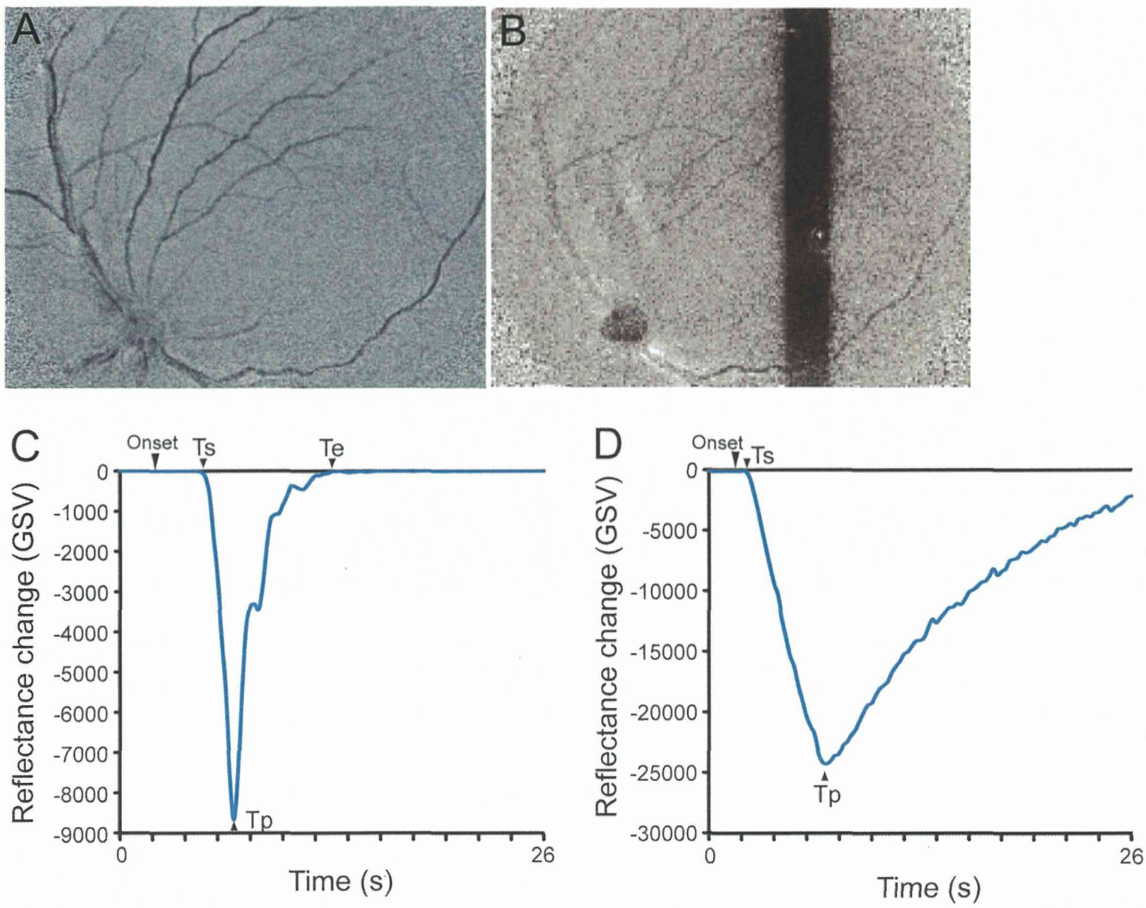

Figure 2. Images of reflectance changes elicited by TES (A) and by photic stimulation (B). The reflectance changes on the OD and over the retinal blood vessels decreased after TES (A), while reflectance changes evoked by light stimulation appeared at the light-stimulated retinal area, the retinal vessels, and OD (B). Plot of the time course of the reflectance changes evoked by TES (C) and light stimulation (D). The latency of the light stimulated retina (D) was shorter than that after TES (C), but the implicit time of the response was same. The time to return to the baseline in the light stimulated retina (D) was significantly later than that after TES (C).
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electrical stimulation system (Stimulator: SEN-7203, Nihon Kohden, Tokyo, Japan; Isolator: BSI-950, Dagan, Minneapolis, MN, USA). To examine the relationship between the stimulus parameters and the intensity of the reflectance changes, the stimulus parameters were changed: current intensities of 0.1,0.5, 1.0, and 2.0 mA of 5 ms/phase at 20 Hz at 20 pulses; pulse durations of 0.5,1.0, 2.0, 3.0, 5.0, and 10.0 ms/phase with constant current intensity at 20 Hz at 20 pulses; frequencies of 5, 10, 20, 30, and 50 Hz at constant current intensity of 5 ms/phase at 20 pulses; and stimulation duration of 0.5, 1, and 4 sec of 5 ms/phase at 50 Hz.

Light Stimulation of Retina

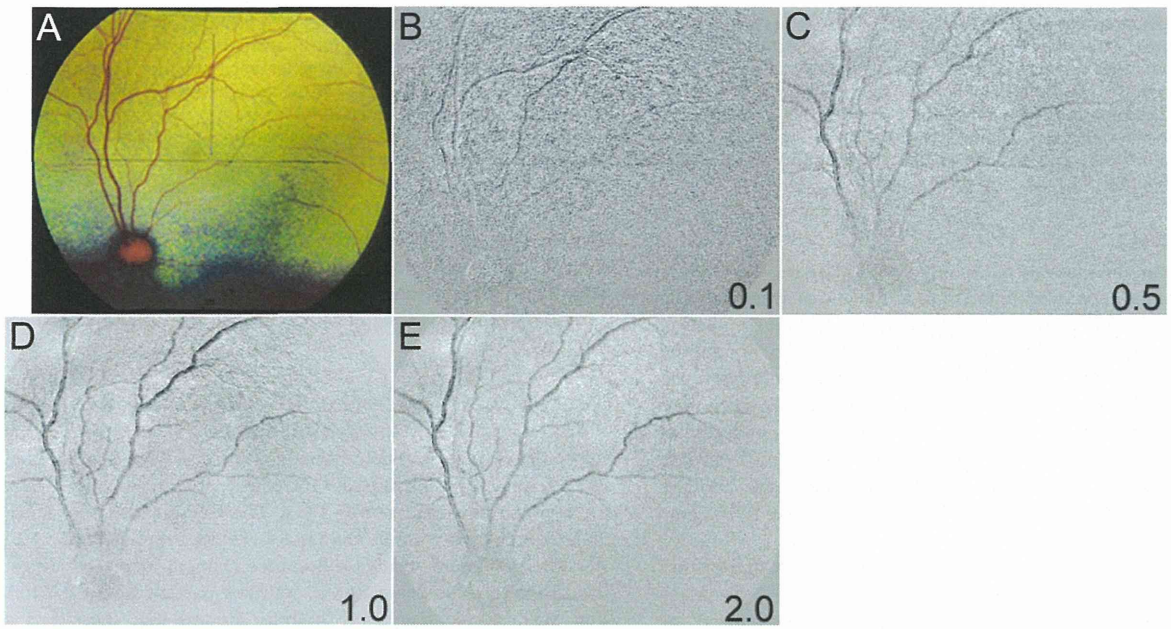
The eye was stimulated with a 4° vertical bar at 8 Hz for 4 s. The bar was presented 6° temporal to the area centralis. The light power was 30 nW.

