Distribution of Retinal Responses Evoked by Transscleral Electrical Stimulation Detected by Intrinsic Signal Imaging in Macaque Monkeys

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Purpose. The distribution of the electrical current over the retina when electrical pulses are delivered transsclerally has not been clearly determined objectively and quantitatively in humans. The purpose of this study was to determine the pattern of electrically evoked neural activity in the monkey retina by using intrinsic signal imaging.

Methods. The intrinsic signals of monkey retinas were recorded as changes in the reflectance of infrared light from the retina after transscleral electrical stimulation by DTL electrodes. The effects of changing the stimulus parameters (e.g., intensity, duration, and frequency) of the electrical current, were investigated.

Results. Electrical stimulation evoked a uniform change in the reflectivity across the posterior pole of the retina; that is, the intrinsic signals changed uniformly. A peak of the intrinsic signal was not observed at the fovea. The threshold of the intrinsic signal was not significantly different for the macula, perimacula, and optic disc, and the threshold did not differ under dark- and light-adapted conditions. The strength of the signals increased with longer stimulus durations, and the maximum signals were obtained when the stimulus frequency was between 15 and 20 Hz.

Conclusions. Intrinsic signals of the monkey retina evoked by transscleral electrical stimulation are elicited uniformly across the posterior pole of the fundus and most likely arise from activation of the inner or middle layers of the retina. These functional measurements could serve as a diagnostic tool for mapping the inner retinal activity, by which the site of a lesion can be noninvasively imaged. (*Invest Ophthalmol Vis Sci.* 2008;49:2193–2200) DOI:10.1167/iovs.07-0727

Electrical stimulation of the retina excites the neural pathway and evokes light sensations, called electrical phosphenes, and electrically evoked responses (EERs) from the occipital lobe.[1–4] Miyake et al.[5–9] have applied this technique for clinical diagnosis and have explored ways to evaluate the function of the inner retina of patients with total retinal detachment, night blindness, central artery occlusion, and optic nerve diseases objectively. With the increasing interest of retinal prosthesis,[10–12] the effects of electrical stimulation of the retina have drawn increasing attention because understanding the neuronal properties of the retina after electrical stimulation is essential for the development of retinal prosthesis[13,14] and determining residual retinal function in patients with inherited and acquired retinal degeneration should be known before a retinal prosthesis is implanted.[15,16] More recently, based on the results of several investigations on the neuroprotective effect of electrical stimulation,[12,17,18] electrical stimulation of the retina has been used to treat optic nerve diseases, such as nonarteritic ischemic optic neuropathy, traumatic optic neuropathy,[19] and longstanding retinal artery occlusion.[20]

Distribution of the electrical current across the retina, however, has not been definitively determined. Although electrical phosphenes are perceived homogeneously and continuously over the visual field when the retina is stimulated with a DTL electrode,[16] there is no way to evaluate the current distribution over the retina objectively, quantitatively, and noninvasively in humans. Electroretinographic techniques cannot be used to evaluate the retinal activity evoked by electrical currents because of the large electrical artifact from the stimulus.

Intrinsic signal imaging is a well-established imaging technique recently applied to translate neural activities elicited by photic or electrical pulses into the minimal visible changes of the appearance of the retina.[21–24] This has been used to assess the cone- and rod-induced responses in the retinas of macaque monkeys[25] and humans.[26,27] Recently, we reported that retinal intrinsic signals are composed of several components with different properties, and some of the components are strongly correlated with the neural activity of the inner retina.[28]

The purpose of this study was to determine the distribution of the electrical currents across the posterior retina after an electrical pulse. To accomplish this, we measured the changes in the retinal intrinsic signals evoked by transscleral electrical stimulation. A DTL electrode was used to deliver the electrical pulses, and different recording conditions, such as light- and dark-adapted states, along with different electrical current parameters, such as intensity, duration, and frequency, were studied. Results indicated that the electrical stimuli elicited responses uniformly across the posterior pole of the ocular fundus and activated mainly the inner or middle layers of the retina.

Methods

The principles of measuring intrinsic signals have been described in detail.[25,28] The experiments were performed on two rhesus monkeys (*Macaca mulatta*) under general anesthesia. After intramuscular injection of a mixture of atropine sulfate (0.08 mg/kg), droperidol (0.25 mg/kg), and ketamine (5 mg/kg), the monkeys were paralyzed with

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vecuronium bromide (0.15 mg/kg per hour) and were artificially ventilated with a mixture of 70% N_2O, 30% O_2, and up to 1.5% isoflurane. Electrocardiograms, expired CO_2, and rectal temperatures were monitored throughout the experiments. Before the recordings, one pupil was fully dilated with topical tropicamide (0.5%) and phenylephrine hydrochloride (0.5%). A hard contact lens was placed on the eye to prevent the cornea from drying.

