Monitoring Retinal Function during Transpupillary Thermotherapy for Occult Choroidal Neovascularization in Age-Related Macular Degeneration

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Purpose. To use focal electroretinography to evaluate changes in retinal function during transpupillary thermotherapy (TTT) for neovascular age-related macular degeneration (ARMD).

Methods. Sixteen eyes of 16 patients with ARMD with occult choroidal neovascularization (CNV) were studied. A 630-nm photocoagulator aiming beam was modified for use as a 41-Hz square-wave focal electroretinogram (fERG) stimulus. The stimulus was presented on a light-adapting background by a Goldmann-type lens (visual angle, 18°; mean luminance, 50 cd/m^2). fERGs were continuously monitored before, during, and after TTT for occult CNV. The amplitude and phase of the fERG's fundamental harmonic were measured.

Results. No suprathreshold or adverse clinical events occurred during the course of the study. fERG amplitude decreased transiently during TTT (23% ± 9% [SE]; $P < 0.05$). The decrease in amplitude was greatest 16 to 20 seconds and 32 to 40 seconds after the onset of TTT. It was followed by a recovery to baseline amplitude during TTT (48 to 60 seconds after TTT was begun). Within 60 seconds after TTT was completed, fERG amplitude was within the range of baseline. TTT did not alter the fERG phase. Mean fERG amplitudes and phases recorded 1 week and 1 month after TTT were comparable to mean pretreatment levels.

Conclusions. fERG amplitude decreases transiently during TTT, despite the absence of ophthalmoscopically apparent lesions. Intraoperative amplitude depression may result from an adaptation effect to laser light energy and/or hyperthermia, resulting in desensitization of cone photoreceptors and bipolar cells. Treatment sites are electrophysiologically functional 1 month after TTT. Detailed parametric study of a larger patient group is needed to determine whether fERG testing is potentially useful for monitoring and perhaps for controlling and optimizing TTT for choroidal neovascularization. (*Invest Ophthalmol Vis Sci.* 2003;44:2133–2140) DOI:10.1167/iovs.02-0716

Retinal photocoagulation is localized heating of the retina and choroid by intense light. A threshold retinal photocoagulation burn may be defined as one that is just barely visible by ophthalmoscopy at treatment time.[1] Subthreshold lesions are retinal burns that are not ophthalmoscopically apparent. It takes less power to produce lesions that are angiographically[2–5] or electrophysiologically[6,7] but not ophthalmoscopically apparent. Subthreshold clinical photocoagulation protocols have been developed to reduce retinal damage during laser photocoagulation.[1]

Subthreshold transpupillary thermotherapy (TTT) has been used to treat choroidal neovascularization (CNV) in age-related macular degeneration (ARMD).[8,9] Conventional retinal photocoagulation uses brief 40°C to 60°C temperature increases to produce lesions that are immediately visible.[1,10,11] TTT uses lower (10°C) temperature increases, but maintains them for 60 seconds to treat CNV.[8,12] A pilot study of TTT for occult CNV in ARMD showed a 94% decrease in exudation over a mean follow-up period of 13 months, with evaluation performed by fluorescein angiography, optical coherence tomography, and/or clinical examination.[8] It also showed a 75% stabilization or improvement in visual acuity.[8] Studies by other investigators have produced similar results.[9,13,14] For instance, in a retrospective, open trial with a mean follow-up of 6.1 months, Newsom et al.[9] found a mean change in vision of −0.66 Snellen lines, a 78% rate of CNV closure, and a recurrence rate of 5.1%. Reported complications or adverse events of TTT for neovascular ARMD include retinal pigment epithelial (RPE) tear[15] and retinal arteriole occlusion.[13] Randomized, prospective controlled clinical trials are under way to compare outcomes of TTT for occult CNV with the natural history of the disease.[16]

The increase in temperature during laser photocoagulation is proportional to retinal irradiance (power density) for a particular chorioretinal pigmentation, exposure duration, spot size, and laser photocoagulator wavelength.[1,10,12] TTT uses large spot sizes to produce low retinal irradiances and temperature increases. Its subthreshold nature is potentially a therapeutic advantage. It is also a practical disadvantage, because some nonvisible lesions may be nonlesions if retinal irradiance is insufficient to produce a therapeutic temperature increase.