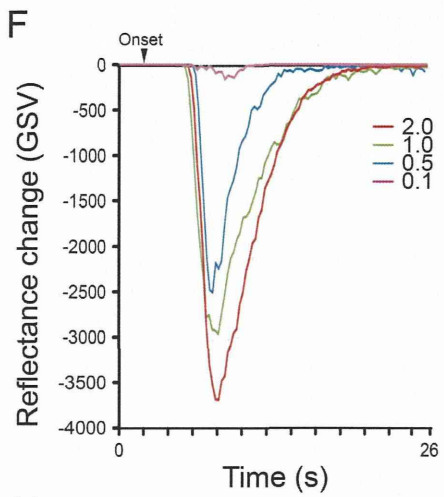
Electrophysiological Recordings from Optic Chiasma

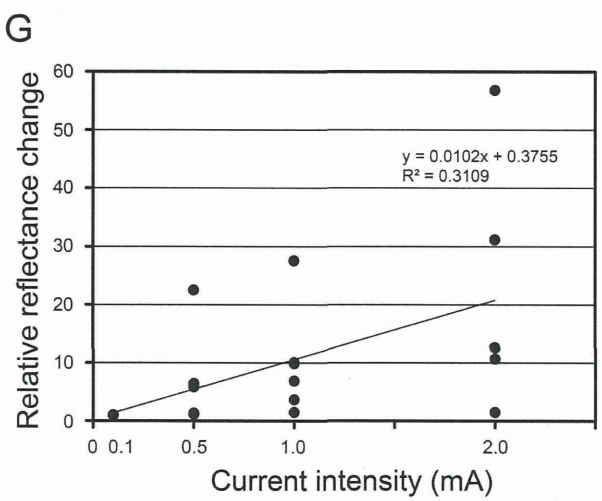
The tips of a pair of stainless steel electrodes was placed in the optic chiasma (OX) stereotaxically to record the electrical potential changes evoked by electrical stimulation of the retina. This was done to record the potential changes in the axons of the retinal ganglion cells elicited by TES. The electrode was inserted from the cortical surface at 13–14 mm anterior to the ear bard and 1–2 mm ipsilateral to the midline. The depth of the electrode tip was 23–26 mm from the cortical surface. Light-evoked responses were recorded from each electrode to be certain that the electrodes were placed in the OX.

To record the electrically evoked potentials (EEPs) by TES, the signal was amplified 10,000 times and bandpass filtered between 300 Hz and 5 kHz with an AC amplifier (Model 1800; Microelectrode AC amplifier; A-M Systems, Inc., Carlsborg, WA) and a signal conditioner (LPF-202A; Warner Instruments, Hamden, CT). Amplified EEPs were fed to a signal processor (Power 1401; Cambridge Electronic Design, Cambridge, UK) and were analyzed with a sampling frequency of 50 kHz offline. Signals were also monitored on an oscilloscope and an audio speaker in real time.

The amplitudes, latencies, and implicit times of the EEPs evoked by TES was measured. The amplitude was measured between the first negative through (N1) to the first positive peak (P1). The P1 latencies were also measured.


A
B
0.1
C
0.5
D
1.0
E
2.0


F
Onset
0
-500
-1000
-1500
-2000
-2500
-3000
-3500
-4000
Reflectance change (GSV)
2.0
1.0
0.5
0.1
0
26
Time (s)


G
60
50
40
30
20
10
0
Relative reflectance change
y = 0.0102x + 0.3755
R² = 0.3109
0 0.1
0.5
1.0
2.0
Current intensity (mA)

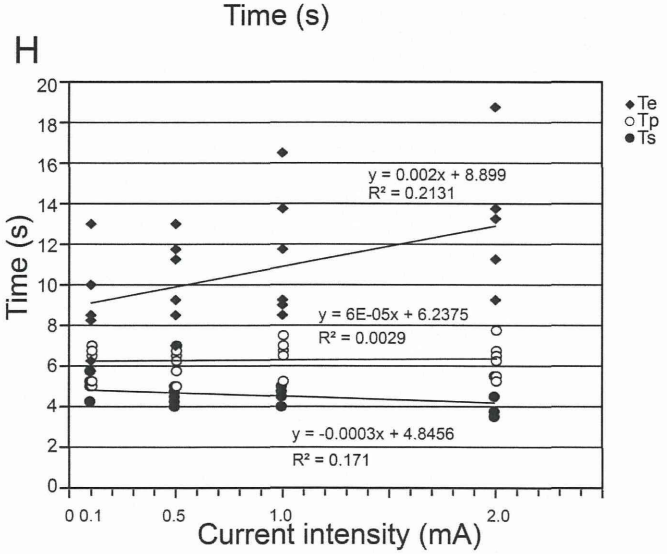

H
20
18
16
14
12
10
8
6
4
2
0
Time (s)
Te
Tp
Ts
y = 0.002x + 8.899
R² = 0.2131
y = 6E-05x + 6.2375
R² = 0.0029
y = -0.0003x + 4.8456
R² = 0.171
0 0.1
0.5
1.0
2.0
Current intensity (mA)

Figure 3. Effect of current intensity of TES on reflectance changes. Fundus photograph (A) and images of reflectance changes elicited by different current intensities (B–E). The GSVs of the reflectance changes (dark signal) decreased as the current intensity increased (F). The relative reflectance changes increase as the current intensities increase (G). The increase in the maximum relative reflectance changes as the current intensities increase was almost linear with stimulus currents up to 2.0 mA (G). There was a significant positive correlation between relative reflectance changes and current intensities ($r^2 = 0.311$, $P = 5 \times 10^{-4}$). A plot of the latency and current intensity is graphed in H. The latency decreases and time to return to baseline increases as the current intensity increases. The changes in the latency is significantly correlated with the current intensities ($r^2 = 0.1333$, $P = 0.045$). The reflectance changes are also significantly correlated with the current intensities ($r^2 = 0.213$, $P = 002$) (H).
doi:10.1371/journal.pone.0092186.g003

Data Analyses

To evaluate the intensity of the reflectance changes, the GSV of each spot of the retina within the fundus image was averaged. The averaged GSV of each spot after the onset of electrical stimulation was subtracted from that before the stimulation to obtain the change in the reflectance of the image. Each recording trial consisted of 300 video frames collected at 30 frames/s for a total recording time of 26 s. The GSVs of 15 video frames collected in 0.5 second were averaged for individual data points to determine the time course of the stimulation-induced reflectance changes. The data were used to plot the time courses of the reflectance changes.

The amplitude was calculated as poststimulus GSV/0.5-second pre-stimulus GSVs pixel by pixel as the relative reflectance changes. The relative reflectance changes were normalized for comparison among the cats, because the magnitude of reflectance changes varied for each cat.

