Phosphene Thresholds Elicited by Transcorneal Electrical Stimulation in Healthy Subjects and Patients with Retinal Diseases

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Purpose. To evaluate electrically evoked phosphene thresholds (EPTs) in healthy subjects and in patients with retinal disease and to assess repeatability and possible correlations with common ophthalmologic tests.

Methods. In all, 117 individuals participated: healthy subjects ($n = 20$) and patients with retinitis pigmentosa (RP, $n = 30$), Stargardt's disease (STG, $n = 14$), retinal artery occlusion (RAO, $n = 20$), nonarteritic anterior ischemic optic neuropathy (NAION, $n = 16$), and primary open-angle glaucoma (POAG, $n = 17$). EPTs were determined at 3, 6, 9, 20, 40, 60, and 80 Hz with $5 + 5$-ms biphasic current pulses using DTL electrodes. Subjects were examined twice (test–retest range: 1–6 weeks). An empirical model was developed to describe the current–frequency relationship of EPTs. Visual acuity, visual field (kinetic + static), electrophysiology (RP, RAO, STG: Ganzfeld-electroretinography [ERG]/multifocal-ERG; POAG: pattern-ERG; NAION: VEP), slit-lamp biomicroscopy, fundus examination, and tonometry were assessed.

Results. EPTs varied between disease groups (20 Hz: healthy subjects: 0.062 ± 0.038 mA; STG: 0.102 ± 0.097 mA; POAG: 0.127 ± 0.09 mA; NAION: 0.244 ± 0.126 mA; RP: 0.371 ± 0.223 mA; RAO: 0.988 ± 1.142 mA). In all groups EPTs were lowest at 20 Hz. In patients with retinal diseases and across all frequencies EPTs were significantly higher than those in healthy subjects, except in STG at 20 Hz ($P = 0.09$) and 40 Hz ($P = 0.17$). Test–retest difference at 20 Hz was 0.006 mA in the healthy group and 0.003–0.04 mA in disease groups.

Conclusions. Considering the fast, safe, and reliable practicability of EPT testing, this test might be used more often under clinical circumstances. Determination of EPTs could be potentially useful in elucidation of the progress of ophthalmologic diseases, either in addition to standard clinical assessment or under conditions in which these standard tests cannot be used meaningfully. (ClinicalTrials.gov number, NCT00804102.) (*Invest Ophthalmol Vis Sci.* 2012;53:7440–7448) DOI:10.1167/iovs.12-9612

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Phosphenes (from Greek *phos* = light and *phainein* = to show) are defined as visual perceptions induced by stimuli other than light. They can be elicited by mechanical force, eye movements, x-rays, magnetic fields, chemicals, or electrical currents.[1] Phosphenes can also appear spontaneously when visual stimuli are lacking, especially when the viewer is subjected to prolonged visual deprivation (known as "prisoner's cinema").[2,3]

Electrically evoked phosphenes (EEPs) were first described in the eighteenth century[4] and have been extensively studied since then.[5–17] To evoke phosphenes, electrical stimulation can be applied to the visual cortex, the optic nerve, the eye ball, or the retina, either from the subretinal, epiretinal, or suprachoroidal space.

Trying to create a visual prosthesis based on electrical stimulation of the visual system, Brindley and Lewin[8] implanted an electrode array connected to a radio receiver into the occipital pole of the right cerebral hemisphere of a 52-year-old blind patient. Upon stimulation the patient reported phosphenes in the left half of the visual field. Dobelle et al.[18] used an array of cortical electrodes enabling a blind patient to recognize Braille letters.

In recent years, the interest in development of visual prostheses increased and several groups worldwide are now performing clinical tests. In those studies electrical stimulation for eliciting phosphenes is applied in a different manner: transcorneal,[19,20] suprachoroidal,[21] transscleral,[22] epiretinal,[23–25] and subretinal.[26–29]

Recently, the noninvasive determination of electrically evoked phosphene thresholds (EPTs) has been used as a screening test for visual prosthesis candidates, providing a safe way to assess electrical excitability in patients before invasive surgical procedures are performed.[19,20,29–31]

Moreover, studies have shown that electrical stimulation produced by visual prostheses can have neuroprotective effects in degenerated retinae,[27,32–34] potentially delaying the disease progress. Some data link electrical stimulation with the upregulation of neurotrophins in the central and peripheral nervous systems.[35–37] In animal experiments electrical stimulation has been shown to be beneficial for the survival of photoreceptors in Royal College of Surgeon's (RCS) rats,[38] to rescue ganglion cells after optic nerve injury[39,40] and to preserve retinal cells after light-induced retinal damage (Zhang H, et al. *IOVS* 2009;50:ARVO E-Abstract 3615).[41] The neuroprotective effects of electrical stimulation have been attributed to upregulation of growth factors, such as insulin-like growth factor-1 (IGF-1),[39,42] fibroblast growth factor-2 (FGF-2),[34,43] ciliary neurotrophic factor (CNTF),[44,45] brain-derived neurotrophic factor (BDNF),[41,46] and to overexpression of neuroprotective genes, such as B-cell lymphoma-2 (BCL-2),[47] BAX, or some tumor necrosis factor genes.[48]

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Transcorneal electrical stimulation (TES) on humans with longstanding retinal artery occlusion,[49] nonarteritic anterior ischemic optic neuropathy, and traumatic optic neuropathy[50] demonstrated positive effects on visual acuity and visual field.

Recently published data showed statistically significant improvement of visual field area and scotopic b-wave amplitude in patients with retinitis pigmentosa,[51] treated with TES.

However, to date very few data are available on where in the retina or the visual system EEPs are generated and how they correlate with processes in different ophthalmic diseases.

To explore the potential of TES as a therapy or for the use of EPTs to detect the residual function in candidates for retinal prostheses, understanding the biological origin of phosphenes, their characteristics, and the appropriate stimulation parameters are crucial.

