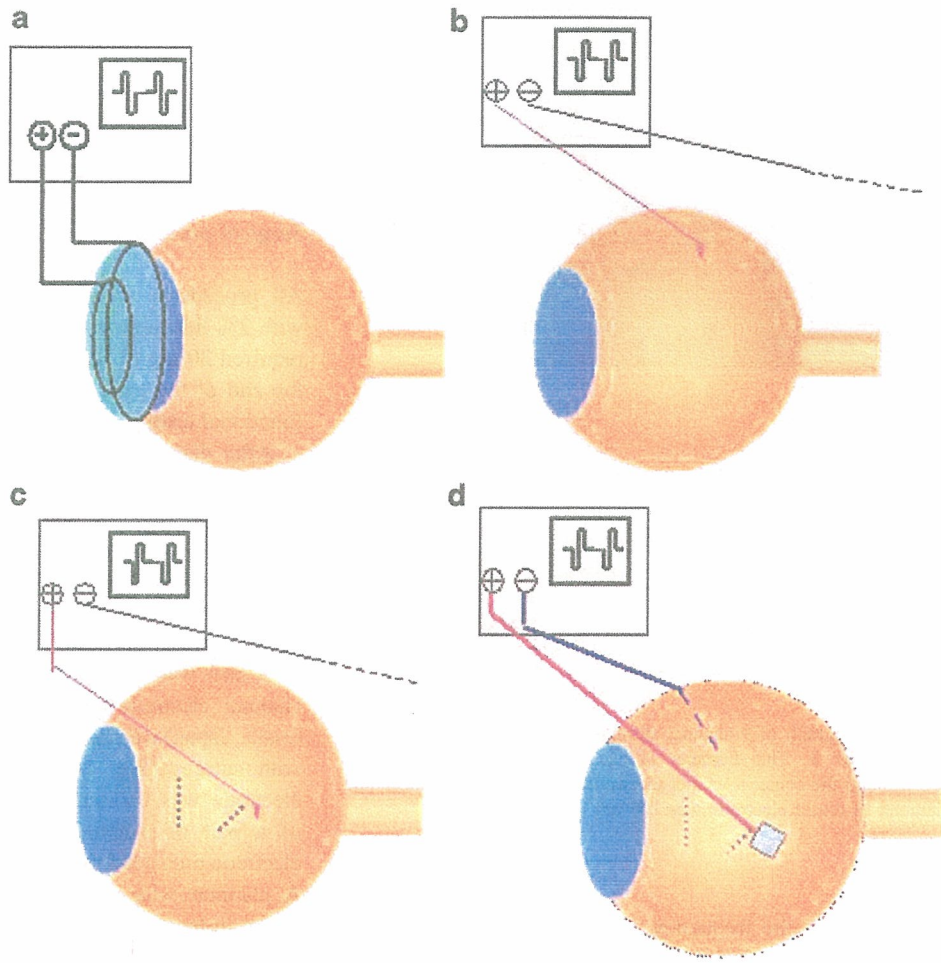

Fig. 1 Schema of different methods to stimulate the retina. **a** Trans-corneal electrical stimulation (TcES); **b** trans-scleral electrical stimulation (TsES); **c** trans-scleral monopolar stimulation in patients; **d** suprachoroidal-transretinal stimulation (STS) in patients

infrared charge-coupled device (CCD) camera and a red light-emitting diode (LED; 660 nm; maximum light power of 10±3 μW; stimulus duration of 0.1 s).

Before inserting the contact lens electrode, the indirect pupillary light reflex was recorded. After inserting the contact lens electrode, the electrically evoked pupillary responses (EEPRs) were recorded from the contralateral eye. The relative amplitude of the EEPR was determined as follows:

$$\text{Relative pupillary constriction(RPC\%)} = 100(a - b)/a$$

where a = pre-stimulus baseline pupil diameter (mm), and b = maximally constricted pupil diameter (mm).

Transscleral electrical stimulation

A stimulating electrode (platinum wire, diameter: 1.0 mm, exposed 1.0 mm at the tip, Unique Medical, Osaka, Japan) was placed on the conjunctiva in the upper temporal quadrant 16 mm to 18 mm from the corneal limbus (Fig. 1b). The conjunctiva was anesthesized with 0.4% oxyburocaine hydrochloride. A return electrode (Ag-AgCl) was placed on the ipsilateral wrist. Pulse trains with charge-balanced biphasic pulses (cathodic first) were applied through the stimulating electrodes.

We examined the relationship between the brightness of phosphenes and the pulse parameters, viz., pulse duration, interpulse delay, frequency and the number of pulse trains, using suprathreshold currents (1.0 to 1.5 mA) (Fig. 2b). Initially, the threshold current to evoke phosphenes was determined with the other parameters fixed at a pulse duration 1 ms, interpulse delay 1 ms, frequency 20 Hz and number of pulses 20. These fixed parameters were chosen based on the results of a preliminary experiment to explore the effective parameters to elicit phosphenes. The relationship between the brightness of the phosphenes and the pulse duration was examined with the injected charge per pulse constant. The pulse duration varied from 0.5 to 4.0 ms while the frequency at 50 Hz and the number of pulses at 20.

The relationship between brightness of the phosphenes and the interpulse delay was examined with the interpulse delay varying from 0 to 4 ms while the pulse duration was

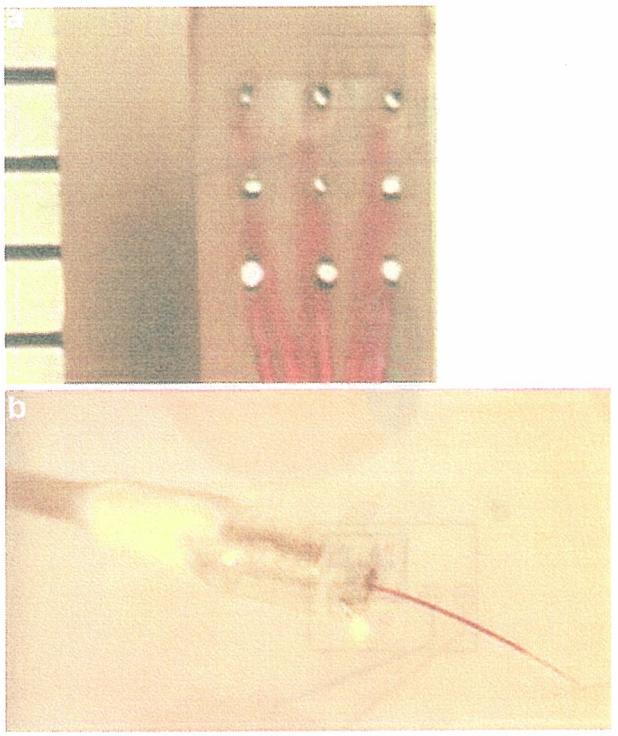

Fig. 2 Photograph of 9-channel stimulating electrode (**a**) and return electrode (**b**). **a**. The diameter of each stimulating electrode was 200 μm and the center-to-center electrode distance was 1 mm. **b** The diameter of return electrode was 100 um

fixed at 1 ms, the frequency at 50 Hz and the number of pulses at 20. The relationship between the brightness of the phosphene and the pulse frequency was examined with the frequency varying from 5 to 100 Hz while the pulse duration was fixed at 1 ms, interpulse delay at 1 ms and the number of pulses at 20. The relationship between brightness of the phosphenes and the number of pulses was also examined with the number of pulses varied from 1 to 50 while the pulse duration was fixed at 1 ms, the interpulse delay at 1 ms and the frequency at 20 Hz.

Subjects were questioned about the brightness and the size of the phosphene for each set of stimulus parameters. The brightness was classified into five grades; the brightest phosphenes during one set of experiments was assigned a value of 5 and the next brightest was 4, and so on. The experiments were conducted systematically with an increase of stimulus parameters (pulse duration, interpulse delay, frequency and the number of pulse trains) with an interval of 5 to 10 s. The number of trials in a unique set of stimulus parameters was generally once, but was two or more when subjects asked for a repetition. Subjects were masked to the test conditions, and the examiner, who was aware of the stimulus conditions, asked questions about the phosphenes. False positive trials (i.e., no stimulus presented) were included to determine the reliability of the responses.

Suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa

Surgical procedure

All procedures were performed under topical anesthesia with 2% lidocaine hydrochloride drops; however, patient 1 required 20 mg of fentanest intravenously at approximately the end of the trial. The total hours including surgery and functional testing was 3 h in patient 1 and 2.5 h in patient 2.

After dissecting the lateral rectus muscle insertion, trial transscleral stimuli were given to determine the scleral area around the insertion of the inferior oblique muscle that consistently evoked low threshold phosphenes (Fig. 1c). The diameter of monopolar platinum electrode was 0.5 mm (Unique Medical, Osaka, Japan). After identifying the low threshold area by monolopar electrode, a scleralpocket of 5×5 mm was created with a crescent knife, and a nine-channel electrode array (size, 4×5 mm, Unique Medical, Osaka, Japan) was inserted into the scleral pocket and secured with sutures (Fig. 1d). The diameter of each platinum electrode was 0.2 mm, and the center-to-center separation of a pair of electrodes was 1 mm. The surface of the electrode protruded from the silicon base by 50 μm (Fig. 2a). A platinum-wire reference electrode (0.1 mm in diameter, 8 mm in length and 3 mm of tip exposed) was inserted into the vitreous cavity through the pars plana (Fig. 2b).

Functional testing

A stimulator was designed to deliver charge-balanced biphasic pulses to individual electrodes simultaneously (Fig. 3a). Biphasic pulses (pulse duration, 0.5 or 1.0 ms; frequency, 20 Hz; interpulse delay, 0.5 ms; number of pulses 20, Fig. 3b) were delivered through the selected channel(s) or combination of multiple channels.

The psychophysical testing was performed under dim room lights. The current stimulation was applied 0.5 s after a conditioning phonic stimulus by a buzzer. The threshold current to perceive a phosphene was determined by increasing the current intensity from 0.1 mA until patients recognized the localized phosphene. The maximum current was limited to 1.0 mA for safety based on the rabbit experiments (Nakauchi et al., ARVO, 2006,47, E-Abstract 3197).

