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Interphase Gap Decreases Electrical Stimulation Threshold of Retinal Ganglion Cells

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***Abstract*—The most common electrical stimulation pulse used in retinal implants is a symmetric biphasic current pulse. Prior electrophysiological studies in peripheral nerve have shown that adding an interphase gap (IPG) between the two phases makes stimulation more efficient. We investigated the effect of IPG duration on retinal ganglion cell (RGC) electrical threshold. We used calcium imaging to measure the activity of RGCs in isolated retina in response to electrical stimulation. By varying IPG duration, we were able to examine the effect of duration on threshold. We further studied this effect by simulating RGC behavior with a Hodgkin-Huxley-type model. Our results indicate that the threshold for electrical activation of RGCs can be reduced by increasing the length of the IPG.**

I. Introduction

RETINITIS pigmentosa (RP) is a leading cause of blindness in adults. It is a genetic disorder affecting more than 1.5 million people worldwide [1]. The disease causes progressive loss of photoreceptors, the light-sensitive cells in the retina. Nearly 50 genes have been implicated in RP; a mutation in any one of them can cause the disease [2].

In healthy retina, visual information is carried from photoreceptors to retinal ganglion cells (RGCs). RGCs transmit the visual signals to the brain through the optic nerve. Once RP damages the photoreceptors, that visual information becomes lost.

While RP causes loss of photoreceptors, other retinal neurons remain largely unaffected. RGCs, for example, have been shown to survive at rates of 30-48% [3]. One treatment strategy for RP circumvents the problem of lost photoreceptors by electrically stimulating surviving RGCs. The so-called epiretinal prosthesis consists of a video camera that wirelessly transmits image data to a microelectrode array (MEA) implanted on the retina. Electrodes stimulate the RGCs in patterns that resemble the camera's images. Clinical trials with a 16-electrode prosthesis have demonstrated patients' abilities to detect objects, count them, and discriminate between object forms [4]. More recently, a 60-electrode device underwent clinical trials in 30 human subjects [5]. As of March 2011, this device has been approved for sale in Europe, making it the first commercially available retinal prosthesis in the world.

The most common pulse shape used in retinal implants is a symmetric biphasic current pulse. The biphasic nature enables charge-balanced pulses to be delivered, which prevents buildup of toxic byproducts that arise from electrochemical reactions at the electrode surface. A drawback to using biphasic pulses is that the trailing phase opposes the depolarization induced by the leading phase, thereby increasing the stimulus amplitude needed to evoke an action potential. Prior electrophysiological studies in peripheral nerve have shown that addition of an interphase gap (IPG) between the two phases reduces this effect [6]. Preliminary tests in retinal prosthesis subjects demonstrated that stimulation with IPGs lowered the amount of current needed to evoke perception. In this study, we used animal electrophysiology and a computational model to investigate the effect of IPG duration on RGC electrical threshold. Our findings indicate that RCG thresholds can be reduced by increasing the length of the IPG.

II. Methods

A. Animal Experiments

We used calcium imaging as a means to report RGC responses to electrical stimulation. Larval tiger salamander (*Ambystoma tigrinum*) RGCs were loaded with the fluorescent calcium indicator, Oregon Green BAPTA-1 dextran 10 kDa (Molecular Probes, Eugene, OR), using a retrograde loading technique developed in our lab [7]. The isolated wholemount retina was mounted on a porous membrane (Millipore, Billerica, MA) and placed ganglion cell-side-down on an MEA designed and fabricated by the authors (Fig. 1). Animals were handled in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Southern California.

Stimulation was performed with a 200 μm diameter transparent disc electrode made of indium-tin-oxide. Cathodic-first biphasic current pulses (460 μs/phase) were delivered to the retina while recording epifluorescence image series. Each stimulus consisted of a 30-pulse burst at 167 Hz. Burst stimuli were delivered 25 times on 2-second intervals and were repeated over ten stimulation amplitudes. Electrically evoked responses were detected by convolving