Here we report on EPTs over a range of stimulation frequencies determined in healthy subjects and patients with retinitis pigmentosa (RP), retinal artery occlusion (RAO), nonarteritic anterior ischemic optic neuropathy (NAION), Stargardt's disease (STG), and primary open-angle glaucoma (POAG). To estimate parameters of the relationship between stimulus amplitude and stimulation frequency an empirical model has been developed. Additionally, the repeatability of EPTs and correlations between EPTs and common ophthalmologic tests such as visual acuity (VA), visual field (VF), Ganzfeld-electroretinography (Ganzfeld-ERG), multifocal electroretinography (mfERG), pattern-electroretinography (PERG), and visually evoked potentials (VEPs) has been assessed.

Materials and Methods

Subjects

The study was performed at the Centre for Ophthalmology, University of Tübingen, Germany. The protocol was approved by the local ethics committee. All procedures were undertaken with the understanding and written consent of each subject, respecting the Declaration of Helsinki. Subjects were recruited from the outpatient clinic and the hospital's personnel.

In all, 117 eyes of 117 individuals were enrolled in this study: healthy subjects ($n = 20$) and patients with RP ($n = 30$), RAO ($n = 20$), NAION ($n = 16$), STG ($n = 14$), and POAG ($n = 17$).

Inclusion criteria were: more than 18 years of age; RP and STG: quantifiable VF and at least one recordable response in Ganzfeld-ERG or mfERG as well as best-corrected visual acuity (BCVA) for RP: 0.02–0.9 and for STG between light perception (LP) and 0.7; RAO and NAION: BCVA between LP and 0.7; POAG: BCVA between LP and 1.0; RAO, NAION, and POAG: a partial, but not complete loss of VF.

Exclusion criteria were proliferative retinal diseases such as diabetic retinopathy, macular edema, retinal or choroidal neovascularization, exudative age-related macular degeneration, silicone oil tamponade, and severe general diseases.

Ophthalmologic Examinations

All subjects underwent extensive ophthalmologic examinations, including BCVA assessment using retroilluminated Early Treatment Diabetic Retinopathy Study charts (ETDRS Visual Acuity Tester; Steinbeis-Transferzentrum, Tübingen, Germany) at 2 m distance and Snellen projector (SCP-660; Nidek, Inc., Fremont, CA) at a viewing distance of 6 m, slit-lamp biomicroscopy, fundus examination, Goldmann applanation tonometry (AT 900; Haag-Streit, Wedel, Germany), and static and kinetic perimetries. In patients with RP, RAO, and STG, Ganzfeld-ERG and mfERG were recorded; in patients with POAG, PERG was recorded; and in patients with NAION, pattern-VEPs were recorded.

Static and kinetic perimetries were performed with full-field perimetry (Octopus 900 perimeter; Haag-Streit). The background luminance was 10 cd/m^2. White-on-white static perimetry was undertaken using a fast threshold strategy (German Adaptive Thresholding Estimation [GATE])[52] up to 85° eccentricity. A test grid consisting of 86 stimulus locations that were condensed toward the center was applied (stimulus size: Goldmann III; duration of presentations: 200 ms; response time: 1200 ms). For kinetic perimetry, white stimuli (Goldmann III4e with constant angular velocity of 3°/s) up to 90° eccentricity were presented every 15°. Isopter and scotoma areas (in deg^2) were quantified using the built-in software algorithm. As quality criterion for kinetic perimetry the blind spot was detected with at least five stimuli Goldmann I4e at 2°/s.

Electrophysiologic examinations were conducted according to the standards of the International Society for Electrophysiology of Vision.[53–56] Ganzfeld-ERG was recorded using an electrophysiology recording system (Espion E2; Diagnosys LLC, Cambridge, UK) in connection with an appropriate light source (ColorDome; Diagnosys LLC). The protocol included four steps with stimulus strengths from 0.1 to 3 phot cd*s/m^2 and 4-ms duration (white 6500K). Two single-flash responses were used under scotopic conditions (stimulus strength 0.1 and 3 phot cd*s/m^2) as scotopic protocol. A single-flash response (3 phot cd*s/m^2 with a background illumination of 30 phot cd*s/m^2 = standard flash [SF]) and a 30-Hz flicker were chosen as cone protocol. The impedance level was <10 kΩ. A wide-range band-pass filter (from 0.3–300 Hz) was applied using the machine's built-in algorithm. ERG potentials < 5 μV were excluded from the analysis.

To carry out mfERG a patient monitoring system (VerisTM, software version Veris Science TM 5.1; Electro-Diagnostic Imaging, Inc., Redwood City, CA) and a 21-inch screen ("UHR21L"; Nortech Imaging Technologies, Plymouth, MN; resolution 1024 × 768) positioned 32-cm distance in front of the subject were used. The stimulus consisted of 61 scaled hexagonal elements presented with alternating black (5 cd/m^2) and white (100 cd/m^2) fields and covered a central visual field of 60 × 55°. The built-in algorithm allowed recordings between 10 and 100 Hz, amplified by a factor of 200,000.

Transient and steady-state reversal PERG and transient and steady-state pattern reversal and onset/offset VEPs were elicited by checkerboard stimuli, with a check size of 0.8° and 0.25° (=15 minutes) at 4 Hz on a 15-inch screen (Elonex [UK] Ltd., Coleshill, UK), with contrast 100% and brightness 35%, on a recording device (Espion E2; Diagnosys LLC).