The experimental protocol was approved by the Experimental Animal Committee of the Riken Institute, and all experimental procedures conformed to the guidelines of the Riken Institute and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Intrinsic Signal Imaging

A digital fundus camera system (NM-1000; Nidek, Aichi, Japan) was modified to record the light reflectance changes of the macaque retina over 45° of the posterior pole. The fundus reflectance was continuously monitored with light from a halogen lamp bulb filtered through an infrared interference filter (840–900 nm). The camera was focused on the macular vessels, and the images were recorded with a charge-coupled device (CCD) camera (PX-30BC; Primetech Engineering, Tokyo, Japan). The images were digitized with an IBM-compatible computer equipped with a video frame-grabber board (Corona I; Matrox, Quebec, Canada: gray level resolution, 10 bits; spatial resolution, 640 × 480; temporal resolution, 1/30 second; Fig. 1A). The respirator was stopped during the recordings to reduce the respiration-induced motion artifacts.

For recordings in the light-adapted condition, half an 80-mm diameter white polyethylene ball was placed between the fundus camera and the eye. The ball was illuminated by two halogen lamps through fiber optics so that the luminance in the center was 30 cd/m^2. The ball was removed a few seconds before data acquisition.

Electrical Stimulation of Retina

DTL electrodes were used to deliver the electrical pulses. The DTL electrode was selected for the measurements of intrinsic signals because the noise of the intrinsic signal is much lower with a DTL electrode than with a contact lens electrode such as the Burian-Allen electrode and because electrical phosphenes were perceived to be more homogeneous and continuous with a DTL electrode than with a contact lens electrode.[16]

The fibers of the DTL electrode were placed on the lower bulbar conjunctiva 5 mm from the corneal limbus. The conjunctiva was covered with 3% hyaluronic acid and 4% chondroitin sulfate (Viscoat; Alcon Japan, Tokyo, Japan), and the reference electrode was placed on the ipsilateral wrist (Fig. 1A).

Biphasic electrical pulses were used for all experiments (Fig. 1A, inset). The stimulus consisted of a positive current for x msec followed by a negative current for x msec and then a rest period for $3x$ msec. In most of the experiments with a pulse frequency of 20 Hz, x was set to 10, but in the experiment in which the pulse frequency was changed, x varied from 40 (5 Hz) to 2 (100 Hz) to keep the total current constant.

Stimuli were delivered 0.5 second after the initiation of data acquisition for 1 second, except in the experiment in which stimulus duration was changed from 0.5 second to 7 seconds. Pulse duration and frequency were controlled by a function generator (Multifunction Synthesizer WF 19443B; NF Corporation, Yokohama, Japan). Timing of the data acquisition and stimulus delivery were under computer control.

To compare the responses evoked by light flashes, white light stimuli were obtained from a xenon strobe (duration, 1 msec) embedded in the fundus camera. The light stimulus was given to the entire posterior pole of the ocular fundus, 0.5 second after the initiation of data acquisition. Flashes were delivered either as flickering flashes (20 Hz, 1 second, Fig. 2A) or as a single flash (Fig. 3). Flash intensity measured at the cornea was 6.07 cd · s/m^2 for the flickering flashes, and 140 cd · s/m^2 for a single flash (measured at 50.2 mm from the object lens by a photoradiometer; IL-1700, International Light Technologies Inc., Peabody, MA).

Data Analysis

After electrical or light stimulus, the light reflectance from the ocular fundus decreased and the fundus image became darker. The intrinsic

