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Physiological Response of Normal and RD Mouse Retinal Ganglion Cells to Electrical Stimulation

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Abstract—**The epiretinal prosthesis aims to restore functional vision by stimulating electrically the retinal ganglion cells (RGCs) in patients afflicted with degenerative diseases that affect the photoreceptors, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). As degeneration progresses, photoreceptor death is followed by pronounced remodeling and rewiring of remaining inner retinal cells. Despite the loss of rods and cones, a considerable population of RGCs remain receptive to prosthetic stimulation. To stimulate effectively a localized population of RGCs, an improved understanding of the anatomical and physiological properties of these cells is required. Additionally, possible alterations in electrical excitability, produced by the effects of retinal degeneration, needs to be assessed. This study investigates the effect of RGC soma size on the threshold for action potential generation in normal and rd mice and its implications for the rescue of visual function.**

I. Introduction

RETINITIS pigmentosa (RP) and age-related macular degeneration (AMD) are two of the most prevalent outer retinal diseases for which there are currently no known cures. These diseases primarily affect the photoreceptors, the class of neurons comprising the sensory retina, loss of these cells can ultimately lead to blindness. Despite the death of rods and cones, numerous studies have shown that inner retinal cells remain; however, the functional and structural integrity of these remaining cells remains poorly understood.

As the photoreceptors die, they no longer make the appropriate structural contacts with bipolar and horizontal cells and they also cease to provide synaptic inputs to these cells; consequently, bipolar cells retract their dendritic processes which results in pronounced structural changes for these cells [1]-[3]. In addition, migration of the remaining cells (amacrine, bipolar, horizontal, ganglion) occurs throughout the retina at very late stages of degeneration, and their processes begin to make improper connections with other cells.

Retinal ganglion cells (RGCs) receive excitatory and inhibitory input from bipolar and amacrine cells and send this visual information, encoded in trains of action potentials, along their axons to higher visual centers in the brain. Rewiring within the inner retina that is induced by degeneration can impact significantly synaptic inputs to ganglion cells; this can ultimately reduce the visual information encoded by RGCs. However, several studies have shown that, despite degeneration, an appreciable number of ganglion cells remain receptive to electrical stimulation with a prosthesis [4], [5].

An epiretinal prosthesis aims to restore some functional vision in patients affected by RP or AMD by stimulating electrically these remaining ganglion cells. This device directly stimulates RGCs, thus bypassing the inner retinal circuitry, and its efficacy depends on the functional viability and structural integrity of these cells. A comprehensive structural assessment of RGC morphology in a mouse model of retinal degeneration (rd10) demonstrated no anatomical differences between retinal degenerate mice and normal controls up to 9 months of age [6]. Analysis of another degeneration mouse model (rd1) showed intrinsic firing properties of RGCs were preserved despite constant oscillatory synaptic input to these cells [7]. Although numerous studies show that the retinal circuitry is significantly affected during degeneration, these results are promising as they demonstrate that, despite varying degrees of degeneration, retinal ganglion cells appear to be functionally receptive to electrical stimulation.

The aim of this study was to investigate the effect of ganglion cell soma size on the electrical threshold for stimulation in normal and retinal degenerate mice. Several studies have shown relationships between spiking threshold and a cell's structural properties, such as dendritic field size and axon diameter [8], [12]. Additionally, a positive correlation between axon diameter and soma size has been identified for several species [10], [11]. We used the rd10 mouse as our model of retinal degeneration. This particular model exhibits a slower progression of the disease than other more aggressive models, such as the rd1 mouse, and more closely mimics the clinical progression of RP. In this study, we want to assess the extent to which soma size affects the electrical excitability of RGCs and also determine if this relationship is altered during retinal degeneration. This study is part of an ongoing investigation to assess physiological differences between the normal and degenerate retina, and potential implications for future retinal

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prostheses.

