Time-Varying Pulse Trains Limit Retinal Desensitization Caused by Continuous Electrical Stimulation

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***Abstract*— An epiretinal prosthesis aims to restore functional vision in patients suffering from retinal degeneration caused by diseases such as Retinitis Pigmentosa (RP) and Age-Related Macular Degeneration (AMD). These diseases result in the loss of photoreceptors but bipolar, amacrine and ganglion cells survive at high rates and can be electrically activate to produce the sensation of light. Continuous application of biphasic stimulus pulses results in desensitization of the retina. In humans, this manifests as decreased brightness and increased stimulus thresholds. This study presents an in vivo model of retinal desensitization caused by continuous electrical stimulation and describes a novel stimulation pattern that limit desensitization.**

I. Introduction

Retinitis Pigmentosa (RP) and Age-Related Macular Degeneration (AMD) are the two common causes of blindness in U.S. and are presently incurable. These diseases cause blindness through the progressive loss of photoreceptors. Morphometric analysis of post-mortem eyes from patients with RP and AMD has revealed that even though photoreceptors are lost, the remaining cells of the retina, including bipolar, ganglion and amacrine cells, survive. Thus the goal of the epiretinal prosthesis is to stimulate the surviving ganglion cells and restore some functional vision.

Psychophysics experiments with epiretinal prosthesis subjects have shown that the brightness of electrically evoked visual percepts tend to decrease over a period of time with continuous electrical stimulation [1]. In order to improve clinical outcome for retinal prosthesis subjects', it would be necessary to precisely control the temporal and spatial pattern of ganglion cell activation. Control of spiking can be accomplished either by direct activation of the ganglion cell, or through activation of neurons presynaptic to the ganglion cells. Direct activation of the ganglion cell has the ability to elicit spike trains at very high rates [2]. However, direct activation is also likely to cause incidental activation of passing axons on the inner retinal surface. This will expand the spatial region of ganglion cell activation, and may smear the elicited percept. Alternatively, the activation of presynaptic neurons is advantageous in that it provides better spatial control over neural activation by avoiding ganglion cell axons. Unfortunately, activation through the synaptic network limits the ability to control the temporal pattern of ganglion cell spiking. For example, in response to repetitive stimulation, ganglion cells respond robustly to the first pulse, but the response decreases for subsequent pulses [3].

This reduction in ganglion cell excitability to repetitive stimulation is termed desensitization. Ray et al have shown a similar decrease in neural excitability using an in vivo model of retinal prosthesis [4]. One reason for the desensitization could be increased inhibition from amacrine cells. Fried et al have shown that in response to electrical stimulation with long pulses, excitatory and inhibitory currents could be measured in ganglion cells [2]. These currents indicate that pulses stimulate both bipolar cells and amacrine cells. They have also shown that prolonged stimulation reduces the excitatory current from bipolar cells proving that inhibition increases from amacrine cells. This inhibition lasted over 100ms.

The objective of this study is to present a new stimulation strategy to limit desensitization. Time-varying pulse trains are defined as pulses where each pulse has a different pulse duration and amplitude when compared to the preceding pulse, while keeping the charge at threshold level for that pulse width. Different pulse widths are known to stimulate different populations of neurons [5,6]. Microsaccades continuously shift the image on the retina and help prevent mechanisms of local adaptation and image fading. By stimulating different population of neurons, the "electrical image" on the retina might shift constantly and help limit image fading. Hence, time-varying pulse trains could help limit desensitization.

II. Methods

A. Animal Preparation and Surgery

Normal Long Evans [postnatal day P90-P120, n = 8] rats were used in the study. Animals were housed in covered cages and fed a standard rodent diet ad libitum while kept on a 12:12-hour light-dark cycle animal facility. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Southern California. All surgeries were performed under general anesthesia induced by intraperitoneal/intramuscular injection of a cocktail of ketamine (100 mg/kg; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (100mg/kg; X-Ject SA, Butler, Dublin, OH) and maintained by sevoflurane (1% in 100% O_2) throughout the entire experiment. Sevoflurane was administered through a mask. The animal's pulse and oxygen saturation were monitored during the surgical procedures. The body temperature was maintained at 37°C with a self-regulated heating blanket (model 50-7053-F; Harvard Apparatus, Holliston, MA). Animals were euthanized after the experiment.