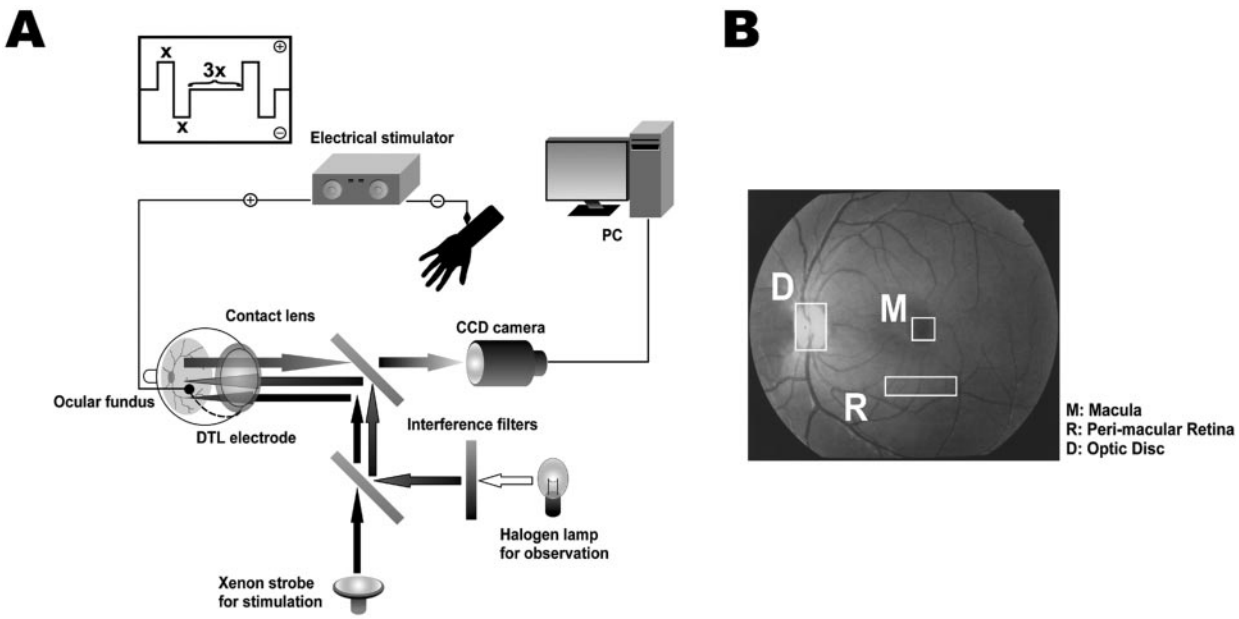

FIGURE 1. Schematic drawing of the experimental setup, pattern of electrical pulses, and fundus photograph. (**A**) Ocular fundus was illuminated by the light from a halogen lamp filtered through an infrared interference filter and monitored by a CCD camera during the recording trial. Stimulus was given by a xenon flash through the objective lens of a fundus camera or by electrical current through a DTL electrode placed on the conjunctiva. *Inset*: biphasic pulse current with a duration of x is followed by a resting period as long as $3x$. As the frequency is changed from 5 to 100 Hz, x is changed from 40 to 2 msec, respectively. (**B**) Fundus photograph of normal retina showing the locations to be analyzed.

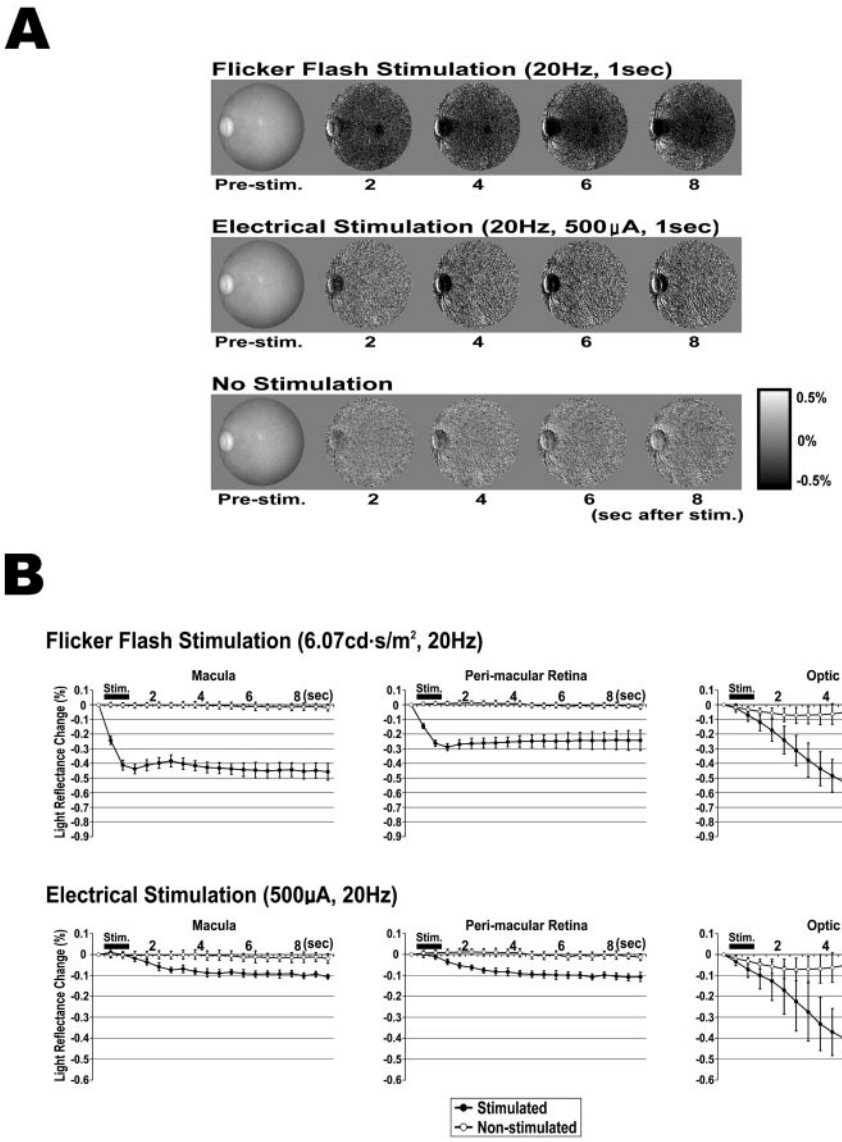

FIGURE 2. Time courses of flash-evoked and electrically evoked intrinsic signals. (**A**) Monochromatic infrared images of the ocular fundus showing the light reflectance changes during 10 seconds with flicker flash stimulation (*top*) or electrical stimulation (*middle*) or without stimulation (*bottom*). Images on the *left* are fundus images taken before the stimulation. Images on the *right* are the differential images showing the light reflectance changes after stimulation. Thirty consecutive video frames collected during 1 second were averaged for one poststimulus fundus image. Darkened regions indicate a decrease of light reflectance after the light stimulus. The data of three consecutive trials are averaged. (**B**) Plot of time courses of the light reflectance changes, evoked by flash (*top*) and electrical (*bottom*) stimulation at three different regions in a normal eye. The period of stimulus delivery (1 second) is indicated by *thick bars*. The time after the initiation of stimulus is shown on the abscissa. Data of 10 consecutive trials were averaged.

signals were measured as the stimulus-evoked changes in light reflectance. The amplitude was calculated as poststimulus grayscale values/prestimulus (0.5-second period) values pixel by pixel. This ratio was rescaled to 256 levels of grayscale resolution to show the stimulus-induced reflectance changes (Fig. 2A).

