27	172
HTG 12	33	15	19	29	20	116
HTG 13	**63**	26	28	30	20	166
HTG 14	57	17	15	13	20	122
HTG 15	39	**49**	21	33	**59**	**201**
Mean ± SD	38.9 ± 15.4	28.7 ± 11.2	29.5 ± 13.3	31.7 ± 10.2	34.1 ± 12.6	162.9 ± 27.6
Normal range						
Min	**14**	**8**	**8**	**12**	**16**	**83**
Max	**60**	**48**	**44**	**49**	**55**	**185**

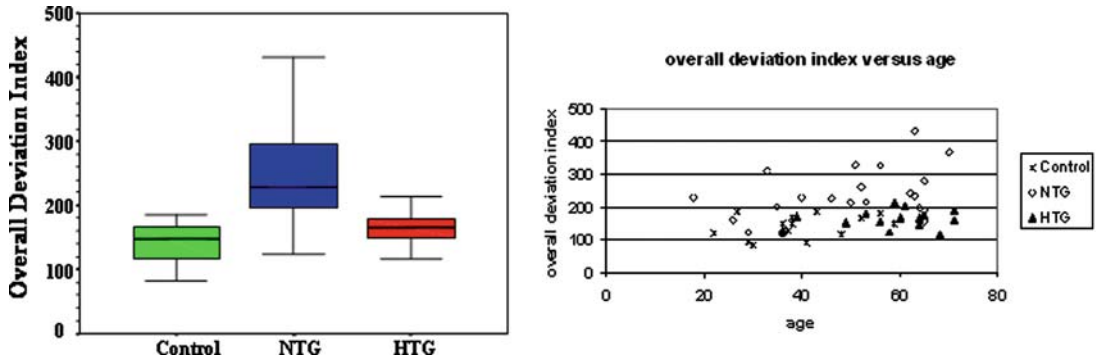

Figure 4. This figure shows the distribution of the overall deviation index. To the left boxplots of the overall deviation index are depicted. The whiskers (upper and lower horizontal bars) represent the range of values, the bold horizontal bar depicts the median. The box represents the interquartile interval, from the 25th to the 75th percentile. To the right a scatter plot of the overall deviation index versus age is shown. There was no significant influence of age on the overall deviation index (Spearman Rank Test). Three of the 15 HTG patients (20%) and 17 of the 20 NTG patients showed an overall deviation index outside the norm, corresponding to a sensitivity of 85% for NTG. The three NTG patients (NTG 10, NTG 12 and NTG 13) with a deviation index inside the range of norm had a highest ever measured IOP of 21 mmHg, that is at the upper range of normal. Thus it cannot be ruled out, that these patients may actually constitute HTG patients, in whom a higher IOP was missed on previous IOP-profiles.

When these mfOPs were isolated by bandpass filtering at 100–300 Hz, the logSP of the glaucoma patients was lower than the logSP of the control group in all response averages analyzed. This reached significance level in the central 7.5° and for NTG patients also in the nasal

Table 6. For NTG (top) and HTG (below) patients, this table shows how often a deviation index was outside the range of normal. This information is shown for each parameter and each response average. NTG patients differed least from the control group in the central response average. The most sensitive parameters were the latencies of the mfOPs. The column on the right depicts the corresponding ranked visual field loss (VF) for the four quadrants, based on the probability plots (Octopus d32). Within the central 50 degrees, visual field loss was distributed evenly

	Amplitudes	Latencies	Oscillatory potentials	Group deviation indices	VF
NTG					
Center	12	5	0	4	
Quadrant A	6	6	11	12	54.5
Quadrant B	11	3	11	9	49.0
Quadrant C	3	9	9	7	51.0
Quadrant D	4	3	13	6	45.5
Sum	36	26	44		
HTG					
Center	7	0	0	2	
Quadrant A	2	3	4	1	43.5
Quadrant B	9	1	3	2	43.5
Quadrant C	0	2	2	0	33.5
Quadrant D	4	0	3	2	29.5
Sum	22	6	12		

field quadrants. Using a stepwise logistic regression on the logSP of the mfOPs, again NTG could be differentiated from normal with a sensitivity of 85% and HTG patients with a sensitivity of 73%.