Evaluating different aspects of the effect of thermal damage on the RPE and neural retina could improve the reproducibility of subthreshold photocoagulation results. Reflectometry has been studied extensively for this application, but practical systems are not available clinically.[17–19] Noninvasive thermometry is under experimental investigation (Brinkmann R, Schuele G, Joachimmeyer E, Roider J, Birngruber R, ARVO Abstract 3749, 2001). Electroretinograms have been measured before and after clinical photocoagulation for ARMD and diabetic retinopathy.[20–26]

The focal electroretinogram (fERG) is a retinal response derived from a localized retinal area.[27] As shown in earlier studies (and reviewed by Biersdorf[28]), the macular fERG has spectral characteristics matching the standard photopic luminous efficiency curve. It is specific for the stimulated retinal area (it is relatively unaffected, depending on the recording technique, by stray-light stimulation of extramacular areas). Its

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amplitude correlates with the number of cones that are present in the stimulated retinal field. When the fERG is recorded to flickering stimuli modulated at different temporal frequencies, the response displays band-pass tuning characteristics, with a broad maximum of approximately 40 Hz and attenuation at lower and higher temporal frequencies.[29] fERG responses to local flicker stimulation can be recorded and evaluated in a statistically reliable fashion by steady state, frequency-domain analysis.[30–32] Responses can be analyzed clinically in real time for specific stimulus parameters (sweep techniques).[29,32,33] The fERG is a sensitive indicator of macular cone system dysfunction in retinal degenerative diseases,[29,33–35] including ARMD.[32,36–38] Depending on stimulus size and retinal location, signal amplitude losses and timing delays can detect dysfunction or loss of foveal or extrafoveal retinal elements.[28]

The fERG is potentially well suited to monitoring TTT, because TTT uses lengthy exposures and large diameter laser spots. The RPE and the choroid are the primary sites of light absorption in retinal photocoagulation, but temperature increase is essentially the same in the RPE and adjacent outer neural retina and choroid, except for the first 0.1 second of the 60-second TTT exposure.[12,39] Thermal effects in photocoagulation depend on the temperature history of exposed tissue. Thus, changes in outer retinal function are potentially useful for monitoring and perhaps controlling TTT. We therefore developed techniques for measuring the fERG during TTT, and used them to determine whether real-time monitoring is feasible during TTT and whether fERG changes are significant and reproducible. The present study was not designed to assess the efficacy of TTT for neovascular ARMD, which is being examined in ongoing randomized prospective controlled clinical trials such as the TTT4CNV study.[16]

Materials and Methods

Patients

Seventeen eyes of 17 consecutive patients (age range, 55–89 years) with occult subfoveal choroidal neovascular membrane due to ARMD were studied. One eye was excluded because of the patient's refusal to participate in the study. Each patient underwent a complete general and ophthalmic examination, which included best-corrected visual acuity by a retroilluminated Snellen chart (that was periodically calibrated and had a mean luminance and contrast of 80 cd/m^2 and 99%, respectively), anterior segment biomicroscopy, fundus examination by direct and indirect ophthalmoscopy, Goldmann lens retinal biomicroscopy, and fluorescein angiography. The latter was performed according to standard techniques.[40] Each patient's iris pigmentation was graded biomicroscopically into one of four categories, ranging from blue (grade A) to brown (grade D).[41]

For inclusion in our study, each patient had to have best corrected Snellen visual acuity (recorded in complete lines and additional letters read) of 0.4 (20/50) or worse in the affected eye, clear optical media, no prior laser photocoagulation or photodynamic therapy, no intraocular surgery within the previous 3 months, and no capsulotomy within the previous 2 months. In 14 of 16 eyes, presumed deterioration of occult CNV lesion had been documented during the 6- to 12-month period that preceded the patient's inclusion in our study. Criteria for deterioration included a loss of one or more lines of visual acuity within 3 months of the baseline examination, or a growth in the greatest linear dimension of the lesion of at least 10% within 3 months of the baseline examination. In the remaining two patients, no prior clinical changes of the lesion had been documented over a 6-month period preceding inclusion in the study.

Each patient had subfoveal occult CNV, defined as a fibrovascular pigment epithelial detachment (V-PED) or late leakage of undetermined source (LLUS) on fluorescein angiography.[8,42] At the time of inclusion in our study, fluorescein angiogram interpretation was performed independently for each patient by two observers. V-PED and LLUS were defined according to the criteria of Stevens et al.[42] The greatest linear dimension (GLD) of each lesion was estimated by means of a standard calibration bar, corresponding to a distance of 1.5 optic disc diameters. The calibration bar was printed on a transparency, which was superimposed on the fluorescein angiogram of the lesion. The presence of blood in the lesion area was documented.

Exudation was defined as an elevation or thickening of the retina or RPE on retinal biomicroscopy and leakage in the late phase of the fluorescein angiogram, graded as mild, moderate, or severe. Late leakage of variable extent was present in all study eyes. Graders did not differ in classification of either the type of occult CNV or the extent of exudation.

Variability in fundus lesion pigmentation due to pigment clumping was classified by retinal biomicroscopy into one of four categories, ranging from 1 (least) to 4 (most). This classification is similar to that reported by Shields et al.[43] Stereo color photographs were not routinely used to classify fundus lesion pigmentation, but they were available in 9 of 16 cases, and their analysis was consistent with our classification. Two observers independently graded iris and fundus pigmentation. They agreed fully on iris pigmentation. The only discrepancy in pigmentation grading was a one-category difference in a single eye (6.25%). This difference was reconciled by a consensus of the graders.