All electrophysiologic examinations and the electrical stimulation were performed with a single-use sterile DTL electrode[57] (Diagnosys LLC; Department of Clinical Engineering, Liverpool, UK) and a gold-plated cup electrode (LKC Technologies, Inc., Gaithersburg, MD) as ground. Cornea and conjunctiva were anesthetized with 0.4% oxybuprocainhydrochlorid (Conjuncain EDO; Bausch & Lomb, Inc., Rochester, NY). Pupils were dilated with 0.5% tropicamide (Mydriaticum Stulln Pharma, Stulln, Germany).

Electrical Stimulation

EPTs were determined at 3, 6, 9, 20, 40, 60, and 80 Hz, with 10-ms biphasic current pulses (5 ms positive followed by 5 ms negative) using a neurostimulator (Twister DN; Dr. Langer Medical GmbH, Waldkirch, Germany). As a working electrode we used a DTL electrode and as a counter electrode a gold-plated cup electrode, fixed on the ipsilateral temple (Fig. 1).

The neurostimulator was modified by the manufacturer to limit current output to 10 mA with increments starting from 1 μA. The small step size and the limited current output allowed a quick and safe determination of EPTs. Stimulation frequencies from 1 to 100 Hz could be set by the examiner. The stimulus waveform could be monophasic, biphasic, or graphically specified. Impedance of the electrodes was tested every time before stimulation and at various intervals during stimulation using the built-in algorithm of the machine and did not exceed 5 kΩ. Measurements were performed in darkness with a very

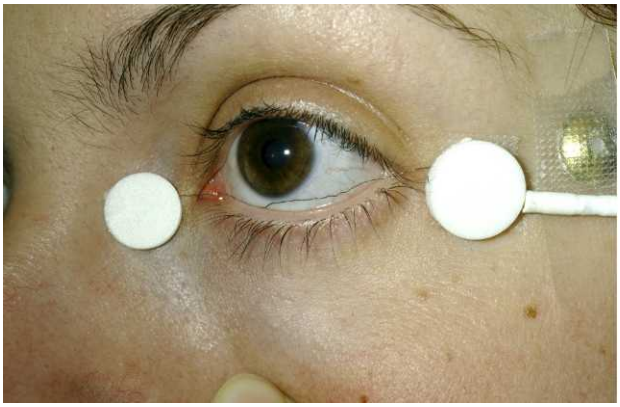

Figure 1. Equipment used for determination of electrically evoked phosphene thresholds. (**A**) A DTL electrode was used as a working electrode and a gold-plated cup electrode, fixed on the ipsilateral temple as a counter electrode. (**B**) Neurostimulator (TwisterDN; Dr. Langer Medical GmbH, Waldkirch, Germany), in which 10-ms (5-ms positive followed by 5-ms negative) current pulses were used.

dim indirect light produced by the shielded computer screen. To avoid dark adaptation, light was switched periodically (approximately every 60–90 seconds).[30] Full darkness was necessary to facilitate perception of the very subtle phosphenes. Thresholds were defined as the minimal electrical current that elicited phosphene perceptions anywhere in the visual field. An alternative forced-choice method was used for verification. The complete procedure was carefully explained before each session. To assess the repeatability all subjects were examined at two visits, test–retest intervals ranging between 1 and 6 weeks, and median 1 week.

Data Analysis

Statistical analyses were performed with commercial interactive software (JMP Software, version 8.02; SAS Institute, Inc., Cary, NC). After analysis of variance (one-way ANOVA) the Tukey's test was used to compare the groups' means of all pairs for all frequencies.

An empirical, Gaussian-shaped model was used to retrieve the parameters for the relationship between stimulus current and frequency for phosphene thresholds: $Thr = Thr_{min} \times \exp[-(Freq - Freq_{min})] - Thr_{max}$, where Thr is the calculated phosphene threshold current, Thr_{min} is the estimated minimal threshold current, Freq is the stimulus frequency, $Freq_{min}$ is the estimated frequency for minimal threshold (= frequency of maximal sensitivity), Thr_{max} is the estimated asymptotic maximal threshold, and h is the difference between Thr_{max} and Thr_{min} (Fig. 2).

Values for Thr_{max}, h, and $Freq_{min}$ of each disease group were compared with one-way ANOVA and Tukey's test.

Pearson's correlation coefficient was used to calculate correlations between EPTs, Thr_{max}, h, $Freq_{min}$, and BCVA, VF, Ganzfeld-ERG, mfERG, PERG, and VEP. To assess the repeatability of EPTs a Bland–Altman-analysis[58] was performed (mean difference $\pm$ 1.96 $\times$ SD) to calculate the repeatability coefficient (RC), adopted by British Standards Institution.[59]

Results

In all individuals, thresholds were safely determined without adverse events. No morphologic alterations were noticed on ophthalmic examination after electrical stimulation. As reported previously,[30] most subjects reported homogeneous, whitish central phosphenes. Three patients with RAO had no phosphene perception despite stimulation currents up to 4 mA, a level at which unpleasant sensations such as a twitch in the lid were noticed. Description of phosphenes was not different between groups. None of the patients reported, on explicit questioning, any difference in phosphene brightness from intact areas in visual field testing and areas of scotomas.

The mean $\pm$ SD values for BCVA determined with ETDRS were in healthy subjects 1.21 $\pm$ 0.14, STG 0.27 $\pm$ 0.2, POAG 0.76 $\pm$ 0.21, NAION 0.37 $\pm$ 0.3, and RP 0.66 $\pm$ 0.22. The mean $\pm$ SD values for BCVA determined with ETDRS in RAO patients could not be calculated, because in six patients BCVA was only counting fingers or lower.

The mean $\pm$ SD values for BCVA tested with Snellen (in logMAR; i.e., logarithm of the minimum angle of resolution) were in healthy subjects –0.08 $\pm$ 0.05, STG 0.7 $\pm$ 0.37, POAG 0.11 $\pm$ 0.12, NAION 0.63 $\pm$ 0.5, RP 0.23 $\pm$ 0.19, and RAO 1.51 $\pm$ 0.5.