Each recording trial consisted of 300 video frames collected at 30 frames per second for a total recording time of 10 seconds. The grayscale values of 15 video frames collected in 0.5 second were averaged for individual data points to determine the time course of the flash-induced reflectance changes (Fig. 2B).

In our previous studies, we showed that the response properties of the intrinsic signals evoked by a brief light flash were distinctive for different regions of the ocular fundus because they arise from different neuronal and vascular components of the eye, though the precise cellular mechanisms of signal production have not been determined.[25,28] To compare the electrically evoked signals with the light-evoked signals, three retinal regions were examined: the macula (30 × 30 pixels, covering 3.5° of the center), the perimacular region between the macula and the inferior-temporal artery (95 × 25 pixels), and the optic disc (40 × 60 pixels; Fig. 1B). To plot the time courses of reflectance changes, grayscale values within each region were averaged (Fig. 2B).

RESULTS

After flickering light or electrical stimuli, the light reflectance of the posterior retina and the optic disc decreased and the image of the ocular fundus became darker (Fig. 2A, top and middle). The time courses of the intrinsic signals, however, were different for these two stimuli. The time courses of the signals in three regions evoked by light flashes (20 Hz, 1 second, 6.07 cd · s/m^2) and electrical pulses (20 Hz, 1 second, 500 μA) under dark-adapted conditions are shown in Figure 2B. With flickering light, the reflectance changes in the macula and the perimacular retina were more rapid than at the optic disc, with the signal reaching its negative peak within 1.5 seconds after the flash. With electrical stimulation, on the other hand, light reflectance changes in the macula and the perimacular retina were as slow as those at the optic disc. Although the onset of light reflectance changes in the perimacular retina slightly preceded that in the optic disc, the signals in three regions reached their negative peaks 5 to 6 seconds after the stimulus. This trend in the signal time course was the same regardless of the current intensity, for a range of 100 to 1000 μA (data not shown).

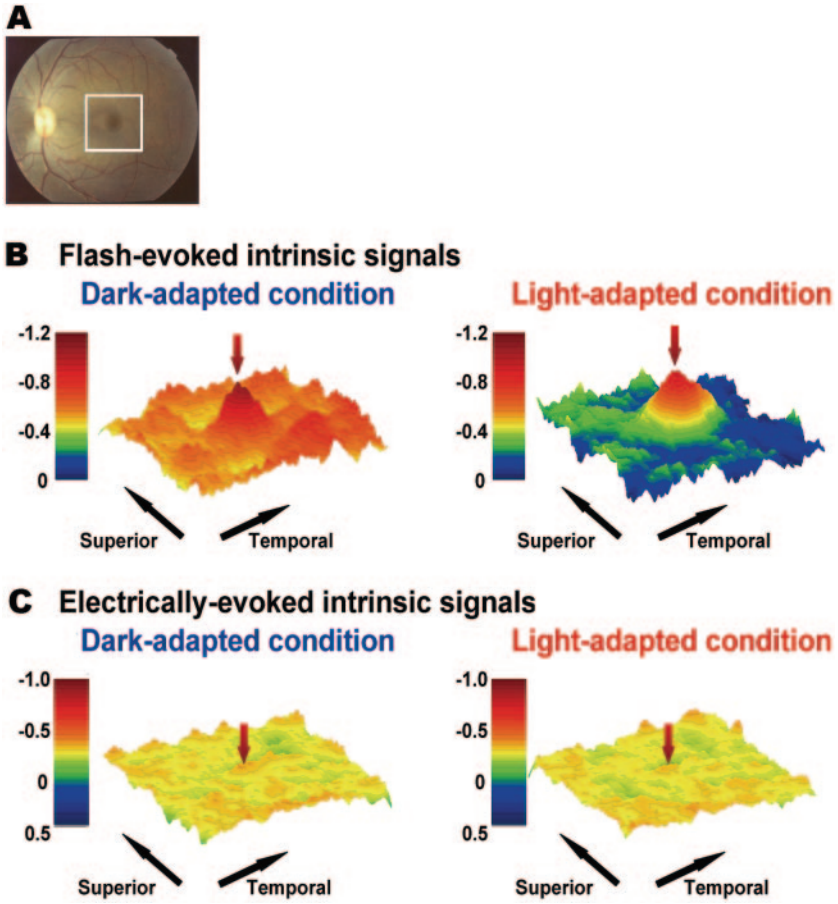

FIGURE 3. Topographic maps of the intrinsic signals elicited by flash-evoked and electrically evoked stimuli in the posterior retina. Pseudocolor topographic maps of light reflectance changes after a single flash (**B**; flash intensity, 140 cd · s/m^2; duration, 1 msec) or electrical stimulus (**C**; current, 500 μA; pulse frequency, 20 Hz; stimulus duration, 1 second) in the posterior pole of retina, under dark (*left*)- or light (*right*)-adapted conditions. Twenty consecutive trials were averaged to construct the topographic map. The location of the fovea is indicated by *red arrows*. The region of interest is shown by a *white rectangle* in (**A**). Note that negative values of light reflectance changes are plotted to indicate the strength of intrinsic signals at each locus.