The size and shape of the phosphene that the patients described were recorded. False positive trials (i.e., no stimulus presented) were included to determine the reliability of the responses. The procedure was repeated to

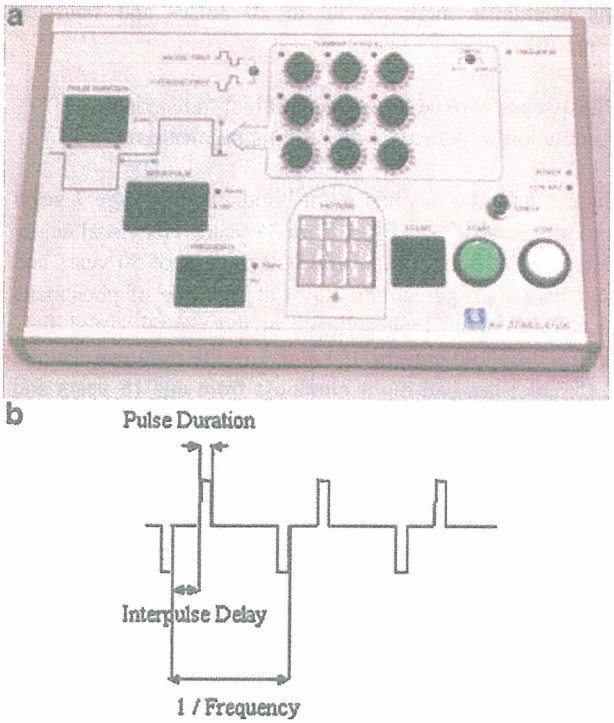

Fig. 3 Photograph of the stimulator for a 9-channel electrode (**a**) and the diagram of stimulating current pulse (**b**). **a** Charge-balanced biphasic pulses were delivered to individual electrodes simultaneously. **b** The first pulse delivers a cathodic current while the second pulse delivers anodic current to balance the charge

identify the threshold current. Care was taken not to influence the description by the patients.

After determining the threshold current of each electrode, simultaneous multi-channel stimulation was performed to examine if patients could achieve two-point discrimination or pattern recognition.

Statistical analyses

Data are presented as the means ± standard error of the means (SEM) and were statistically analyzed with the SPSS 10.0J program (SPSS Inc, Chicago, IL). Comparisons between two groups were made by the Student's *t* test when data were normally distributed or by the Mann-Whitney U test when data were not normally distributed. The degree of correlation was evaluated by the coefficient of correlation (r) calculated using Pearson's correlation coefficient. Comparisons between three groups or more were made by one-way ANOVA followed by the Tukey test when data were normally distributed or by the Dunn's test when data were not normally distributed. The probability level is represented as the value *P*; statistical significance was set at $P<0.05$.

Results

Electrically evoked and light-evoked pupillary responses in normal subjects

Typical pupillary responses from a normal subject are shown in Fig. 4. The waveform of the EEPR was similar to that of the light response, but the amplitude of the EEPR was smaller than that of the light response. The mean latency of the EEPR (at 150 μA; 10 ms duration; 20 Hz; 20 pulses) was 0.29±0.01 s and that of the light response (660 nm; maximum light power of 10±3 μW; stimulus duration of 0.1 s) was 0.33±0.01 s. This difference was significant ($P<0.01$, Student's *t* test).

The relationship between the current intensity and EEPR was examined with stimuli of 20 Hz, 10 ms duration, and 20 pulses while the current intensity was varied from 25 μA to 250 μA. A RPC greater than 3% was obtained at currents ≥150 μA and the mean RPC was 6.3±1.1%. A highly significant positive correlation was found between the current intensity and the RPC amplitude (r=0.98 and $P<0.01$; Pearson's correlation coefficient, Fig. 5a).

We then examined the relationship between the frequency of the pulses and the EEPR at 150 μA, 10 ms duration and 20 pulses while the frequency of pulses varied from 5 Hz to 50 Hz. Although the mean RPC amplitude of the EEPR was very small at frequencies lower than 10 Hz, the mean RPC reached a peak of 7.9±1.9% at 20 Hz. Increasing the frequency up to 50 Hz resulted in a decrease in the RPC to 3.9±1.5% at 50 Hz (Fig. 5b).

The subjective phosphenes were brighter at frequencies between 15 and 33 Hz compared with those elicited by frequencies lower than 15 Hz or higher than 33 Hz.

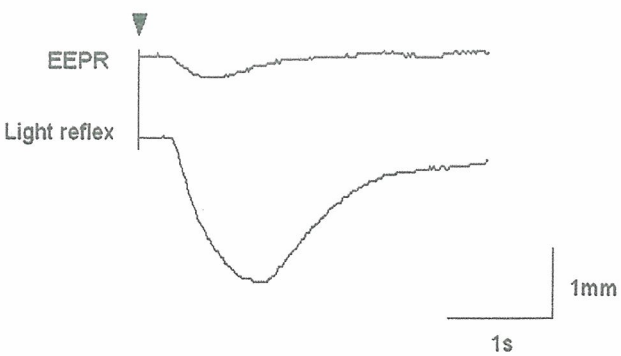

Fig. 4 Electrically evoked (upper) and light-evoked (lower) indirect pupillary responses elicited by electrical stimulation or light stimulation. The amplitude of the EEPR was smaller than that of light reflex, but the shape of the waveform is similar to that of light response

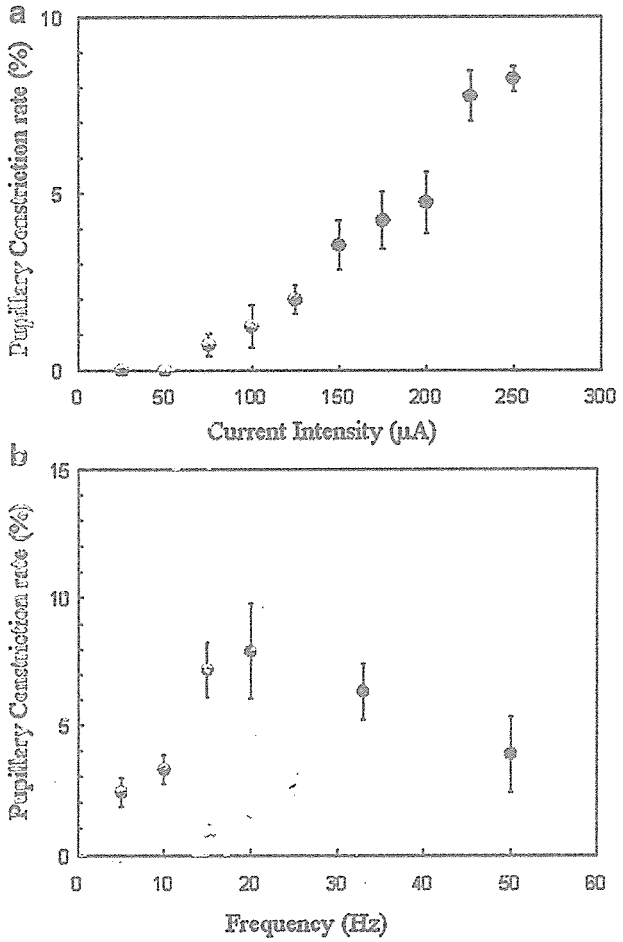

Fig. 5 Characteristics of pupillary constrictions elicited by transcorneal electrical stimulation. a Relative pupillary constriction amplitudes are plotted against the current intensity in normal subjects. The average pupillary constriction amplitude increases as the current intensity increases. b Relative pupillary constriction amplitudes are plotted against the frequency of electrical stimulation in normal subjects. A bandpass-shaped increase of pupillary constriction amplitude is observed. Bar represents standard error

Phosphenes evoked by trans-scleral electrical stimulation

All subjects reported a localized, round-shaped phosphene in response to TsES. The position of the phoshenes corresponded to the retinal loci where phoshenes were evoked by indenting the scleral electrode. Generally, shorter pulse durations elicited more localized phosphenes. The brightness of the phosphenes decreased with an increase of pulse duration in which the injected charge per pulse was kept constant (Fig. 6a). An increase in the interpulse delay also increased the perceived brightness of the phosphenes and was almost saturated at 1 ms (Fig. 6b). The brightness of the phosphenes increased with an increase in the pulse frequency up to 20 Hz and peaked at 50 Hz, but decreased at 100 Hz (Fig. 7a). With an increase in the pulse number, the brightness increased up to 20 pulses and saturated (Fig. 7b).

Phosphenes evoked by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa

Patient 1 (male) has had night blindness since age 7 years and progressive visual loss from 35 years. His visual acuity decreased to hand motion (OU) at the age of 50 years and was bare light perception (OU) at the time of phosphene test. TcES elicited phosphenes in the central visual field with a threshold current of 1.4 mA in the right eye. Patient 2 (female) has had night blindness from age 15 years and progressive visual loss from 27 years. Her visual acuity decreased to hand motion (OU) at the age of 55 years and was bare light perception (OD), and was 0 (OS) at the time of phosphene test. TcES elicited phosphenes that were perceived in the central visual field with a threshold current of 1.1 mA in the right eye.