We also analyzed the time course of reflectance changes to determine the latency as the start of the rise of the wave of the reflectance changes, the implicit time as the time to the peak of reflectance changes, the time when the reflectance change returned to the baseline.

Statistical Analyses

Data were analyzed with the JMP (ver. 9.0; SAS Institute Inc., NC) program. The data are expressed as the means ± standard error of the means. Regression analyses between the stimulus parameters and the intensity and latency of the reflectance changes or the amplitude and latency of the EEP at the OX was evaluated by JMP. Statistical significance was set at $P<0.05$.

Results

Characteristics of Retinal Reflectance Changes after TES

Two-dimensional maps of the reflectance changes after TES are shown in Figure 1. The reflectance changes appeared at the optic disc (OD) and retinal blood vessels about 2.0 s after the onset of stimulation (4.0 s after the onset of recording). The intensity of the reflectance changes continued to increase for 7.0 s and then gradually decreased for 12.0 s after the onset of the recordings.

Images of the reflectance changes after TES and light stimulation are compared in Figures 2A and 2B. The time course of the reflectance changes evoked by TES and light stimulation is shown in Figures 2C and 2D. There were some differences of images and time courses in the reflectance changes between TES and light stimulation. The GSV of the reflectance changes evoked by TES began to decrease about 5.0 s after the onset of recording and continued to decrease for 7.0 s (Fig. 2C). The GSV returned to the baseline at about 13.0 s (Fig. 2C).

The reflectance changes evoked by light stimulation appeared at the light-stimulated retinal area, the retinal vessels, and optic disc (OD) (Fig. 2B). The time course of the GSV of the reflectance changes evoked by light stimulation are plotted in Figure 2D. The GSV of the reflectance changes decreased just after the onset of light stimulation, and the latency of the reflectance changes (Ts) after the light stimulation was shorter than that after TES, but the implicit time of the peak of the reflectance changes (Tp) was the same. However, the time to return to the baseline (Te) in the light stimulated retina was significantly longer than that after TES (Figs. 2C, 2D).

Effect of Current Intensity of TES on Reflectance Changes

The effect of the strength of the electric current on the reflectance changes was determined for currents from 0 to 2.0 mA. The stimulus duration was 5.0 ms/phase, frequency was 20 Hz, and stimulation duration was 1.0 s. The GSV of the reflectance changes increased with an increase of electric current (Figs. 3A to 3F). The maximum relative reflectance changes increased almost linearly with an increase in the stimulus current up to 2.0 mA (Fig. 3G). Simple regression analysis showed that there was a significant positive correlation between relative reflectance changes and current intensities ($r^2 = 0.311$; $P = 0.005$; Fig.3G).

Three measurements of the time course of the reflectance changes are plotted in Figure 3H. It can be seen that the latency of the reflectance changes (Ts) and the time to return to the baseline (Te) were depended on the current intensity. There were significant correlations between these latencies and current intensities (Ts, $r^2 = 0.133$, $P = 0.045$; Te, $r^2 = 0.213$; $P = 0.023$; Fig. 3H).

Effect of Pulse Duration on Reflectance Changes

The effect of pulse duration on the reflectance changes was determined for pulse durations of 0.5 to 10.0 ms/phase. These experiments were done with a pulse frequency of 20 Hz; current intensity of 0.1 to 0.5 mA depending on the response; and stimulus duration time of 1.0 s. Our findings showed that the reflectance changes decreased with increasing pulse durations up to 10.0 ms/phase (Figs. 4A to 4G). The increase in the amplitudes of the relative reflectance changes depended on the pulse duration (Fig. 4H). Simple regression analysis showed that there was a significant positive correlation between the relative intensities of the reflectance changes and pulse durations ($r^2 = 0.432$; $P< 0.0001$).

The latency of a response (Ts) and time to the return to the baseline (Te) depended on the pulse duration (Figure 4I). The implicit times did not change significantly with pulse duration. There were significant correlations between the latency and time to return to baseline of the reflectance changes and pulse durations (Ts, $r^2 = 0.171$, $P = 0.009$; Te, $r^2 = 0.246$, $P = 0.001$; Fig. 4I).

Effect of Stimulation Duration on Reflectance Changes

The effect of stimulation duration on the reflectance changes was determined for stimulus durations of 0.4, 1.0, and 4.0 s. For these experiments, the pulse frequency was 50 Hz, current intensity was 0.1 to 0.5 mA depending on the response, and the pulse duration was 5 ms/phase. The relationship between the reflectance changes and the stimulation time showed that the reflectance changes also decreased almost linearly with the stimulation durations up to 4.0 s (Figs. 5A to 5E). The amplitudes of the relative reflectance changes increased and was depended on