The baseline characteristics of each patient are reported in Table 1. A V-PED–type lesion was present in each study eye. V-PED and LLUS were both present in one eye (patient 10), with the former type of lesion located subfoveally. Subretinal blood was present in six eyes. The area of hemorrhage or blocked fluorescence did not exceed that of visible CNV in any study eye.

All patients signed an informed consent form after a careful explanation of the goals of the study. They were given a thorough explanation of the risks and benefits of TTT and alternative forms of CNV management including photodynamic therapy and observation. Our study was performed in accordance with the tenets of the Declaration of Helsinki. The research was approved by the institutional review board and ethics committee of Catholic University.

fERG Stimulation and Recording Methodology

fERG stimulus, recording, and analysis technologies were based on clinical systems used in the Visual Electrophysiology Laboratory of the Institute of Ophthalmology (Catholic University, Rome, Italy). The flicker stimulus was the red diode laser aiming beam of an infrared diode laser photocoagulator (OcuLight SLx; Iridex Corporation, Mountain View, CA), square-wave modulated at 41-Hz at 50% duty cycle. Stimulus wavelength and minimum and maximum luminance were 630 nm and 0 and 100 cd/m^2, respectively. The stimulus was centered on the fovea and subtended a field that was 18° in diameter (6 mm, approximately) and was presented in Maxwellian view by a standard Goldmann-type lens. Stray-light effects were minimized by using the photocoagulator's slit lamp light source to produce a large (60°), light-adapting background at the same mean luminance (50 cd/m^2) as the stimulus. Under these experimental conditions, the response can be considered cone driven and focal.[28,34,35] Preliminary control experiments in two untreated patients showed that the fERG amplitude decreased substantially when the stimulus was centered on the optic disc. Response amplitude also decreased to noise level when the stimulus was centered on a large retinal scar including part of the foveal region, thus confirming the focal origin of the ERG signal.

fERG recording, sampling, and analysis protocols have been published previously.[32,35,38,44] Retinal signals were recorded with skin electrodes and an interocular reference.[32,44] Although a better signal-to-noise ratio can be achieved by conductive fiber electrodes such as DTL (see for example Ref. 24), preliminary trials indicate that, in a typical patient, skin electrodes produce an acceptable signal stability and signal-to-noise ratio (discussed later). Signals were amplified, band-

TABLE 1. Summary of Clinical Results and Laser Treatment Parameters in Patients with ABMD

Patient	Age, Sex, Iris Pigment	Size of Lesion (μm) (Pigment Score)	Exudation Grade and Blood (Y/N), Pre-Rx‡	Acuity, Pre-Rx‡	Laser Power (mW), Duration (s)
1	64, F, hazel (B)	3600 (2)	Severe, N	0.02	600,60
2	79, F, hazel (B)	2700 (1)	Moderate, N	0.3	400,60
3	74, M, blue (A)	900 (1)	Severe, Y	0.2	450,60
4	83, F, blue (A)	2700 (1)	Moderate, N	0.01	800,60
5	72, M, brown (C)	3600 (3)	Moderate, Y	0.1	400,60
6	55, F, brown (C)	3600 (1)	Severe, N	0.2	400,60
7	77, M, brown (C)	2700 (4)	Moderate, Y	0.02	600,60
8	80, F, blue (A)	3600 (1)	Moderate, Y	0.02	500,60
9	84, F, blue (A)	1200 (1)	Moderate, N	0.2	600,60
10	80, M, brown (C)	2700 (1)	Mild, N	0.4	450,60
11	77, F, hazel (B)	3600 (1)	Moderate, N	0.02	550,60
12	71, F, brown, (C)	2700 (1)	Moderate, N	0.1	450,60
13	89, F, hazel, (B)	1800 (1)	Moderate, N	0.1	550,60
14	68, F, hazel, (B)	2400 (2)	Mild, N	0.1	420,60
15	74, F, dark brown, (D)	2700 (2)	Moderate, N	0.2	400,60
16	81, F, brown, (C)	3600 (2)	Moderate, Y	0.01	450,57

* Iris color classification system according to Seddon et al.[40]

† Size of lesion: greatest linear dimension. Grading of fundus lesion pigmentation, ranging from 1 (least) to 4 (most) and based on retinal bio microscopy performed by two independent observers.

