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Scanning laser ophthalmoscope-evoked multifocal ERG (SLO-mfERG) in patients with macular holes and normal individuals

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CLINICAL STUDY

Abstract

Aims A scanning laser ophthalmoscope (SLO) has been used for multifocal electroretinography (mf ERG) measurements under simultaneous fundus monitoring. The aim of this study was to prove if the SLO-mfERG measurement reflects reliably the clinically registered underlying disease, and to demonstrate the importance of its main advantage, fixation monitoring.

Methods In all, 10 patients with macular hole stage II/III were included in the study, and 19 normal individuals served as the control group. The mf ERG device was combined with an SLO, which was used both as a stimulus and trigger unit as well as a fundus-monitoring system. Monitoring of the fundus was guaranteed by an infrared laser (780 nm). The stimulus matrix consisted of 61 hexagonal elements, covering 24° of the posterior pole. We examined both, patients with macular holes and healthy individuals.

Results Compared to normal controls, patients with a macular hole (Gass stage III) showed a significant decrease in response density in the centre of the stimulus array, which correlated well with the morphological alteration observed by clinical examination. However, variation of response density of the central hexagonal area has been proved to be high.

Conclusions SLO-mfERG is a feasible and reliable new technique to investigate macular function under simultaneous fundus control. The main advantage is that control of fixation can be used in order to obtain more reliable results that correlate well with visible fundus abnormalities such as in patients with macular holes. However, further investigations have to

be performed in order to overcome sufficiently the problem of fixation instability.

Introduction

Generalized diseases of the retina such as, for example, retinitis pigmentosa are detected by standardized Ganzfeld electroretinography (ERG) with high sensitivity, even in early stages of the disease.[1–3] In localized retinal diseases, for example, in macular diseases, the Ganzfeld ERG is a less useful technique, as its global response is only affected in proportion to the size of the altered retinal area. The pattern ERG (PERG) improved at that point our ability to examine localized retinal diseases.[4,5] However, attempts to obtain ERG responses from small retinal areas never overcame the main problem of small signal-to-noise ratio.[6,7] The multifocal ERG allows topographic mapping of retinal function of about 50° of the central retina.[8,9] A stimulus array is presented on a monitor and the stimulated areas are changing independently alternating between black and white. The patient has to fixate on a small fixation spot presented in the middle of the stimulation matrix during the whole examination. A major problem of this technique is that no sufficient control of fixation can be performed by the examiner. Therefore, in patients with fixation problems, results may be unreliable, such as in patients with reduced

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compliance, in children or patients with eccentric fixation. To overcome these difficulties, Kondo *et al* [10] used an infrared fundus camera combined with a stimulus array of 19 light-emitting diodes (LEDs), allowing the performance of a multifocal ERG under simultaneous fundus control. Seeliger *et al* [11] showed the application of scanning laser ophthalmoscope-evoked mfERG (SLO-mfERG) measurements in animals, using a green laser (515 nm). In many animals, the only way to obtain satisfactory results exists in using a short-wave laser because of the lack of long-wavelength cones.[11]

In this study, we used an SLO-mfERG to examine patients with macular hole (Figure 1) and normal individuals. This new technique offers the possibility to generate a stimulus array with a helium–neon laser under simultaneous control of fixation.[12–17] The purpose of this study was to assess the feasibility of this method to measure focal retinal function or dysfunction under simultaneous control of fixation.