Spatial Distribution of Intrinsic Signals

Distribution of the intrinsic signals evoked by a flashed light stimulus represents the responses of cone and rod photoreceptors.[25] After 30 minutes of dark adaptation, a topographic map of the intrinsic signal elicited by a flashed light stimulus had a steep peak at the fovea, and the perimacular region was moderately activated (Fig. 3B, left). The strong response at the fovea reflects cone-induced activities, and the response at the perimacular region reflects both cone- and rod-induced activities.[25] In the light-adapted condition, the topography of the response had a steep peak at the fovea, but the response in the perimacular region was strongly reduced because of suppression of rod function (Fig. 3B, right).

The distribution of the electrically evoked signals, on the other hand, did not have a foveal peak in dark- or light-adapted conditions (Fig. 3C). In addition, the perimacular response under dark-adapted conditions did not differ significantly from that under light-adapted conditions. The intrinsic signals evoked by electrical stimulation were roughly homogeneous in the posterior pole, and the spatial distribution did not reflect the anatomic distribution of cone and rod photoreceptors as it did with light stimulation.

Effect of Changes in Stimulus Current

The effect of currents ranging from 0 to 1000 μA on the intrinsic signals was determined under dark- and light-adapted conditions (pulse frequency, 20 Hz; stimulus duration, 1 second; pulse duration, 10 ms; Fig. 4). The peak light reflectance value obtained during the 10-second recording was used for the signal amplitude for each current (same as in Figs. 5 and 6), and the results of three trials were averaged. Results measured at the macula, perimacular retina, and optic disc are shown for two monkeys (M1 and M2). Response properties appear to be approximately the same in each region under both dark- and light-adapted conditions. Change in reflectance as a function of the electrical current was sigmoidal; weak responses were recorded at low currents from 100 to 400 μA, stronger and faster rising signals were recorded above 400 μA, and maximum signals were recorded above 600 μA. The threshold of the electrically evoked intrinsic signals might have been lower than 100 μA in each of the three regions, but it was technically difficult to determine the peak value of the signal when the absolute light reflectance changes became smaller than 0.05%. A small difference of signal amplitudes between dark- and light-adapted conditions in the perimacular area can be noted (Fig. 4, middle graphs); however, this difference was negligible in amplitude and threshold when compared with that in the flash-evoked response, in which twofold to fivefold differences in signal amplitude and a 3-log difference in the threshold of flash intensity were observed between dark- and light-adapted conditions.[28]

Effect of Stimulus Duration

We measured the intrinsic signals evoked by different stimulus durations in the dark-adapted condition. Stimulus durations varied from 0.5, 1, 3, 5, and 7 seconds, pulse frequency was 20 Hz, and stimulus current was 500 μA for a pulse duration of 10 msec (Fig. 5). Results of four trials were averaged in the two monkeys (M1 and M2). Response properties seem to be almost the same in each region; the intrinsic signals increased with longer stimulus durations.