‡ Pre-rx; before TTT treatment (final result).

pass filtered (1–250 Hz, −6 dB/octave) and sampled at 12-bit resolution with a 2-kHz sampling rate. Six blocks of 150 events each were averaged. Single sweeps exceeding a threshold voltage were rejected to minimize noise coming from blinks or eye movements. Discrete Fourier analysis[45] was performed online to isolate the fERG's fundamental harmonic, the amplitude (in microvolts) and phase (in degrees) of which were estimated. Standard errors of the amplitude and phase estimates, derived from the block averages, were calculated to determine response reliability.[32,46] Background noise at the fundamental component was estimated by real-time averaging and Fourier analysis of signals sampled asynchronously at 1.1 times the temporal frequency of the stimulus.[32,46]

Procedure

fERG testing was started after a 20-minute period of preadaptation to the mean stimulus luminance (50 cd/m^2). Patients' pupils were dilated at least to 8 mm with 1% tropicamide. After preadaptation, an fERG was recorded by collecting six block averages of 150 events, each over a period of 8 to 10 seconds. The total recording time was 48 to 60 seconds (the baseline fERG). Response amplitude and phase, as well as response reliability and signal-to-noise ratio, were estimated on-line. Data were stored on disc for subsequent off-line analysis.

TTT was then performed with a protocol similar to that of Reichel et al.[8] Laser parameters are listed in Table 1. We used a 3-mm diameter (subtending approximately 11° on the retina, taking into account the Goldmann lens magnification) treatment spot centered on the fovea in all patients. It did not cover the entire lesion in six eyes. Laser power was 400 to 800 mW, similar to that used in the study of Reichel et al.[8] Power selection for individual patients was based on clinical criteria that include patient acuity, iris pigmentation, and CNV lesion characteristics, such as the amount of exudation and pigmentation. For instance, the typical 800-mW power setting used for a 3-mm diameter spot, was reduced by 50% in eyes with heavily pigmented iris and/or fundus lesions (grades 3 and 4). A similar reduction was applied in eyes with an acuity of 0.2 (20/100) or better. The power setting was instead increased by 10% to 25% if the lesion showed a moderate to severe grade of exudation. The mean laser power setting used in our patients was 507 mW, not significantly different (independent *t*-test) from the average power of 572 mW (range: 380–800 mW) used by Reichel et al.[8] for a 3-mm spot size.

fERGs were recorded during treatment by collecting six block averages of 150 events, each over a period 8 to 10 seconds (total recording time: 48 to 60 seconds; the "treatment" fERG). The response amplitude and phase of the various blocks were estimated on-line, as well as response variability and signal-to-noise ratio. Data were stored on disc for further analysis. At the end of treatment, fERGs were recorded again by collecting six block averages of 150 events during a total recording time of 48 to 60 seconds (the "recovery" fERG). Response amplitude and phase of the various blocks were again estimated online, as well as response variability and signal-to-noise ratio.

Follow-up Evaluations

Electrophysiologic testing was repeated in all patients 1 week and 1 month after treatment. No further recordings were performed, because the scope of this study was limited to the intraoperative effects of TTT on the fERG. Clinical evaluations including fluorescein angiography were performed for all patients at 1 month and then every 3 months after treatment. Clinical follow-up duration was, on average, 7.5 months (range, 6–12 months).

Data Analysis

Data analysis was performed on the block averages recorded individually from each patient. After several pilot recordings, we determined that the best compromise between signal reliability and temporal resolution was achieved by using the average of two consecutive blocks (a total of 300 events) as our main outcome measure. This average is referred to as an experimental block. It is used in all subsequent analyses and figures, and has a temporal resolution of 16 to 20 seconds (mean, 18 seconds) throughout monitoring periods.

Baseline, treatment, and recovery fERGs were compared statistically to determine whether fERG amplitude and/or phase varied significantly across the different experimental conditions. Two-way, repeated-measures analyses of variance (ANOVAs) were performed on amplitude, phase, and signal-to-noise ratio data. Subject factors included experimental time (the baseline, TTT, and recovery recordings) and blocks (the three blocks of responses recorded during each experimental time). This analysis allowed evaluation of the specific effects of treatment (experimental time) while checking for possible

changes in within-blocks variability (blocks and interaction of block by experimental times).

Changes in fERG amplitudes and signal-to-noise ratios were quantified as the percentage change from baseline. A correlation analysis (Spearman correlation) was performed using relative fERG changes as dependent variables and laser power and fundus lesion pigmentation (scored from 1 to 4 by means of retinal biomicroscopy) as the independent variables, to determine whether fERG changes in patients were significantly dependent on these factors. In all the analyses, $P < 0.05$ was considered to be statistically significant.

Results

At the end of clinical follow-up, 15 (94%) of our 16 patients had stabilization (no change or one line increase) or improvement (two or more lines increase) of their visual acuity. No patient had a decrease in visual acuity of two or more lines. Mean visual acuity before treatment was 0.12 ± 0.12 (SD). It increased at the end of follow-up to 0.18 ± 0.16). Eight of our 16 patients (50%) had a decrease in exudation as evaluated by biomicroscopy and fluorescein angiography. Suprathreshold neural retinal effects did not occur in any patient. No patient showed development of postoperative retinal arteriole occlusion, subretinal hemorrhage, or classic CNV.