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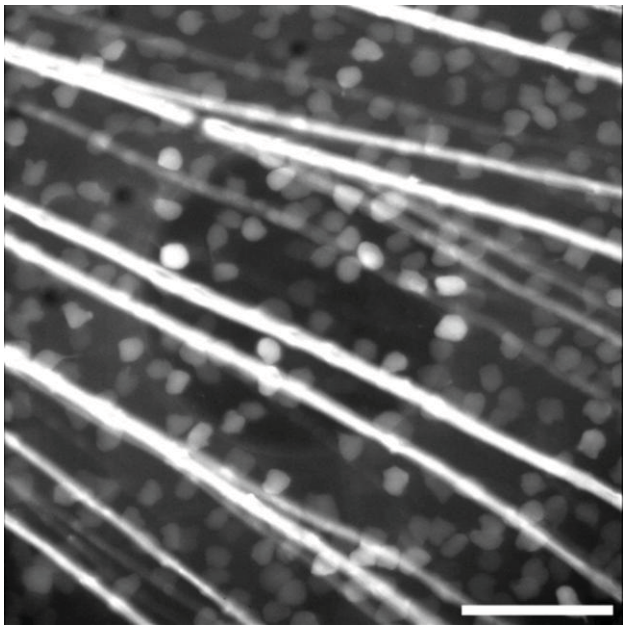

Fig. 1. Salamander retina loaded with calcium indicator and mounted on an MEA. Retinal ganglion cell bodies and axon bundles are clearly visible. A 200 µm diameter indium-tin-oxide electrode is centered in the field of view. Scale bar is 100 µm.

the fluorescence intensity of each cell body with a difference filter and identifying rapid changes in fluorescence temporally correlated with the stimuli (Fig. 2). A dose-response curve was generated for every RGC by plotting the fraction of the 25 stimuli that elicited a response at each amplitude. A sigmoidal function was fit to this curve, and threshold was defined as the stimulation amplitude that yielded a 50% response. All post-processing steps were performed automatically in MATLAB (MathWorks, Natick, MA).

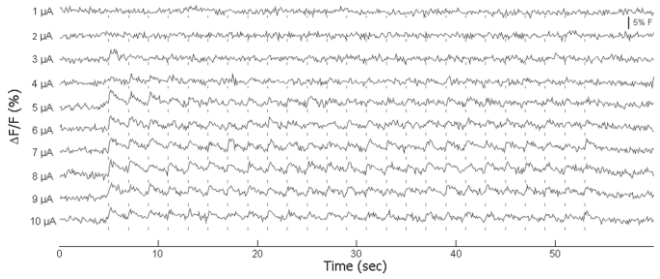

Fig. 2. For each RGC, the time derivative of fluorescence intensity is formed by convolution with a difference filter. As stimulation current is increased (downward), there are more responses to the 25 stimulus events. Threshold for this RGC was calculated to be 4.4 µA.

By varying the IPG duration, we were able to study the effect of duration on threshold. We tested seven different gap lengths ranging from 120 µs (26% of pulse width) to 1840 µs (400% of pulse width). Each cell's threshold at a particular gap length was compared to its threshold when no IPG was used. As a control, we ran two trials with no IPG in a single animal to investigate repeatability of the threshold measurements. In total, we examined thresholds of 303 RGCs from five salamanders.

B. Computational Model

We generated a Hodgkin-Huxley-type model to further study the effect of IPG on ganglion cell electrical threshold. Equations and parameters were taken from a prior physiology and modeling study of tiger salamander RGCs [8]. As shown in Fig. 3, the model incorporates four types of voltage-gated ion channels (Na^+, delayed rectifier K^+, A-type K^+, and L-type Ca^{2+}), as well as Ca^{2+}-activated K^+ channels. We used current clamp to simulate extracellular stimulation. Differential equations were solved in MATLAB using the forward Euler method with a time step of 0.01 ms.

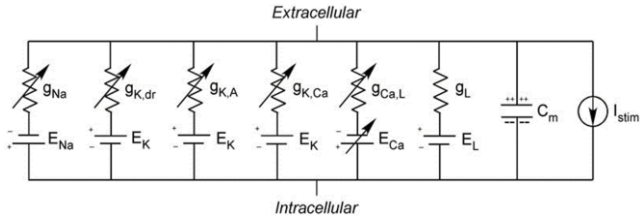

Fig. 3. Components of the Hodgkin-Huxley-type model include four types of voltage-gated ion channels (Na^+, delayed rectifier K^+, A-type K^+, and L-type Ca^{2+}), Ca^{2+}-activated K^+ channels, leak channels, a membrane capacitance, and a current stimulation source.