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B. SC Exposure and Recording Electrode Positioning

Electrically evoked responses (EERs) were recorded from the superior colliculus (SC) in rats. In rats, axons of 90% of the RGCs synapse onto the superficial layers of the SC, thus recording from the SC provides a convenient measure of retinal output [7,8]. In order to access the SC, the skull was exposed and a craniotomy was performed on the right side ((caudal-medial corner: ~4 mm caudal and ~3 mm lateral to lambda) using a hand-held drill. The overlying cortex was aspirated approximately 4mm deep from the dura mater until the SC surface was exposed. Epoxy-coated tungsten microelectrodes (10 MOhms, FHC) were positioned within the superficial layers of the SC at a depth of 300-350 µm from the SC surface. This recording technique was described in detail in previous work [9].

C. Stimulation Electrode

The stimulation electrode was a concentric bipolar Pt-Ir electrode (model CBDFG74, FHC, Bowdoin, ME) with a flat tip. The diameter of the inner pole was 75 µm and that of the outer pole was 300 µm. The electrode was used in a monopolar configuration: the inner pole was used for stimulation and a large surface area platinum needle inserted in the skin adjacent to the nose was used as the return electrode. The stimulating electrode was mounted in a 1-ml syringe for handling and attached to a single-axis linear translational micromanipulator (model NT33-475, Edmund Optics, Barrington, NJ) on a magnetic based articulating arm.

D. Stimulation Electrode Insertion

The surgical procedure to insert a stimulation electrode into the rat eye was reported in previous work [10,11]. The left eye was dilated with a few drops each of 1% tropicamide (Tropicacyl, Akorn, Buffalo Grove, IL) and 2.5% phenylephrine (AK-Dilate, Akorn). The dilated eye was proptosed using a small piece of a surgical glove. Slightly flattening the cornea using a glass coverslip covered with gel (Goniosol, Gonak) allows focused viewing of the fundus through an operating microscope. A scleral incision was made using a 25-guage needle near the limbus. The needle was inserted at a 45 ◦ angle with respect to the scleral surface in order to avoid damaging the lens. The stimulation electrode was inserted through the incision site along the path made by the needle. The electrode was positioned in the ventral temporal quadrant without contacting the retina. Final positioning of the stimulation electrode to ensure close proximity utilizes impedance feedback described Chan et al [9].

E. Electrical Stimulation

Figure 1 illustrates the experimental protocol. Continuous stimulation consisted of two types of pulses: test stimuli and probe pulses. Test stimuli were a train of pulses (60uA, 0.5ms) delivered at 20Hz for 1sec. Test stimuli were preceded by a single probe pulse (0.5ms, 60uA). During the first part of the experiment (figure 1A), probe pulses interleaved with test stimuli examined the effect of continuous stimulation on EERs recorded in the SC. The probe pulse and test stimuli combination was delivered for 2 min and this part of the experiment was referred to as "stimulation phase". In the second part of the experiment after the probe pulse and test stimuli combination ended (figure 1B), only probe pulses were delivered for 3 min to monitor the recovery of the EERs. The second part of the experiment is referred to as "recovery phase". Probe pulses were delivered at 0.2Hz after the continuous stimulation ended.

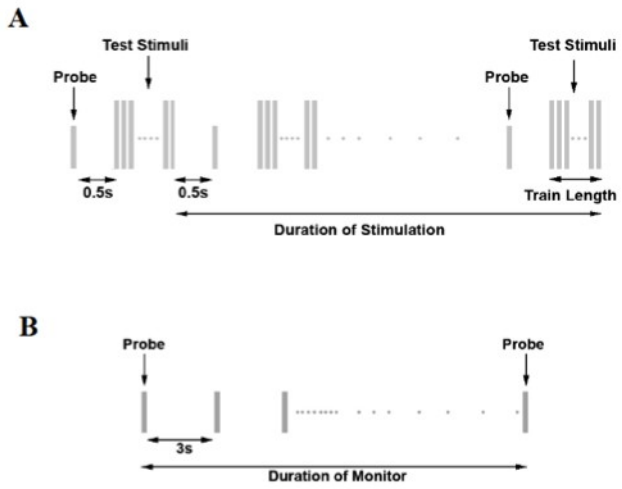

Figure 1. Stimulation protocol used in control and experimental groups.

Two types of experiments were performed: "control experiments" in which the test stimuli consisted of stimulus pulses that did not vary in time (all the stimulus pulses had the same amplitude and pulse duration i.e. 60uA and 0.5ms) and "time-varying" pulse train experiments in which the test stimuli consisted of pulse train where each consecutive pulse had different amplitude and pulse duration when compared to the preceding pulse. The pulse duration of the time-varying pulses was randomly chosen from a set of pulse widths between 0.1ms and 20ms. The amplitude of the pulses was chosen such that it was three times the threshold amplitude for that particular pulse duration. In order to determine the threshold amplitude, a strength-duration curve was constructed from 3 additional experiments. Both control and time-varying pulse experiments were performed in the same animals.