Examination of the ocular fundus of the two patients with RP revealed extensive retinal degeneration including

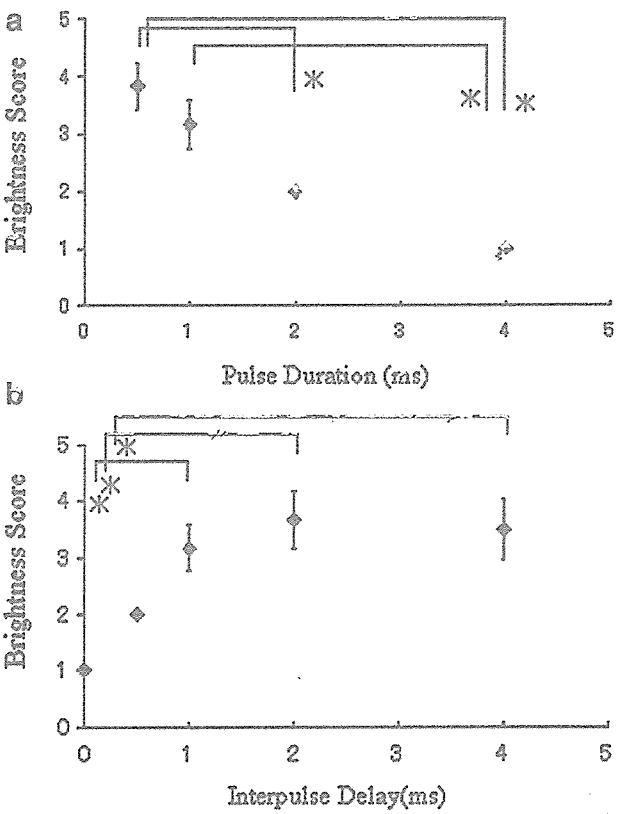

Fig. 6 The relationship between the brightness of phosphenes and pulse duration (a) and interpulse delay (b). a The brightness of phosphenes decreases with an increase of pulse duration (the total injected charge was constant). b The application of interpulse delay increased the perceived brightness of phosphene and almost saturated at 1 ms

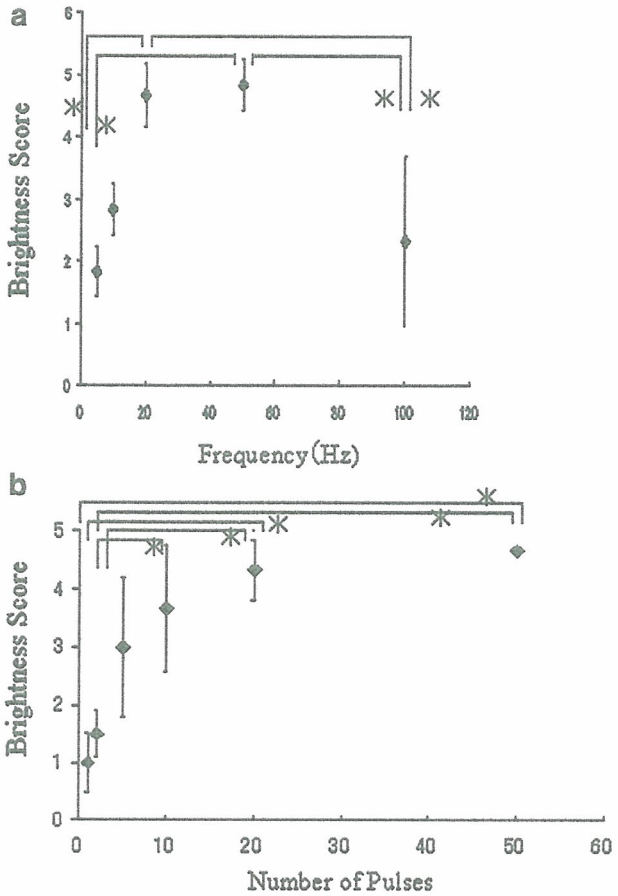

Fig. 7 The relationship between the brightness of the phosphenes and the pulse frequency (**a**) and the number of pulses (**b**). **a** The brightness of the phophenes increased with the increase of pulse frequency up to 20 Hz and peaked at 50 Hz, but decreased at 100 Hz. **b** With the increase of pulse number, the brightness increased up to 20 pulses and saturated. Bar represents standard error. $*P<0.05$

the macular and peripheral retina (Fig. 8). Trans-scleral monopolar stimulation revealed a confined low threshold area (0.4 mA, duration 1 ms) about 2 mm posterior to the inferior muscle insertion in patient 1. In patient 2, phosphenes were evoked from only a confined area about 2 mm posterior to the inferior muscle insertion with the maximum electrical current (1 mA, duration 1 ms).

By stimulating a single channel of nine electrodes with STS, localized phosphenes were obtained with stimuli of 0.3–0.5 mA (duration,0.5 ms: 0.48–0.80 mC/cm2) in patient 1 and 0.4 mA (duration,1.0 ms: 1.27 mC/cm2) in patient 2. Phosphenes were not reported from false-positive trials. Due to the difficulty in obtaining the results, only qualitative data can be provided in psychophysical experiments. The size of phosphene varied from a dime to a quarter coin at a distance of a stretched arm depending on the channel stimulated in both patients. Dumbbell-shaped phosphenes were perceived when the stimuli were delivered through two adjacent channels in both patients. Two dumbbell-shaped phosphenes oriented in different directions were perceived by stimulating different pairs of channels in patient 1.

Discussion

We have determined the efficient parameters to stimulate the retina by extraocular electrical stimuli. For TcES, two concentric ring electrodes were placed on the corneal surface and a current between the two rings has been reported to stimulate the peripheral retina by lower electrical currents and the macular area by higher currents [11]. The EEPR was used to evaluate the frequency dependence of TcES.

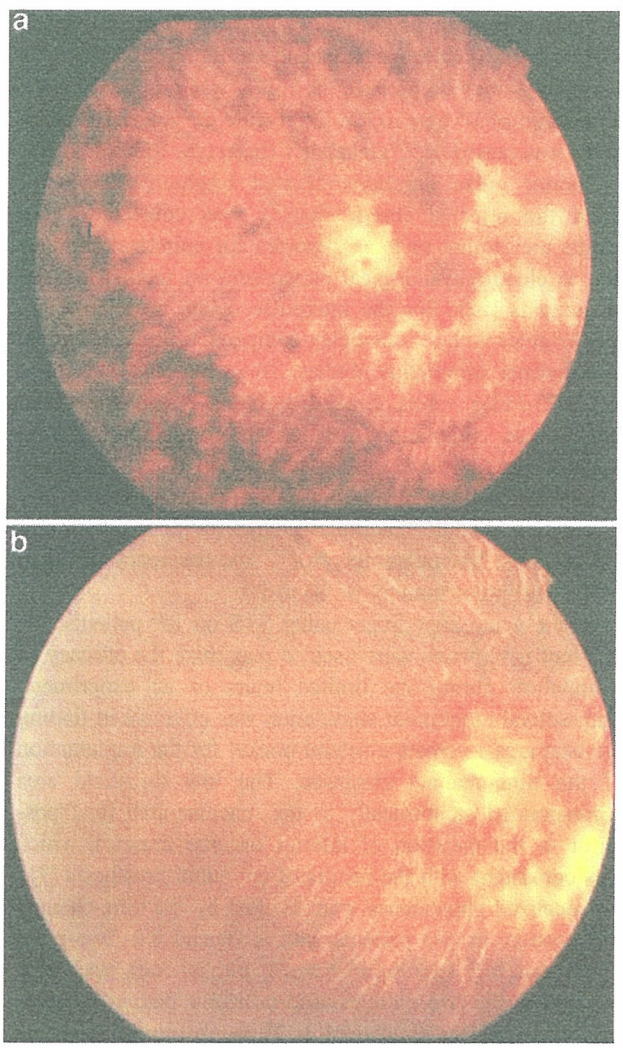

Fig. 8 Fundus photos of patient 1 (**a**) and patient 2 (**b**). In both patients, retinal degeneration can be seen in the macular area as well as in the peripheral retina

The relative amplitude of constriction of the EEPR increased as the current intensity increased, and the amplitude was largest at 20 Hz (Fig. 5). The perceived phosphene also became brighter around this frequency. These results together with previous reports [18, 28] suggest that a frequency around 20 Hz is the most effective frequency to stimulate the retina. The frequency dependency of the intensity of the phosphenes has not been explicitly reported, but for epiretinal stimulation, 20 Hz was used [22].

A possibility that a direct current affected the pupil efferent and elicited the EEPR was eliminated by the report that an EEPR cannot be elicited from the contralateral healthy eye from electrically stimulating an eye with optic atrophy [28].

The efficient parameters for localized extraocular stimulation for TsES were determined in normal subjects. The frequency dependence of the phosphene intensity showed a bandpass-shaped curve (Fig. 7), which was similar to the result of TcES. The peak EEPR was elicited by 20 Hz for TcES, while that for a subjective phophene was 50 Hz for TsES. This discrepancy may be because the EEPR was produced by the stimulation of W type RGCs [26], while the subjective phosphene was caused by X (alpha) or Y (beta) type RGCs [12, 26].

Shorter pulse durations elicited brighter phosphenes when the injected charge density was constant. These results are similar to those reported for epiretinal stimulation in electrophysiological experiment on rabbits [9] and on humans [22]. For the interpulse delay, the phosphene was brighter with a 1-ms delay compared with 0 or 0.5 ms. In epiretinal stimulation studies, 10 us to 2 ms were used [6, 22]. Pulse trains evoked brighter phosphenes than single-pulse stimulation. A pulse train (duration, 1.5 s) was also used in the epiretinal stimulation study [22]. From these findings, the efficient pulse parameters for extraocular stimulation in acute experiments may be a pulse duration of 0.5 to 1 ms, interpulse delay of 1 ms, frequency of 20 to 50 Hz and pulse number of 10 to 20.

In the acute experiment using STS on RP patients, the efficient parameters were used to maximize the efficacy of stimulation during the limited hours of an experiment. Transscleral monopolar stimulation was effective in finding the best area to create a scleral pocket for the implantation of the nine-channel electrode. The low threshold area corresponded anatomically to the macular area (just posterior to the insertion of inferior oblique muscle), which was consistent with the results of epiretinal prosthesis [7].

Localized phosphenes were elicited by the nine-channel STS system in two patients with advanced RP. Two-point discrimination (dumbbell-shaped phosphene) was also attained in the two patients, and primitive pattern recognition was obtained in patient 1. These results suggest that STS has the potential for being the basis for a pattern recognition system and be a feasible artificial vision system.

The charge densities to evoke phosphene by STS in two RP patients were 0.48 to 1.27 mC/cm2, which were comparable to the reported data of epiretinal stimulation, 0.28 to 2.88 mC/cm2 in RP patients using 400-um electrode [22].

In summary, biphasic pulses with a duration of 0.5–1.0 ms, interpulse delay of 1 ms, frequency of 20–50 Hz and trains of 10 to 20 were optimal for evoking phosphenes from localized extraocular stimulation. The STS using these parameters for advanced RP elicited localized phosphenes and enabled two-point discrimination, suggesting that STS is feasible as an approach to retinal prosthesis.

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Commercial interest Hiroyuki Kanda and Motoki Ozawa are employees of the Nidek Company.

