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Physiological Response of Mouse Retinal Ganglion Cells to Electrical Stimulation: Effect of Soma Size

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Abstract—**An epiretinal prosthesis aims to restore functional vision by stimulating electrically the retinal ganglion cells (RGCs) in patients affected by photoreceptor degenerative diseases, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). During retinal degeneration, photoreceptor death is followed by pronounced remodeling and rewiring of inner retinal cells. Despite these changes, a considerable population of RGCs remain receptive to prosthetic stimulation. To target selectively a localized subset of RGCs, an improved understanding of the anatomical and physiological properties of these cells is required. Additionally, potential alterations in electrical excitability produced by the retinal degeneration needs to be assessed. This study investigates the effect of RGC soma size on the threshold for action potential (spike) generation 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 common outer retinal diseases for which there are currently no known cures. These degenerative diseases primarily affect the photoreceptors, whose death ultimately causes total blindness. Although all rods and cones may die completely, numerous studies have shown that inner retinal cells remain; however, the functional and structural integrity of these remaining cells remains poorly understood.

Significant rewiring and remodeling as well as individual structural changes in inner retinal cells have been observed for various animal models and stages of degeneration [1]-[3]. As the photoreceptors die, they no longer provide synaptic inputs to bipolar and horizontal cells; consequently, bipolar cells retract their dendritic processes resulting in pronounced structural changes for these cells. In addition, migration of bipolar, horizontal, and amacrine cell bodies occurs throughout the retina at very late stages of degeneration, and their processes begin to make improper connections with other cells.

Retinal ganglion cells receive input from inner retinal cells (bipolar and amacrine) and send this visual information, encoded in trains of action potentials, along their axons to higher visual centers in the brain. Inner retinal rewiring resulting from degeneration can impact significantly synaptic inputs to ganglion cells; this may ultimately reduce the visual information encoded by RGCs. However, an appreciable number of ganglion cells remain despite degeneration, which can be stimulated electrically by the retinal prosthesis, allowing some rescue of vision [4], [5].

An epiretinal prosthesis aims to restore some functional vision in blind patients by stimulating electrically the remaining ganglion cells. This device bypasses inner retinal processing by stimulating directly RGCs, and its effectiveness depends on the functional viability and structural integrity of this class of cells. An extensive anatomical assessment of RGC structure in a mouse model of retinal degeneration (rd10) demonstrated no difference in cell structure between retinal degenerate mice and normal controls up to 9 months of age [6]. Additionally, in another degeneration mouse model (rd1), intrinsic firing properties of RGCs were preserved despite oscillatory synaptic input to these cells [7]. The results are promising as they demonstrate that, despite varying degrees of degeneration, retinal ganglion cells appear to be functionally receptive to electrical stimulation.

In this study, we investigated the effect of RGC cell body size on the threshold for electrical stimulation in mouse retina. A previous observation by Fried, et al. found a correlation between spiking threshold and dendritic field size [8]. The mammalian retina contains ~13-17 classes of ganglion cells, each characterized by distinct morphological features [9] and furthermore, a positive correlation between RGC soma size and axon diameter has been identified for certain species [10], [11]. Additionally, observations seen during peripheral nerve fiber recruitment have shown that large diameter axons are recruited first to externally applied current [12].

The aim of this study was to assess the extent to which the size of RGC soma affected threshold for electrical stimulation. This study is part of an ongoing investigation to assess physiological differences between the normal and degenerate retina, and to determine how these differences might need to be accounted for in a retinal prosthesis.

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II. METHODS

A. Retinal Preparation and Physiological Recordings

C57BL/6 mice ranging from 6-10 weeks in age were dark-adapted for several hours and euthanized in accordance with protocols approved by the IACUC of the University of Southern California. A section of retina, approximately 1mm x 1mm, was mounted onto filter paper with the ganglion cell side facing up as shown in Fig. 1a. These retinas were superfused with heated Ames media (35-37^{o}C) at a rate of 4-5 ml/min, and a glass pipette was used to carefully tear away a portion of the inner limiting membrane (ILM) to expose several retinal ganglion cell bodies for electrophysiological recordings. Patch clamp electrodes were filled with K-aspartate internal solution and had open tip resistances ranging from 8-10 MΩ. Whole-cell current-clamp recordings were made from the exposed ganglion cell somas, allowing the membrane potential to be recorded while currents from an extracellular electrode were applied (see below).