F. Data Acquisition and Analysis

EERs were recorded for all the probe pulses in both control and time-varying pulse experiments. The most sensitive response region within the SC was determined and EERs were recorded from that region. Details about the determination of the most sensitive region were discussed in previous work [9]. Briefly, the recording electrode was moved in a grid pattern while a standard pulse was applied. The area that responds most robustly, on visual inspection, is deemed the most sensitive area.

G. Quantification of EERs

For all the probe pulses, the resulting EERs were recorded. The strength of the EER was calculated using equation 1.

$$\text{Signal\_Strength} = \sum_{i=0}^{N} \{X(T_i)\}^2 \qquad (1)$$

T_i

defines the time window within which the signal strength is calculated. It was the first 50 ms after the stimulus pulse was delivered. X (T_i) is the amplitude of the EER measured in $\mathcal{V}$ and N is the number of samples with the first 50ms. Stimulus artifact was excluded from this calculation. In order to illustrate how the strength of the varied over time, the strength of EERs from all the probe pulses were normalized to the strength of the EER from the first probe pulse. The normalized EER strength data from all the experiments were averaged and plotted against time. Standard error was indicated as a shaded region. In all the plots, the normalized EER strength data from 0-120s is from the stimulation phase and from 120-300s is from the recovery phase.

In order to determine statistical significance, we randomly selected 10 time points and analyzed the normalized EER strength data from probe pulses at those time points. The probe pulses were selected from both stimulation phase and recovery phase (5 time points in each phase). Student t-test was performed to determine statistical significance between normalized EER strength at a given time from control and time-varying experimental groups. A p-value < 0.05 was considered to indicate significance.

III. Results

Normalized EER strength was plotted against time to illustrate the effect of continuous electrical stimulation on the EER strength. Note the difference in the time scale (x-axis) when the continuous stimulation was on and after the continuous stimulation ended. The difference exists because the probe pulses were delivered at different frequencies during and after continuous electrical stimulation. Figures 2, 3 and 4 represent average data from all the eight experiments. In figure 2, the normalized signal strength is averaged across all the experiments and is plotted against time. The averaged signal strength data were compared between a control experiment and a time-varying pulse experiment conducted in the same animal. The shaded area in the plot indicates standard error.

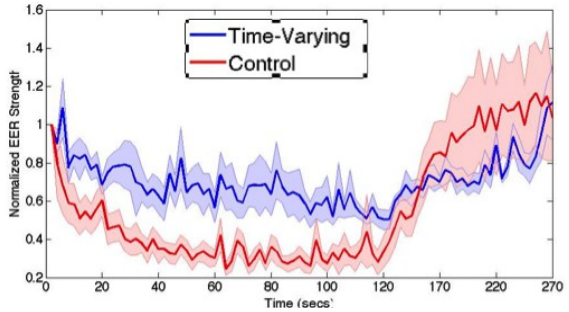

Figure 2. Average normalized EER strength is plotted against time for both control and time varying pulse experiments. The shaded regions represent standard error.

As is evident from figure 2, the strength of the EERs does not decrease as much when time-varying pulses were used. The strength of the EERs recover to pre-stimulation level after continuous stimulation ends in the case both time-varying pulses and control pulses.

We performed student t-test to determine statistical significance. Statistical analysis indicated that the average EER strength was significantly different between the control and experimental groups (p-values < 0.05). In particular, the normalized EER strength delivered by probe pulses in the experimental group during the stimulation phase was significantly higher than those delivered by probe pulses in the control group. During the recovery phase, the normalized EER strength data from the experimental group was statistically equivalent to that from the control group. This indicates that during the stimulation phase, the time-varying pulses do not desensitize the retina as much as the control pulses. In the recovery phase, the normalized EER strength recovers rapidly when both control and time-varying pulses were used for stimulation. The range of p-values for the data points is listed in Table I.