II. Methods

A. Animal Model

In this study, we used wildtype and rd10 mice purchased from Jackson Laboratories (Bar Harbor, Maine). Both strains of mice were bred on the same C57BL/6 background. The rd10 mouse carries a missense mutation on the gene encoding for the PDE-β subunit, which is part of the phototransduction cascade [13]. In this particular mouse model of degeneration, peak photoreceptor death occurs around P21 with almost complete loss of rods and cones by the end of 2 months. The rd10 mutation is an autosomal recessive form of RP (arRP) which represents about 50-60% of all clinical cases of retinitis pigmentosa [14].

B. Retinal Preparation and Physiological Recordings

C57BL/6 and rd10 mice ranging from 6-10 weeks in age were euthanized in accordance with protocols approved by the IACUC of the University of Southern California. A 1 mm x 1 mm section was mounted onto Millipore filter paper with the ganglion cell side facing up as shown in Fig. 1a. The retina was superfused with heated and oxygenated Ames media (35-37^{o}C; 95% O_2,5% CO_2) at a rate of 4-5 ml/min. An empty glass pipette was used to carefully remove a section of the inner limiting membrane (ILM) to expose several RGC bodies for electrophysiological recordings. The ILM in rd10 retina was more difficult to remove so a weighted horseshoe was used to secure the retina. Patch clamp electrodes were filled with K-aspartate internal solution and had open tip resistances ranging from 6-10 MΩ. All recordings were done in whole-cell mode which allowed us to record changes in the membrane potential in response to external electrical stimulation.

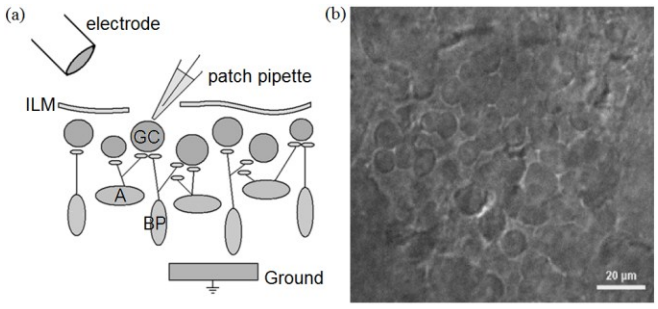

Fig. 1. (a) Schematic showing cross-section of retinal preparation. ILM – inner limiting membrane, GC – ganglion cell, A – amacrine, BP – bipolar. (b) Infrared image of RGCs using a 40x water-immersion objective.

C. Visualization and Measurement of RGC Somas

A Nikon 40x water-immersion objective (0.75 NA) was used to visualize cells under infrared (IR) illumination (Fig. 1b). Targeted ganglion cell somas typically ranged from 10-20 µm in diameter. Prior to recording, the targeted cell was identified and the length of the soma was measured along its major and minor axes; IR images using Nikon software were also taken for each cell.

D. Extracellular Electrode

The external electrode used for stimulation was a 75 µm diameter Pt-Ir disk electrode that was positioned ~50-60 µm away from the center of the RGC cell soma (x-y plane) and 50 µm away along the z-axis. The ground electrode was placed behind the retina on the photoreceptor side while the extracellular electrode was positioned above the ganglion cell layer (Fig. 1a); the placement of the ground was chosen to maximize the amount of current flowing through the tissue rather than shunting through solution to ground.

E. Stimulus Parameters

Multi-Channel Systems (Germany) stimulus software was used to deliver current pulses through the external electrode. Charge-balanced biphasic current pulses (cathodic-phase first) were delivered at a frequency of 10 Hz (interpulse period = 100 ms).

Amplitudes for current stimuli ranged from 5 to 50 µA and pulse duration was kept constant at 500 µs/phase. After breaking into the cell and establishing a high resistance seal, resting membrane potential and spontaneous spike activity were recorded for several minutes; during this time, the internal solution dialyzed the cell to ensure resting potential was stabilized prior to delivering any stimuli. Threshold for each cell was defined as the current level at which a spike was elicited in at least 50% of delivered pulses. An elicited action potential (spike) was defined as occurring within 2 ms of the simulus onset.

III. Results

Baseline activity was recorded to observe each cell's spontaneous firing rate. Fig 2 shows the spontaneous spike rates for a representative wildtype and rd10 RGC.