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Threshold suprachoroidal–transretinal stimulation current resulting in retinal damage in rabbits

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Abstract

The purpose of this study is to determine the threshold suprachoroidal–transretinal stimulation (STS) current that results in retinal damage in rabbits. Biphasic STS pulses (anodic first, frequency 20 Hz) were used to stimulate the retina of pigmented rabbits ($n = 18$) continuously for 1 h using a 100 μm diameter platinum wire electrode. The STS current that induced retinal damage after 1 h was determined by ophthalmoscopy or by fluorescein angiography (FA) independently. The effect of the pulse duration on the threshold current was investigated. Histological studies were performed after electrical stimulation experiments. The threshold for a safe current to the retina was 0.6 mA for a duration of 0.5 ms. The threshold for a safe charge increased approximately linearly with an increase of stimulus duration but the threshold for a safe current decreased logarithmically with an increase of duration. The threshold for a safe electrical energy remained almost constant for all durations. Histological examination showed severe retinal damage when the current exceeded the threshold, with more damage in the inner layers compared with the outer layers of the retina. The threshold for the safe current was higher than that reported for direct stimulation of neural tissues, suggesting that the STS method was safe and able to be used with a retinal prosthesis. Because the threshold for the safe charge was lower with shorter pulse durations, care should be taken using pulses of short durations.

Introduction

Stimulating the retina electrically with an implanted retinal prosthesis in eyes with very limited vision is one of the promising approaches for providing some degree of vision to visually impaired patients. For this, suprathreshold currents are used to evoke phosphenes by stimulating still functioning neurons in the retina. Although biphasic, charge-balanced pulses are used, the retina can still be damaged if high electric currents are used. Several studies have determined the safe current for cortical cells when needle-type electrodes are used [1–4]. Epiretinal and subretinal types of electrodes are being intensively investigated as retinal prostheses, but only a few studies have reported the safe limits of the applied currents or voltages with these types of electrodes [5, 6].

We have developed a new method to stimulate the retina called suprachoroidal–transretinal stimulation (STS) [7] and have shown that STS can elicit electrically evoked potentials (EEP) from the rabbit cortex in acute experiments [8]. We have investigated the effects of chronic implantation of electrodes without electrical stimulation [9] or with stimulation by biphasic pulses of 100 μA, 20 Hz for 1 h/day [10]. EEP

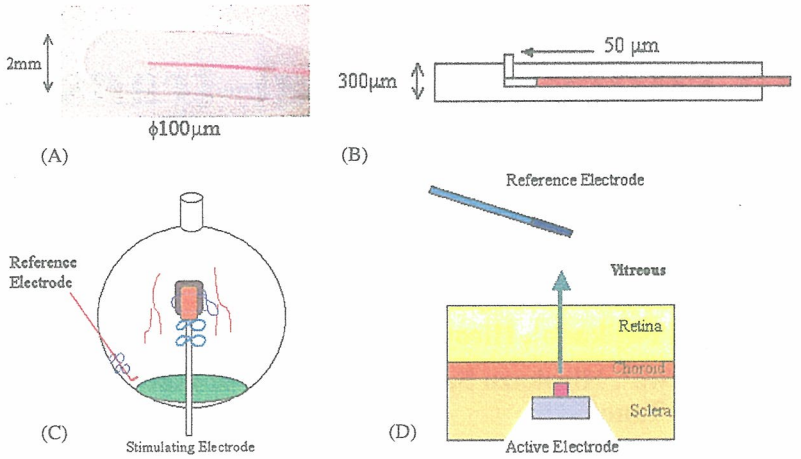

Figure 1. Photograph (A) and diagram (B) of the stimulating electrode, diagram of the placement of the electrodes in the rabbit eye (C) and diagram of suprachoroidal–transretinal stimulation used in this experiment (D). (A) and (B) A wire electrode (diameter = 100 μm) was embedded in a 2 mm × 4 mm × 0.3 mm silicone plate. The electrode wire was coated with polyurethane, and 0.5 mm of the tip was exposed. The tip protruded 0.05 mm from the plate surface. (C) Sclerotomy was performed over the visual streak of rabbits until the choroid was observed. The silicone plate with the electrode was fixed to the fenestrated sclera with 8-0 Vicryl and 5-0 Dacron sutures. The return electrode was placed in the vitreous cavity and fixed at the ora serrata with 8-0 Vicryl. (D) The stimulating electrode was set in the fenestrated sclera with a residual thickness of 50–100 μm. The return electrode was placed in the vitreous and the electric current passed through the retina.

responses could be elicited even 2 weeks after the implantation, and no retinal damage was observed under either condition.

One advantage of the STS method is that the stimulating electrode is not in direct contact with the retina as with epiretinal and subretinal electrodes. We showed that after 1 h of continuous biphasic 20 Hz stimulation (pulse duration 0.5 ms), the ophthalmoscopically determined safe current was 1 mA with a 100 μm diameter platinum (Pt) electrode and 1.5 mA with a 200 μm diameter Pt electrode [11].

Before applying the STS method to patients, the threshold current that does not injure the retina must be determined more accurately. Thus, the purpose of this study was to determine the threshold current delivered by the STS method that did not injure the retina. Ophthalmoscopy and fluorescein angiography (FA) were used to evaluate the retina.

Materials and methods

Animals

Eighteen pigmented, Dutch-belted rabbits (weight 1.5–2.2 kg) were used for the experiments. Twenty-eight retinal loci of 20 eyes (10 rabbits) were used to determine the threshold current that just caused a visible alteration of the retina as detected by ophthalmoscopy, and 30 retinal loci of 16 eyes (8 rabbits) were used to identify the threshold current using fluorescein leakage during FA as the end point. The procedures used on the animals conformed to the Institutional Guidelines of Osaka University and the ARVO Resolution on the Use of Animals in Research.

Stimulating electrodes

A 100 μm diameter platinum wire was coated with polyurethane and embedded in a 2 mm horizontal × 4 mm vertical × 0.3 mm thick silicone plate (figures 1(A) and (B)) (Unique Medical, Tokyo Japan). Then, 0.5 mm of the tip of the wire was bent to 90° and protruded from the surface of the plate by 0.05 mm. The polyurethane coating was scraped away from the tip to expose a surface area of 2.36×10^{-4} cm^2. The return electrode was made of the same polyurethane-coated platinum wire, and 2 mm of the tip was exposed and inserted into the vitreous. This electrode configuration was found to be effective in stimulating the rabbit retina by STS [8].

Surgical procedures

The rabbits were anesthetized by an intramuscular injection of a mixture of ketamine (50 mg kg^{-1}) and xylazine (20 mg kg^{-1}), and if additional anesthetics were needed, one-half of the initial dose was injected. Sclerotomy was performed over the visual streak region, and the sclera was dissected away until the choroid was visible. The stimulating electrode was fixed to the sclera with 8-0 Vicryl and 5-0 Dacron sutures. When the electrode was moved to stimulate another retinal loci, sutures were cut and re-fixed to the corresponding scleral loci. The return electrode was pushed into the vitreous cavity through the ora serrata and placed approximately in the middle of the vitreous body. The electrode was secured with 8-0 Vicryl sutures (figure 1(C)).

Electrical stimulation by STS

A diagram of the direction of current flow delivered by STS is shown in figure 1(D). The stimulus consisted of biphasic pulses (anodic first) with a frequency of 20 Hz penetrating through the retina. The biphasic pulses were generated with a signal processor (SEN-7203, Nihon Kohden, Tokyo, Japan), and the electric current was delivered through a stimulus isolation

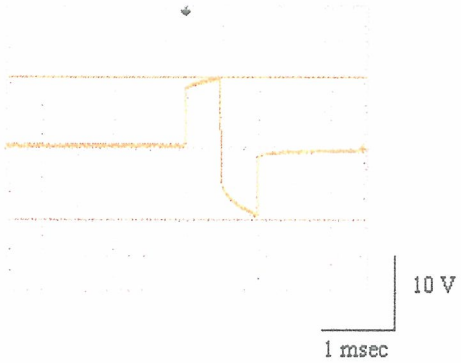

Figure 2. Photograph of oscilloscope monitor showing voltage between the active and the reference electrode. With biphasic rectangular pulse currents, the voltage increases slowly because of the effect of capacitance.

unit (WPI-A365, WPI, Sarasota, USA). The potential changes between the active and return electrodes were monitored with a storage oscilloscope and one cycle is shown in figure 2 (TPS-2014, Tektronics, Beaverton, OR, USA).

Evaluation of voltage between the active and return electrodes

When rectangular pulses are delivered, the waveform of the response was not rectangular due to the capacitance effect (figure 2). Thus, we calculated the integrated value of the area under the pulse and found that the integrated voltage was about 85% of the peak voltage. In addition, the value of the area under the voltage trace is used to calculate the energy per phase by multiplying the area with the current amplitude.

Determination of the threshold current that does not injure the retina

To determine the threshold current that did not injure the retina, the pulse duration and current intensity were changed, while the frequency was fixed at 20 Hz. Preliminary experiments showed that 1 mA biphasic pulses of 0.5 ms duration or a charge of 0.5 μC/phase did not injure the retina [11]. Thus, total charge for the stimulation was initially kept constant at 0.5 μC/phase, i.e., for a duration of 0.1 ms, the current was 5 mA, for 0.25 ms—2 mA, for 0.5 ms—1 mA and for 1 ms—0.5 mA, respectively.

The ophthalmoscopic sign of retinal damage was defined as the appearance of a whitish spot around the electrode (figure 3). After 1 h, the retina was examined ophthalmoscopically, and if fundus changes were detected, the stimulating electrode was moved and a different retinal locus in the same eye or in the opposite eye was tested. The current was reduced by 0.1–0.5 mA units, and the procedure was repeated and the retina was examined ophthalmoscopically. This procedure was repeated until no retinal changes were detected ophthalmoscopically. When no fundus changes were detected, the electrode was moved to a different retinal locus in the same eye or in the opposite eye and repeated with 0.1–0.5 mA stronger currents and the fundus was examined.