Materials and methods

The scanning laser ophthalmoscope (Rodenstock; München, Germany) was used both as a stimulus and trigger device as well as a fundus-monitoring unit. To record an mf ERG, a confocal scanning laser ophthalmoscope is connected to an mfERG device (RETIscan, Roland Consult, Wiesbaden, Germany). The visual stimulus, consisting of a matrix of hexagonal areas flickering concurrently according to a short m-sequence, is displayed directly onto the retina covering a visual angle of about 12° or a diameter of 24°, respectively (Figure 2).[18,19] It consists of a matrix of 61 hexagonal elements and is generated by a helium-neon laser (632.8 nm) modulated via an acousto-optic modulator. The matrix has been presented according to a scaling factor 1:4, so that the hexagonal size would be accordingly scaled with eccentricity in order to evoke focal responses of approximately the same amplitude per stimulus element in the response arrays of normal individuals.[20] With this setting the outermost hexagonal areas were approximately four times larger than the central one. The elements change from red to black or *vice versa* according to a complete cycle of a binary m-sequence.[21] The contrast is about 90%.[22] The Roland system uses 'short-m-sequences' and a complete measurement cycle consists of 511 steps lasting 40.8 sec (511×80 ms). Scan time is 20 ms prohalf-frame (50 half-frames/s, 25 frames/s). The examined persons were asked to gaze at a centrally positioned cross. If loss of fixation occurred, the measurement would be interrupted. In cases of unstable fixation the measurement cycle was discarded. The signal of each hexagonal field is calculated by cross-correlation analysis between the m-sequence and the response. The signals are bandpass filtered (10–100 Hz) and amplified by a two-channel amplifier with a common mode reaction of >110 dB and a sensitivity of 1–20 μV/div. Simultaneous funduscopy and control of fixation is guaranteed by illumination with an infrared diode laser (730 nm).

Patients and controls

In all, 10 patients (ages 46–77 years, mean 64 years) with unilateral diagnosis of an idiopathic macular hole

Figure 1 Fundus photography of a patient with a macular hole (Gass II).
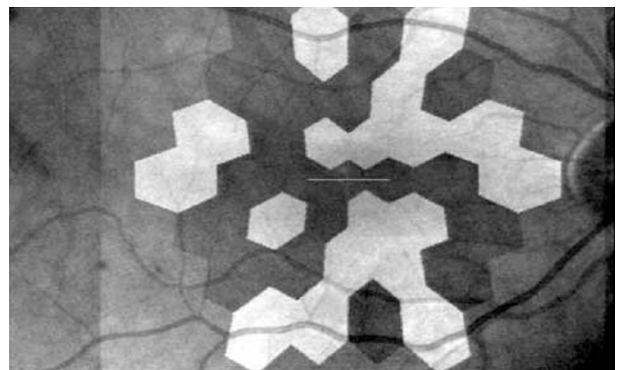
Figure 2 The stimulus pattern on the posterior pole of the eye: the figure displayed was derived from the screen that is used for real-time fundus monitoring, where the fundus picture and the stimulation matrix appear in black and white. Actually, the stimulation pattern generated by the helium–neon laser consists of red light.

were compared with 19 healthy volunteers
(ages 18 to 63 years, mean 40 years) contributing
to the study with one eye each. Normal eyes of
patients did not serve as controls. Altogether
29 persons were examined with SLO-mfERG using a
scaling factor of 1:4. Ophthalmic examination included
slit-lamp examination, measurement of visual acuity,
funduscopy, and fundus photography. Macular holes
were diagnosed by biomicroscopy using a 78 dioptre lens
(Volk) and by optical coherence tomography (OCT). Two
patients with a macular hole stage II according to Gass
showed central fixation, while eight patients with
macular hole stage Gass III demonstrated slightly
paracentral fixation, but still in the central hexagonal
element. Patients with more eccentric fixation were
excluded from the study. The visual acuity range was
0.05–0.4.

The recordings were analysed by using concentic ring
grouping with evaluation of amplitude density and by
displaying the plots. ERGs retrieved from the individual
hexagonal areas would also be plotted. Special traces
could be selected for detailed data analysis. Amplitude
density (nV/deg^2) represents the amplitudes for each
grouping adjusted for the distance angle of the stimulus
element, giving a more accurate view of the actual
response amplitudes of each group.[23] To construct the
two and three-dimensional plots the mean of amplitude
density was used.[9]

The criteria for the normal controls included in the
study were an age of over 18 years, visual acuity of 1.0
with or without correction and contact lens tolerance for
both groups. Exclusion criteria for both groups were
media opacities, refractive errors of more than 3 dioptres,
a history of ocular trauma, diabetic retinopathy, age-
related macular degeneration, or glaucoma. The pupils
were not dilated. Refractive errors would be
compensated from an integrated system of the SLO. This,
generally described, consists of movable mirrors that
automatically can be moved, shifting therefore the light
beam. Jet contact lens electrodes (Universa SA, La
Chaud-de-Fonds, Switzerland) were used. The reference
electrode was placed in line with ocular canthi behind
the orbital ridge. This research adhered to the tenets of
the Declaration of Helsinki and informed consent was
obtained from all persons.