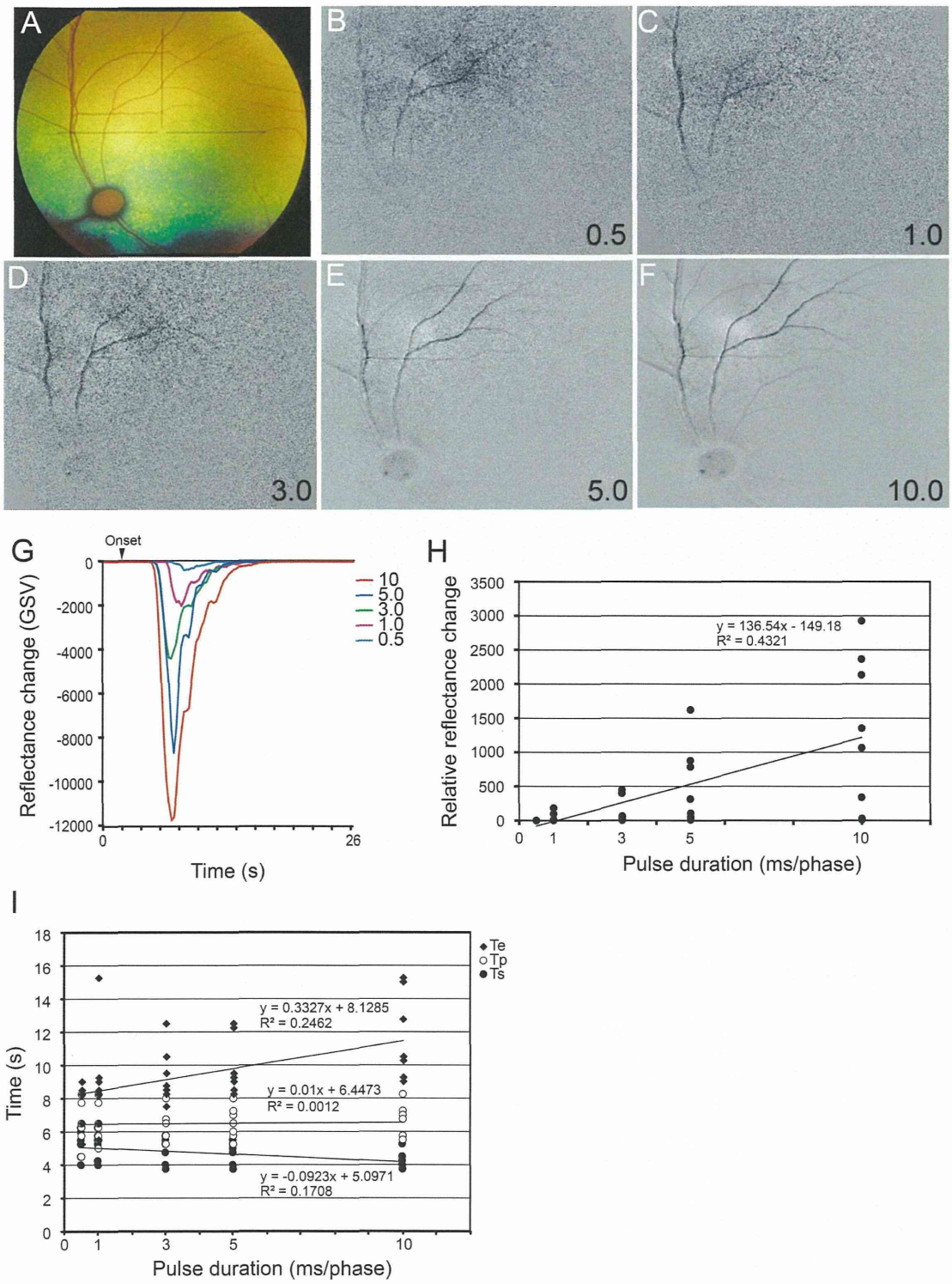


Figure 4. Effect of stimulus pulse duration on reflectance changes. Fundus photograph (A) and images of reflectance changes elicited by different pulse durations (B–F). The GSV of the reflectance changes (dark signal) decreases as the pulse duration increases (G). The relative reflectance changes increases as the pulse durations increase (H). The relative reflectance changes increases almost linearly with an increase of the pulse duration from 0.5 to 10.0 ms/phase (G). There is a significant positive correlation between the relative reflectance changes and pulse durations ($r^2 = 0.432$, $P < 1 \times 10^{-4}$). Plot of the relationship between the latency and pulse duration (H). The latency decreases as the pulse duration increases, but the time to return to the baseline increases as the pulse duration increases. The latency is significantly correlated with the pulse duration ($r^2 = 0.171$, $P = 0.009$), and the time to return to baseline was significantly correlated with the pulse durations ($r^2 = 0.246$, $P = 0.001$) (I).
doi:10.1371/journal.pone.0092186.g004

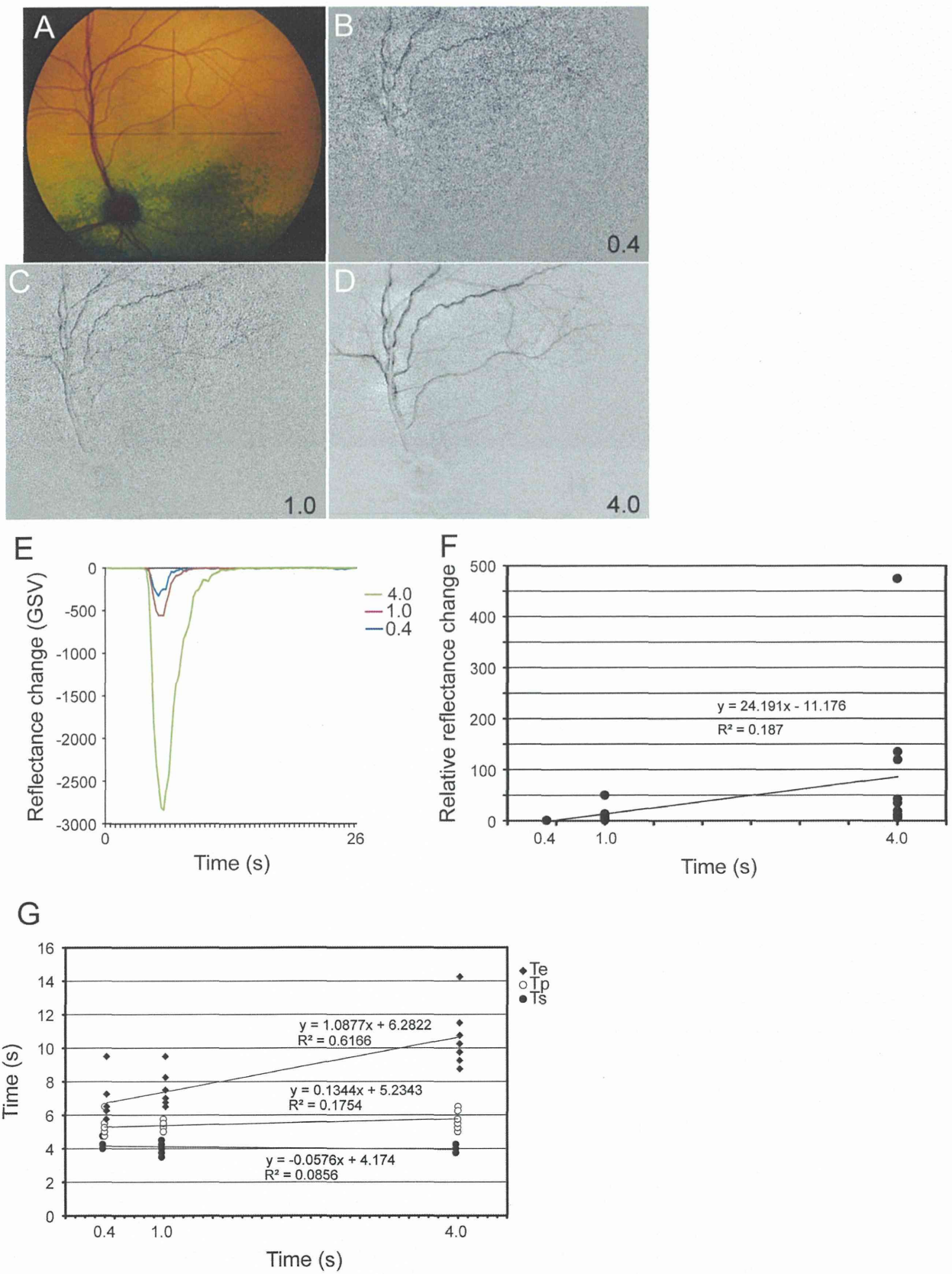

A
B
0.4
C
1.0
D
4.0
E
Reflectance change (GSV)
0
-500
-1000
-1500
-2000
-2500
-3000
0
26
Time (s)
4.0
1.0
0.4
F
Relative reflectance change
500
400
300
200
100
0
y = 24.191x - 11.176
R² = 0.187
0.4
1.0
4.0
Time (s)
G
Time (s)
16
14
12
10
8
6
4
2
0
y = 1.0877x + 6.2822
R² = 0.6166
y = 0.1344x + 5.2343
R² = 0.1754
y = -0.0576x + 4.174
R² = 0.0856
0.4
1.0
4.0
Time (s)
Te
Tp
Ts