We set the model's stimulation parameters to match our experimental conditions as closely as possible. A burst of 30 pulses was delivered at 167 Hz. Pulse width was 460 µs/phase, and IPGs ranged from 120 µs to 1840 µs. Membrane potential was calculated in response to each stimulus burst. Action potentials were identified as peaks in the membrane potential that exceeded 20 mV. Threshold was defined as the minimum stimulation amplitude that caused at least 50% of the pulses in the burst to elicit action potentials. Fig. 4 shows the membrane voltage and intracellular calcium concentration, $[Ca^{2+}]_i$, of a model RGC computed in response to a stimulus burst at threshold. As was the case with our animal experiments, we compared threshold at a particular IPG length to threshold when no IPG was used.

III. Results

A. Animal Experiments

Fig. 5 summarizes the effect of IPG duration on RGC electrical threshold, for both our animal experiments and computational model. In the animal data, IPG durations 240 µs and longer caused a significant reduction in threshold versus no IPG ($p < 0.05$, Student's t-test). With an IPG duration equivalent to the pulse width (460 µs), RGC threshold was 21.1±13.9% (mean±SD, $p < 0.01$) lower than when no gap was used. Gap lengths longer than 460 µs further decreased threshold, but only marginally: With 920 µs, 1380 µs, and 1840 µs gaps, mean change in threshold fell only by an additional 3.8%. Gaps shorter than 460 µs were less effective at stimulating RGCs. For example, an IPG duration of 240 µs (52% of pulse width) lowered threshold by only 12.4±16.4% ($p < 0.05$) versus no gap.

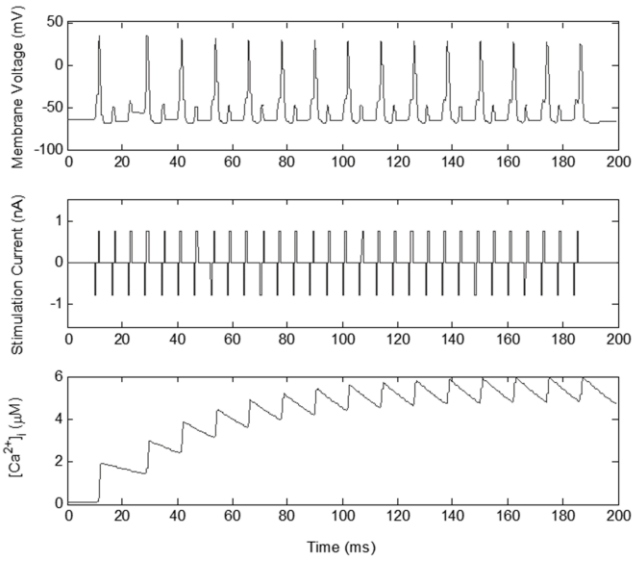

Fig. 4. Membrane voltage (top) and intracellular calcium concentration (bottom) of a model RGC in response to a stimulus burst (middle). Fifteen of the 30 stimuli in the burst evoked action potentials, a condition we defined as threshold.

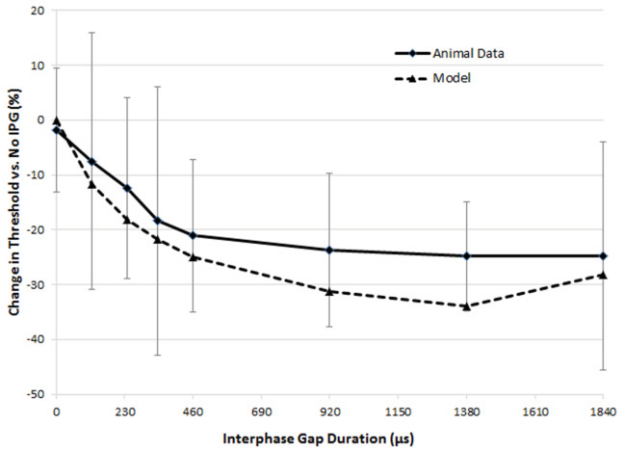

Fig. 5. Effect of interphase gap duration on retinal ganglion cell threshold. Error bars represent standard deviation.

