In Vivo Study of Response Threshold in Retinal Degenerate Model at Different Degenerate Stages

L. H. Chan, A. Ray, B. B. Thomas, M. S. Humayun, and J. D. Weiland, Senior Member, IEEE

Abstract—Retinal prostheses are being developed to apply electrical stimulation to the retina in order to restore vision of individuals who suffer from diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). Various electrical stimulus parameters have been extensively studied in both experimental and clinical settings. Both electrophysiological and psychophysical results have shown that outer retina disease exhibit higher stimulus threshold in one degenerate group versus the control group. Fewer studies have been conducted to investigate the change in threshold currents as a function of different degenerate stages. We propose to study the electrophysiological change in degenerate rat retinas by using an in vivo recording method. We recorded retinal-driven superior colliculus cells response in two control groups and four degenerate groups. Current pulses of seven different stimulus pulse durations were applied to the retinas to obtain strength duration curve per group. Preliminary results showed that for the postnatal (P) day 90 and 180 degenerate groups, threshold currents were not significantly different from the normal control group (P90 and P250). For P300 degenerate group, the threshold currents progressively increased. For P760 degenerate group, threshold currents were significantly elevated across all the stimulus pulse durations tested. Charge densities calculated for P760 degenerate group exceeded the safe limit of the stimulating electrode. Cell morphology in all control and degenerate groups is still under investigation for a correlation study.

I. INTRODUCTION

Two most prevalent outer retinal diseases are retinitis pigmentosa and age-related macular degeneration. The worldwide prevalence of RP is about 1:4000 for a total of more than 1 million affected individuals [1]. It is a hereditary disorder characterized by dysfunction and loss of photoreceptors and retinal pigment epithelium. AMD affects between 20 and 25 million people worldwide [2], primarily individuals over 65. Existing therapies to slow the progression of AMD include macular translocation surgery [3], photodynamic therapy [4] and intravitreal injection of antiangiogenic drugs [5]. However, none of these approaches can restore the lost functionality of photoreceptors. There is no clinically accepted treatment for RP. Potential treatments of RP include gene therapy [6], stem cells [7], and RPE and retinal transplantation [8]. In recent years, retinal prostheses has become a promising approach for RP and AMD treatments and several groups worldwide have conducted clinical trials [9, 10].

Many studies have demonstrated that electrical stimulation of neurons in the visual pathway produces the perception of light. In an experiment involving nine patients with either RP or AMD, the retina was stimulated with multiple electrodes of an electrode array [9]. More recently, chronic retinal stimulation in 4 RP patients has shown that simple patterns, such as bar and cross, originated via activation of electrodes can be distinguished by the patients [11]. Other studies also suggest a correlation between degeneration state and stimulus threshold. Subjects with vision deficiency due to RP and AMD exhibited higher perceptual thresholds than the normal sighted subjects [12]. Several animal studies also confirmed lower threshold values in healthier retinas compared to degenerate retinas [13, 14]. However, these studies do not show a qualitative connection between the degenerate state and the threshold. Which degenerate state will cause an increase in the threshold? Which retinal cells or remodeled synaptic connectivity will give rise to threshold changes due to degeneration? Other anatomical studies have shown that progressive inner retina remodeling occurs in response to photoreceptor loss [15, 16]. These studies showed that deafferentation activates remodeling which starts by subtle changes in neuronal structure and alter by large scale reorganization. However, clinical studies cannot include retinal histology, and also studies on the electrophysiological properties of degenerate retina are few. Here, we studied the change of response threshold to the extent of retinal degeneration. Our results indicate that an increase in electrophysiological threshold occurs with age. We will correlate the electrophysiology data that we have collected to the morphology data that is currently under investigation. By understanding the qualitative relationship between the response threshold and the degenerate state, optimal electrical stimulation could be applied to the degenerate retina for an effective stimulation in retinal prostheses development.
II. MATERIAL AND METHODS

A. Animal Model and Surgical Procedures

Epiretinal electrical stimulation was performed in wild type pigmented rats (Copenhagen, n=3, P90; n=3, P230) and S334ter-line-3 RD rats (n=3, P90; n=3, P180; n=5, P300; n=3, P760). S334ter is a transgenic rat model which inherits forms of rhodopsin truncations [15] and possesses genetic mutations comparable to human autosomal dominant RP. Line-3 expresses retinal degeneration at a faster rate, compared to the other 4 lines. The transgenic rats were bred in house by crossing between pigmented Copenhagen rats and line-3 homozygous albino rats provided by Dr. Matthew LaVail, SF. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) in University of Southern California.