Results

To quantify the electrophysiological results, the
responses were grouped by retinal eccentricity in five
ring areas. To summarize the results, boxplots showing
the 25th and 75th percentile of amplitude density were
calculated for both groups of examined persons (healthy
individuals and patients with macular holes) (Figure 3).
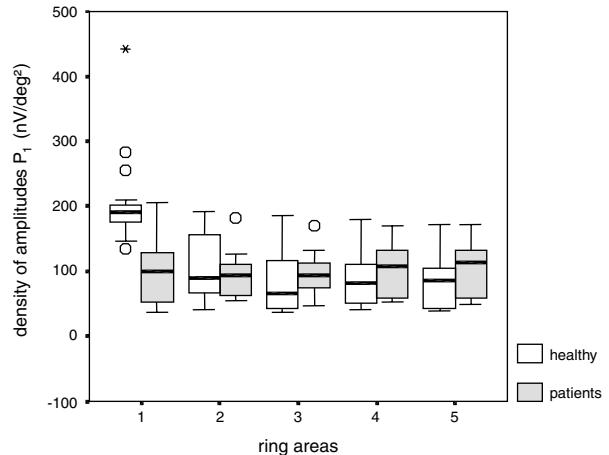
Figure 3 Bold lines in the boxes indicate median, upper and
lower edges of the boxes indicate 25th and 75th percentiles
accordingly. Upper/lower ends of bars indicate highest/lowest
data that is not an outlier. Circles on the graph represent
'outliers'. Outliers are cases with values between 1,5 and 3 box
lengths from the upper or lower edge of the box. The box length
is the interquartile range. Small stars on the graph represent
'extremes'. The latter are cases with values more than three box
lengths from the upper or lower edge of the box.

Fifth, 25th, 75th, 95th percentiles for the normative data
and the patients' individual responses can be seen in
Table 1. The mean and median values as well as the
standard deviation (SD), the interquartile range,
and the coefficient of variation of the central
hexagonal element and the rings were calculated
for each group (Table 2). Our data were tested for and
were found inconsistent with the normality assumption
(using the Kolmogorov–Smirnov test). The significance
level was set at $P = 0.05$ and the Mann–Whitney test was
applied. Two- and three-dimensional plots of amplitudes
P1 both for the healthy individuals as well as for the
patients were derived and compared (Figures 4a,b
and 5a,b).

The amplitudes of the first ring area (central hexagonal
element) were found statistically significantly higher in
the group of healthy individuals ($P < 0.05$), M
(normals) = 198.6, SD = 74.3; M (macular holes) = 104.8,
SD = 54.0. The amplitudes and latencies of rings 2–5
areas showed no significant differences (Figures 6a,b and
7a,b, Table 2).

The coefficient of variation (or percentage error)
measures the relative scatter of data with respect to the
mean and therefore the imprecision in our estimates. It is
calculated as 100 (SD)/(mean value of set). In our data set,
the coefficient of variation of the response density of the
central hexagonal element is relatively higher centrally in
the patients with a macular hole as compared to the
healthy individuals.

Table 1 Individual responses of the patients and the 5th, 25th, 75th, and 95th percentiles for the normative data

| | Amplitude density (nV/deg2) | | | | |
Concentic areas	R1	R2	R3	R4	R5
Normwerte					
5th Percentile	62.0	42.0	37.0	42.0	39.0
25th Percentile	174.0	65.0	41.0	45.0	42.0
75th Percentile	204.0	172.0	122.0	115.0	112.0
95th Percentile	283.0	192.0	151.0	160.0	168.0
Patient 1 (Gass III)	52	81	113	123	126
Patient 2 (Gass III)	37	60	48	53	49
Patient 3 (Gass III)	79	63	86	96	111
Patient 4 (Gass II)	167	107	100	135	136
Patient 5 (Gass II)	206	182	170	170	172
Patient 6 (Gass III)	126	127	132	132	132
Patient 7 (Gass III)	88	106	111	108	116
Patient 8 (Gass III)	113	110	80	106	105
Patient 9 (Gass III)	128	68	75	59	59
Patient 10 (Gass III)	52	54	47	52	49