Initial studies applying the mfERG to detect glaucomatous retinal dysfunction used fast stimulation recordings with high luminance and differing contrast settings, [3]. While changes in the visual field parameters correlated with changes in

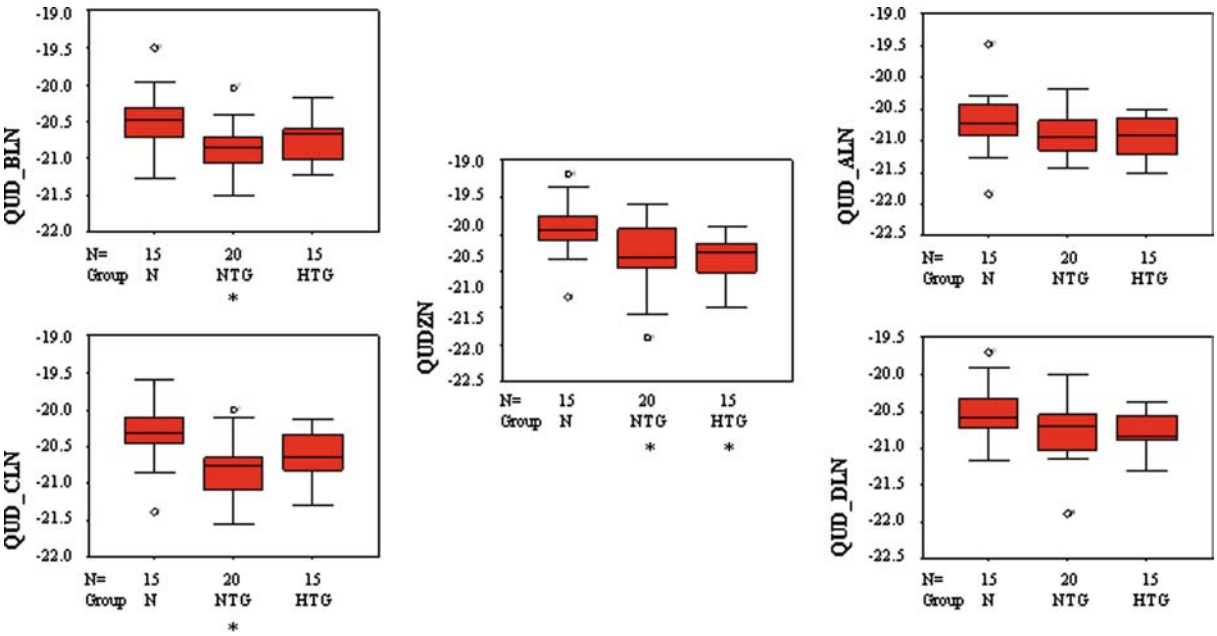

Figure 5. This figure depicts the boxplots of the log scalar product of the mfOPs for the control group (N), the NTG and the HTG group. Each response average shows a reduced SP in the mfOPs of glaucoma patients. When compared to the control group, * depicts a difference at a significance level of $p < 0.05$. The whiskers (upper and lower horizontal bars) represent the range of values, the bold horizontal bar depicts the median. The box represents the interquartile interval, from the 25th to the 75th percentile.

Table 7. For NTG patients, a stepwise logistic regression showed quadrants C and A to contain the most relevant parameters, allowing 85% of NTG patients to be differentiated from normal

Group	Classified as normal	Classified as NTG	Percent correctly classified
Control	11	4	73.3
NTG	3	17	85
Overall percentage			80

Table 8. For HTG patients, a stepwise logistic regression showed the central response average to contain the most relevant parameters, allowing 73% of patients with HTG to be separated from normal

Group	Classified as normal	Classified as HTG	Percent correctly classified
Control	13	2	86.7
HTG	4	11	73.3
Overall percentage			80.0

the mfERG parameters [9], a considerable overlap between the mfERG response parameters of glaucoma patients and a control group, prevented the reliable characterization of an individual's mfERG response as glaucomatous [4, 5, 10].

Recently, the sensitivity of the mfERG to detect inner retinal dysfunction in open angle glaucoma has been studied using global flash stimulation sequences, where for example, three bright flashes follow each m-sequence step regardless of it's polarity. A response induced by the interposed bright flashes can only be seen in the presence of adaptive mechanisms which are generally attributed to the inner retina. With such a stimulation sequence, the changes in the relative contribution of the response to the second of three global flashes increased the sensitivity to detect early retinal dysfunction in open angle glaucoma (OAG) to 50% [11]. When only one global flash was introduced into the m-sequence, changes in an induced oscillatory component increased the sensitivity of the mfERG in primary OAG patients to 88% [12].

The results of the mfERG may be compared to the pattern ERG (PERG), which has also been shown to detect glaucomatous dysfunction in 50% of glaucoma patients [13]. In the PERG [14] as well as in the mfERG [4] of glaucoma patients the ERG is affected more diffusely and thus the changes seen do not correspond too well with areas affected in the visual field [4, 14, 15]. This is in agreement with our results showing that the group deviation index or the mfOPs did not correlate well with the visual field quadrants affected.