Repeatability of the threshold measurements was examined by running two trials with no IPG in a single animal. RGC thresholds between these two trials varied by -1.8±11.3%, indicating that the threshold-lowering effects of IPGs are likely due to the IPGs themselves, rather than trial-to-trial variability. The standard deviation in repeatability provides an estimate for the noise in the measured threshold of each RGC. Its relatively large value may be a consequence of pooling data from multiple animals and RGC subclasses. Furthermore, some cells are larger than others and have different distributions of voltage-gated Na^+ channels [9].

B. Computational Model

The predictions from our model fit the experimental data remarkably well. As shown in Fig. 5, predicted change in threshold falls within the standard deviation of the animal data for every IPG duration we studied. Adding 10% uniformly distributed pseudorandom noise to the model parameters had minimal effect on predicted threshold changes.

The model revealed an interesting behavior that may have implications for high-frequency stimulation with long IPGs. As shown in Fig. 5, 1380 μs pulses were more effective at stimulating the model RGC than 1840 μs pulses. We hypothesized that this effect was due to interactions between successive pulses; as IPG becomes longer, pulses become closer in time. This causes a blocking effect, in which the anodic phase of one pulse raises the threshold of action potential initiation for the next pulse. It has been shown previously that this block likely results from the anodic phase depolarizing the cell membrane, which causes inactivation of Na^+ channels [10].

To test our hypothesis, we solved the model using lower stimulation frequencies (125, 100, and 83 Hz), spacing pulses farther apart in increments of 2 ms. As expected, we found that the blocking effect is only present at longer IPG lengths and is reduced as stimulation frequency decreases. Results are summarized in Fig. 6.

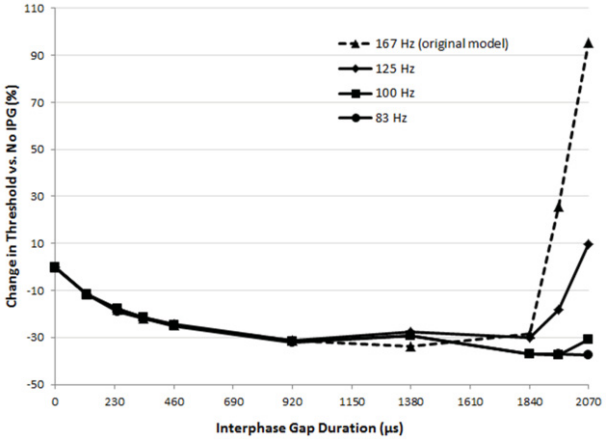

Fig. 6. Modeling the effect of stimulus frequency and IPG length on anodic block. The blocking effect is most pronounced when pulses are spaced close together (i.e., at high frequencies and long IPG durations).

IV. Discussion

Our results indicate that the threshold for electrical activation of RGCs can be reduced by increasing the length of the IPG. We found that once the IPG duration exceeds a certain length (460 μs in our experiments), change in threshold further decreases only marginally. This is likely due to the timing of the trailing phase of the stimulation pulse and where it occurs in time with respect to the rising phase of the action potential.

The leading phase of the stimulation pulse depolarizes the cell, which may lead to an action potential. The trailing phase opposes that depolarization and may prevent the action potential from firing. At a certain point during the rising phase of the action potential, positive feedback from voltage-gated Na^+ channels causes the action potential to become self-sustaining. If the trailing phase of the

stimulation pulse occurs before this happens, it can cause the action potential to cease. If the trailing phase occurs after the action potential is self-sustaining, it will continue firing.

While our pooled animal data shows a trend that matches the model predictions, results from individual animal experiments were less consistent. In cases where data from a single animal did not follow the trend, change in IPG threshold versus no gap was usually not significant. This is likely due to the sample size of RGCs being too small, as change in threshold became significant when pooling data from multiple animals.

Our results suggest a means for more effective stimulation in human subjects fitted with retinal prostheses. Use of IPGs would decrease power consumption of the devices and increase safety of stimulation. In the future, we plan to compare the effects of IPG duration in human subjects to the effects observed in our animal data and computer simulations.

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