Table 2 Amplitude density (nV/deg^2): statistical values of normal individuals and patients with macular holes

	Ring 1	Ring 2	Ring 3	Ring 4	Ring 5
Normals					
Mean	198.6	109.3	86.6	90.0	86.6
median	191.0	90.0	65.0	81.0	85.0
SD	74.3	53.0	47.3	43.8	46.6
Coeff.Var.	37.4	48.4	54.6	48.6	53.8
Macular holes					
mean	104.8	95.8	96.2	103.4	105.5
median	100.5	93.5	93.0	107.0	113.5
SD	54.0	39.3	37.7	39.2	41.0
Coeff. Var.	51.5	41.0	39.1	37.9	38.8
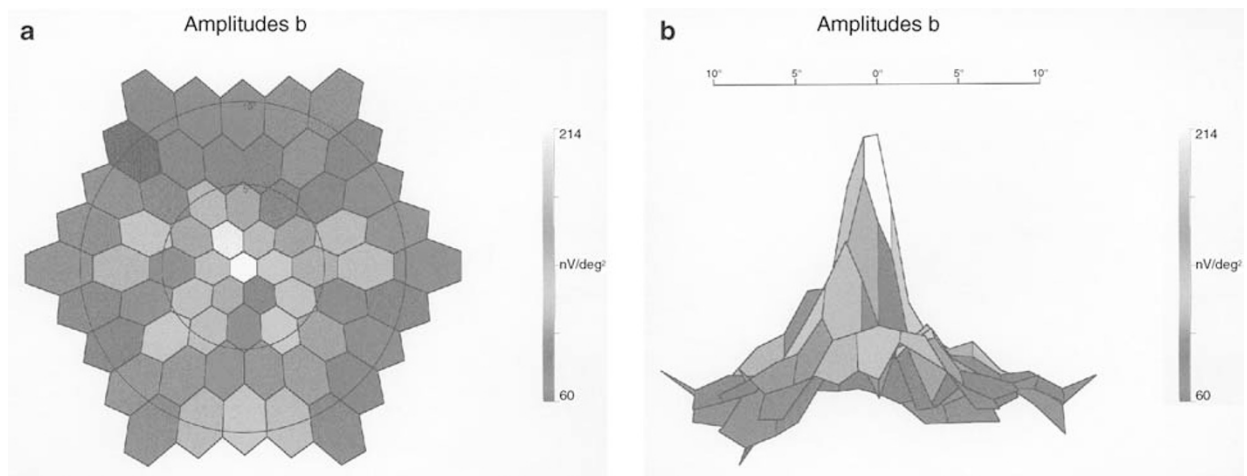