In the PERG, there is a large inter-individual variability preventing characterization of individual patients as glaucomatous when only the absolute amplitudes are analyzed. However, when the relative difference in the PERG response to different check sizes was studied, the overlap between OAG and control could be decreased [16]. Under these circumstances, the sensitivity of the PERG to differentiate between primary OAG and a control increased to 82.7% [17]. The study by Pfeiffer and Bach [17] also included eyes with an intraocular pressure <21 mmHg in the presence of additional risk factors such as diabetes mellitus without retinopathy or cardiovascular disease. However, HTG and NTG patients were not analyzed separately.

In the mfERG an induced component also becomes increasingly apparent, when the stimulus sequence is slowed down. This results in less overlap between the response to the initial m-sequence step and the response induced by the following m-sequence step.

In our study, the mfOPs follow the first response complex N1–P1–N2 by a latency of about one stimulus base interval. The calculation of the first order response component (Figure 1) shows, that a flash following the preceding m-sequence step by one stimulus base interval will only contribute to the first order response component in the presence of adaptation. This effect can be shown by shortening the stimulus base interval of the m-sequence stimulation from 53.3 to 13.3 ms by reducing the number of the interposed black frames. Under such conditions the mfOPs' latencies will be shortened corresponding to the stimulus base interval until this complex contributes to N2 at a base interval of 13.3 ms. Thus, in analogy to the presence of a second order response component, the mfOPs constitute a nonlinear contribution to the first order response of the mfERG [6, 18, 19].

At a base interval of about 53.3 ms (three dark frames interposed after each m-sequence step, MBBB) the induced component, the mfOPs,

shows a marked naso-temporal asymmetry [6]. This asymmetry may be attributed to the misalignment and partial cancellation of the retinal component with the ONHC in the nasal retina and the relative alignment and enhancement in the temporal retina [6]. Thus an impairment of mfOPs would be expected to be more easily seen in the temporal retina (nasal field) than in the nasal retina (temporal field) as well as in changes in the relation between nasal and temporal responses.

The oscillatory potentials of the photopic ERG receive a strong contribution from the inner retinal layers [20]. Glycine, GABA and TTX suppress the function of the inner retina and result in reduced or missing oscillatory potentials of the photopic ERG [21]. In mfERG recordings these substances also affect nonlinear contributions to the mfERG which under faster and brighter stimulation conditions are mainly apparent in higher order response components [22, 23]. Therefore the observation of major differences in the mfOPs points toward an inner retinal damage occurring in NTG, and also in HTG. In agreement with our results Turno-Krecika et al. [24] has reported the oscillatory potentials of the Ganzfeld ERG to be especially affected in NTG.

The three groups examined here differed in age (control: 39.5 ± 10.7 years, NTG: 50.8 ± 15.5 years and HTG: 58.0 ± 10.5 years). However, there was no significant correlation between age and the overall deviation index (Spearman Rank Test, control: $r = 0.342$, $p = 0.213$; NTG: $r = 0.234$, $p = 0.322$; HTG: $r = 0.123$, $p = 0.661$). Figure 4 (right) shows a scatter plot of the overall deviation index versus age indicating that the influence of age on our findings, seems to be negligible. Also, age did not influence the results of the ANOVA when the logSP of the mfOPs were analyzed.

To our knowledge, this study reports the highest sensitivity of the mfERG to detect glaucomatous retinal dysfunction in patients with NTG. To a lesser degree, differences between NTG and HTG, have previously been observed in the fast stimulation mfERG obtained at a contrast of 50% [5]. The fact that the sensitivity of this stimulus differs between the two groups of glaucoma suggests that retinal dysfunction varies between NTG and HTG and is in support of a differing underlying pathomechanism, that could possibly consist of differences in the neurovascular coupling: Flickering light is known to cause changes in retinal blood flow [25]. This coupling is affected in glaucoma. A recent study showed reduced vasodilatation following flicker stimulation in patients with glaucoma [26]. In this study, differences between HTG and NTG patients were not analyzed. In other areas of the body, differences in the vascular response of NTG and HTG patients have been described previously. For instance, a study by Gasser et al. reported a significantly reduced nail-fold capillary blood flow velocity in patients with normal-tension glaucoma. Cold provocation resulted in a capillary perfusion stop > 12 s in 25 of 30 patients with NTG but only 3 of 30 control subjects and 4 of 30 HTG patients [27]. Decreased blood flow velocities for NTG compared to HTG eyes have been reported in short posterior ciliary arteries, peak systolic and end diastolic velocities [28]. For the retina, a recent pilot study has also indicated that the flicker stimulation of the slow mfERG stimulus used in the present study may result in a reduced dilation of the retinal vessels that seems more apparent in NTG than in HTG [29].

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