Phosphene Thresholds

EPTs in healthy subjects were 0.062 $\pm$ 0.038 mA at 20 Hz (mean $\pm$ SD), 0.085 $\pm$ 0.053 mA at 40 Hz, 0.098 $\pm$ 0.061 mA

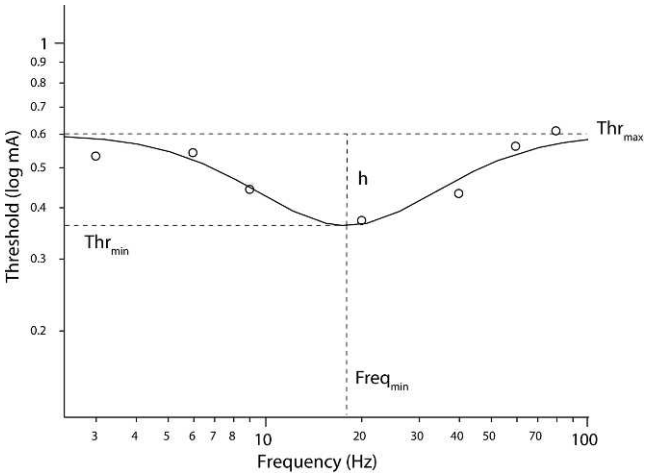

Figure 2. Empirical, Gaussian-shaped model to estimate parameters of the relationship between stimulus current and frequency for electrically evoked phosphene thresholds: $Thr = Thr_{min} \times \exp[-(Freq - Freq_{min})] - Thr_{max}$, where Thr_{min} is the estimated minimal threshold current, Freq is the stimulus frequency, $Freq_{min}$ is the estimated frequency for minimal threshold (= frequency of maximal sensitivity), Thr_{max} is the estimated asymptotic maximal threshold, h is the difference between Thr_{max} and Thr_{min}, and Thr is the actual estimated phosphene threshold current.

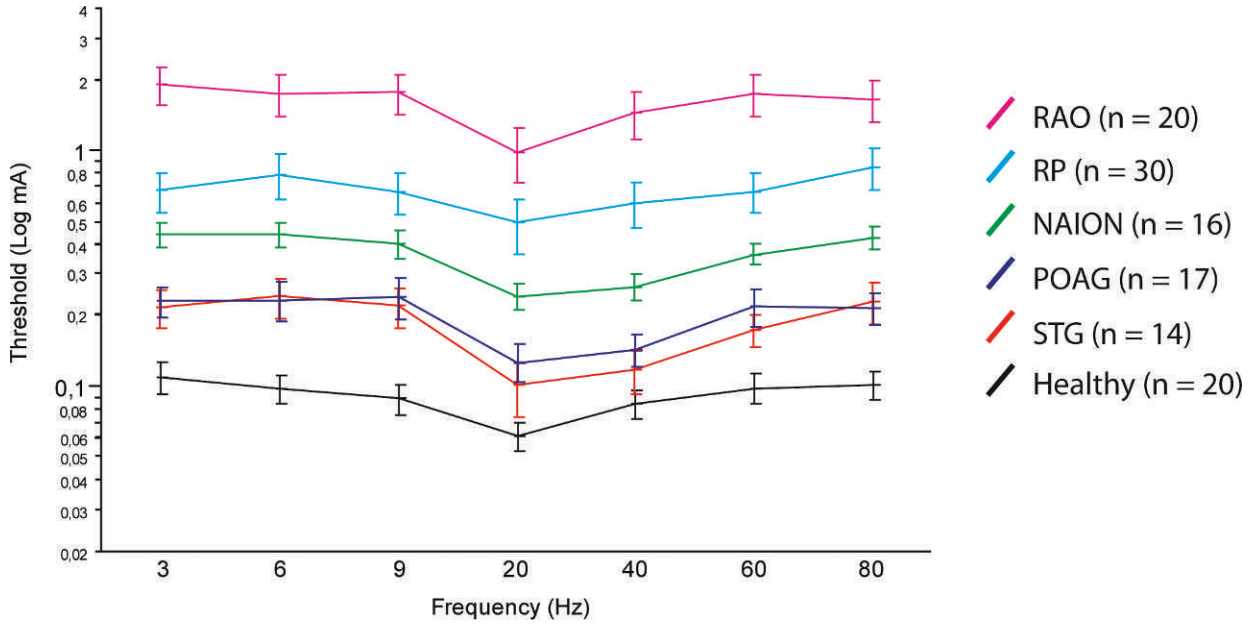

Figure 3. Electrically evoked phosphene thresholds (EPTs) in healthy subjects and in patients with retinal diseases (mean + SE) over all tested frequencies. EPTs at all frequencies were lowest in healthy subjects and highest in RAO, and midrange in increasing order were STG, POAG, and NAION. In all groups the lowest EPTs were found at 20 Hz.

at 60 Hz, 0.102 ± 0.061 mA at 80 Hz, 0.109 ± 0.076 mA at 3 Hz, 0.097 ± 0.061 mA at 6 Hz, and 0.089 ± 0.056 mA at 9 Hz.

EPTs at all frequencies were lowest in healthy subjects and highest in RAO; midrange in increasing order were STG, POAG, and NAION (e.g., at 20 Hz mean ± SD in healthy subjects: 0.062 ± 0.038 mA; STG: 0.102 ± 0.097 mA; POAG: 0.127 ± 0.09 mA; NAION: 0.244 ± 0.126 mA; RP: 0.371 ± 0.223 mA; RAO: 0.988 ± 1.142 mA) (Fig. 3).