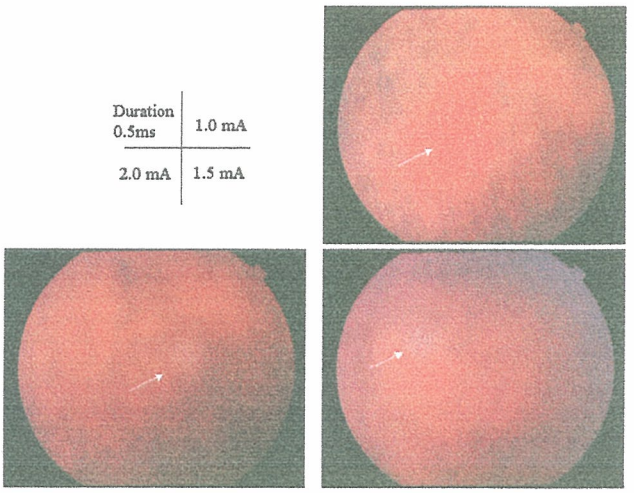

Figure 3. Fundus photograph 1 h after electrical stimulation. No change is observed with 1 mA (upper right) and a whitish spot is observed with 1.5 mA (lower right) and 2 mA (lower left). The white arrows indicate the retinal area where the electrode was attached to the sclera.

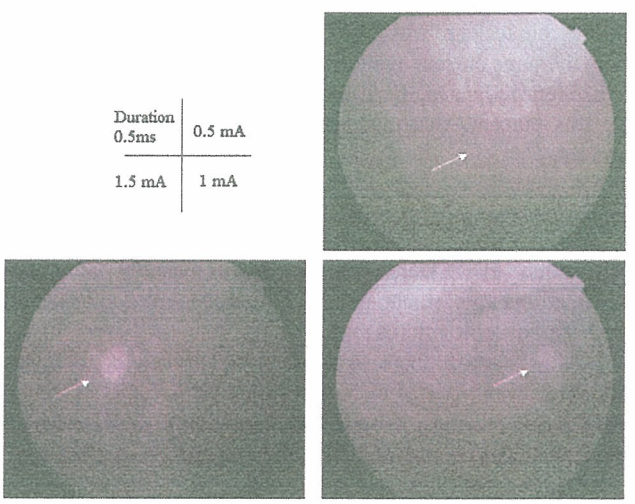

Figure 4. Fluorescein angiography 1 h after electrical stimulation. No change is observed with 0.5 mA (upper right) and leakage of fluorescein dye is observed with 1.0 mA (lower right) and 1.5 mA (lower left). The white arrows indicate the retinal area where the electrode was attached to the sclera.

This was repeated until the whitish spot on the retina was observed. The threshold current was defined as the strongest current that did not produce retinal damage as assessed by ophthalmoscopy.

For FA, the sign for electrically induced damage to the retina was the appearance of dye leakage or hyperfluorescence around the retinal area where the electrode was placed (figure 4). The baseline parameters of stimulation were similar to those used during the ophthalmoscopic assessment, and the total injected charge/phase was kept constant. After stimulation for 1 h with one current intensity and duration setting, FA was performed, and if the retinal leakage change was observed (figure 4), the current was decreased by 0.1 mA

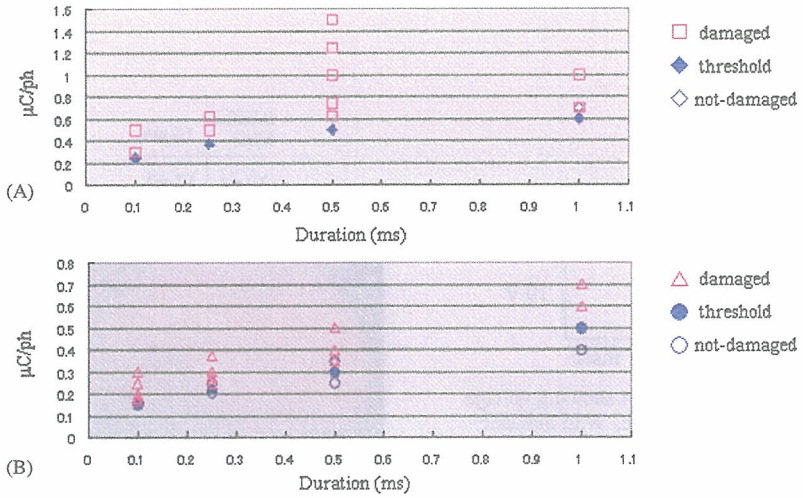

Figure 5. Graphs showing the relationship between charge per phase and duration of the pulses as assessed by ophthalmoscopy (A) or by fluorescein angiography (B). Threshold charge was defined by the highest charge that did not damage the retina. The threshold charge increases logarithmically or almost linearly with an increase of duration in both graphs.

after changing the retinal locus, and the procedure was repeated. When the current intensity that did not lead to fluorescein leakage was found, the retinal change was checked with the same current again and with a current 0.1 mA lower at a different retinal locus. If retinal changes were not observed by this current, this current was defined as the threshold current.

Histological studies

All rabbit eyes were enucleated immediately after the stimulation experiments, fixed in glutaraldehyde and observed with stereoscopic microscope.

After marking the scleral site in which the electrode was attached, eyes ($n = 8$) were embedded in paraffin, sectioned with 3 μm thickness around the marked scleral site with an interval of 20 μm and stained with hematoxylin–eosin.

Results

Threshold charge as a function of stimulus duration

Initially, we examined the effect of the pulse duration on the threshold charge (coulombs/phase or C/ph). The retina was examined by ophthalmoscopy (figure 3) and by FA (figure 4) after each set of stimuli. These data plots showed that the threshold for a safe charge increased logarithmically or almost linearly with an increase in the stimulus duration (figures 5(A) and (B)).

Threshold current as a function of stimulus duration

Next, we examined the relationship between the duration and the threshold current (mA) (figures 6(A) and (B)). These plots seemed to be lined on a logarithmic curve. When the data were plotted on a log–log scale, the threshold current decreased linearly with an increase in the pulse duration (figures 7(A) and (B)). The slope of the regression line was −0.61 by ophthalmoscopy and −0.52 by FA.

Table 1. The threshold currents for each duration and other accompanying parameters determined by ophthalmoscopy (A) and fluorescein angiography (B).

	(A)			
Duration (ms)	0.1	0.25	0.5	1.0
Current (mA)	2.5	1.5	1.0	0.6
Charge (μC/ph)	0.25	0.38	0.50	0.60
Voltage (V)	19.7	13.0	9.0	6.6
Energy consumption (μJ/ph)	4.9	4.9	4.5	4.0
	(B)			
Duration (ms)	0.1	0.25	0.5	1.0
Current (mA)	1.6	0.9	0.6	0.5
Charge (μC/ph)	0.16	0.23	0.30	0.50
Voltage (V)	11.0	5.8	6.0	4.2
Energy consumption (μJ/ph)	1.8	1.3	1.8	2.1

The charge was calculated by multiplying duration with current. The voltage was the integrated value of the area under the pulse with Excel. The energy consumption was calculated by multiplying duration with current and voltage, and remained constant at around 5 μJ/ph (ophthalmoscopy (A)) and 2 μJ/ph (fluorescein angiography (B)).

Threshold energy as a function of stimulus duration

An examination of the relationship between the threshold electrical energy (J/ph) and the duration (figures 8(A) and (B) and table 1) showed that the threshold electrical energy remained almost constant for all durations.

Histological examinations

A photomicrograph of a retinal site stimulated by biphasic pulses with a duration of 0.5 ms and a current of 1.0 mA that did not lead to an ophthalmoscopic alteration but lead to FA alterations is shown in figure 9(A). The retinal cells

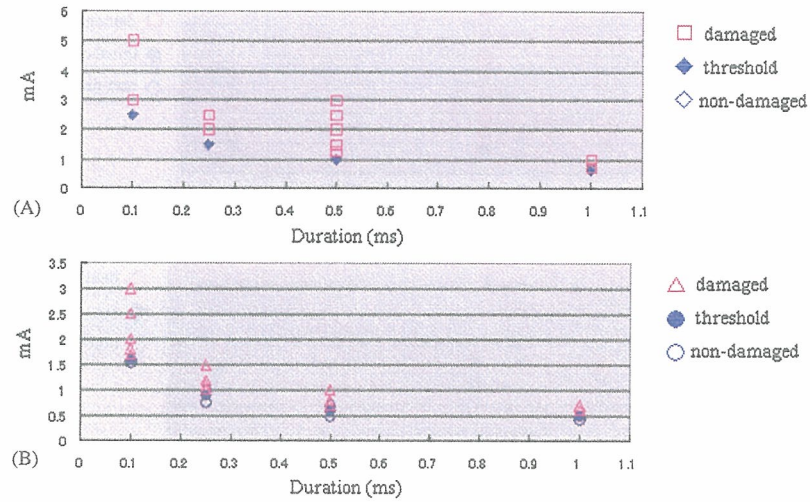

Figure 6. Graphs showing the relationship between current per phase and duration as assessed by ophthalmoscopy (A) or by fluorescein angiography (B). The threshold current decreases logarithmically with an increase of duration in both graphs.

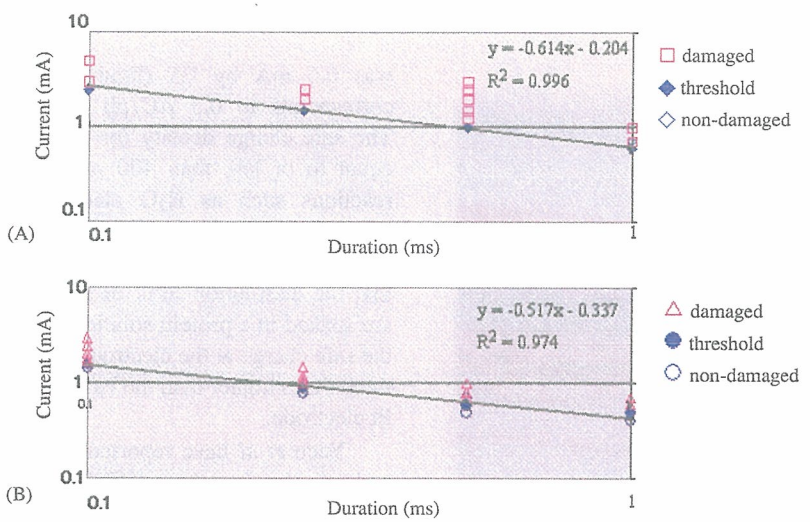

Figure 7. Graphs showing the relationship between the effect of current per phase and the duration by ophthalmoscopy (A) or by fluorescein angiography (B) with *x*–*y* axes plotted on a logarithm scale. The threshold current decreases linearly with an increase of duration in both graphs. The slope of the regression line is −0.61 by ophthalmoscopy (A) and −0.52 by FA (B).

and architecture are normal. An example of the histological damage of the retina induced by biphasic pulses (duration 0.5 ms, current 1.5 mA) is shown in figure 9(B). An enlargement of the choroidal vessels, disruption of outer nuclear layer cells, condensation of the inner nuclear layer and vacuoles in the outer plexiform, inner plexiform and nerve fiber layers can be seen.