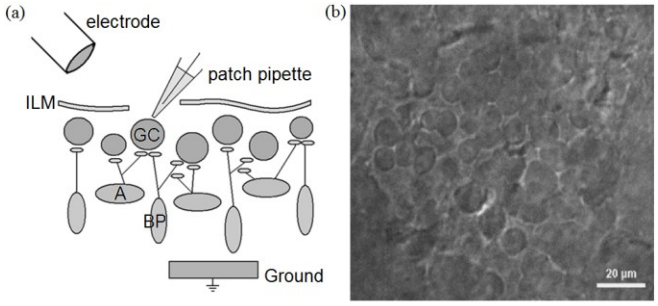

Fig. 1. (a) Diagram showing side-view of retinal preparation. ILM – inner limiting membrane, GC – ganglion cell, A – amacrine, BP – bipolar. (b) Infrared image of retinal ganglion cells taken with 40x objective.

B. Visualization and Measurement of RGC Somas

A Nikon 40x water-immersion objective (0.75 NA) was used to visualize cells under infrared (IR) illumination. Targeted ganglion cell somas typically ranged from 10-20 µm in diameter.

Prior to recording, the cell body was identified and the length of the soma was measured along its major and minor axes since these cell bodies were elliptical. Additionally, images for cells were captured using Nikon imaging software and measurements of the soma diameter along both axes were taken (Fig. 1b).

C. Extracellular Electrode

The electrode used for external stimulation was a Pt-Ir disk electrode with a diameter of 75 µm and 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.

D. Stimulus Parameters

Multi-Channel Systems (Germany) stimulus software was used to deliver current stimuli through the extracellular electrode. Biphasic current pulses (cathodic-phase first) were delivered at a frequency of 10 Hz (interpulse period = 100 ms); this frequency was appropriate since it was low enough to avoid depression of cellular activity which can occur with higher frequency stimulation.

Amplitudes for current stimuli ranged from 5 to 35 µA and pulse duration was kept constant at 500 µs/phase. Immediately after establishing the whole-cell recording, resting membrane potential and spontaneous spike activity were recorded prior to delivering stimuli. Threshold for each cell was defined as the current level at which a spike was elicited in at least 75% of trials where an electrical stimulus was delivered. An elicited action potential (spike) was defined as occurring within 2 ms of the simulus onset.

III. RESULTS

Baseline spike activity for each cell was measured prior to delivering electrical stimuli to distinguish between spikes elicited by electrical stimulation and spontaneous spikes. Fig 2 shows the spontaneous spike rate for a representative large diameter RGC. All recorded cells exhibited either no spontaneous spikes or a very low spike rate at rest.

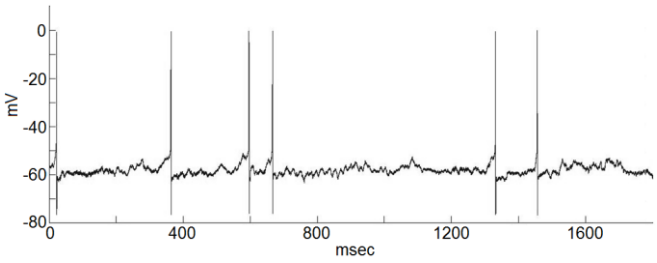

Fig. 2. Spontaneous spike activity for a large-diameter cell.

Elicited action potentials at threshold for a small diameter cell (15.3 µm) and large diameter cell (21.5 µm) are shown in Fig. 3. Thresholds for the small and large diameter cells were 25 µA and 15 µA, respectively. Resting membrane potential was -64.5 mV for the smaller cell and -58.1 mV for the larger cell (cells were from the same retinal preparation).