Table 1. Estimated Parameters of Electrically Evoked Phosphene Thresholds in Relation to Frequency (the model is described in Fig. 2)

				Confidence Interval	
	Group	**Mean**	**SD**	**95% Lower**	**95% Upper**
Thr_{max}, mA	Healthy	0.11	0.07	0.08	0.15
	STG	0.25	0.17	0.15	0.35
	POAG	0.26	0.18	0.17	0.35
	NAION	0.48	0.21	0.37	0.60
	RP	0.79	0.74	0.51	1.07
	RAO	1.99	1.62	1.24	2.76
h, mA	Healthy	0.05	0.04	0.03	0.07
	STG	0.15	0.10	0.09	0.22
	POAG	0.13	0.09	0.08	0.17
	NAION	0.26	0.14	0.18	0.33
	RP	0.30	0.38	0.16	0.44
	RAO	0.90	1.01	0.42	1.37
$Freq_{min}$, log	Healthy	1.34	0.18	1.26	1.42
	STG	1.41	0.06	1.37	1.44
	POAG	1.38	0.09	1.33	1.43
	NAION	1.38	0.11	1.32	1.44
	RP	1.33	0.10	1.29	1.37
	RAO	1.35	0.11	1.30	1.40
$Freq_{min}$, Hz	Healthy	21.9	1.51	18.2	26.3
	STG	25.7	1.15	23.4	27.5
	POAG	23.9	1.23	21.4	26.9
	NAION	23.9	1.29	20.9	27.5
	RP	21.4	1.26	19.5	23.4
	RAO	22.4	1.29	19.9	25.1

In all groups the lowest EPTs were found at 20 Hz (Fig. 3).

In all groups with retinal diseases and across all frequencies, EPTs were significantly higher than those in the control group, except STG versus healthy patients at 20 Hz ($P = 0.09$) and at 40 Hz ($P = 0.17$).

The values for Thr_{max}, h, and $Freq_{min}$ for each group are shown in Table 1. Thr_{max} and h were lowest in healthy volunteers (mean ± SD: Thr_{max}: 0.11 ± 0.07 mA and h: 0.05 ± 0.04 mA) and highest in RAO (mean ± SD: Thr_{max}: 2.00 ± 1.62 mA and h: 0.90 ± 1.01 mA) and showed significant differences between RAO and all other groups. Additionally, Thr_{max} showed significant differences in RP and healthy patients ($P = 0.038$). There was no significant difference for the Thr_{max} and h between healthy patients and STG, POAG, and NAION. $Freq_{min}$ was lowest in healthy subjects and highest in patients with STG, in all groups in average between 21.4 and 25.7 Hz (range: 18.2–27.5 Hz). The differences between the groups were not statistically significant (Fig. 4).

Correlations

Correlation analysis was performed between phosphene parameters (EPTs, Thr_{max}, h, $Freq_{min}$) and common ophthalmologic tests (BCVA, VF, Ganzfeld-ERG, mfERG, PERG, and VEP) using Pearson's correlation.

We found a strong correlation between Thr_{max} and VA in RAO ($r = 0.73$, $P = 0.0002$ for ETDRS and $r = 0.71$, $P = 0.0004$ for Snellen) and between $Freq_{min}$ and VA in NAION ($r = 0.76$, $P = 0.0006$ for ETDRS and $r = 0.70$, $P = 0.003$ for Snellen). In RP Thr_{max} and h correlated strongly with amplitude and implicit time in dark- and light-adapted ERG, but they did not reach statistical significance (e.g., $r = -0.79$, $P = 0.2$ for Thr_{max}/amplitude, and $r = -0.82$, $P = 0.18$ for Thr_{max}/implicit time). In STG, Thr_{max} and $Freq_{min}$ showed negative correlation with the P1-latency of the central ring of mfERG (e.g., $r = -0.62$, $P = 0.03$ for $Freq_{min}$). In NAION and RAO we found a strong correlation between Thr_{max} and h with VF ($r = -0.7$, $P = 0.002$ for static MS and $r = 0.7$, $P = 0.005$ for static MD to Thr_{max} in NAION and $r = 0.92$, $P = 0.02$ for kinetic area to h in RAO). N95-Latency and P50-Amplitude in PERG in POAG correlated