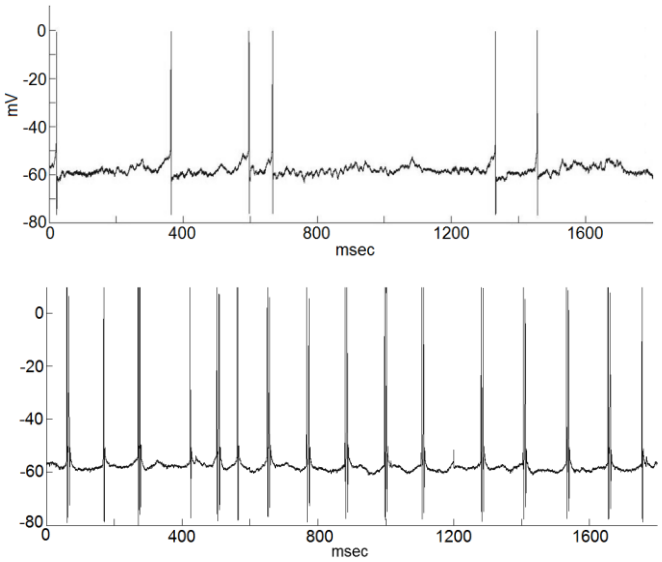

Fig. 2. Spontaneous spike activity for a wildtype (top) and rd10 (bottom) retinal ganglion cell.

A majority of wt RGCs displayed very low frequency spontaneous spiking. Although some rd10 RGCs also displayed low frequency firing rates, there were several rd10 ganglion cells that exhibited rhythmic baseline spiking activity (Fig 2).

Elicited action potentials at threshold for a wt and rd10 RGC are shown in Fig. 3, with soma diameters of 15.3 µm and 12.87 µm, respectively.

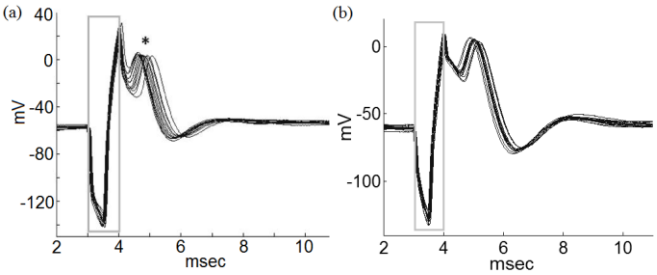

Fig. 3. Elicited spikes (indicated by asterisk) for (a) wildtype and (b) rd10 RGC. Gray boxes delineate duration of delivered biphasic pulse. Electrical thresholds were 25 µA (wt) and 10 µA (rd10).

Table 1 shows the measured experimental parameters for rd10 ganglion cells, which include electrical threshold, soma diameter, and resting membrane potential. Spontaneous firing rates for each cell were classified as high if greater than the stimulus pulse frequency (10 Hz).

TABLE I

Cell	Threshold (uA)	Diameter (um)	Vm (mV)	Spontaneous rate (Hz)
a	40	12.9	-54.240	0 (low)
b	10	12.87	-54.840	15.83 (high)
c	30	14.62	-54.500	5.83 (low)
d	52	13.3	-59.280	3.33 (low)
e	12	14.52	-61.829	12.5 (high)
f	12	15.07	-61.645	11.25 (high)
g	49	12.5	-69.255	0 (low)

Fig 4 shows the relationships in wt RGCs between threshold and soma size, and threshold and resting membrane potential (open circles, data previously shown [15]). The same relationships (threshold vs. soma size, threshold vs. resting potential) were plotted for the rd10 ganglion cells analyzed in this study (Fig. 4a,b).

Threshold values in the rd10 mice ranged from 10 to 50 µA; soma diameters for this study encompassed a much narrower range (12-15 µm) compared to measured soma sizes in wt mice.