In the most severely damaged area where the center of the electrode was attached, all retinal layers were destroyed. In the periphery of the stimulated area, the changes were less severe with relatively more damage in the inner layer than the outer layers including RPE or photoreceptors. The sclera and choroid were not altered.

Histological examination of the electrode sites showed that the scleral thickness was 50–100 μm in all preparations. When the scleral thickness was less than 50 μm, the choroid was easily ruptured in the fenestrated area, so scleral tissue with a minimum thickness of about 50 μm was needed for structural integrity.

Discussion

Our results showed that the threshold charge per phase increase with an increase of duration was similarly assessed by ophthalmoscopy and by FA, although the threshold charge was about 20% lower when assessed by FA than by ophthalmoscopy (figure 5). This difference indicates that FA is more sensitive than ophthalmoscopy in detecting retinal injury caused by electrical STS.

The threshold current with a duration of 0.5 ms, a common duration used for human STS experiments,

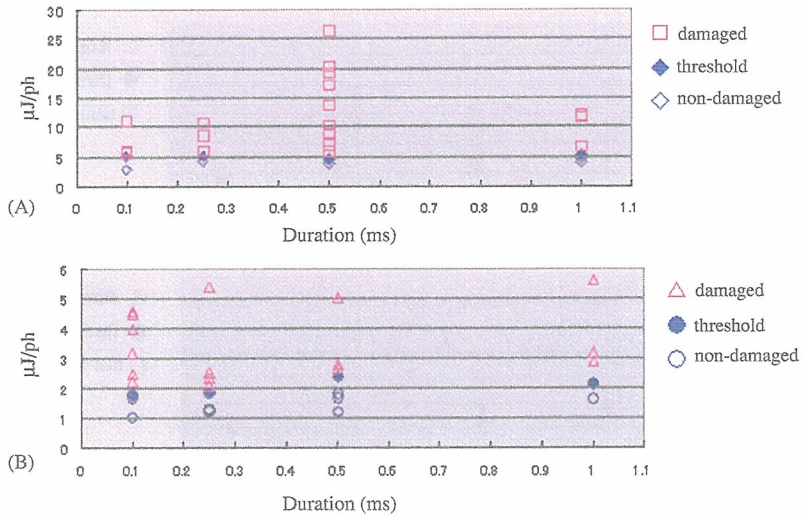

Figure 8. Graphs showing the relationship between the effect of energy consumption per phase on the retina and the duration by ophthalmoscopy (A) or by fluorescein angiography (B). Multiplying voltage with current and duration yields the electrical energy consumption per phase (J/ph). The threshold energy consumption was almost constant for all durations in both graphs.

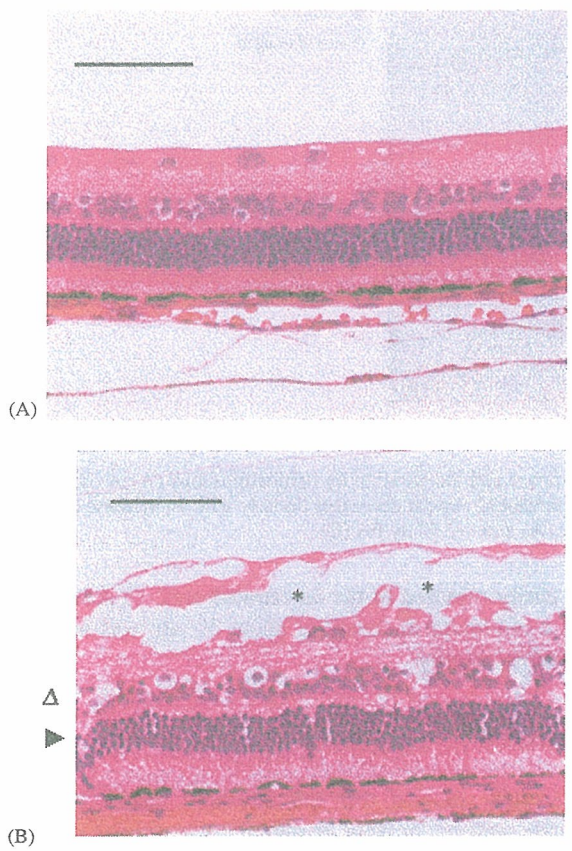

Figure 9. Histology of the retina 1 h after stimulation with currents of 1.0 mA (A) and 1.5 mA (B). (A) Except a small enlargement of choroidal vessels, no change was observed. Bar represents 100 μm. (B) Enlargement of choroidal vessels, disarray of outer nuclear layer cells (▲), condensation of inner nuclear layer cells (△) and vacuoles in the outer plexiform, inner plexiform and nerve fiber layers (*) can be seen.

was 0.6 mA by FA (figure 6(B) and table 1(B)). This corresponds to 0.3 μC/ph for a 0.5 ms duration pulse. The safe charge density for Pt electrodes is reported to be equal to or less than 400 μC cm^{-2}/ph to avoid Faradaic reactions such as H_2O electrolysis or Pt dissolution in inorganic saline [12, 13]. This corresponds to about 0.1 μC/ph for our electrode. Although there was a report that the dissolution limit of Pt was higher if the electrodes are soaked in a protein solution than in inorganic saline [14], the safe charge at the electrode surface for the STS method is three times higher than the Faradaic or dissolution limit of the Pt electrode.

Yuen *et al* have reported that with platinum electrodes, a charge density/phase (QD/ph) of 40 μC cm^{-2}/ph did not affect cortical neural tissues even after 50 h of continuous stimulation at 50 Hz [1]. McCreery *et al* have determined the thresholds for neuronal damage at a QD/ph of 50–100 μC cm^{-2}/ph after 7 h of continuous cortical stimulation at 50 Hz [4]. Recently, Harnack *et al* also reported that a QD/ph of up to 26 μC cm^{-2}/ph did not cause any neuronal damage after 4 h of stimulation at 130 Hz with Pt/Ir electrodes [15]. Although stimulation electrode size, hours and frequency were different, these values are quite comparable, and the threshold for neuronal damage by direct stimulation may be 26–100 μC cm^{-2}/ph.

The threshold for QD/ph in our experiment was 678–2119 μC cm^{-2}/ph, which is about 20 times higher than that of previous reports. Donaldson and Donaldson reported that several factors could be the cause of tissue damage when direct electrode stimulation is used, e.g., pH changes, gas evolution, bleaching products, $Mg(OH)_2$ deposits and electrode metal dissolution [16]. The safe charge in the present work may be higher than that of previous reports because the retina is protected by the sclera against electrochemical reactions

occurring at the electrode interface. Abundant blood flow in the choroid may also protect the retina.

McCreery *et al* investigated the relationship between charge density/phase (QD/ph) and charge/phase (Q/ph) that caused neural damage by Pt electrodes [4]. More recently, Vankov *et al* reported on the cellular damage produced by an electric field using glass pipette electrodes [17]. 7500 pulses were applied at 25 Hz during 5 min of stimulation. The pulse duration ranged from 6 μs/phase to 6 ms/phase and the electrode diameter ranged from 0.1 to 1 mm. The area of cellular damage was measured by propidium iodide fluorescent staining. The threshold current density was proportional to the reciprocal of the square root of pulse duration. In contrast to earlier reports, cellular damage by the pulsed electric current was not necessarily determined by charge density.

Our results (figure 5) showed that the threshold for a safe charge was not constant but decreased with shorter pulse durations, which is consistent in terms of electrochemical reaction limit with the result of Rose *et al* [18]. This indicates that although phosphenes are elicited effectively by pulses of short duration [19, 20], care should be taken using pulses of short duration.

Our results combined with those of Vankov *et al* [17] suggested that the threshold for a safe current density/ph (mA cm^{-2}/ph) is a reciprocal of the square root of duration (figure 7). If the electrode size is fixed, current/ph (mA/ph) is also a reciprocal of the square root of duration. This relationship is expressed as

$$I^2t = A, \tag{1}$$

where I is the current, t is the pulse duration and A is a constant.

Energy consumption (J) is expressed as

$$J = IVt = I^2Rt,$$

where I is the current, V is the voltage, R is the impedance and t is the pulse duration. The results, indicating that the threshold energy consumption is almost constant (figure 8), are consistent with equation (1), when R does not change much relative to the duration.

Donaldson and Donaldson further stated that the stimulating current produced negligible heating effect [16] and Mortimer *et al* reported that the lesion of blood–brain barrier of cortex was estimated by watt per square inch [21].

Our results that the threshold energy consumption was almost constant for all pulse durations (figure 8) may suggest that heat affects the retina with suprathreshold energy. But our histological observations showing that inner retinal layers were more damaged than the outer layers (figure 9) suggest that heat is not the main cause of retinal damage because heat may affect the proximal layers of retina (RPE, photoreceptors) more than distal layers. Mechanisms other than heat or electrochemical reaction at the electrode tissue surface may play a role in the retinal damage caused by suprathreshold STS.

In conclusion, the threshold for a safe current for 1 h of STS was higher than that reported by the direct stimulating methods for neural tissues and points to the safety of the STS system. The finding that the threshold for a safe charge was not constant but was lower with short pulse durations indicated that care should be taken using pulses with short duration although phosphenes are generated more effectively with short pulses.