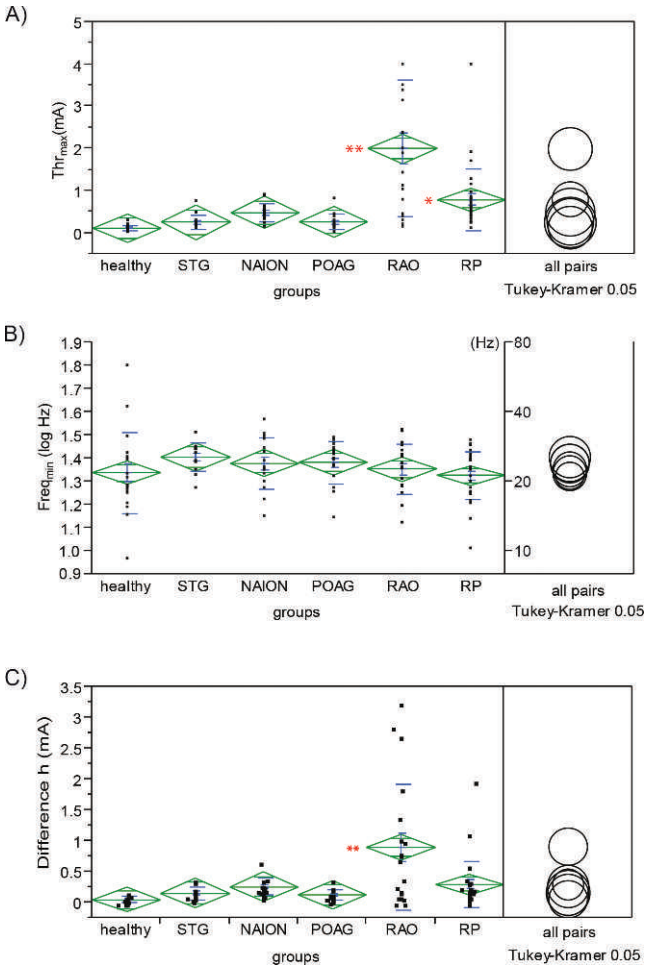

Figure 4. Estimated values for Thr_{max} (maximal threshold), $Freq_{min}$ (frequency of minimal threshold), and h (difference between maximal and minimal threshold) of each disease group against healthy subjects, compared using one-way ANOVA and Tukey's test. (**A**) Thr_{max} was lowest in healthy subjects and highest in RAO; **RAO was significantly higher compared with all other groups ($P < 0.0001$); *RP was significantly higher only compared with healthy subjects ($P = 0.038$). (**B**) $Freq_{min}$ was not statistically significantly different between all groups; mean, SD, 95% lower and 95% upper confidence intervals (CIs) are shown in Table 2. (**C**) h was significantly higher in RAO than that in all other groups ($P < 0.0001$).

significantly with Thr_{max}, h, and $Freq_{min}$ (e.g., $r = 0.82$, $P = 0.01$ for Thr_{max} and $r = -0.83$, $P = 0.01$ for h). Also N75- and P100-Latency were correlated with Thr_{max} and h in VEP in NAION (e.g, $r = -0.73$, $P = 0.09$ for Thr_{max} and $r = 0.78$, $P = 0.06$ for h with N75-Latency).

Repeatability

The mean difference between two determinations of EPTs (visit 2 − visit 1) was at 20 Hz in healthy condition −0.006 mA, in STG 0.003 mA, in POAG −0.0003 mA, in NAION 0.007 mA, in RP −0.04 mA, and in RAO −0.03 mA. Table 2 shows the values for mean difference ± SD in all groups for all frequencies. In RAO and RP we found large SDs, leading to high values for the repeatability coefficient (RC): in healthy subjects 0.031 mA, in STG 0.084 mA, in POAG 0.087 mA, in NAION 0.140 mA, in RP 0.370 mA, and in RAO 0.502 mA (Fig. 5).

Discussion

This study describes the behavior of EPTs over a range of frequencies in patients with various ophthalmologic diseases and compares them to values of healthy subjects. An empirical mathematical model was developed to describe the relationship between stimulus frequency and threshold current. The repeatability of phosphene measurements was assessed and correlations between phosphene threshold parameters and findings of common ophthalmologic tests were tested. Furthermore, this is the first study describing EPTs in patients with STG.

In analogy to previous studies[20,31] EPTs in our study showed significantly lower values in healthy volunteers than those in patients with retinal diseases. EPTs found in healthy subjects and patients with RP and RAO match well to previously published data, for example, Morimoto et al.,[20] who studied EPTs in healthy patients and patients with RP and cone–rod dystrophy using contact lens electrodes and biphasic 10-ms current pulses at 20 Hz, and also to Inomata et al.,[49] who treated patients with longstanding RAO using TES (contact lens electrodes, biphasic pulses, 10 ms, 20 Hz). Despite differences in the measurement technique (contact lens electrode versus DTL electrode) our results are very similar to their data: mean ± SD at 20 Hz in healthy patients: 0.065 ± 0.008 mA (Morimoto[20]) vs. 0.062 ± 0.038 mA (our results); EPTs in RAO patients ranging from 0.800 to 1.100 mA (Inomata[49]) versus mean ± SD 0.988 ± 1.142 mA (our results). This fact indicates that elicitations of EEPs and determinations of EPTs are robust and reliable, also across different and nonstandardized recording electrodes.

In our assessment we have shown that measurements of EPTs are also well repeatable and that this repeatability is comparable in healthy condition and in retinal disease, for example, the mean difference between two measurements of EPTs at 20 Hz in healthy subjects was only −0.006 mA and in the groups with retinal diseases between 0.003 and 0.04 mA. Considering the mean EPTs (e.g., at 20 Hz 0.244 mA for NAION

Table 2. Mean Differences between Two Determinations of Electrically Evoked Phosphene Thresholds in All Groups at All Tested Frequencies

	Test–Retest Difference (mean ± SD, in mA)					
	Healthy	**STG**	**POAG**	**NAION**	**RP**	**RAO**
Frequency, Hz						
3	−0.017 ± 0.007	−0.02 ± 0.02	−0.003 ± 0.01	0.06 ± 0.03	−0.04 ± 0.06	−0.56 ± 0.28
6	−0.01 ± 0.004	−0.05 ± 0.04	0.005 ± 0.02	0.05 ± 0.04	−0.22 ± 0.12	−0.41 ± 0.20
9	−0.007 ± 0.005	−0.02 ± 0.03	−0.01 ± 0.01	0.07 ± 0.03	−0.08 ± 0.06	−0.56 ± 0.26
20	−0.006 ± 0.015	0.003 ± 0.04	−0.003 ± 0.05	0.007 ± 0.07	−0.04 ± 0.19	−0.03 ± 0.26
40	−0.01 ± 0.004	0.001 ± 0.01	0.01 ± 0.02	0.07 ± 0.03	−0.12 ± 0.06	−0.26 ± 0.18
60	−0.017 ± 0.005	0.008 ± 0.02	−0.004 ± 0.02	0.07 ± 0.04	−0.1 ± 0.05	−0.38 ± 0.23
80	−0.01 ± 0.005	−0.01 ± 0.03	0.01 ± 0.02	0.04 ± 0.04	−0.08 ± 0.05	−0.14 ± 0.09