Rats were anesthetized by intraperitoneal injection of ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) and placed into a stereotoxic device with gas inhalant anesthetic (2% sevoflurane in 100% O₂). Craniotomy (5 mm A-P and 3 mm D-V) was performed next to lambda. The right side of the superior colliculus (SC) was exposed after cortex aspiration using an electric vacuum pump.

B. Microelectrode Insertion and Stimulation

A flat concentric bipolar stimulating Pt/Ir electrode (FHC Inc., ME) was inserted in the eye through a sclerotomy and positioned on the central part of the retina. The inner pole (diameter, 75 µm) of the stimulating electrode acted as the working electrode and the outer cannula (diameter, 300 µm) acted as the return electrode. A constant current source was triggered by an analog pulse from a stimulus system (DataWave Technologies, CO). Charge-balanced, biphasic current (1-100 µA) with seven different pulse durations (0.1, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0 ms) were applied. The inter phase interval was 100 µs. The proximity of the stimulating electrode to the retina was indirectly estimated by monitoring the electrical impedance. Our pilot studies have shown that electrode impedance of 6.5-8 kΩ (at a high frequency) results in a low threshold and focal SC response without mechanically damaging the retina. Impedance was monitored and measured by an impedance spectroscopy (Gamry Instruments, PA).

C. Electrophysiological Recording

Retinal input to SC is densest in the stratum griseum superficial (SGS), which exclusively processes visual information [17]. Multi-unit or single unit responses were recorded in the SGS layer. Recording electrode (FHC Inc., ME, 8-10 MΩ) was penetrated vertically at a depth of 100 to 200 µm from the SC surface. Responses were amplified 20X in a head stage unit, further amplified 50X, and then high pass filtered at 0.15-9 kHz (Plexon Inc., TX). The amplified and filtered data was digitized at 40 kHz using a data acquisition system (DataWave Technologies, CO) and stored.

D. Data Analysis and Statistical Method

Stored data was processed off-line using a custom program (SciWorks, DataWave Technologies, CO). Raster plots (50 trials) and post-stimulus time histograms (PSTH, bin size 1 ms) of the retinal-driven superior colliculus cell response was obtained. Signal-to-noise ratio was chosen to be 5:1 for good response selection. Threshold currents were determined when responses were obtained 75% of the time in 50 trials. SAS V9.1 programming language (SAS Inst, Cary NC) was used for all statistical analyses. Independent samples t-tests were used to compare means between the two normal groups. Analysis of variance was used to test for the overall significance between groups, with multiple comparison t-tests with Bonferroni adjusted p-values for pairwise comparisons. The accepted level of significance for all tests was p<0.05.

III. RESULTS

A. Retinal-driven Superior Colliculus Cell Response to Electrical stimulation in Degenerate Retina

Epiretinal stimulation with seven different pulse durations in the range of 0.1 to 2.0 ms consistently evoked retinal-driven responses from the contralateral SC. All recorded positions from the SC surface of the electrically evoked responses corresponded to the retinal regions in which the retinal ganglion cells were excited electrically. The stimulating electrode was positioned in the ventral-temporal quadrant of the left retina, near the optic disc. Retinal-driven SC responses were located in the medial-rostral quadrant of the contralateral SC due to the orderly retinocollicular projections [18].

![Fig. 1. Example of spikes recorded from SGS neurons to electrical stimulation at 0.3 ms pulse duration. A: No response below threshold current (6 µA). B: Response at current 7 µA. C: Raster plot of 50 trials and PSTH at 7 µA, bin size 1ms. Scale bar = 200 µV (vertical), 20 ms (horizontal). Arrow represents stimulus artifact.](image-url)

Electrically-evoked responses are shown in Fig. 1. Threshold current was determined when response appeared 75% of the time in all trials with 5:1 signal-to-noise ratio. Early latencies of retinal-driven SC spikes were about 8 ~ 10 ms.
B. Strength Duration Curves of Electrical Stimulation in Control and Degenerate Retina

Current pulse durations were changed from 0.1 to 2.0 ms with test current levels ranging from 1 to 100 µA. Threshold current was coarsely estimated using a single biphasic pulse per current level. Three current levels (1 or 5 µA resolution) were then chosen for finer estimation. Final test of threshold current was determined by repeating the biphasic current pulse 50 times with the response appearing 75% of the time in all trials.