IV. Discussion

Although some cells in the rd10 retinas exhibited rhythmic baseline spiking activity, this intrinsic behavior did not appear to interfere with the stimulus' ability to consistently elicit spikes in these cells. Action potentials in rd10 mice looked similar in appearance to action potentials elicited in

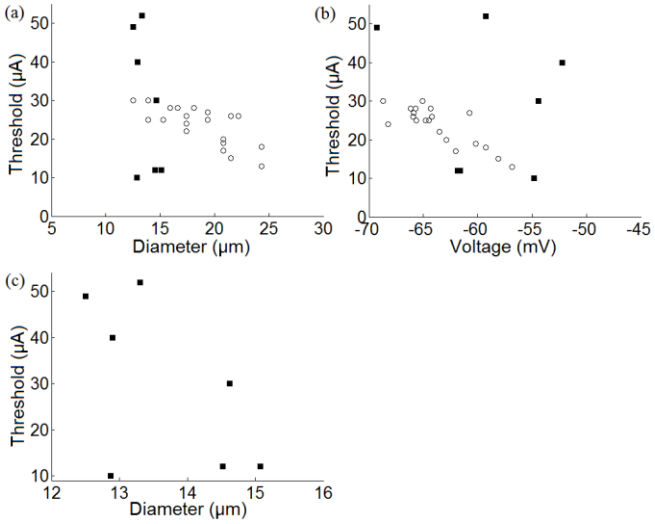

Fig.4. Plots showing (a) relationship between threshold and soma diameter and (b) relationship between threshold and resting membrane potential, V_m, in wt (open circle) and rd10 mice (filled square). (c) Rd10 data from (a) viewed over diameter range of 12-16 µm.

wt mice. The onset of the elicited action potentials were also similar between wt and rd10 mice (1-2 ms after stimulus onset).

In previous work on wt retina, we found that there appeared to be a correlation between electrical threshold and soma size, as well as between threshold and resting membrane potential. From a biophysical perspective, this relationship makes intuitive sense since a cell that has a slightly depolarized resting potential will be sitting closer to threshold, thus requiring less current to elicit a spike.

We did not observe these same relationships in rd10 retina (Fig. 4a,b). However, there are several factors that should be noted before making comparisons between wt and rd10 data. There was a much smaller sample size for the rd10 data compared to wt RGCs (7 rd10 cells) which may influence our ability to draw any conclusions about the relationships between the various parameters. Additionally, the range of soma diameters in rd10 mice was much narrower than those collected in wt mice (12-16 µm). Compared to wt data, the rd10 data showed significant variability in electrical threshold and spontaneous rate. The 3 cells that exhibited high spontaneous firing rates (Table 1) also had the lowest thresholds for excitation, although their resting membrane potentials did not appear more depolarized. This result suggests that higher spontaneous rates may increase sensitivity to extracellular stimulation, thus resulting in lower thresholds. Since we did not employ pharmacological agents for this study, we were unable to determine whether the source of higher spontaneous firing rates in rd10 retina was due to intrinsic RGC physiology or synaptic inputs to the ganglion cells.

Fig 4c shows the relationship between soma size and threshold in rd10 mice (fig. 4a) but plotted over the range of measured soma diameters, 12-16 µm. There appears to be a

trend showing larger diameter cells eliciting action potentials at lower extracellular current; however, the limited sample size prevents us from drawing any meaningful conclusions.

Our findings indicate that the resting membrane potential is related inversely to RGC soma size in wt retina, but this relationship may not necessarily exist in rd10 retina. As degeneration progresses, ganglion cells receive aberrant synaptic input from bipolar and amacrine cells which may influence the electrical excitability of RGCs. In addition to geometrical properties, other factors that influence the intrinsic excitability of RGCs may also need to be considered.

V. Conclusion

We find that the threshold to elicit spikes in wt retina is influenced by RGC soma size, but this relationship may not hold in rd10 mice. Currently, the sample size for rd10 mice may be too small to draw any conclusions about the relationship between threshold and soma size. Future studies will be conducted to determine whether these relationships truly exist in degenerate retina by accounting for a larger range of soma sizes and accounting for factors that influence intrinsic excitability in degenerate RGCs.

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