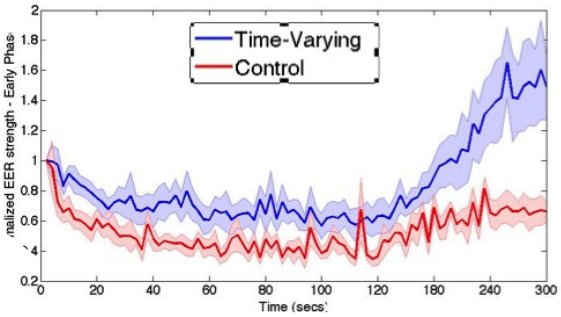

Figure 3. Early phase of the average normalized EER strength plotted against time for both control and time-varying pulse experiments. The shaded regions represent standard error.

A single EER was further divided into early (latency: 3 ~ 12 ms) and late (latency: 12 ~ 40 ms) response. In figure 3, the normalized EER strength for early phase was averaged across all experiments and compared between control and time-varying pulse experiments. Statistical analysis indicated that the average EER strength in the early phase was significantly different between the control and experimental groups (p-values < 0.05). In particular, the normalized EER strength delivered by probe pulses in the experimental group during the stimulation phase was significantly higher than those delivered by probe pulses in the control group. During the recovery phase, the normalized EER strength data from the experimental group was statistically equivalent to that from the control group. This indicates that during the stimulation phase, the time-varying pulses do not desensitize the early phase response as much when compared to control pulses. The recovery phase is statistically equivalent when control and time-varying pulses were used for stimulation. The p-value range is listed in Table I.

In figure 4, the normalized signal strength for late phase was compared between control and time-varying pulse experiments. The late phase response doesn't desensitize appreciably when both control and time-varying pulses were used for stimulation. Statistical analysis indicated that there is no statistical difference between normalized EER strength data from control and time-varying experimental groups (p-value listed in Table I).

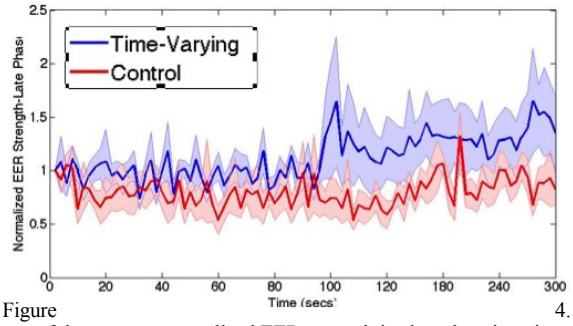

Figure 4. Late phase of the average normalized EER strength is plotted against time for both control and time-varying pulse experiments. The shaded regions represent standard error.

TABLE I. RANGE OF P-VALUES

	Stimulation phase p-value range	*Recovery phase p-value range*
Overall Normalized EER Strength	0.0006-0.0148	0.2061-0.3419
Early Phase: Normalized EER Strength	0.0002-0.0225	0.076- 0.184
Late Phase: Normalized EER Strength	0.0862-0.6518	0.0776-0.8936

IV. DISCUSSION

The results of the study exhibit that continuous epiretinal stimulation causes a depression in the EER strength in the SC over a short period of time. The strength of the EERs was observed to decline in the first 20-30s of continuous stimulation. This effect was more pronounced in the early component of the EERs compared to the late component. Freeman et al showed that continuous electrical stimulation causes suppression in ganglion cell responses [12]. Freeman et al also categorized ganglion cell responses into early and late phase responses. They showed that there is some desensitization in both the early and late phase components and that the early phase desensitization was not as pronounced as the late phase desensitization. Our results also indicate that both early and late phases undergo desensitization. However, our results indicate that early phase desensitization is more pronounced than late phase desensitization. We also showed that subsequent application of stimulus pulses does not cause any further decrease in the EER strength. Once the continuous stimulation is stopped, a relatively rapid recovery of EER is observed.

We investigated whether time-varying pulse trains limit desensitization. As shown in figure 2, desensitization is not as pronounced when time-varying pulse trains were used for epiretinal stimulation. We further divided the responses into early and late phases. The early phase represents the initial signal from the retina (to the SC). Thus, attenuation of the early phase indicates desensitization of the retina with continued pulsing. Time varying pulse trains maintain greater early phase EER signal strength, compared to constant pulse trains. In contrast, late phase EER signal strength diminishes less and the decrease is the same for time varying pulse trains and constant pulse trains. It is unclear if the late phase EER in this model is due to late activity generated in the retina or SC neurons.

V. CONCLUSION

Continuous electrical stimulation causes the retina to desensitize and in humans it leads to a reduction in the brightness of phosphenes and also increases threshold. We have developed an in vivo model of desensitization and presented a strategy that limits desensitization. We showed that time-varying pulses could be used to limit retinal desensitization.

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