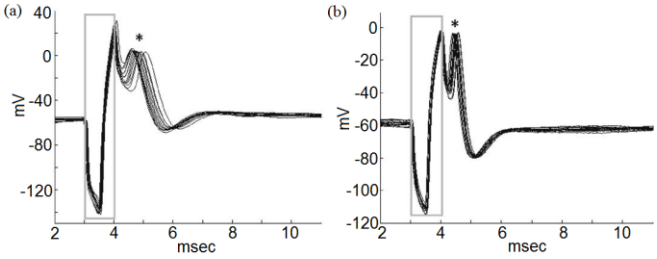

Fig. 3. Elicited spikes (indicated by asterisk) for (a) small diameter cell and (b) large diameter cell. Gray boxes delineate duration of delivered biphasic pulse.

Table 1 documents the derived experimental parameters

for the cells analyzed including values for electrical threshold, resting membrane potential, and soma diameter.

TABLE I

Cell	Threshold (uA)	Diameter (um)	Vm (mV)
a	25	15.3	-64.49
b	15	21.5	-58.1
c	25	13.9	-64.78
d	26	21.5	-70.1
e	26	22.2	-65.92
f	25	19.4	-65.64
g	17	20.8	-62.01
h	24	17.4	-68.24
i	28	16.6	-65.68
j	22	17.4	-63.53
k	20	20.8	-62.89
l	27	19.4	-65.89
m	30	12.5	-68.69
n	30	13.9	-65.08
o	26	17.4	-64.19
p	27	19.4	-60.77
q	18	24.3	-59.27
r	13	24.3	-56.86

Threshold values ranged from 13 µA to 30 µA, soma diameters from 12.5 µm to 24.3 µm, and resting membrane potential (V_m) from -70.1 mV to -56.9 mV. The relationships between threshold vs. soma diameter and threshold vs. V_m are shown in Fig. 4.

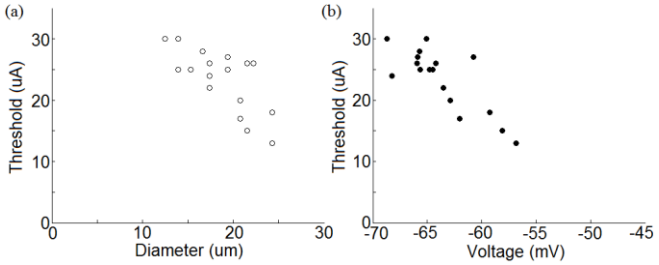

Fig.4. Plots showing relationship between (a) threshold and soma diameter and (b) threshold and resting membrane potential, V_m.

IV. Discussion

Cells used in this study displayed low levels of spontaneous spiking in baseline measurements. Cells with a higher spontaneous spike rate (> 20-30 Hz) would make it difficult to distinguish between elicited and spontaneous spikes, and were thus excluded. Action potentials elicited at threshold for each cell occurred at ~ 1-2 ms following the stimulus and were reproducible in amplitude and duration.

The relationship between threshold and soma size demonstrates that larger diameter cells elicit action potentials at lower extracellular current amplitudes than smaller diameter cells (Fig. 4a). Measurements of the resting membrane potential also follow this trend, where more depolarized RGCs are excited sooner with a lower current threshold (Fig. 4b). These relationships may make intuitive sense in that lower stimulus currents would be needed to evoke a response in a cell with a resting membrane potential closer to the threshold for spike generation.

RGCs receive both inhibitory and excitatory synaptic input from bipolar and amacrine cells. In addition, there are many different classes of ganglion cells each with distinct morphological characteristics, including differences in soma size. If excitatory and inhibitory inputs to these cells are evenly distributed [13], we expect that smaller diameter RGCs with higher input impedences would display lower spike thresholds than for larger RGCs.

Our findings indicate that the resting membrane potential is related inversely to RGC soma size; it appears that larger diameter cells have lower spike thresholds than smaller cells. Such an organization might allow large cells to compensate for their geometry (low input impedance, high capacitance) by positioning their resting membrane potential closer to spike threshold. This scenario would create a balanced level of excitability for all cells independent of soma size.

V. Conclusion

We find that the threshold for spike generation in RGCs is related to the size of the cell's soma and their resting membrane potential. In particular, larger RGCs display a more depolarized resting membrane potential. We propose that such control of resting membrane potential based on soma size (i.e. cell's input impedence) allow RGCs to maintain fixed excitability. Future studies will also be conducted to determine whether these relationships remain in degenerate retina.

Acknowledgment

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