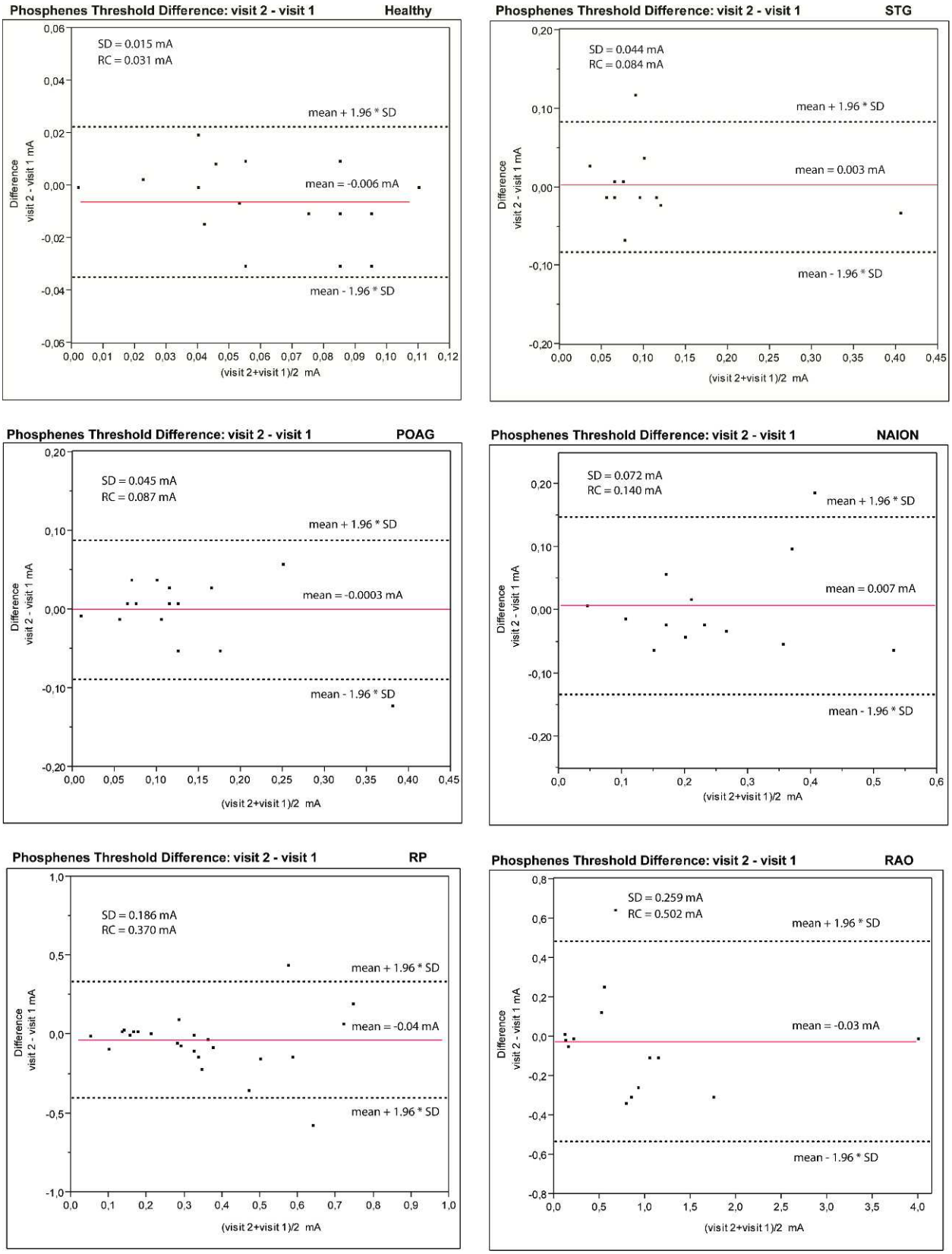

Figure 5. Assessment of repeatability of two measurements of electrically evoked phosphene thresholds for each group represented in a Bland–Altman diagram (RC). *Top left*: Healthy subjects ($n = 20$). *Top right*: Patients with STG ($n = 14$). *Middle left*: Patients with POAG ($n = 17$). *Middle right*: Patients with NAION ($n = 16$). *Bottom left*: Patients with RP ($n = 30$). *Bottom right*: Patients with RAO ($n = 20$).

or 0.127 mA for POAG), these differences (e.g., 0.007 mA for NAION or 0.003 mA for POAG) constitute only 2% to 3%.

By repeatedly including the presented data,[30] we have shown that eliciting EEPs with TES, applied with DTL electrodes, is safe, painless, and simple to perform. None of the individuals reported any signs of pain and the ophthalmologic examination after electrical stimulation showed no morphologic alterations. Most patients reported homogeneous white flashes in the central visual field. In patients with visual field loss, no correlation was found with the remaining visual islands.

Up to now, although a number of studies have been designed to explore the origin of phosphenes, it has not been conclusively answered as to which type of retinal cells are the major contributors to the perception of phosphenes. Potts et al.[12] investigated the effect of dark adaptation and RP on the electrically evoked response (EER) and concluded that the structure sensitive to the electrical pulse lies proximal to the photoreceptors. One year later, Potts et al.[13] conclusively eliminated the photoreceptors as the site of origin of EER by showing that rats with complete loss of receptor segments and nuclei, where light-evoked responses and ERGs were absent, had normal EER. Potts and colleagues[13] subsequently suggested that electrical current is stimulating the retina in the region of bipolar, horizontal, and amacrine cells with its inhibitory connections, rather than ganglion cells or their axons. This is in accordance with our own findings in patients of the subretinal implant project in whom well-recordable EERs have been found despite completely extinct light perception and ERG responses (unpublished data). Miyake et al.[15–17] have also shown that EERs can be nearly normal in patients with a dysfunctional rod or cone visual pathway, although in patients with central RAO or optic nerve diseases EERs were reduced. Slaughter and Miller[60] found that a glutamate agonist, DL-2-amino-4-phosphonobutyric acid (APB), eliminates light-triggered responses of depolarizing ON bipolar cells with no effect on any other retinal cells. After APB application, responses of retinal ganglion cells to TES were significantly reduced. This suggests that the ON bipolar cells and their related synaptic sites are involved in the origin of phosphenes.