Figure 1A shows representative fERGs recorded before, during, and after TTT for patients 2 and 10 in Table 1. Signal amplitudes and phases recorded at the different experimental times in both subjects are plotted in Figure 1B. Noise amplitudes throughout the experiment are represented by rectangular boxes indicating minimum–maximum ranges. In both patients, fERG amplitude decreased from baseline during TTT. It recovered to pretreatment levels during the 60 seconds after TTT was stopped. In our patients who had a mean signal-to-noise ratio of 9.4 dB at baseline, the signal-to-noise ratios of 12 dB for patient 2 and 6.5 dB for patient 10 were the largest and smallest recorded ratios, respectively. Data of patient 3 were excluded from further analysis, because the fERG signal could not be reliably separated from noise level at baseline, during or after TTT.

Figure 2 shows mean fERG amplitudes and phases (±SEM) recorded before, during, and after TTT in the 15 of 16 patients who had reliable baseline responses. A two-way, repeated-measures ANOVA showed that amplitudes were affected significantly by experimental time ($F_{(3,5)} = 2.47$, $P < 0.05$). Mean amplitude decreased during TTT by 23% ± 9% at 16 to 20 seconds and 13.5% ± 9% at 32 to 40 seconds. It increased again, essentially to baseline, at 48 to 60 seconds (Fig. 2, 52-second mark) after the onset TTT. Immediately after TTT, mean amplitude was within baseline range (within one SEM of the mean value). There was no significant effect of block (indicating no change in amplitude across blocks recorded at various experimental times) or interaction of block by experimental time (indicating no dependence of within-block amplitude changes on experimental time). The coefficient of variation for within-blocks amplitude variability did not change significantly across experimental times. Its value, averaged across patients, was 18%. Mean phase did not change significantly throughout the experiment, nor were there changes in its intrablock variability. The within-blocks phase SD, averaged across patients, was 14°.

Figure 3 shows the relative changes in fERG amplitude recorded in individual patients at the various experimental times and expressed as percentage changes from the amplitude of the first block recorded at baseline. The first block recorded during TTT showed a decrease in amplitude from the baseline in 13 of 15 eyes. In 3 of those 13 eyes, the percentage decrease in amplitude exceeded that expected from normal variability

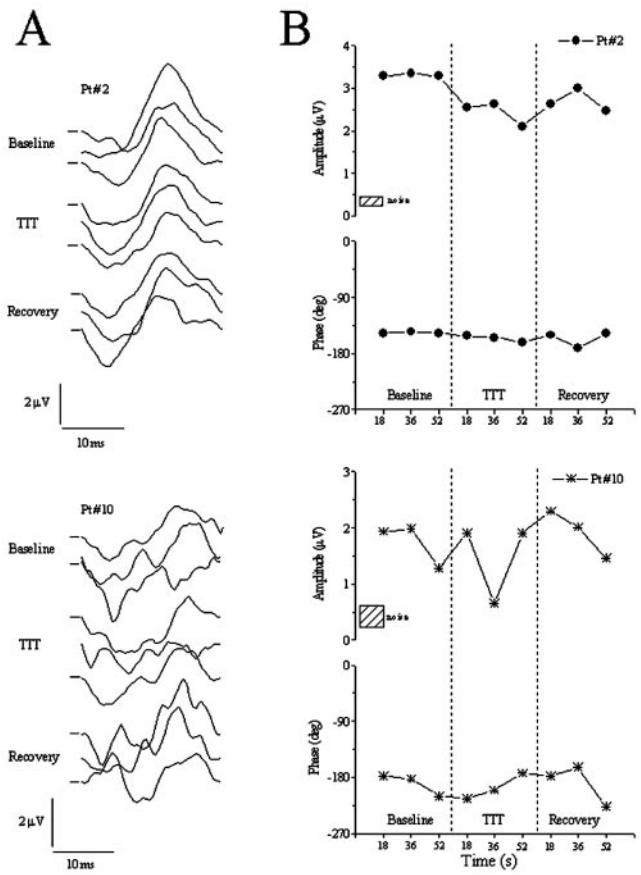

Figure 1. (**A**) Representative fERGs before (Baseline), during (TTT) and after (Recovery) TTT in patients 2 and 10 in Table 1. (**B**) Signal amplitude and phase levels were recorded at different experimental times (average temporal resolution: 18 seconds) in both subjects. Noise amplitude throughout the experiment is represented by a rectangular box displayed in the amplitude plot indicating the minimum maximum range. fERG amplitude decreased from baseline during TTT in both patients and recovered to pretreatment levels within 60 seconds after TTT was stopped. Patients 2 and 10 had the largest and smallest recorded signal-to-noise ratios, respectively.