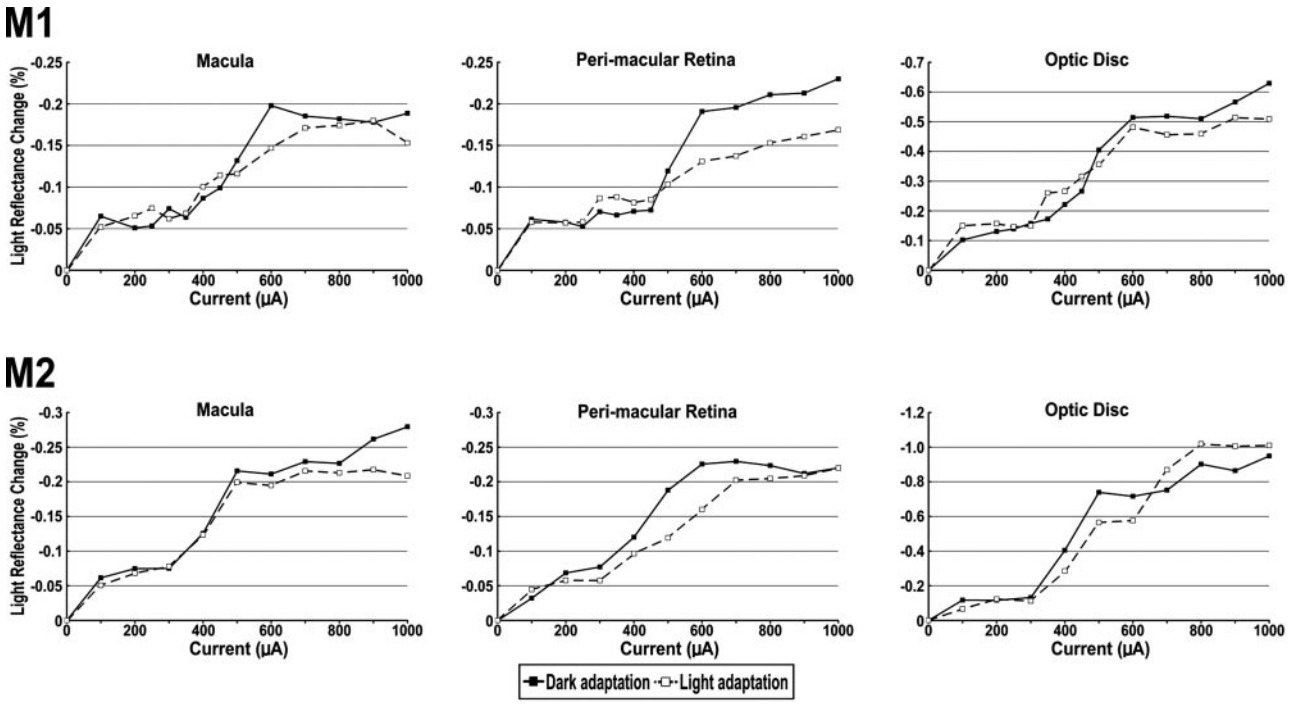

FIGURE 4. Stimulus intensity and intrinsic signals. Changes in the intrinsic signals of three regions after increasing electrical currents (current, 0–1000 μA; total stimulus duration, 1 second; pulse frequency, 20 Hz; pulse duration, 10 msec) in dark- and light-adapted conditions are shown as light reflectance changes in two monkeys (M1 and M2). The peak value of light reflectance decrease during a 10-second recording period was used for the signal amplitude for each current (as in Figs. 5, 6). Note that negative values of light reflectance changes are plotted to indicate the strength of intrinsic signals, and that the vertical scaling is different in three recording regions (as in Figs. 5, 6).

Effect of Stimulus Frequency

We measured the intrinsic signals evoked by different stimulus frequencies under dark-adapted conditions (stimulus current, 500 μA; stimulus duration, 1 second; pulse frequency (Hz)/pulse duration (msec), 5/40, 10/20, 15/13.3, 20/10, 40/5, 60/3.33, 80/2.5, and 100/2; Fig. 6). Results of five trials were averaged for each monkey (M1 and M2).

Response properties seem to have been almost the same in each region; intrinsic signals were maximal when the current frequency was 20 Hz, with one exception in M1 at the perimacular region (15 Hz). The signal was reduced when the frequency was increased or decreased from 20 Hz.

DISCUSSION

Results of this study showed that electrical stimulation through a DTL electrode resulted in a homogeneous change of light reflectance (intrinsic signals) within the vascular arcades of the retina. Unlike the intrinsic signals induced by light stimuli, a peak of the intrinsic signal was not observed at the fovea, and the threshold of the electrically evoked intrinsic signal was not significantly different for the macula, perimacula, and optic disc. In addition, the threshold did not differ under dark- and light-adapted conditions. The strength of the intrinsic signals increased with longer stimulus durations, and maximum signals were obtained when the stimulus frequency was between 15 and 20 Hz.

There are a number of studies, mainly in vitro experiments using isolated retinas, in which the retinal site activated by electrical stimuli was investigated. Results of most of the studies showed that the site activated—e.g., synaptic terminals of the photoreceptor cells,[29–31] bipolar cells,[32–35] horizontal cells,[36,37] amacrine cells,[38] retinal ganglion cells—was more proximal than the photoreceptors.[14,34,35]

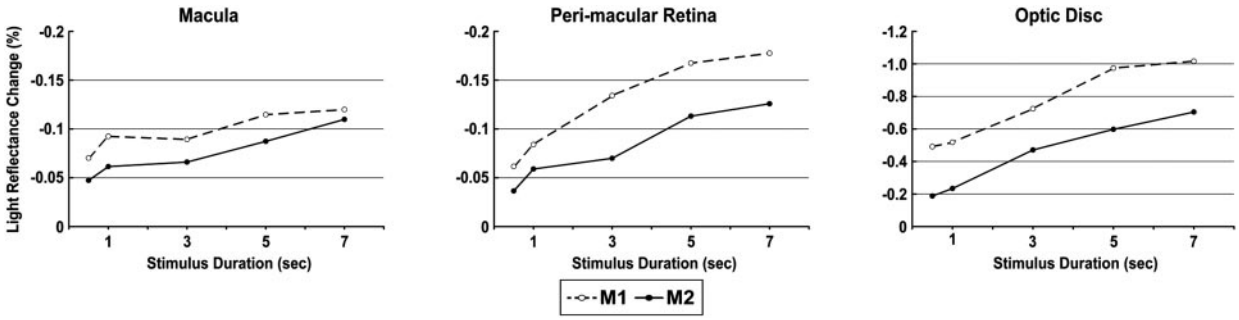

FIGURE 5. Stimulus duration and intrinsic signals. Intrinsic signals of three regions to increasing stimulus durations (total stimulus duration, 0.5 second and 1, 3, 5, and 7 seconds; pulse frequency, 20 Hz; current, 500 μA; pulse duration, 10 msec) in dark-adapted conditions are shown as light reflectance changes for two monkeys (M1 and M2).