Certainly, ganglion cells must be intact to conduct visual perceptions of any kind (including EEPs) generated anywhere within the retina to the brain. Accordingly, in patients with NAION, POAG, and RAO in our study EPTs at all tested frequencies were significantly higher than those in healthy subjects. In RP patients EPTs were significantly higher than those in healthy subjects (although lower than those in RAO patients). This might be explained with the secondary damage to the more proximal layers such as the ganglion cell layer in RP[61] or with the fact that, in addition to other cells, remaining intact photoreceptors can be used (while not being required) to perceive EEPs. The presence of a difference in EPTs in light- and dark-adapted retinae[30] (with lower thresholds in the light-adapted state) suggests that cones are more involved in the origin of phosphenes than rods. This would, however, be in contradiction to the similarities of EPTs found in patients with STG and healthy subjects in our study.

In the evaluation of correlations between parameters of phosphenes and of common ophthalmologic examinations we have found a strong correlation between VA and phosphenes' maximal threshold and frequency of maximal sensitivity in RAO and NAION, but no such correlation in RP, STG, and POAG. We do not have a unifying explanation for this distribution among these different diseases, but the result should be taken with caution since VA is extremely low in RAO and NAION. Furthermore, we have found a strong correlation between the maximal threshold and VF in NAION and RAO and N95-Latency and P50-Amplitude in PERG in POAG, as well as N75- and P100-Latency in VEP in NAION. Other studies[30,62] have also described a correlation between VA and phosphene thresholds in patients with RP. Morimoto et al.[20] found no correlation between EPTs and VA, but a correlation between EPTs and area and location of the residual VF as well as the type of disease: EPTs (20-Hz, 10-ms biphasic current) in patients with RP or cone–rod dystrophy (CRD) were significantly higher than those in healthy subjects (Morimoto et al.[20]: mean ± SD in healthy condition: 0.065 ± 0.008 mA, in RP: 0.640 ± 0.101 mA, and in CRD: 0.163 ± 0.038 mA cf. mean ± SD in our study in healthy condition: 0.062 ± 0.038 mA, in RP: 0.371 ± 0.223 mA, and in STG: 0.102 ± 0.097 mA).

We assessed the correlations between EPTs and ophthalmologic tests with the expectation to learn more about electrical phosphenes. So far, the question about the origin of phosphenes is still open and further studies are needed to help to establish clarity.

We have determined EPTs over a range of stimulation frequencies (in Hz: 3, 6, 9, 20, 40, 60, and 80) and have developed an empirical model to estimate parameters of the interrelation between stimulus amplitude and frequency. Across all groups and all tested frequencies we found the lowest EPTs around 20 Hz (range: 18.2–27.5 Hz).

Modeling data for EPTs across a frequency range allowed us to show a similar pattern for the interrelation between EPTs and stimulus frequency in healthy subjects and patients with retinal diseases. Overall, EPTs are maximal (lower sensitivity) for lower frequencies (e.g., 3 Hz), then continuously decrease as the stimulus frequency increases, until minimal EPTs are reached at approximately 20 Hz. The model proposed in this work can be considered as a simple but reasonable approximation to describe mathematically the higher sensitivity found for EPTs at approximately 20 Hz, because the model could be fitted for all individuals in all different retinal conditions. The four parameters estimated describe the minimal threshold current across frequencies (Thr_{min}), the frequency for minimal threshold ($Freq_{min}$), the maximal threshold (Thr_{max}), and the difference between Thr_{max} and Thr_{min} (h), which quantifies how much lower the threshold is for stimulus frequency equal to $Freq_{min}$.

It is well known that the critical fusion frequency (CFF) is dependent on light intensity (Ferry–Porter law)[63,64]: under scotopic conditions the rods respond at frequencies approximately 22–25 Hz; under photopic conditions the cones are capable of achieving higher CFF than rods. The clear trough of EPTs at 20 Hz (i.e., where thresholds are lowest in all diseases) indicates that the rod system is more involved in the generation of electrically evoked phosphenes. This frequency might also be most apt for testing since currents can be kept minimal and unpleasant sensations at higher current values in advanced disease stages can thus possibly be avoided. This finding also has potential implications in the design of visual prostheses, where stimulation parameters still have not been definitely determined. This frequency range from 20 to 25 Hz could potentially lead to lower thresholds, thus also allowing reduced energy consumption and probably minimized damage due to long-term electrical stimulation.

Accordingly, by analyzing parameters of EPTs, we have confirmed that RAO and RP eyes show higher EPTs (Thr_{max}), but the difference between maximal and minimal EPTs (h) found at $Freq_{min}$ (~20 Hz) is also higher (Fig. 4). As a consequence, even eyes with very high EPTs for lower and high frequencies might show EPTs in relatively low ranges at $Freq_{min}$.

Considering the fast, safe, and reliable practicability of testing EPTs we suggest that it could be used more often under clinical circumstances than it is presently done. Determination of EPTs could be potentially useful in elucidation of the progress of ophthalmologic diseases, either in addition to standard clinical

assessment or under conditions in which these standard tests cannot be used meaningfully. This can be the case when visual acuity is below a quantifiable measure, visual field, or electrophysiology are not recordable, in patients with nystagmus, or in handicapped patients if fixation is problematic.

Our normative data for many ophthalmologic conditions might help to set the standard for therapeutic electrical stimulation in these ocular diseases, to promote scientific evaluation of this approach, and thereby help to treat patients more efficiently in the future.

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