Acknowledgments

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CLINICAL INVESTIGATION

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Evaluation of residual retinal function by pupillary constrictions and phosphenes using transcorneal electrical stimulation in patients with retinal degeneration

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Abstract *Background:* To evaluate inner-retinal function by pupillary constrictions and phosphenes evoked by transcorneal electrical stimulation (TES) in patients with hereditary retinal degeneration. *Methods:* Consecutive 20 eyes of 20 patients (16 with retinitis pigmentosa (RP); and four with cone-rod dystrophy (CRD)) whose visual acuity was equal to or worse than 20/2000 at Osaka University Hospital and eight eyes of eight healthy subjects were enrolled. TES was performed on with a contact lens stimulating electrode. The electrically evoked pupillary response (EEPR) was recorded by a pupillometer, and the phosphenes by the subjective responses. Three electrical current thresholds were determined: T1, threshold current for initial phosphene; T2, threshold for eliciting a phosphene extending into the central field; and P, threshold for a relative pupillary constriction ≥3%. The EEPR and phosphene thresholds were compared with the visual acuity or the visual field. *Results:* All T1, T2 and P were significantly higher in patients than in normals (Mann-Whitney, $P<0.001$). Both T1 and T2 were not correlated with visual acuity but depended on the area and location of the residual visual field. T1 and T2 in RP eyes with a EEPR was significantly lower than that in RP eyes without an EEPR. During TES, all subjects and patients had no pain, and no complications except for a slight corneal superficial punctuate keratopathy. *Conclusions:* The safety and the efficacy of TES to estimate the residual inner-retinal function in patients with retinal degeneration indicate that TES can be used as one of the most important test to select candidates for retinal prostheses.

Keywords Retinitis pigmentosa · Cone-rod dystrophy · Pupillary reflex · Phosphene · Transcorneal electrical stimulation

Introduction

Retinitis pigmentosa (RP) is one of the leading causes of blindness in the world. RP includes a group of heredity retinal degenerations that primarily affects photoreceptor (PR) function [18, 23]. In the last stage of the disease, RP patients have little or no functional vision.

To restore some vision to patients with RP, the strategy of replacing the degenerated photoreceptors by a bionic device called a "retinal prosthesis" is under serious study [17, 36]. Various types of retinal prosthesis have been proposed and tested in animals [2, 8, 11, 13, 16, 28, 34] and patients [3, 10, 26, 33]. A typical retinal prosthesis consists of an array of electrodes that is implanted on the retinal surface and is used to deliver electrical current to the retina to evoke a light sensation called a phosphene.

Another approach to restore vision in RP patient is to transplant retinal progenitor cells (RPCs) into the retina [7, 36]. The success of an artificial retina or the transplantation of RPCs to restore vision depends on the presence of

physiologically intact retinal ganglion cells (RGCs) which can transmit visual signals to the brain.

Morphometric studies of the retinas in RP patients have shown the preservation of some of the RGCs [9, 27, 29]. Postmortem studies of RP eyes have shown that the number of RGC was approximately 30% of that in normal age-matched eyes in the macular area but only 20% in extramacular regions [9, 27]. On the other hand, it must be remembered that remodeling and ectopic retinal structures develop in RP retinas [5, 15]. Retinal remodeling and retinal circuit corruption may prevent the surviving RGCs from transmitting visual signals.

Given these pieces of evidence, a small number of RGCs are certainly present in the eyes of RP patients. However, it is difficult to determine to what extent these RGCs are functional compared with those in an intact retina because the method to evaluate the residual RGC function is limited.

Electroretinography (ERG) and visually evoked potentials (VEPs) are of little value when only a small number of PRs remain in the degenerated retina. On the other hand, electrical stimulation to evoke phosphenes is a potential useful method to evaluate the function of residual RGCs in humans. Phosphenes generated by galvanic or faradaic currents passed through the orbit by various electrode arrangements have been reported since the mid-20th century [1, 6, 19, 20]. More recent studies have used transcorneal electrical stimulation (TES) using corneal electrodes under local anesthesia to evoke phosphenes and to obtain electrically evoked responses (EER) in healthy subjects [21, 24] and RP patients [22, 25].

Although it is not conclusive what kind of retinal neurons are primarily stimulated by TES, the RGCs must be finally activated to transmit visual signals to the brain when a phosphene is evoked. Thus, TES could be one way to estimate the function of the residual RGCs in patients.

The area of the perceived phosphene may correspond to the area where functional RGCs are present and the extent of residual inner retinal function in a degenerated retina. However, it is difficult to assess the RGC function based on the evaluation of phosphene, because phosphene is a subjective sensation. Delbeke et al. tried to compare the phosphenes to somatosensory sensation or pain of the eyelid evoked by electrical stimulation through the eyelid to assess the function of RGCs in patients [4]. Because the somatosensory sensation is also a subjective parameter and is not directly related to phosphenes, the assessment of RGC function based on the somatosensory sensation is limited for candidates of retinal prosthesis [3, 10, 26], even though it is effective for candidates of optic nerve stimulation [33].

Direct and indirect pupillary constrictions can be evoked by TES by stimulating the afferent pupillary pathway and are called electrically evoked pupillary responses (EEPR) [30, 31]. The EEPR can be an objective parameter to be compared with phosphene; however, the relationship between phosphenes and EEPR has not been determined.

Thus, the purpose of this study was to investigate the phosphenes and EEPR in healthy subjects and in patients with retinal degeneration, and to compare these findings to the visual function in these eyes. The long term goal of our studies is to develop a simple and safe method to evaluate the function of the residual RGCs by combining phosphenes and EEPR to select candidates for the retinal prosthesis implant.

Materials and methods

Setting These studies were performed at the Osaka University Medical School, Osaka, Japan.

Patients The characteristics of all subjects are shown in Tables 1 and 2. Eight eyes of eight male volunteers (34±6 years, mean age±SD) with no ocular disorders, and consecutive 20 eyes of 20 patients (51±13 years) with hereditary retinal degeneration [16 patients had RP and four patients had cone-rod dystrophy (CRD)] who visited Osaka University Hospital between January 2003 and December 2003 were studied. The diagnosis was confirmed by independent ophthalmologic and ERG examinations. The inclusion criteria for patients was that the visual acuity was equal or lower than 20/2000, which was lower than the intended resolution of our project of artificial retina. The exclusion criteria were those patients with cardiac pacemaker or the presence of corneal diseases.

All subjects gave an informed consent after the purpose of this study and the procedures to be used were explained. They were free to withdraw at any time. This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Osaka University Hospital.

The slit-lamp examination of the corneal was performed just after the TES examination in all subjects.

Table 1 Characteristics of normal subjects

No	Age	Sex	T1 (μA)	T2 (μA)	P (μA)
1	49	M	75	100	125
2	34	M	50	100	125
3	27	M	50	75	125
4	32	M	75	125	150
5	31	M	75	125	150
6	32	M	75	125	150
7	32	M	100	150	200
8	33	M	25	75	75

T1 threshold current of initial perceptual phosphene; *T2* threshold current of phosphene expanding over the center of visual field; *P* threshold current of EEPR

Table 2 Characteristic of patients

No	Age	Sex	VA	T1 (μA)	T2 (μA)	P (μA)	RPC (%)	CVF (deg^2)	PVF (deg^2)	Diagnosis
1	60	F	HM	200	250	600	N	0	1.5×10^3	CRD
2	9	M	20/2000	200	250	250	13	0	1.9×10^4	CRD
3	31	M	20/2000	50	600	600	N	0	7.9×10^3	CRD
4	32	F	20/2000	200	700	300	15	0	1.4×10^4	CRD
5	42	F	20/2000	550	550	N	5.2	1.7×10^2	3.0×10^3	RP
6	51	F	NLP	650	650	NR	NR	0	0	RP
7	50	F	NLP	150	550	NR	NR	0	0	RP
8	23	M	CF	400	400	400	N	0	2.8×10^3	RP
9	45	F	HM	300	350	550	N	3.4×10	0	RP
10	66	F	20/2000	550	700	800	8	4.6×10^2	0	RP
11	44	F	20/2000	600	600	NR	NR	2.9×10	0	RP
12	50	M	20/2000	150	150	600	N	2.5×10	0	RP
13	56	M	HM	550	700	N	2.1	4.1×10	0	RP
14	55	M	HM	1,500	1500	N	4.6	6.1×10	0	RP
15	56	F	20/2000	500	500	1500	7.6	2.0×10^2	0	RP
16	62	F	HM	500	500	900	N	3.4×10	0	RP
17	65	M	LP	1,000	1400	N	N	0	0	RP
18	66	M	LP	1,100	N	N	N	0	0	RP
19	62	F	20/2000	350	700	N	N	2.4×10^2	0	RP
20	66	M	LP	1,400	1400	N	N	0	0	RP

F female, *M* male, *VA* visual acuity; *NLP* no light perception; *HM* hand motion; *CF* counting fingers; *LP* light perception; *T1* threshold current of initial perceptual phosphene; *T2* threshold current of phosphene expanding over the center of visual field; *P* threshold current of EEPR; *RPC* relative pupillary constriction by flash-light stimulation measured by pupillography; *N* not responded; *NR* non-recordable due to nystagmus; *CVF* area of preserved central visual field within a radius of 30°; *PVF* area of peripheral visual field left outside the radius of 30°; *CRD* cone rod dystrophy; *RP* retinitis pigmentosa

Assessment of visual function

The best-corrected visual acuity was measured by a certified orthoptists with a standardized Landolt visual acuity chart. The visual field was quantitatively determined by kinetic perimetry using a Goldmann perimeter. The V/4e target with a luminance of 320 cd/m^2 was projected on a background with a luminance of 10 cd/m^2. The area of the visual fields was calculated using the Scion Image program (Scion Corporation, Frederick, Mass., USA).

Transcorneal electrical stimulation

TES was performed on eight healthy subjects and 20 patients. Before the TES, the cornea was anesthetized with 0.4% oxyburocaine hydrochloride, and the cornea was covered with 3% hyaluronic acid and 4% chondroitin sulfate (Viscoat, Alcon Japan Ltd, Tokyo, Japan) to protect it from injury by the contact lens electrode. A concentric bipolar contact lens electrode (Burian-Allen; Hansen Ophthalmic Laboratories, Iowa City, Iowa, USA) was placed on the cornea, and electric current pulses (20 pulses) were delivered from a stimulator SEN-7203 (Nihonkoden, Tokyo, Japan) and stimulus isolator unit A395 (WPI, Sarasota, Fla., USA) through the two electrodes embedded in the contact lens.

The electrical stimuli were rectangular, biphasic (anodic first) pulses of 10 ms/phase duration, frequency with 20 Hz, and train of 20 paired pulses. These parameters were chosen based on the psychophysical experiment on normal volunteers to elicit phophesene effectively (Matsushita K et al., ARVO abstract 2003). The current intensity ranged from 50 μA to 2 mA with a step of 25 μA up to 100 μA, 50 μA up to 1000 μA, and 100 μA above 1000 μA.