(2-SD range of baseline variability: ±36%). A lower proportion of eyes showed similar behavior in the second and third block recorded during TTT. None of the three eyes showing a significant ($P < 0.05$) amplitude loss during TTT exhibited borderline ophthalmoscopic changes. In the third block of recordings during TTT, most of the eyes showed an increase in signal amplitude compared with that recorded during first and second blocks. In the recovery phase, some eyes had recordings within the baseline variability, whereas others showed amplitudes that were below or exceeded the 2-SD range of baseline variability.

Figure 4 shows the percentage change from baseline in fERG amplitude in the first block during treatment, plotted as a function of TTT laser power and fundus lesion pigmentation score (as determined by biomicroscopic grading, see Materials and Methods). In individual patients, the relative percentage change in amplitude during TTT did not correlate significantly with laser power. However, amplitude changes were inversely related (Spearman correlation, $P < 0.01$) to fundus lesion pigmentation, indicating that the amplitude loss in individual eyes tends to increase with increasing fundus lesion pigmentation.

Mean fERG amplitudes and phases obtained from the patients 1 week and 1 month after treatment were computed and

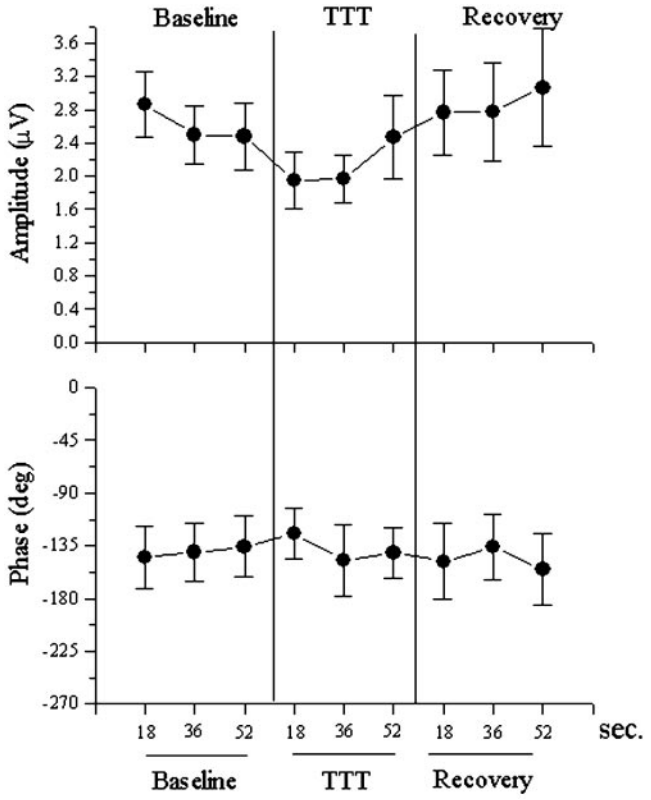

FIGURE 2. Mean fERG amplitudes and phases (±SEM) before (Baseline), during (TTT), and after (Recovery) TTT for the 15 of 16 patients who had reliable baseline responses.

compared with the corresponding pretreatment levels. Mean fERG amplitudes (±SEM) were 2.45 ± 0.30 and 2.67 ± 0.53 and mean fERG phases were −88.7 ± 23.48 and −92.3 ± 5.73, 1 week and 1 month after treatment, respectively. These values were comparable to the mean fERG amplitudes (2.34 ± 0.32) and phases (−86.7 ± 15.45), averaged across blocks, at baseline.

DISCUSSION

The 41-Hz square-wave modulated aiming beam of a diode laser photocoagulator provided an effective fERG stimulus for monitoring clinical TTT for CNV. Baseline responses were reliably above background noise levels in all but one patient. Within-blocks average variability, for the coefficient of variation of response amplitude and for the SD of response phase, was reasonably small and within the range of previously reported fERG responses in patients with ARMD.[32,38] Variability did not change significantly during TTT, allowing detection of relatively small amplitude changes (approximately 25% on average) with statistical confidence. Although the amplitude variability tended to increase after TTT (Fig. 3, third block), this change did not reach statistical significance.

Our current fERG technique has limited spatial resolution.[32] Nonetheless, in comparison to small-field focal ERG[36,37] or multifocal ERG,[47,48] it is well suited for monitoring retinal function during large field, subthreshold laser photocoagulation. Indeed, by exploiting the steady state analysis of the main harmonic response component (i.e., the fundamental), a large amount of data can be collected in a relatively short period. This feature is critical for tracking functional changes in the neural retina with adequate temporal resolution. In addition, the reliability of data can be evaluated unambiguously by monitoring the signal-to-noise ratio of the fundamental harmonic. Other focal ERG techniques may require lengthy recording sessions to produce dependable signals, thereby reducing temporal resolution to an unacceptable level.