Figure 5. Effect of stimulation duration on reflectance changes. Fundus photograph (A) and images of the reflectance changes elicited by different stimulation durations (B–D). The GSV of reflectance changes (dark signal) decreases as the stimulation duration increases (E). Relative reflectance changes increases as the stimulation duration increases (F). There was an almost linear increase in the maximum relative reflectance changes and the stimulation duration from 0.4 to 4.0 s (F). There was a significant positive correlation between relative intensities of reflectance changes and the stimulation duration ($r^2 = 0.187$, $P = 0.017$). The relationship between latency and the stimulation time (G). The time to return to baseline increases as the stimulation duration increases. There was a significant correlation between the time to return to baseline and the stimulation duration ($r^2 = 0.086$, $P < 1 \times 10^{-4}$) (G).
doi:10.1371/journal.pone.0092186.g005

the stimulation duration (Fig. 5F). Simple regression analysis showed that there was a significant positive correlation between the relative intensities of the reflectance changes and stimulation duration ($r^2 = 0.187$, $P<0.017$). The time course of the reflectance changes are plotted in Figure 5G. The latency (Ts) and time to baseline (Te) increased as the stimulation duration increased. There was a significant positive correlation between the times (Ts, Te) and the stimulation duration (Ts, Te: $r^2 = 0.086$, $P<0.0001$).

Effect of Stimulus Frequency on Reflectance Changes

The effect of the stimulus frequency ranging from 5 to 50 Hz on the reflectance changes was determined with a current intensity of 0.1 to 0.5 mA depending on the response, pulse duration of 5 ms/phase, and pulse number of 20. The relationship between reflectance changes and the stimulus frequency showed that the GSV of the reflectance changes was also dependent on the stimulus frequency (Figs. 6A to 6G). The amplitudes of relative reflectance changes also changed depending on the frequency (Fig. 6H). Non-linear polynomial regression analysis showed that these data were best fitted by a non-linear equation (quadratic term; $P = 0.001$; Fig. 6H). The latencies of Ts, Tp, and Te are plotted in Figure 6I. There was no significant correlation between all of these measures and the stimulus frequencies.

Electrophysiological Recordings from Optic Chiasm after TES

We examined the relationship between the EEP amplitude recorded in the OX and the intensity of the stimulus currents. The EEP amplitude increased linearly with an increase of stimulus current (Figs. 7A, 7B), and linear regression analysis showed that there was a significant positive correlation between amplitudes of EEPs and the current intensities ($r^2 = 0.289$; $P = 0.001$). However, there was no significant correlation between the P1 latencies and the current intensities (Fig. 7C).

These results indicate that TES with the parameters used can activate retinal ganglion cells (RGCs) directly or indirectly and the degree of activation was significantly correlated with the intensity of electrical current. In addition, the EEP amplitudes were significantly correlated with the GSV of the reflectance changes of the retina ($P = 0.0011$; $r^2 = 0.4962$; Fig. 7D). This suggests that the reflectance changes represented the neuronal changes in the retina, i.e., the excitation of the RGCs.

Discussion

Our results showed that there were specific reflectance changes of the retina in response to the TES. The GSVs of the reflectance changes were significantly increased with higher current intensities, longer pulse durations, and longer stimulation durations. These findings indicate that the increase in the GSVs of the reflectance changes was due to the increase of the electrical flux. In addition, there was a frequency specificity with the maximum signals obtained with a stimulus frequency of 20 Hz, if the electrical flux was same at each frequency. Our findings are similar to those obtained from monkey eyes [24,28].

The relationship between the time course and stimulus parameters on the RCs has not been determined. We have determined the relationship between the time course and stimulus parameters on the RCs. The time course of the responses was also altered by the parameters of the TES. The latency of response (Ts) was shorter and the time to return to the baseline (Te) was longer with higher current intensities and longer pulse durations, but the time of the peak of the response (Tp) was not changed (Figs. 3H, 4I). When the current intensity and the pulse duration were the same, only the time to return to baseline (Te) was longer with longer stimulation durations (Fig. 5G).

An increase in the electrical flux by stronger currents, longer pulse durations, and longer stimulation durations should increase the number of retinal neurons activated by TES. Therefore, the latency of the reflectance changes are shorter and the return to the baseline longer. Thus, the time course of the response might be related to the electrical flux. On the other hand, the peak implicit time of the response (Tp) was not changed by the different parameters of the TES. This indicates that the reflectance changes occurred in response to a certain process, such as a neurological change to a vascular change.

What do Intrinsic Reflectance Changes Represent?

The reflectance changes evoked by TES appeared over the retinal vessels and optic disc, and these were different from those elicited by photic stimulation (Fig. 2s). Mihashi et al reported that the inhibition of the action potentials of retinal ganglion cells (RGCs) by tetrodotoxin (TTX) abolished the reflectance changes elicited by TES or by OX stimulation, but TTX did not induce significant changes in the retinal intrinsic signals elicited by photic stimulation [31]. These results suggest that the reflectance changes after either TES or OX stimulation were caused by the activation of the RGCs.

Although there was a significant correlation between the activity of RGCs and the reflectance changes, this does not directly indicate that they are due to the activation of the RGCs, because the latency of the response was much longer than that of action potentials of RGCs. The RCs represent the responses associated with hemodynamic changes in either the blood volume and flow or the oxygenated state of hemoglobin that is caused by the activity of the RGCs [32–35].

The existence of a fundamental relationship between neural activity, blood flow, and metabolism, called a neurovascular coupling, was suggested by Roy and Sherrington [32]. A neurovascular coupling in the optic nerve and retina was postulated by Riva et al [33]. The blood flow and local partial pressure of oxygen in the optic nerve have been shown to be modulated by intermittent light stimuli, and both were coupled to the activity of RGCs [34,35]. These findings indicate that the blood flow and metabolism in the retina and optic nerve can be modulated by local neural activity. Moreover TES has been shown to increase the chorioretinal blood flow in normal subjects with minimal effects on the systemic blood circulation and the intraocular pressure [36]. The increased retinal blood flow elicited by photic stimulation measured by laser Doppler flowmetry is