Figure 4 (a) Two-dimensional presentation of amplitudes P_1 of a healthy person. (b) Three-dimensional presentation (side view) of amplitudes P_1 of a healthy person showing the central peak.
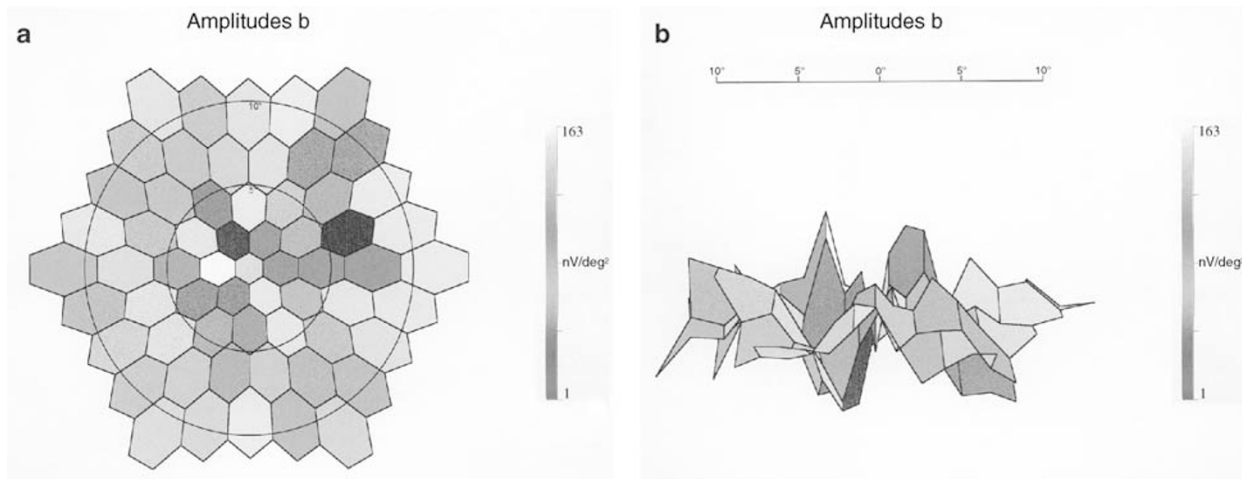
Figure 5 (a) Two-dimensional presentation of amplitudes P_1 of a patient with macular hole. (b) Three-dimensional presentation (side view) of amplitudes P_1 of a patient with macular hole showing the reduction of the central peak.
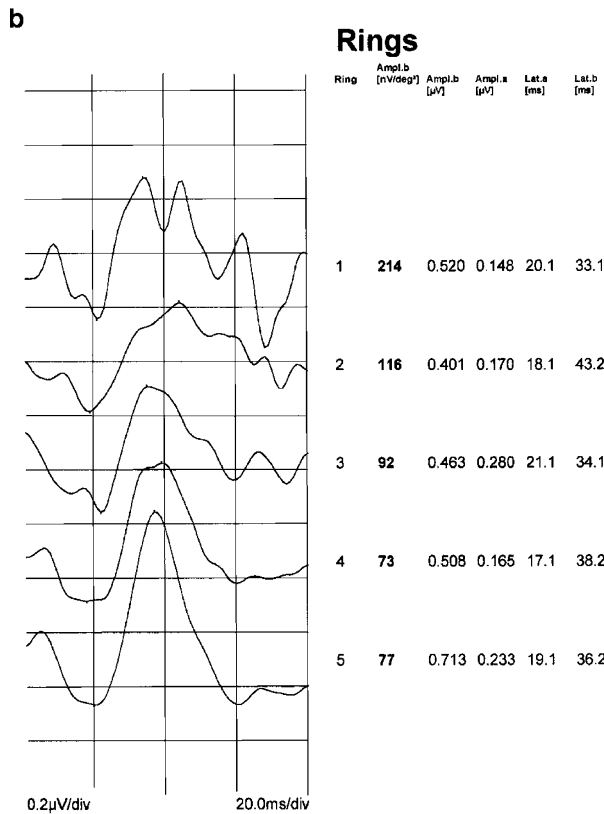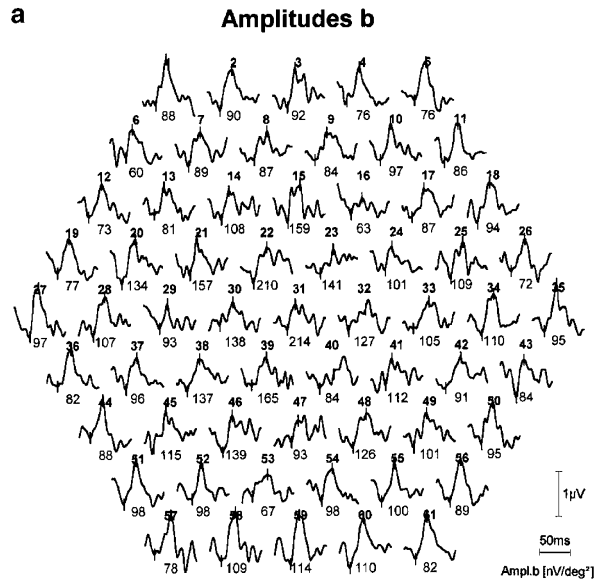
Discussion