Macular fERG amplitudes declined significantly during TTT in our patients. However, they tended to recover to baseline quickly, at 48 to 60 seconds during TTT. Within 60 seconds after TTT was completed, mean fERG amplitudes were comparable to baseline, and no significant amplitude changes were observed 1 week and 1 month after treatment. The amount of intraoperative suppression of amplitude varied between patients. It was significant (>36%, i.e., the limit of baseline fERG test–retest variability, similar to that reported for the full-field ERG[49]) in only a small fraction of treated eyes (3/15). These eyes, compared with the remaining study eyes, tended to have a higher fundus pigmentation score (Table 1). However, none of them showed borderline ophthalmoscopic changes during TTT. The fERGs elicited by flickering uniform fields reflect the activity of outer and middle retinal layers.[33,50–52] Recent monkey experiments[53] suggest that on and off cone bipolar cells make a major contribution to the fERG. Thus, the changes we observed in fERG amplitude during TTT may be related to functional changes in bipolar and/or cone photoreceptor cells.

We found that much of the recovery in fERG amplitude occurred during TTT. It is therefore unclear whether observed response changes are related to increases in temperature or some other effect during the procedure. Rapid temperature changes can alter photoreceptor sensitivity and kinetics in vivo

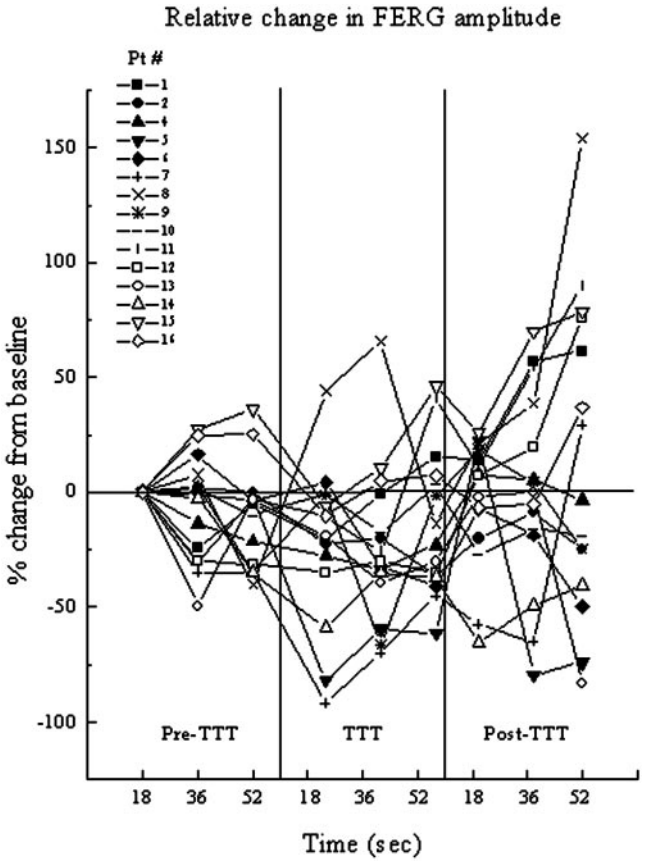

FIGURE 3. Relative changes in fERG amplitude recorded in individual patients at the various experimental times and expressed as percentage changes from the amplitude of the first block recorded at baseline. The first block recorded during TTT showed a decrease in amplitude from the baseline in 13 of 15 eyes.