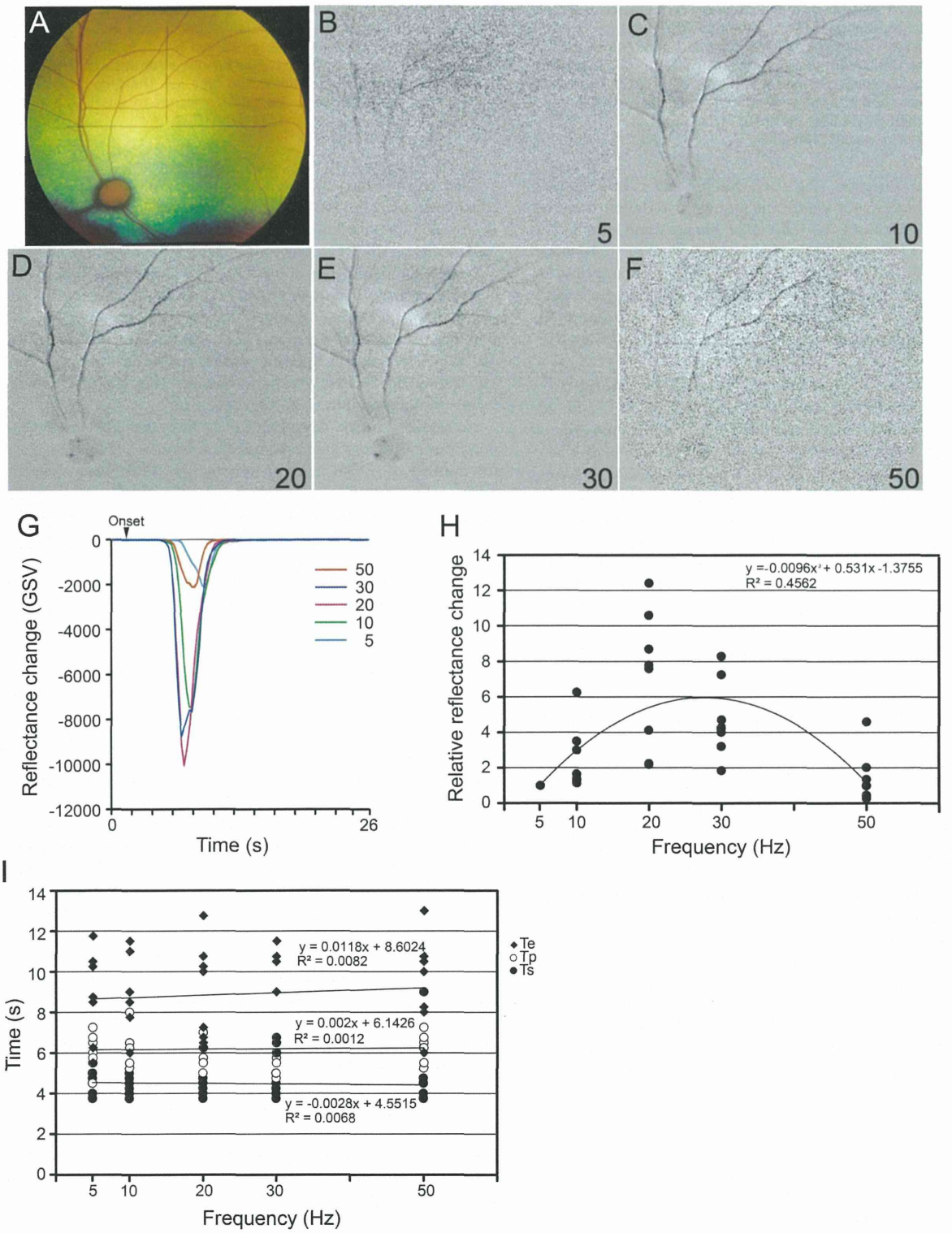


Figure 6. Effect of stimulus frequency on reflectance changes. Fundus photograph (A) and images of reflectance changes elicited by different stimulus frequencies (B–F). The GSV of the reflectance changes (dark signal) depended on the frequency (G), with the relative reflectance changes depended on the stimulus frequency (H). The maximum relative reflectance changes was best fit to the stimulus frequency by a non-linear curve (quadratic term; $P = 0.001$). The relationship between the latency and the frequencies (I). There was no significant correlation between latencies and stimulus frequencies.

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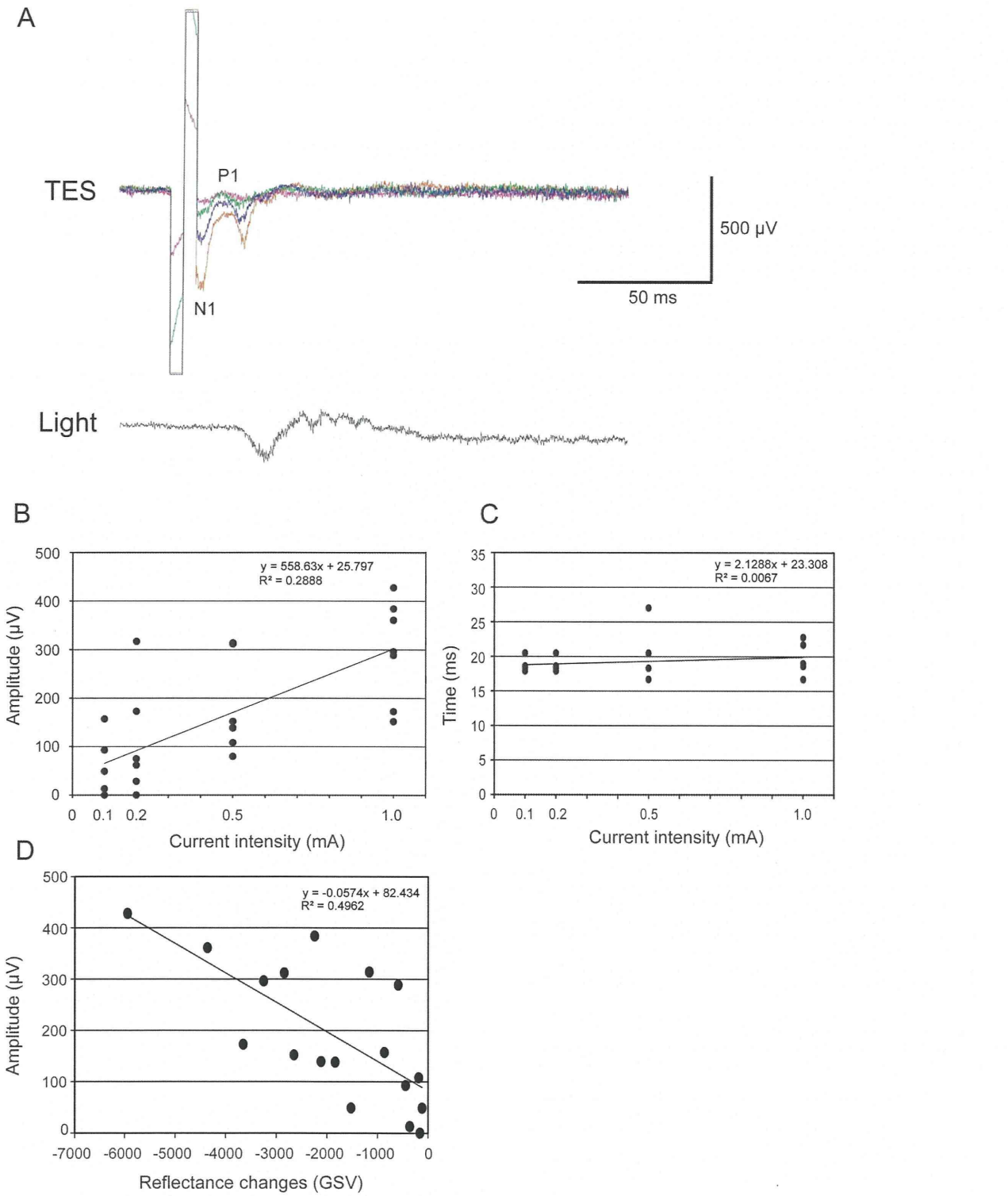