The Ganzfeld ERG produces a generalized response because of the diffuse stimulation of the retina and therefore neglects the effect of limited lesions. Conventional, monitor stimulated mfERG is a technique capable of measuring retinal function of the posterior pole with high spatial resolution. By multifocal stimulation and by the simultaneous measurement of all the focal responses, a strict correlation of any given signal to a corresponding field exists.[24–26] By using short m-sequences, the quality of each measurement cycle can be evaluated separately, and any cycle in which significant artefacts occur can be discarded. The SLO-system we have employed is also a significant technical advance. It allows the direct projection of the laser-generated stimulus pattern onto the retina. Moreover, the presence of an infrared diode laser allows simultaneous funduscopy, control of fixation, and imaging of the hexagonal matrix during the whole examination. This is important for all patients with instability of fixation. The importance of an appropriate fixation for a reliable stimulation–response correlation analysis has been lately shown using mfERG recordings and SLO-microperimetry.[27,28] Moreover, in a recent study where mfPERG recordings were obtained with an eye tracker from normal individuals, 49% of the recordings were acquired, while fixation was inadequate.[29] Loss of fixation would lead to an artificial correlation of the recorded amplitudes to the expected measured retinal areas. The SLO allows continuous monitoring of fixation. The patient can be encouraged and epochs in which control is poor can be discarded. Ultimately, software that compensates for eye movements could be introduced.

Although the main advantage of the mfERG is its spatial resolution, in accordance with Parks et al,[30] who concluded that repeatability of mfERG can be further enhanced by forming regional groups, concentric rings were grouped around the innermost hexagon of the stimulation array. In concentric macular diseases such as macular holes, an averaging of concentric rings allows a better comparability of responses obtained from the central retina to those derived from more eccentric parts.[30] As known from the conventional mfERG, response density decreases markedly from the foveal region towards the periphery. This distribution of response density approximately corresponds to the anatomical occurrence of cone distribution of the posterior pole.[18–20,31] The control group in this study was not age matched, which could have an influence on the results because it is known that an age-related effect exists. Other studies showed a decrease of amplitudes in older patients.[32,33]
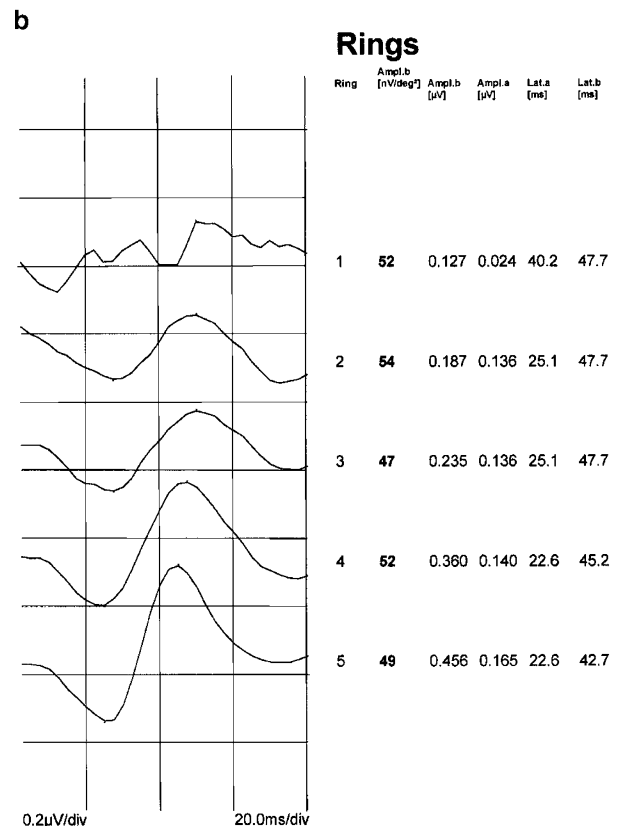
In patients with macular hole, a significant reduction of response density could be demonstrated in the centre of the stimulus array. Retinal dysfunction leads to profound amplitude reduction of the central hexagonal element in the SLO-mfERG results of these patients. In the areas corresponding to the elevated margins adjacent to the hole, no significant reduction could be seen in the retinal electrical response. As expected, the strength of the recorded amplitudes of the more peripheral areas does not differ significantly in these patient in comparison with normals.[34–38]