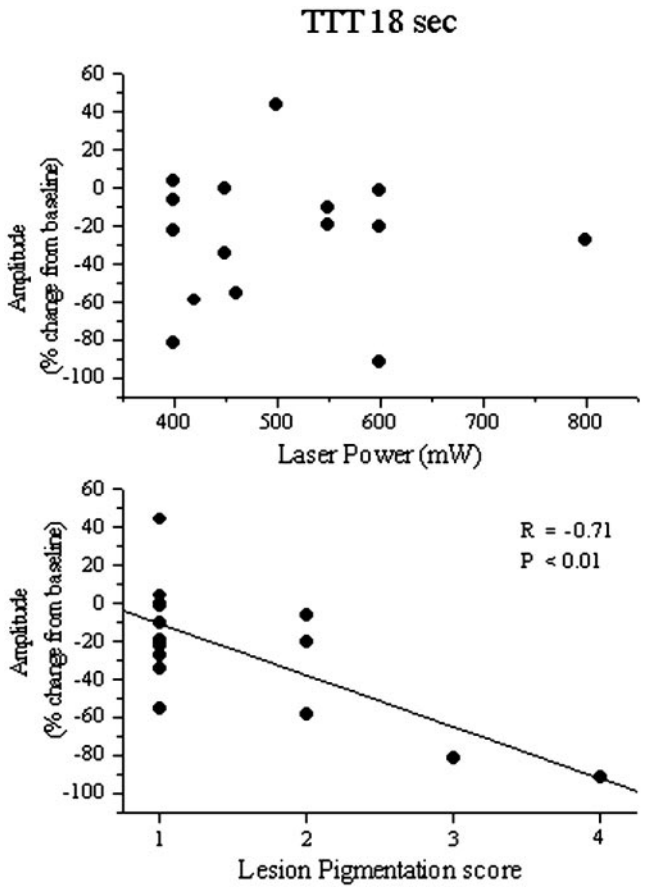

FIGURE 4. Percentage change from baseline in fERG amplitude in the first block during treatment, plotted as a function of TTT laser power (*top*) and fundus lesion pigmentation score (*bottom*, as determined by biomicroscopic grading). In individual patients, the relative percentage changes in amplitude during TTT were inversely related (Spearman correlation, $P < 0.01$) to fundus lesion pigmentation, indicating that the amplitude loss in individual eyes tends to increase with increasing fundus lesion pigmentation.

and in vitro,[54–57] so transient desensitization of cone photoreceptors and, consequently, of bipolar cell responses are potential mechanisms underlying suppression of fERG amplitude during TTT. It is also possible that some of the electrophysiological effects we observed were due to the brightness of the 810-nm diode laser treatment beam rather than just the chorioretinal temperature increase that it produces. Although the photopic spectral luminous efficiency of an 810-nm radiation is only[58] 0.0000018, a typical TTT retinal irradiance[12] of 7.5 W/cm^2 produces a significant photopic retinal illuminance of roughly 92 lux. Indeed, it is possible to see even longer-wavelength 1064 nm infrared radiation from an Nd:YAG laser.[59] Thus, an increase in the background brightness from the treatment beam may have produced some photoreceptor/bipolar cell desensitization that contributed to the fERG amplitude suppression response. In a separate experiment, performed in a normal control subject by increasing the background brightness of our stimulus configuration to an amount similar to that estimated to occur during TTT, we found a response amplitude reduction of 12% with a phase advance of 15°, compared with the levels recorded under standard conditions, thus confirming the possible role of light-adaptation on the observed effects during TTT. We believe that no other possible causes of light adaptation, such as the slit lamp light beam while the patients were being set up to start the procedure, could have been involved in the observed fERG effect. Indeed, preadaptation for each patient was performed under reasonably controlled and uniform conditions.

The decrease in fERG amplitude that occurred 16 to 20 seconds after the onset of TTT depended on fundus lesion pigmentation. It was more prominent in eyes with increased lesion pigmentation. In our patients, iris pigmentation was also used as an indirect method of estimating fundus pigmentation, because it correlates with fundus pigmentation in individual subjects.[60] However, such a relationship may not be strict, because it is quite evident from the data reported in Table 1. A particular retinal irradiance produces a higher increase in chorioretinal temperature in individuals with darker fundus pigmentation.[12] This could also explain the lack of correlation of intraoperative fERG amplitude changes with the laser power settings used for individual treatments. Thus, the eyes with more darkly pigmented fundus lesions in our study that showed greater fERG amplitude reductions, probably also experienced higher chorioretinal temperature increases. The validity of this correlation awaits confirmation by a more detailed, parametric study that can evaluate the effects of laser power and fundus pigmentation, taking into consideration lesion size and the clarity of ocular media in individual eyes.

In conclusion, fERG may be a useful measure of neural retinal function during TTT.[32,33,54–52] Despite the absence of ophthalmoscopically apparent lesions, transient suppression of fERG amplitude documents physiological events during TTT that affect the outer and/or middle retina. These events produce objective, measurable responses that may be useful for guiding and perhaps optimizing subthreshold photocoagulation. Treatment sites are electrophysiologically functional 1 month after TTT, with fERG responses that are comparable to those at baseline. In most eyes the area under the treatment beam was large enough to include functioning retina (Table 1), so that the results are not simply a record of retina that was outside the treatment beam. In addition, the neural retina over the CNV lesion showed, in all but one patient, a significant functional response before TTT, and this response did not change significantly after treatment. The results of ongoing randomized prospective trials will help determine the role of TTT in managing CNV. The identification of transient changes in fERG amplitudes during TTT is a first step in evaluating the potential role of fERG in monitoring and controlling subthreshold photocoagulation. The possible use of the present technique for providing feedback control of the laser power used for TTT could be tested independently in animal models. However, as far as human subjects are concerned, only a detailed study of a larger patient group can reveal whether characterization of outer retinal function by fERG can help guide TTT for particular eyes and macular abnormalities.

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