Figure 7. Electrophysiological recordings from the optic chiasm (OX) after TES. The electrically evoked potentials (EEPs, upper) and the light evoked potentials (VEPs, lower) are shown. Graph showing the relationship between the EEP amplitude and electric current (B) and the relationship between the peak latency (P1) and electric current (C). B. There was a significant positive correlation between amplitudes of EEPs and current intensities ($P = 0.0007$, $r^2 = 0.2888$). C. The relationship between the peak latency (P1) and electric current. There was no significant correlation between latencies and current intensity. D. EEP amplitude was correlated with the GSV of reflectance change in the retina. There was a significant positive correlation between intensities of reflectance changes and EEP amplitudes ($r^2 = 0.496$, $P = 0.001$).
doi:10.1371/journal.pone.0092186.g007

similar to that of the reflectance changes induced by the same stimuli [28]. Thus, the reflectance changes investigated in this study represented the hemodynamic responses of the retina and optic nerve to the increased retinal neural activity, which are secondary to the activation of the neural activity of the retina. We suggest that the RCs represent the response of neurovascular coupling and TES might stimulate the neurovascular coupling in the retina and optic nerve.

In conclusion, the intensities of the reflectance changes were dependent on the stimulus parameters of TES. The reflectance changes represent changes of the retinal vessels and optic disc, and these results indicate that TES influenced neurovascular coupling, i.e., RGCs and retinal hemodynamics. More experiments are necessary to determine the retinal neurovascular coupling, i.e., the relationship between the activated RGCs and the retinal hemodynamics. However, we conclude that this imaging technique might be a method to investigate the neurovascular coupling.

Author Contributions

Conceived and designed the experiments: T. Morimoto TF. Performed the experiments: T. Morimoto HK T. Miyoshi YH T. Mihashi YK TF. Analyzed the data: T. Morimoto HK YH T. Mihashi. Contributed reagents/materials/analysis tools: T. Morimoto HK T. Miyoshi YH T. Mihashi YK TF. Wrote the paper: T. Morimoto KN TF.

References

1. Potts AM, Inoue J (1968) The electrically evoked response of the visual system (EER). Invest Ophthalmol 7: 269–278.
2. Potts AM, Inoue J (1969) The electrically evoked response of the visual system (EER) II. Effect of adaptation and retinitis pigmentosa. Invest Ophthalmol 8: 605–613.
3. Miyake Y, Yanagida K, Yagasaki K (1980) Clinical application of EER (electrically evoked response). (1) Analysis of EER in normal subjects. Nippon Ganka Gakkai Zasshi 84: 354–360.
4. Fujikado T, Morimoto T, Matsushita K, Shimojo H, Okawa Y, et al. (2006) Effect of transcorneal electrical stimulation in patients with nonarteritic ischemic optic neuropathy or traumatic optic neuropathy. Jpn J Ophthalmol 50 : 266–273.
5. Inomata K, Shinoda K, Ohde H, Tsunoda K, Hanazono G, et al. (2007) Transcorneal electrical stimulation of retina to treat longstanding retinal artery occlusion. Graefes Arch Clin Exp Ophthalmol 245: 1773–1780.
6. Gall C, Fedorov AB, Ernst L, Borrmann A, Sabel BA (2010) Repetitive transorbital alternating current stimulation in optic neuropathy. NeuroRehabilitation 27: 335–341.
7. Schatz A, Röck T, Naycheva L, Willmann G, Wilhelm B, et al. (2011) Transcorneal electrical stimulation for patients with retinitis pigmentosa: a prospective, randomized, sham-controlled exploratory study. Invest Ophthalmol Vis Sci 23: 4485–4496.
8. Delbeke J, Pins D, Michaux G, Wanet-Defalque MC, Parrini S, et al. (2001) Electrical stimulation of anterior visual pathways in retinitis pigmentosa. Invest Ophthalmol Vis Sci 42: 291–297.
9. Morimoto T, Fukui T, Matsushita K, Okawa Y, Shimojyo H, et al. (2006) Evaluation of residual retinal function by pupillary constrictions and phosphenes using transcorneal electrical stimulation in patients with retinal degeneration. Graefes Arch Clin Exp Ophthalmol 244: 1283–1292.
10. Fujikado T, Morimoto T, Kanda H, Kusaka S, Nakauchi K, et al. (2007) Evaluation of phosphenes elicited by extraocular stimulation in normals and by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa. Graefes Arch Clin Exp Ophthalmol 245: 1411–1419.
11. Naycheva L, Schatz A, Röck T, Willmann G, Messias A, et al. (2012) Phosphene thresholds elicited by transcorneal electrical stimulation in healthy subjects and patients with retinal diseases. Invest Ophthalmol Vis Sci 53: 7440–7448.
12. Shimazu K, Miyake Y, Watanabe S (1999) Retinal ganglion cell response properties in the transcorneal electrically evoked response of the visual system. Vision Res 39: 2251–2260.
13. Shah HA, Montezuma SR, Rizzo JF 3rd (2006) In vivo electrical stimulation of rabbit retina: effect of stimulus duration and electrical field orientation. Exp Eye Res 83: 247–254.
14. Knighton RW (1975) An electrically evoked slow potential of the frog's retina. I. Properties of response. J Neurophysiol.; 38(1): 185–97.
15. Grinvald A, Lieke E, Frostig RD, Gilbert CD, Wiesel TN (1986) Functional architecture of cortex revealed by optical imaging of intrinsic signals. Nature 324: 361–364.
16. Frostig RD, Lieke EE, Ts'o DY, Grinvald A (1990) Cortical functional architecture and local coupling between neuronal activity and the microcirculation revealed by in vivo high-resolution optical imaging of intrinsic signals. Proc Natl Acad Sci USA 87: 6082–6086.
17. Ts'o DY, Frostig RD, Lieke EE, Grinvald A (1990) Functional organization of primate visual cortex revealed by high resolution optical imaging. Science 249: 417–420.
18. Fukuda M, Rajagopalan UM, Homma R, Matsumoto M, Nishizaki M, et al. (2005) Localization of activity-dependent changes in blood volume to submillimeter-scale functional domains in cat visual cortex. Cerebral Cortex 15: 823–833.
19. Cohen LB (1973) Changes in neuron structure during action potential propagation and synaptic transmission. Physiol Rev 53: 373–418.
20. Tsunoda K, Oguchi Y, Hanazono G, Tanifuji M (2004) Mapping cone- and rod- induced retinal responsiveness in macaque retina by optical imaging. Invest Ophthalmol Vis Sci 45: 3820–3826.
21. Abràmoff MD, Kwon YH, Ts'o D, Soliz P, Zimmerman B, et al. (2006) Visual stimulus-induced changes in human near-infrared fundus reflectance. Invest Ophthalmol Vis Sci 47: 715–721.
22. Okawa Y, Fujikado T, Miyoshi T, Sawai H, Kusaka S, et al. (2007) Optical imaging to evaluate retinal activation by electrical currents using suprachoroidal–transretinal stimulation. Invest Ophthalmol Vis Sci 48: 4777–4784.
23. Hanazono G, Tsunoda K, Shinoda K, Tsubota K, Miyake Y, et al. (2007) Intrinsic signal imaging in macaque retina reveals different types of flash-induced light reflectance changes of different origins. Invest Ophthalmol Vis Sci 48: 2903–2912.
24. Inomata K, Tsunoda K, Hanazono G, Kazato Y, Shinoda K, et al. (2008) Distribution of retinal responses evoked by transscleral electrical stimulation detected by intrinsic signal imaging in macaque monkeys. Invest Ophthalmol Vis Sci 49: 2193–2200.
25. Hanazono G, Tsunoda K, Kazato Y, Tsubota K, Tanifuji M (2008) Evaluating neural activity of retinal ganglion cells by flash-evoked intrinsic signal imaging in macaque retina. Invest Ophthalmol Vis Sci 49: 4655–4663.
26. Schallek J, Li H, Kardon R, Kwon Y, Abramoff M, et al. (2009) Stimulus-evoked intrinsic optical signals in the retina: spatial and temporal characteristics. Invest Ophthalmol Vis Sci 50: 4865–4872.
27. Schallek J, Kardon R, Kwon Y, Abramoff M, Soliz P, et al. (2009) Stimulus-evoked intrinsic optical signals in the retina: pharmacologic dissection reveals outer retinal origins. Invest Ophthalmol Vis Sci 50: 4873–4880.
28. Tsunoda K, Hanazono G, Inomata K, Kazato Y, Suzuki W, et al. (2009) Origins of retinal intrinsic signals: a series of experiments on retinas of macaque monkeys. Jpn J Ophthalmol 53: 297–314.
29. Ts'o D, Schallek J, Kwon Y, Kardon R, Abramoff M, et al. (2009) Noninvasive functional imaging of the retina reveals outer retinal and hemodynamic intrinsic optical signal origins. Jpn J Ophthalmol 53: 334–344.
30. Schallek J, Ts'o D (2011) Blood contrast agents enhance intrinsic signals in the retina: evidence for an underlying blood volume component. Invest Ophthalmol Vis Sci 52: 1325–1335.
31. Mihashi T, Okawa Y, Miyoshi T, Kitaguchi Y, Hirohara Y, et al. (2011) Comparing retinal reflectance changes elicited by transcorneal electrical retinal stimulation with those of optic chiasma stimulation in cats. Jpn J Ophthalmol 55: 49–56.
32. Roy CS, Sherrington CS (1890) On the regulation of the blood supply of the brain. J. Physiol 11: 85–108.
33. Riva CE, Logean E, Falsini B (2005) Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. Prog Retin Eye Res 24: 183–215.
34. Riva CE, Harino S, Shonat RD, Petrig BL (1991) Flicker evoked increase in optic nerve head blood flow in anesthetized cats. Neurosci Lett 128: 291–296.
35. Riva CE, Cranstoun SD, Petrig BL (1996) Effect of decreased ocular perfusion pressure on blood flow and the flicker-induced flow response in the cat optic nerve head. Microvasc Res 52: 258–269.
36. Kurimoto T, Oono S, Oku H, Tagami Y, Kashimoto R, et al. (2010) Transcorneal electrical stimulation increases chorioretinal blood flow in normal human subjects. Clin Ophthalmol 4: 1441–1446.