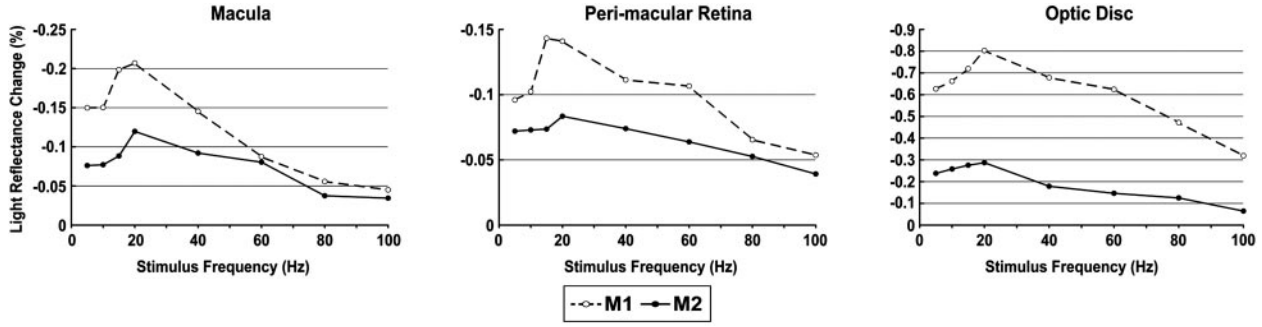

FIGURE 6. Stimulus frequency and intrinsic signals. Intrinsic signals of three regions to increasing stimulus durations (pulse frequency, 5, 10, 15, 20, 40, 60, 80, and 100 Hz; total stimulus duration, 1 second; current, 500 μA) in dark-adapted conditions are shown as light reflectance changes for two monkeys (M1 and M2).

Another method used to identify the site of electrical activation of the retina objectively was the examination of the EER recorded from visual cortex. Thus, Potts et al.[2–4] demonstrated that EER could be recorded in patients with advanced retinitis pigmentosa. They concluded that the site of activation was more central than the photoreceptors.[2–4] Miyake et al.[5–8] showed that the EER is nearly normal in patients with dysfunctional rod or cone visual pathways but that it was extremely abnormal in patients with central artery occlusion. These findings indicate that the retinal origin of EER lies in the middle layer of the retina or close to the retinal ganglion cell layer.[5–8]

The mechanism by which the electrical current is distributed across the retina, however, has not been clearly determined, and the distribution had been estimated mainly by the spatial brightness and extent of phosphenes.[16,39] No study has been reported that estimates the distribution of neural responses over the retina, directly and objectively. In the present study, the current from the DTL electrode enters the eye through the lower anterior part of the sclera and may travel through the vitreous, retina, choroid, or bloodstream to reach the posterior retina. It was not the purpose of this study to investigate the actual pathway of the current. We think a significant amount of the current enters the eye through the sclera and passes through the vitreous body, which also has very low impedance. Brindley[39] designed various types of electrodes that were placed on various locations in the bulbar conjunctiva to investigate the current distribution in the eye by carefully examining the strength and extent of the phosphenes evoked by these electrodes. He concluded that all the electrical phosphenes obtained under the wide range of conditions of his experiments were due to stimulation of the retina by currents flowing perpendicularly to its surface (radial currents through the vitreous humor).[39] Moreover, by observing that the phosphenes were lost as early as 40 seconds from the onset of firm pressure to blind the eye, he concluded that the electrical phosphenes did not result from stimulation of the optic nerve fibers.[39]

When the electrical current is applied from the inferior sclera, one would expect the gradient of stimulation to vary from the inferior retina to the superior retina. Although the current, which spreads radially through the vitreous humor, may not be distributed over the retina in a homogeneous way, the recording region in which quantitative analysis can be reliably conducted is limited to the central 25° in diameter. Thus, we could not measure differences in the signal distribution between the superior and inferior retina outside the vascular arcade.

The retinal intrinsic signals evoked by light stimuli are composed of several components with different properties.[28] Although the precise cellular mechanisms of signal production have not been determined, it is generally believed that the fast signals in the posterior retina (peak time, approximately 150–200 msec) reflect the light-scattering changes after activation of neurons in the outer retina and that the slow signals observed at the posterior retina and the optic disc (peak time, approximately 5–6 seconds) reflect changes in blood flow after neural activation of the cells in the middle or inner layer of the retina. In the later phase, the focally stimulated region showed a focal decrease in light reflectance, with the region corresponding to the location of the stimuli.[28] These findings indicate that the slow components of the intrinsic signals measured in the posterior retina may have a spatial resolution fine enough to indicate the local region of inner retina and can be used for mapping regions made dysfunctional by, for example, glaucoma.

Recently, we showed that the time course of the slow components was strongly correlated with that of blood flow changes measured by laser Doppler flowmetry and that the signals are strongly suppressed by TTX injection into the vitreous cavity, indicating that the slow component of the intrinsic signal are predominantly derived from the stimulus-evoked blood flow increase, which is triggered by the inner retinal activities (Hanazono G, et al. *IOVS* 2007;48:ARVO E-Abstract 528).