Recording the pupillary constriction

An infrared pupillometer, the IRISCORDER C7364 (Hamamatsu, Hamamatsu, Japan), was used to measure the pupillary responses evoked by TES. The pupillometer is equipped with an infrared charge-coupled device (CCD) camera and recorded the pupillary diameters at a 60 Hz sampling rate. Normal subjects and patients wore a goggle equipped with the CCD camera and the red light-emitting diode (LED) stimulus light (660 nm; maximum light power of 10±3 μW; stimulus duration of 0.1 s). Before inserting the contact lens electrode, the direct and consensual pupillary light reflex of normal subjects and patients were recorded. After inserting the contact lens electrode,

the EEPRs were recorded from the fellow eye. The relative amplitude of the pupillary constriction was determined by calculating the relative amplitude of pupillary constriction starting from the baseline diameter at the stimulus onset to the peak of the pupillary constriction as follows:

$$Relativepupillaryconstruction(RPC\%) = 100(a-b)/a$$

where a=pre-stimulus baseline pupil size (mm), and b=maximally constricted pupil size (mm).

The threshold current for a relative pupillary constriction was determined by the minimal electrical current necessary to elicit an EEPR of ≥3 %.

Psychophysical procedures

We recorded the characteristics of the phosphenes (e.g. location, size, color, brightness, shape) for each electrical current intensity in the dark room. Subjects were masked to the test conditions, as this allowed each subject to provide a non-biased descriptions of their perception. The examiner, who was aware of the stimulus conditions, asked questions about the phosphene. False positive trials (i.e. no stimulus presented) were included to determine the reliability of the responses.

Two thresholds were determined; threshold 1 (T1) was defined as the value of the electrical current that elicited the first perceived phosphene anywhere in the visual field, and threshold 2 (T2) was the value at which the subjects perceived a phosphene extending into the center of the visual field. We determined the two thresholds by starting with a current intensity below threshold and increasing the stimulus strength stepwise until a perception of a phosphene was first perceived (T1a), and the current at which the phosphene extended into the central visual field (T2a). Next, the stimulus strength was started well above threshold and reduced along the same steps until the same perceptions were obtained, i.e. disappearance of the phosphene from the central visual field (T2b), and the complete disappearance of a phosphene (T1b). T1a and T1b are usually the same but if the values differed, we averaged the two values to determine the threshold (T1). The same procedure was taken to determine the value of T2. For each step, the patient was asked to describe his/her perceptions in detail which were recorded on audio tape.

Statistical analyses

Data are presented as the means±standard error of the means (SEM), and statistically analyzed with the SPSS 10.0J program (SPSS Inc, Chicago, Ill., USA). Comparisons between two groups were made by the student *t*-test when data were normally distributed, or by the Mann-Whitney *U*-test when data were not normally distributed. The degree of correlation was evaluated by the coefficient of correlation (r) calculated using Pearson correlation coefficient. Comparisons between the three groups were made by one-way ANOVA followed by the Tukey test when data were normally distributed. The probability level is represented as the value "*P*"; statistical significance was set at $P<0.05$.

Results

Characteristics of phosphenes in normal subjects and patients

In normal subject, phophene was first perceived in the upper or lower peripheral field with the mean threshold of 65±8 μA (T1). With a further increase in the current intensity, phosphene spread into the center of the visual field with the mean threshold of 109±9 μA (T2). With a further increase in the current intensity, a pupillary constriction was evoked with a mean intensity to evoke a RPC of just >3% was 138±13 μA (P). A comparison of the three thresholds, T1, T2, and P, showed that they were significantly different from each other ($P<0.001$, one-way ANOVA) (Table 1, Fig. 1).

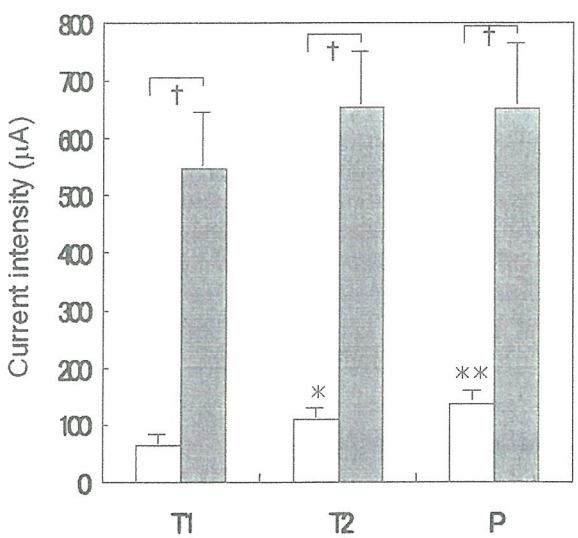

Fig. 1 Average threshold current intensities of phosphenes and pupillary constrictions in normals (*open bar*) and in eyes with retinal degeneration (*filled bar*). *T1* threshold current intensity of initial phosphene, *T2* threshold current intensity for phosphene covering the center of visual field, *P* threshold current intensity of electrically evoked pupillary response (*EEPR*) . Data are presented as mean± SEM. There was a significant difference among three thresholds in normals (one-way ANOVA, $P< 0.01$; Tukey test, * $P<0.05$, ** $P<0.01$, vs T1). No significant difference among three thresholds was obtained in eyes with retinal degeneration. There was a significant difference in each threshold between normals and retinal degeneration (Mann-Whitney Rank Sum Test,† $P<0.001$)

The distribution of the T1, T2, and P thresholds in patients are shown in Table 2. A phosphene was elicited by TES in all patients. However, the threshold currents were much higher than normal subjects and varied considerably among patients. The mean T1 threshold was 545±411 μA. With an increase in the current intensity, many patients reported that bright light sensation spread toward the center of visual field. The mean T2 threshold was 723±479 μA. However patient 18, mentioned that the phosphene did not spread into the center with maximum current intensities (2000 μA). The threshold of P was higher than T2 in most cases but in some patients, pupillary reflex was not evoked with the maximum current intensities (2000 μA) (Table 2, Fig. 1). The false positive rate was 0% in the subjective phosphene test.

Relationship between thresholds (T1 and T2) in patients and normal subjects

Although the thresholds of the normal subjects were quite comparable, the thresholds of the patients varied considerably. A scatter plot of T2 as a function of T1 in normal subjects and patients is shown in Fig. 2. In the normal subjects, a highly significant positive correlation was observed between the T1s and T2s (r=0.900 and P=0.002; Pearson's correlation coefficient).

The patients, on the other hand, were divided into two groups from the scatter plot. One group was made up of patients whose thresholds were distributed tightly around the linear regression line of normal subjects, and the thresholds of other group of patients were shifted above the line. These results lead us to examine whether the thresholds in the patients were dependent on the visual acuity or the residual visual field or the type of disease.

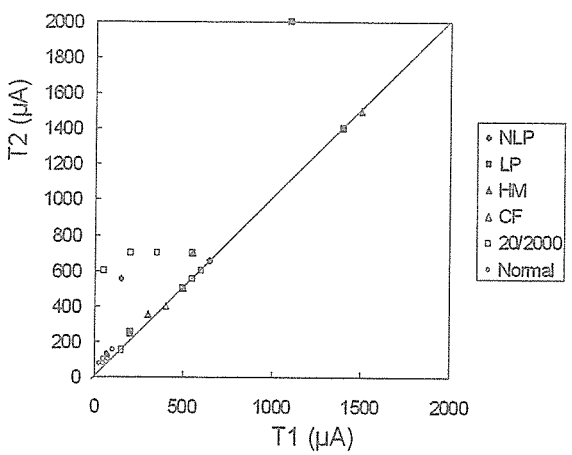

Fig. 2 Comparison of T1 with T2 in normal subjects and patients. Normal subjects (*open circles*) and patients (*filled circles*) are plotted. Patients were divided into two groups. One group includes patients with a closer fit to the linear regression line of normal subjects, another group included patients distributed above the line

Relationship between thresholds and visual acuities in patients

The visual acuities were converted to the logarithm of the minimum angle of resolution (logMAR) units for the statistical analysis. For visual acuities less than 20/2,000 (2.0 logMAR units), the following log MAR values were assigned [12]: 2.6 logMAR for counting finger (CF); 2.9 logMAR for hand motion (HM), 3.1 logMAR for light perception (LP); and 3.4 logMAR for no light perception (NLP). The relationship between the electrical phosphene thresholds and logMAR visual acuities is shown in Fig. 3. There was no significant relationship (T1,r=0.433; P=0.056: T2,r=0.417; P=0.067) between log MAR visual acuities and thresholds (Fig. 3a,b). For example, although patients 6 and 7 were NLP, their thresholds were lower than those of 11 and 14, whose visual acuities were 20/2000 and HM, respectively (Table 2).

Relationship between thresholds of phosphenes and residual visual fields in patients

The patients were classified into three groups on the basis of the location of residual visual field: type C, visual field present within the central 30° radius (n=10); type P, peripheral visual field left beyond the central 30° radius (n=5); and type N, complete loss of visual field (n=5). A patient who had two islands of visual field with one located within 30° radius was categorized as type C.

The relationship between the thresholds and type of residual visual fields is shown in Fig. 4. There was a significant difference in the thresholds for a phosphene in the three groups (one-way ANOVA, T1, T2; P<0.05). The mean current intensities of T1 and T2 for type P patients were the lowest among the three groups: T1=210±56 μA; T2=440±91 μA, and for type C: (T1; 555±114 μA, T2; 625±112 μA), and the mean intensities in type N were much higher (T1; 860±214 μA, T2; 1200±269 μA) (Fig. 4).

We further analyzed the relationship between the area of residual visual field and thresholds in each groups, and no significant correlation was found.

Relationship between thresholds of phosphenes and type of disease

In eyes with CRD, the cones are predominantly damaged and the loss of cones result in a loss of the central visual field, while in RP, the rods are predominantly damaged and the loss of rods result in a loss of the peripheral visual field. We therefore divided eyes with retinal degeneration into the CRD group and RP group (Fig. 5, Table 2).

The mean current intensity of T1 in the RP patients was significantly higher than that in CRD patients (640±101 μA vs 163±38 μA, P<0.05; Fig. 5e). Although the