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LABORATORY INVESTIGATION

Evaluation of electrochemically treated bulk electrodes for a retinal prosthesis by examination of retinal intrinsic signals in cats

Hiroyuki Kanda · Toshifumi Mihashi · Tomomitsu Miyoshi · Yoko Hirohara · Takeshi Morimoto · Yasuo Terasawa · Takashi Fujikado

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Abstract

Purpose Our goal was to determine the feasibility of using electrochemically treated bulk platinum electrodes with large charge injection capacity for a retinal prosthesis.

Methods Seven eyes of seven cats were studied. Small retinal areas were focally stimulated with electrochemically treated bulk electrodes ($\varphi = 500$ μm) placed in a scleral pocket. Fundus images with near-infrared (800–880 nm) light were recorded, and a 2D map of the reflectance changes elicited by the electrical currents was constructed by subtracting the images taken before stimulation from those taken after stimulation. The impedance of each electrode was measured at 1 kHz. The degree of retinal elevation by the electrode was measured by optical coherence tomography. Scleral thickness where the electrode array was inserted was measured in histologic sections.

Results The diameter of reflectance changes (full width at half maximum) was 0.42 ± 0.22 mm [mean ± standard deviation (SD)] in minor axes and 1.46 ± 0.82 mm in major axes. The threshold current decreased with a reduction in the residual scleral thickness ($R^2 = 0.9215$; $P = 0.0002$); it also decreased with an increase in retinal elevation ($R^2 = 0.6259$; $P = 0.0111$).The threshold current also decreased with an increase in electrode impedance ($R^2 = 0.2554$; $P = 0.0147$).

Conclusions Electrochemically treated porous platinum electrodes can stimulate localized retinal areas. The threshold current necessary to stimulate the retina was influenced by residual scleral thickness and the electrode tightness of fit against the sclera.

Keywords Optical imaging · Intrinsic signal · Porous electrodes · Electrical stimulation · Retinal prosthesis

H. Kanda · T. Morimoto · T. Fujikado (✉)
Department of Applied Visual Science, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: fujikado@ophthal.med.osaka-u.ac.jp

T. Mihashi
Department of Innovative Research Initiatives, Tokyo Institute of Technology, Yokohama, Japan

T. Miyoshi
Department of Integrative Physiology, Osaka University Graduate School of Medicine, Osaka, Japan

Y. Hirohara
Optical Engineering Laboratory, Topcon Corporation, Tokyo, Japan

Y. Terasawa
Vision Institute, NIDEK Company, Gamagori, Japan

Introduction

Retinitis pigmentosa (RP) is one of the leading causes of blindness and is characterized by degeneration of photoreceptors. At the advanced stage, RP patients have little or no functional vision, and no effective treatment exists for this disease. To restore some vision to these patients, the strategy of stimulating residual retinal neurons using a retinal prosthesis has been extensively studied [1–10]. A retinal prosthesis is a medical device that has the potential of restoring vision by stimulating the retina with electrical pulses. Retinal prostheses are classified into three types: subretinal [1–3], epiretinal [4, 5], and suprachoroidal–transretinal stimulation (STS) [6–11]. In the epiretinal and subretinal types, the implanted stimulating electrode is attached directly to the retina, so the