In a series of experiments, we have found some discrepancies between the properties of light-evoked and electrically evoked intrinsic signals. First, in the electrically evoked signals, the fast components, which are thought to reflect outer retinal activities, were not observed in the macular and perimacular regions; only slow components were observed (Fig. 2B). Second, the peak of the intrinsic signals in the foveal region evoked by light flashes, which is thought to reflect the activation of foveal cone photoreceptors, could not be observed in the electrically evoked signals, and the response topography in the posterior retina seemed almost homogeneous under dark- and light-adapted conditions (Fig. 3). These findings indicate that the electrical stimuli applied transsclerally do not affect the outer segments of the photoreceptors. We thus believe that the homogenous appearance of the electrically evoked signal may primarily reflect changes induced by the activation of neurons in the inner or middle retinal layers. The most plausible source of the signal is a change in blood flow in the capillaries after activation of the neural cells, although there may be some other cellular mechanisms that can change the light reflectance after electrical stimulation.

When the relationship between the electrical current and the intrinsic signal intensity was examined, we found the response properties seemed to be almost the same under dark- and light-adapted conditions. This is consistent with the previous findings by Miyake,[5] who showed that the amplitude of the EER in humans did not change under dark- and light-adapted conditions. The perceived phosphenes were not altered by the

state of adaptation, and the results of a recent study showed that the threshold of phosphenes is even lower under light-adapted conditions.[16] Taken together with our results, electrical stimulation seems not to be altered by the phototransduction process in the outer segment of photoreceptors.

The relationship between the electrical current and the intrinsic signal intensity was similar in different retinal regions. Under dark- and light-adapted conditions, changes in the intrinsic signal intensity as a function of the electric current were sigmoidal for the three regions studied, and neither the current threshold nor the current giving the maximum intrinsic signal was significantly different. This was, however, not true for the relationship between light intensity and intrinsic signal intensity.[28] In the experiments with light stimuli, the thresholds of intrinsic signals were different, depending on the location of measurement, and the graphs obtained in different regions were completely different. Moreover, there was a shift in threshold to the higher flash intensity to the right after light adaptation. With electrical stimulation, however, the graphs obtained in the three retinal regions were similar and resembled those from the optic disc evoked by light stimulation. This indicates that the electrically evoked intrinsic signals in three regions are related to the blood flow increase after stimulation, though there may be some other mechanisms to induce these signals that are unrelated to blood flow.

With changes in the stimulus frequency of the electrical pulses, the maximal signals were obtained when the current frequency was 20 Hz regardless of the recording region in the ocular fundus. Toi et al.[40] presented an achromatic checkerboard pattern to anesthetized cats and found that the stimulus-related blood flow increase measured by laser Doppler flowmetry was maximum when the stimulus frequency was 20 Hz. The blood flow increase at the optic nerve head after diffuse luminance flicker had physiological properties similar to those of magnocellular retinal ganglion cell neural activities.[41,42] Based on this idea, Riva et al.[43] measured the blood flow increase after 15-Hz flicker stimulus in patients with ocular hypertension and early glaucoma and found that the flicker-evoked blood flow change was abnormally reduced in these patients. These studies suggest a potential in our imaging system to map the dysfunctional regions of the inner retina, such as Bjerrum scotoma in patients with glaucoma. Interestingly, psychophysical studies using flickering stimuli,[44] electrical phosphene,[45] visually evoked potentials,[46] and electrically evoked pupillary reflexes[47] show maximal sensitivities or responses at a frequency of 15 to 20 Hz. The frequency-to-response curves in these studies are similar to those in our study, though the actual sites that regulate this response property are unknown.

In our recording protocol, as the frequency was increased from 5 to 100 Hz, the pulse duration was decreased from 40 to 2 msec, respectively, to keep the total current constant (Fig. 1, inset). There is, however, an in vitro study using isolated salamander retinas, that indicates that the pulse duration is an important factor by which the targeted layer of retina can be determined.[35] The effect of changes in pulse duration in our recording protocol might have influenced the depth of current propagation to some extent.

The resolution of the intrinsic signal topography evoked by electrical stimulation appears to be worse than that evoked by flash stimuli because of the smaller signal amplitudes in the posterior retina. Another factor that might deteriorate the quality of data is the artifacts induced by the electrical current. In a preliminary experiment, we found that currents greater than 1000 μA produce significant artifacts that appear as a mosaic pattern in the posterior pole, possibly because of the muscular contraction of the choroidal arteries by the electrical currents. We found that intrinsic signals could be recorded by transcorneal electrical stimulation by a Burian-Allen contact lens electrode but that the image quality was worse than with transscleral electrical stimulation. This is because the electrical current vibrates the corneal epithelium or the tear film on the cornea, which deteriorates the fundus image observed through the cornea. In our present experimental protocol, we applied the current transsclerally, and it was set lower than 1000 μA to reduce the artifacts.

In conclusion, the results of intrinsic signal imaging indicated that transscleral electrical stimulation is distributed homogeneously over the ocular fundus and represents the activities of neurons mainly in the inner or middle layer of the retina. With further modification of the stimulus protocol and the recording apparatus, it should be possible to record the electrically evoked intrinsic signals in patients. This functional measurement may have potential as a new diagnostic tool for mapping the lesion site of the inner retinal activity, such as Bjerrum scotoma in a patient with glaucoma.

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