Strength duration curves are plotted in age matched group, except for the control group P180 and degenerate group P760, in Fig 2. Strength duration curves measured degree of responsiveness of the test tissue structure. Electrically elicited excitation is a function of the amount of the charge injected into the tissue. As pulse duration increases, less current is needed to excite the tissue.

Charge densities of the control and degenerate groups were also calculated as a function of pulse durations. Strength duration curves are plotted as charge densities in Fig 3. There was no significant difference in the threshold charge densities between control groups P90 and P230 (p>0.05). There was no significant difference between degenerate groups P90, P180 and P300 (p>0.05). There was a progressive elevation of threshold charge density at degenerate group P300. However, a significant difference was observed between degenerate group P760 and the remaining degenerate groups (p<0.05) as well as the control groups (p<0.05). Asterisk represents the significant difference between P760 degenerate group and the remaining control and degenerate groups in Fig 2 and Fig 3.

All applied charge densities were within the safe limit of the stimulating electrode (Pt/Ir), 0.35 mC/cm², except for those applied in degenerate group P760.

Our preliminary results showed that progressive elevation of threshold occurred at degenerate group P300 and significant elevation of threshold currents and charge densities occurred at degenerate group P760 for all tested pulse durations.

IV. DISCUSSION

We observed an increase in threshold current in the degenerate group compared to control group which has been also shown in other clinical and physiological studies [12-14]. Most of the physiological studies have been done in rd1 mouse in vitro [13, 14]. O’Hearn et. al. reported that the outer segments were completely degenerated in 8-12 week rd1 mouse, using hematoxylin and eosin stain in rd1 mouse retinas. They examined thresholds in the degenerate retina at one age group only and showed a higher threshold current. Ye et. al. reported that evoked ganglion cell responses were rarely observed in rd/rd1 mouse retina when the same stimulus evoked ganglion cell responses in normal mouse retina. This study did not mention the age or the stage of the degenerate retina. Here, we studied the change in threshold currents and charge densities as a function of different degenerate stages of rat retina.

The charge densities were within the safe limit of the Pt/Ir stimulating electrode in the degenerate group P90, P180 and P300. In degenerate group P760, the charge densities (>0.7 mC/cm²) were above the safe limit of the stimulating electrode (0.35 mC/cm²) for all tested pulse durations. (Please note that although a Pt/Ir stimulating electrode was used in our experiment, it carried the same charge density as a Pt stimulating electrode because the coating of Iridium is mainly used to enhance the mechanical stability of the electrode. It plays a minor role in contributing to the electrochemical property of the electrode.) This suggests that for stimulating severely degenerated and remodeled retinas with microelectrodes may require charge densities higher than what can be supported safely by platinum. A large number of densely packed electrodes are being developed to provide a high acuity image. It requires individual electrodes to safely inject a large amount of charge to the degenerate retina. Therefore, a material which possesses a higher charge capacity may need to be developed to safely stimulate degenerate and remodeled retina.

We performed experiments in an in vivo setting because there was little effect of the stimulus artifact on the
responses. In general, in vitro electrophysiological threshold is lower than the in vivo electrophysiological and psychophysical thresholds [19-21]. Therefore, the threshold currents obtained in later stages of the visual pathway are more similar to perceptual threshold currents in vivo setting. However, care needs to be taken during in vivo recording on the SC surface. The recording electrode needs to be positioned on the SGS layer (100 – 200 μm) as most of the retinal ganglion cell axons terminate in this layer.

At present, the reason behind the elevation of threshold in degenerate group P760 has not been investigated. Only gross retinal morphologies have been compared that have provided limited information about the morphological contribution to the physiological changes [13]. By examining the detailed morphology in correlation with electrophysiological changes, effectiveness of electrical stimulation in the degenerate retina, and knowledge about the contribution of remodeled structure to threshold change can be identified. Low stimulation threshold will enable an efficient design for retinal prosthesis. This characteristic will also help to determine how much power will be necessary for the development of the retinal prosthesis.

V. CONCLUSION

We have performed a detailed study on the change of response threshold at different age groups of degenerate rat retinas. Our preliminary results showed that at degenerate group P90 and P180, the threshold current has no obvious change compared to the control group P90 and P230, whereas at degenerate group P300, progressive elevation of threshold current occurred. At degenerate group P760, significant elevation of threshold current occurred in the seven tested pulse durations. Detailed morphology of all control and degenerate retinas is under investigation.

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REFERENCES