The higher coefficient of variation of amplitudes of the central hexagonal element in patients with macular holes (Table 2) could be explained because of loss of fixation in these patients. However, it raised questions about the

a Amplitudes b

measurements' reliability. Fixation loss (through foveal displacement) shifts the central hexagonal element to a more peripheral retinal area, and for a short but variable time period destroys correlation between recorded amplitude and field location. Part of the response attributed to the inner ring will also be generated by the

a Amplitudes b

b Rings

Ring	Ampl.b [nV/deg²]	Ampl.b [µV]	Ampl.a [µV]	Lat.a [ms]	Lat.b [ms]
1	214	0.520	0.148	20.1	33.1
2	116	0.401	0.170	18.1	43.2
3	92	0.463	0.280	21.1	34.1
4	73	0.508	0.165	17.1	38.2
5	77	0.713	0.233	19.1	36.2

0.2µV/div   20.0ms/div

b Rings

Ring	Ampl.b [nV/deg²]	Ampl.b [µV]	Ampl.a [µV]	Lat.a [ms]	Lat.b [ms]
1	52	0.127	0.024	40.2	47.7
2	54	0.187	0.136	25.1	47.7
3	47	0.235	0.136	25.1	47.7
4	52	0.360	0.140	22.6	45.2
5	49	0.456	0.165	22.6	42.7

0.2µV/div   20.0ms/div

Figure 6 (a) Plots of amplitudes P_1 from the 61 hexagonal elements of a healthy person. The number of the hexagonal elements is given at the top of the curves while at the bottom appears the amplitude of each field (nV). (b) Concentric ring grouping of a healthy person showing the amplitudes and latencies for each ring. In the first columns is given the amplitude density in nV/deg². In the second and third column are the summarized amplitudes P_1 and N_1 accordingly per ring grouped area in µV. In the fourth and fifth columns are the latencies P_1 and N_1 for the summarized curves in ms.

fovea and *vice versa*, which would be otherwise covered by the central hexagonal element. Our measurements were made under simultaneous fundus visualization and direct fixation control, and still the coefficient of variation implies that such results contain significant errors. Not only in patients but also in healthy individuals, the fixation can pose a real problem. When performing the examination with the monitor version, these possibilities of fixation control are not available. Eyetracking, among other efforts, to solve this problem cannot be a reliable solution, because absence of eye movement does not absolutely mean proper fixation of the eye to the presented target (fixation cross). Thus, when the above-mentioned precautions are not available, it is important to remember that scaling may introduce significant difficulties in obtaining reliable data.[11,29,39]

While fixation loss cannot be prevented, the value of our fixation monitoring system depends on the possibility of continuous visualization of fixation during the examination. Fixation depending on concentration, motivation, and retinal function can be, on the one hand, positively affected and on the other objectively evaluated by using the SLO direct fundus control method in order to record mfERGs. In conclusion, it seems that the SLO-mfERG is a useful technique to investigate retinal and in particular macular function under control of fixation, and further studies will be helpful to improve this diagnostic tool.

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Figure 7 (a) Plots of amplitudes P_1 from the 61 hexagonal elements of a patient with macular hole. The number of the hexagonal elements is given at the top of the curves while at the bottom appears the amplitude of each field (nV). No recognizable amplitudes in the fields 14, 22, 25, and 48 may be caused by shifts because of microsaccades. (b) Concentric ring grouping of a patient with macular hole showing the amplitudes and latencies for each ring. In the first column is given the amplitude density in nV/deg^2. In the second and third columns are the summarised amplitudes P_1 and N_1 accordingly per ring grouped area in μV. In the fourth and fifth columns are the latencies P_1 and N_1 for the summarised curves in ms.

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